June 2009 *Volume 5 Issue 3*

Trade Science Inc.

Materials Science

An Indian Journal

Review

MSAIJ, 5(3), 2009 [277-287]

Diffusion cells for measuring skin permeation *in vitro*

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Received: 15th April, 2009 ; Accepted: 20th April, 2009

ABSTRACT

The assessment of percutaneous permeation of molecules is one of the main steps in the initial design and later in the evaluation of dermal drug delivery systems. The literature reports numerous ex vivo, in vitro and in vivo models used to determine drug skin permeation profiles and kinetic parameters, some studies focusing on the correlation of the data obtained using these models with the dermal absorption in humans. This paper reviews various in vitro skin models for study of permeation studies which can correlate to *in vivo* release.

INTRODUCTION

Traditional skin models from animals have been used for *in vitro* and *in vivo* studies. Practically, it would be advantageous to use human cadaver skin for permeation studies but, for most investigators, human cadaver skin is not readily available. Also, the skin samples are typically obtained from a variety of anatomical sites and after many different disease states, which might alter the percutaneous permeability of the drug[1].

Most permeation testing is performed using hairless mouse skin. However, other models are sometimes used including rat, guinea pig, rabbit and shed snake skin, artificial composite membranes, and, more recently, living skin equivalents[2-5]. Although there are many similar features between these models and human cadaver skin, no model has yet been tested that fully mimics the results obtained with human cadaver skin.

Hairless mouse skin

The permeability of other animal models presents a problem when extrapolating *in vitro* data to make dosing predictions. The hairless mouse is used predominantly because it is economical, attainable, easy to house and hairless[6]. However, the permeability and lipid composition of hairless mouse skin are very different to those found in human cadaver skin. Hairless mouse skin tends to be very thin with a small stratum corneum and the permeability of hairless mouse skin in some studies has been found to be 30-40-fold higher than human cadaver skin[7-9]. A recent report compared the permeation of three model drugs of varying lipophilicity (tamoxifen, hydrocortisone and caffeine) using human cadaver and hairless mouse skin[10]. All of the percutaneous permeability parameters obtained from the two skin models were significantly different (p, 0.05). All three drugs had a higher permeability through hairless mouse skin.

Pig skin

Weanling pig skin (i.e. skin from a pig that has recently been weaned) is recognized as the closest alternative to human cadaver skin in its permeability and

lipid composition. However, there are some slight structural differences between weanling pig and human skin, including bristles, more subcutaneous fat and less vasculature[11,12]. Gore et al. evaluated the permeability of tacrine (a centrally acting acetylcholine-esterase inhibitor) using pig skin and human cadaver skin. The data obtained for domestic pigs shows low intra and inter animal variability. In addition, the intrinsic permeability, partition coefficient and diffusivity of pig skin are very similar to those of human cadaver skin[13].

Living skin equivalents

Recently, the use of living skin equivalents and epidermal equivalents has become popular for permeation and *in vitro* toxicity studies[14]. The skin equivalents used for permeation testing are typically epidermal or full-thickness skin.

Full-thickness skin equivalents are composed of both dermal and epidermal tissues, with the dermis being constituted from a collagen matrix. The epidermal substitutes are composed mainly of a sheet of normal human keratinocytes that have been cultured at the air-liquid interface to insure proper development and terminal differentiation[15]. These skin equivalents have many advantages, including the ability to eliminate animal experimentation. Also, they use human skin cells, which provide skin properties similar to those found in native human skin. In this context, it is interesting that all of the lipids found in the native human skin are found in skin equivalents, but in reduced quantities15. These skin models have very diverse permeability characteristics depending on the tissue culture protocols. A study by Ogiso et al. examined drug penetration through a living skin equivalent, Wistar rat and human cadaver skin[16]. The penetrations of 5-fluorouracil, Indomethacin and Dexamethasone across the living skin equivalent were approximately 15-54 times higher than across human skin, with a significantly shorter lag time. For all of the drugs tested, the living skin equivalent also had a higher permeability than the Wistar rat skin. For example, with 5-fluorouracil, the flux across Wistar rat skin was 112.4 6 0.3 mg cm22 hr21, whereas that across the living skin equivalent was 199.9 6 17.4 mg cm22 hr21. A commonly used epidermal equivalent for permeability testing is EpiDerm™ (Matech, Boston, MA,USA).This consists of normal, human-derived epidermal keratinocytes that have been cultured to form a multilayered, highly differentiated model of the human epidermis. El-Kattan et al. evaluated the permeation of chlorhexidine digluconate (an antimicrobial agent used widely to disinfect skin burns) in two skin models (hairless mouse skin and EpiDerm™)[17,18]. The lag time for EpiDerm™ was close to zero whereas, for hairless mouse skin, it was ~16 h. The cumulative amount of chlorhexidine digluconate in the receptor phase after 50 h for EpiDerm was significantly higher than that of hairless mouse skin using a similar application method. El-Kattan et al. concluded that EpiDerm™ was more permeable to chlorhexidine digluconate than was hairless mouse skin. Polymeric membranes and other artificial membranes have also been used for transdermal experiments even though these membranes lack the complex histological structures present in the human skin[19]. These membranes showed higher permeation relative to animal and human skin models.

In vitro permeation methods

The use of invitro permeation studies to control drug permeation across the skin has seen extensive research in the past two decades. The permeation rate of the drug across the skin has been measured using several different kinds of *in vitro* skin permeation apparatus. A typical apparatus has three main components[20]. The first is the donor compartment, where the drug is applied uniformly. From the donor compartment, the drug passes through a permeation barrier or membrane (i.e. skin), which is the second compartment, and into the receptor solution, which is the third compartment. Properties of the receptor solution, such as temperature and buffer composition, can have a significant effect on drug permeation through the skin. Typically, physiological saline or a phosphate-buffered solution maintained at 37^0C is used. This will keep the skin surface at approximately 32^0C, which simulates the temperature of the human skin. Generally, antibiotics and preservatives are added to the receptor solution to prevent microbial growth, enzymatic degradation, and to stabilize the skin. Drug permeation across the skin is evaluated using different *in vitro* models. These include horizontal-type skin permeation system, Franz diffusion cell and the flow-through diffusion cell (discussed below).

Horizontal-type skin permeation system

This has been widely used for the evaluation of drug permeation across skin. The cell is divided into receptor and donor compartments with a low solution volume (3.5 ml) for each compartment and a small membrane area (0.64 cm2).They are continuously stirred by a matched set of star-head magnets, which are rotated at a speed of 600 rpm. The system is controlled by circulated thermostated water through a water jacket surrounding the two compartments[21].

Franz diffusion cell

The cell is composed of two compartments: donor and receptor. The receptor compartment has a volume of 5-12 ml and an effective surface area of 1.0-5.0 cm^2.The diffusion buffer is continuously stirred at 600 rpm by a magnetic bar. The temperature in the bulk of the solution is maintained by circulating thermostated water through a water jacket that surrounds the receptor compartment[22,23].

Flow-through diffusion cells

Flow-through diffusion cells have the advantage that they can be used when the drug has lower solubility in the receptor compartment. In addition, these cells can be fully automated and connected directly to HPLC. They have a large-capacity donor chamber to allow appropriate loading of the applied compound and a low volume (0.3 ml) receiving chamber that ensures rapid removal of penetrant at relatively low (1.5 ml h21 or less) pumping rates. Furthermore, various sized support disks allow skin pieces as small as 4 mm in diameter to be used[24]. Choosing a system The percutaneous permeation of a drug across the skin is measured by collecting receptor fluid using either static or continuous flow-through collection. The solubility of the drug in the receptor fluid determines the diffusion-cell apparatus to be used. A static diffusion cell such as the Franz diffusion cell or horizontal-type skin permeation system can be used if the permeation of the drug across the skin will not result in a concentration of 10% of the maximal solubility in the receptor fluid. By contrast, if the drug has a low solubility in the receptor fluid, a flow-through apparatus is recommended. A further consideration is that the activity of hydrolytic enzymes released during tissue isolation might be higher with the static apparatus than the flow-through apparatus[25].

Percutaneous absorption

Animal skin

Researchers have for many years used skin excised from rodents and other animals. Skin from animals is much easier to obtain, the age and sex of the animals can be controlled, and large numbers of samples can easily be obtained. The primary problem with using rodent skin as a model for human skin is that it can overestimate permeation relative to that in human skin[26-29]. This problem is partly associated with the effects of hydration wherein prolonged (Generally2 4 h or longer) exposure of rodent skin (hairless mouse skin is the most notorious) to aqueous donor and receptor phases brings about a marked diminution in the barrier properties of the skin[15]. The primary difference between human skin and rodent skin is the lipid composition and organization in the stratum corneum. Some species of rodent skin may be useful in studying permeation of compounds if the total exposure time is I2 h or less. It has been suggested that hairless mouse skin can, when using limited amounts of acetone, be used to provide relevant guidelines for risk assessment calculations and bioavailability determinations[18] although this conclusion is limited to using acetone in small volumes to deposit a penetrant onto skin. Interestingly, many patents are issued based on data collected using rodent skin; it is possible that the utility of these patents may be limited in clinical practice.

Other models

A reliable model for human skin has been a highly desirable goal for a number of years. The goal remains elusive: nonetheless, significant advances are being made in the area of tissue culture. For example, human keratinocyte cultures grown at the air liquid interface have been found to develop substantial barrier properties to water diffusion[19], Reconstructed human epidermis has been used to examine the nitroglycerin and sucrose permeability[20]. Houk and Guy have reviewed the literature on various membrane models such as eggshell membranes, composites, laminates, zeolites, silastic and organic liquid membranes[21]. Reconstituted stratum corneum films have also been examined as a potential model for skin transport[22]. However, prepara-

tion of a reconstituted membrane using a surfactant to disaggregate stratum corneum into cells requires intact human skin, which could be used directly in permeability experiments. At present, the most common means to evaluate the *in vitro* permeability of skin involves t he use of excised human skin obtained during plastic surgery or cadavers and from common laboratory animals such as rodents.

Diffusion cells for measuring skin permeation *in vitro*

Key design and use parameters

A wide variety of diffusional systems have been developed for use with rate limiting membranes (e.g., excised skin from a laboratory animal or from a human). These diffusion cells generally have common elements: two chambers, one containing the active agent (donor vehicle) and the other containing a stirred receptor solution, separated by a piece of excised skin or other membrane..The cells are generally arranged in side by-side or vertical configurations. In the case of side-by-side chambers, both chambers should be mixed homogeneously. Mixing is most commonly accomplished by magnetic stir bars; however, adequate mixing in diffusion cells can be a problem in several types of diffusion cell design.

Controlling the temperature has been accomplished using water-jackets or simply submerging the entire cell assembly into a water bath. Temperature gradients can be introduced if the membrane flange is not properly heated; such temperature gradients could be exacerbated by systems with incomplete fluid agitation. However, small temperature variations will probably not significantly affect relative rates of penetration. A key concern in the use of diffusion cells is whether there is sufficient agitation to prevent local concentrations of drug and to minimize static diffusion boundary layers. The goal, as far as possible, is to maintain sink conditions during the experiment. It has been shown that as agitation in the receptor phase increases, the thickness of the unstirred boundary layer decreases: the permeation rate therefore increases[25,26]. Sufftcient agitation of the receptor fluid can minimize the unstirred boundary layers, thereby minimizing diffusional resistance. Under in vivo conditions, it is generally assumed that diffusional boundary layers in dermal capillaries are insignificant. The calculation of diffusion coefficients will include a contribution from the boundary layer barrier and hence result in an apparent diffusion coefficient. The problem of boundary layer effects can be exaggerated when relatively hydrophobic drugs and chemicals are studied *in vitro*. When full thickness rodent skins are used in in-vitro permeation experiments, the results indicate that permeation is underestimiated compared with that observed *in vivo*[27-29]. It has been hypothesized that compounds of low water solubility have low partition coefficients from the lipoida1 domains of the stratum comeum and the aqueous environment of the viable epidermis or the aqueous receptor fluid[27]. Low partition co-efftcients lower the overall permeation rate accordingly. In vivo, the drug is absorbed by the blood in capillaries that lie at a depth of about I50-200μm from the surface of the skin. Bronaugh and Stewart[27] have suggested that compounds having an aqueous solubility of 1O mg/l or less may demonstrate limited partitioning *in vitro* in aqueous receptor solutions. A technique used to obviate the problem of poor *in vitro*/in vivo correlation when using full thickness skin *in vitro* to evaluate hydrophobic compounds is the addition of solubilizing agents to the receptor solution, Examples of useful solubilizing agents include PEG-20 oleyl ether, octoxynol- 9 (Triton X-100)[27], bovine serum albumin (3% in buffer), Poloxamer 188,PEG400,and ethanoI .An important requirement of solubilizing agents is that they must not alter the inherent permeability orooerties of the skin. The addition of PEG 400 to the receptor solution reportedly leads to a significant alteration of the inherent barrier properties of human skin. Also, methanolic and ethanohc receptor solvents can damage full thickness rat skin *in vitro* as assessed by variable cortisone fluxes. It has been suggested that isopropyl myristate is a potential receptor-phase solubilizing agent. While certain properties (bipolarity, inertness) make isopropyl myristate attractive as a solubilizing agent, it is immiscible with water, can potentially extract lipophilic components of skin, and has been found to increase the flux of theophyliine in propylene glyco1 following pretreatment of hairless mouse skin.

Hydration is another variable to consider in long-term permeation experiments under *in vitro* conditions. While some studies have found the effects of hydration in long-term permeation experiments to be negligible,

other studies have demonstrated that certain rodent skins, in particular hairless mouse skin, are susceptible to hydration leading to changes in permeation rates over time.

Diffusion cells for measuring *in vitro* permeation

A myriad of cell designs have been used over the past 30 years. However, most designs fall into one of two general categories: side-by-side diffusion cells and in vivo mimic diffusion cells. The examples provided herein are far from exhaustive: they were chosen to illustrate some of the basic design considerations and some potential problems encountered during the routine use of diffusion cells for *in vitro* skin permeation studies

Side by side diffusion cells

Side-by-side diffusion cells usually comprise two chambers wherein one chamber contains the permeant in solution and the other contains the receptor solution. These two chambers are separated by a membrane (skin in percutaneous permeation experiments). The contents of one or both chambers can be agitated to ensure adequate dispersion of the drug molecules and to minimize the static diffusion boundary layers. Configurations of side-by-side diffusion cells include T-shapes[30] and identical L shapes[31,32,33]. Most cells are composed of glass as illustrated by the modified conical flask design of Wurster et al.[34] (see figure 2).

This cell has several drawbacks,most notably the inability to agitate the solutions internally although the authors propose gently shaking the entire apparatus in the plane of the membrane. The apparatus is suspended in a water bath for temperature control. While this design was shown to be adequate for rapidly diffusing compound (sarin), its broader applicability is unknown.

A considerably more complex design is that of Flynn and Smith[35] (see Figure 2). The chambers, unlike those used by Wurster et al.[34], are manufactured from brass. These chambers are held together with a nut-and-thread assembly. The membrane is held clamped between Orinas seated in the flanze surfaces. Relativelv large Teflon stirrers are mounted vertical to the membrane; these stirrers are in turn mounted onto shafts which protrude from each chamber. These protruding stirrer shafts are attached to gears interlinked with a synchronous

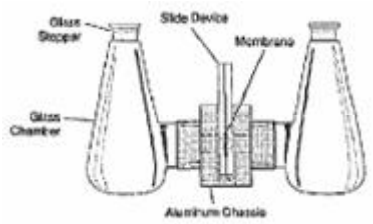

Figure 1: Permeability cell for study of solution and gel phase donors.the slide device holds the membrane (skin) between a relatively narrow tube connecting larger tubes.the two chambers are held together with an aluminum chassis. Redran from wuster et al.[34].

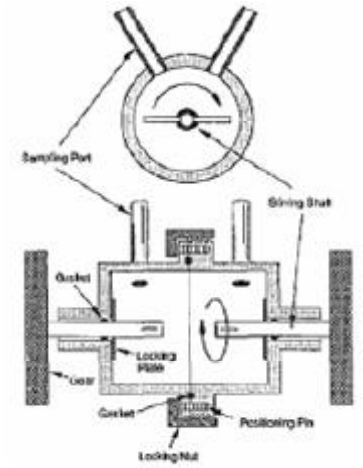

Figure 2 : Side by side permeability cell showing general features .mixing is accomplished through rotation of Teflon stirrers.mounted perpendicular to the membrane. A synchronous motor drives the stirrers in each chamber at the same rate. Redrawn from flynn and smith[36]

motor. Removal of the chamber fluid is accomplished through sampling ports in both chambers. A perforated screen can be used to support the membrane if required. The entire apparatus can be immersed in a water bath for control of temperature. As designed, the device requires relatively large amounts of membrane, which limits its usefulness to skin available in large amounts. While many of the features of this side-by-side are well-suited for assessing skin permeation under *in vitro* conditions, the complexity of the design (motors, shafts, cogs) make the widespread use of this system limited despite its advantages over many other *in vitro* diffusion cells.

Another design for side-by-side diffusion cells is shown in figure 3[37]. Two glass chambers with a relatively small diffusional area are temperature controlled by immersion in a water bath. Both chambers are stirred with Teflon-coated magnetic bars; a magnetic stirrer is

positioned below the side-by-side cell system to produce synchronous stirring of the bars in both cells. AS with the example shown in figure 2, the membrane can be supported with a stainless steel mesh. This mesh can also be used to prevent distention of the membrane if the receptor chamber is used to measure vapor diffusion through skin. Receptor and donor sampling is accomplished, through ports located on each chamber; each port can be sealed with Parafilm if needed. Depending on the height of the fluid in the sampling ports, mixing may be inadequate if a substantial portion of the receptor (or donor) solution is within the sampling port.

Another side-by-side diffusicn cell, the so called Valia-Chien cell, has been studied extensively by Chien and coworkers[38]. These cells are composed of two identical horizontal chambers, 0.9 cm in diameter and 3.8 cm in length: the stoppered sampling ports are vertical (see Figure 5). The active surface area, sufficiently small for use with biomembmaes such as human skin, is 0.64 cm'. As can be seen in figure 5. The cylindrical portions of the ceils are enclosed in a water jacket although the membrane connecting flange is open to ambient temperatures. Fluid mixing is reduced in the constricted region of the flange; however, mixing and temperature equilibrium are reached relativelv ranidlv in this cell. As noted by Smith and Haigb[39], the design of cells as shown in figure 5 has led to the apparent requirement of a computational correction to account for the nonideal design. Again, such corrections lead to, at best, only minimal changes in the interpretation of data obtained with Valia Chien diffusion cells

A combination of a magnetically-stirred donor chamber and a flow-through receptor chamber has also been developed (see Figure 5). A unique feature of this design is the location of the receptor fluid inlet positioned at the center of the membrane while the effluent is collected from the periphery. This diffusion cell was reportedly capable of efficiently removing the penetrant from the membrane/receptor solution interface thus maintaining maximum sink conditions.

Despite the dissimilarity between side-by-side designs and assessing skin permeation under in vivo conditions, a large amount of the skin permeation data has been collected skin using the side-by-side chambers. Side-by-side designs expose the membrane (skin) to solvent on both sides throughout the experiment leading to potential salvation effects. Nonetheless, the data collected in side-by-side chambers is useful if the limitations of the design are acknowledged. Measurement of permeation rates under conditions similar to those encountered in *vivo* requires a different cell design as explained below.

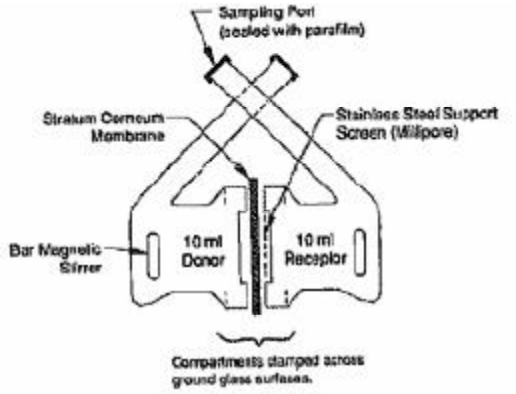

Figure 3 : Glass diffusion cell for study state permeation experiments. A stainless stel screen is used as a support of the biological membrane, in this case the stratum corneum. Stirring is accomplished by Teflon coated magnetic stirring bars. Redrawn from southwell barry[37]

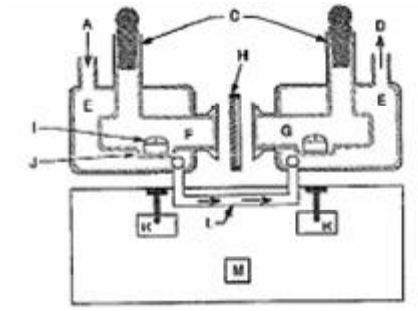

Figure 4: Skin permeation system (valia-chien). key: A,inlet; B.glass stopper, c,samplingports; D,outlet; E,water jacket; F,donor compartment(4ml); I,magnetic stirring; J,stirring platform; K,synchronous motor; L,connecting tube; M,on off button.redrawn from chein and valia[38]

Diffusion cells designed to mimic *in vivo* conditions

Systems that parallel conditions found in vivo are normally vertical with the bottom chamber designed to hold a receptor fluid. The bottom chamber is agitated or recycled in an attempt to maintain sink conditions throughout the experiment. An advantage of the vertical cell design is the ability to vary the nature of the donor vehicle. A film of material can be applied by solvent evaporation; ointments, pastes, synthetic mem-

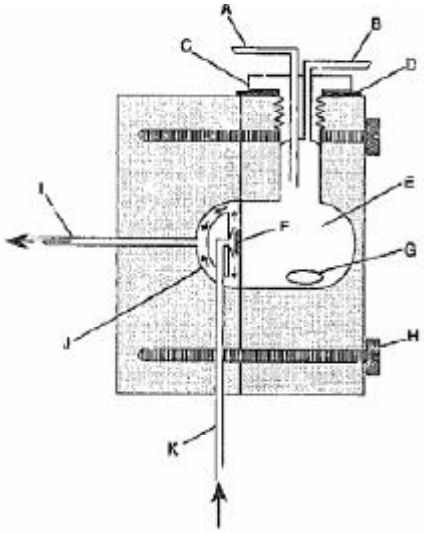

Figure 5: Flow throuh diffusion cell key:A,application tube;B,removal tube;C,donor compartment stopper; D, neoprenerubber washer,E,donor compartment;F,skin specimen clampd between perspex blocks; G,stirrer; H,clamping screw; I,eltlet tube;J,acceptor compartment; K,inlet tube, with drawn from astley and levine[39]

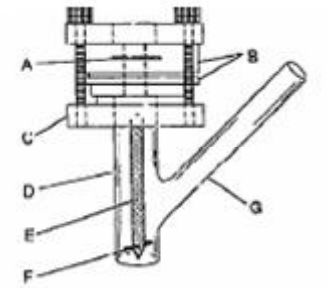

Figure 6: Glass diffusion cell consisting of a lower chamber with a side arm for sampling of receptor phase.key, A,skinspecimen,B.teflon pieces holding skin, C.clamp, D.receptor chamber, E.polyetjylene sail, F.teflon coated magnaetic stirrer, Redrawn from coldman et al.[40]

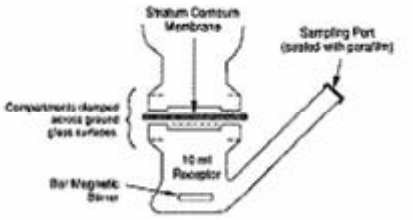

Figure 7: Vertical diffusion cell for steady state permeation experiments. The stainless steel screen is ued to support fragite biologic membrane,such as the stratum corneum.redrawn from southwell et al.[40]

branes in series with skin, and entire transderma1 devices can also be studied. Atmospheric conditions (e.g., humidity) can be controlled in these cells as well. Se-

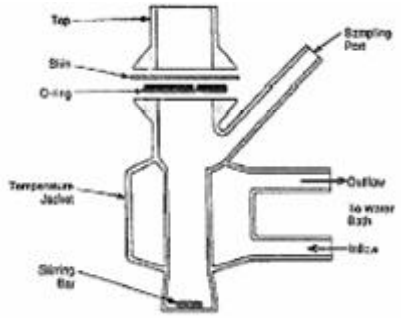

Figure 8: The franz diffusion cell redrawn from franz

quential treatments, such as pretreatment with an enhancer followed by deposition of a drug, are easily accomplished using vertical cells. It is also possible to conduct infinite and finite dose experiments. The actual experimental design will vary depending on the type of formulation under investigation. For instance, testing of drug permeation from a topical vehicle to deliver corticosteroids is accomplished through semi infinite dose or finite dose techniques. There have been a number; of vertical cells designed and tested over the past 25-30 years. One of the earlier cells is that of Coldman et al.[40].

shown in figure 7. This static cell is composed primarily of glass with a side arm for sampling. A Teflon-coated stirring bar is attached to a polyethylene sail to provide mixing of the receptor solution. The skin is held in place by a Teflon disk on a flat ground glass surface at the top of the receptor chamber. The exposed surface area of this cell was 0.30 cm^2 and the receptor chamber volume was IO ml, a portion of which is located in the side-arm (mixing may not be adequate in the side-arm). Fluid mixing and mass transfer characteristics of this diffusion cell have not been fully investigated.

Barry and coworkers have, in addition to a side-by-side design, examined a vertical cell (see Figure 8). This cell is merely one half of the side-by-side chambers (see figure 2) turned vertically with a donor chamber placed on top of the half-cell. As expected, cell components are those of the cell shown in Figure 2 (glass chambers, Teflon magnetic stir bar, glass side arm sealable with Parafilm, stainless steel membrane support). Similar to the diffusion cell of Coldman et al.[41], a relatively large portion of the receptor solution is located in the poorly stirred side-arm. The Franz diffusion cell is one of the most widely used systems for *in*

vitro skin permeation studies. First disclosed in 1978 and subsequently marketed, this cell has a small donor compartment and a dumbbell-shaped receptor chamber (see Figure 9). The bottom portion of the dumbbell-shaped chamber communicates with a narrower cylindrical tube which widens in the upper part of chamber near the area of contact with the membrane. In the original design, the cell was static and therefore had a single sampling port (unstoppered). The central part of the receptor chamber is enclosed in a water jacket for temperature control. Portions of the receptor chamber and the entire donor compartment are open to ambient conditions. As with most vertical systems, the receptor chamber is agitated with a Teflon-coated magnetic stir bar. A number of modifications have been introduced into the original design by Franz. O-Ring flanges have been added; a second side-arm has been added to permit flow-through operation, the donor compartment can be sealed, and it can be made in a variety of active surface area diameters. While the Franz cell is widely used, it has several potential draw backs, most notably relatively poor mixing hydrodynamics. Poor mixing results from the fact that agitation in the lower bulb must be transmitted through the narrow cylinder. There is considerable resistance to laminar fluid flow through the constricted portion of the receptor chamber leading to a static boundary layer at the interface between the membrane and the receptor solution.

The poor mixing properties in the receptor chamber of Franz cells have been studied. It was found that the time to complete mixing, as measured by homogeneous dye dispersion, was inadequate in the side-arm and the upper portion of the dumbbell-shaped receptor cell in that homogeneity was not reached until 30 mm had passed in some cases. Based on the data collected with the Franz cell, two flow-through *in vitro* penetration cells were designed to obviate the problem of poor mixing (see Figure 10). Two types of cells were prepared to accommodate two different surface areas. The central design feature of these cells is the receptor chamber. Its diameter is wider than that of the Franz cell to achieve rapid and even stirring. As can be seen in Figure 10, these cells feature a flow-through receptor chamber. O-Rings are absent in this design the skin is sandwiched between two areas of ground glass. The authors report that no leakage of material was observed under any experimental conditions used.Using the time

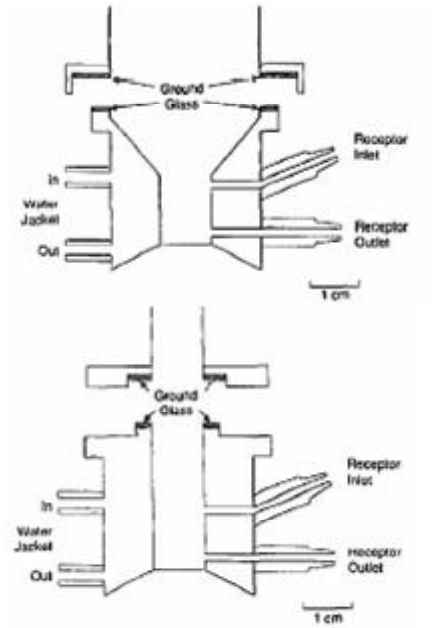

Figure 9 : Flow through diffusion cells. The upper cell has a receptor volume of 5.3ml;lower cell has a receptor volume of 3.0ml.redrawn from Gammer et al.

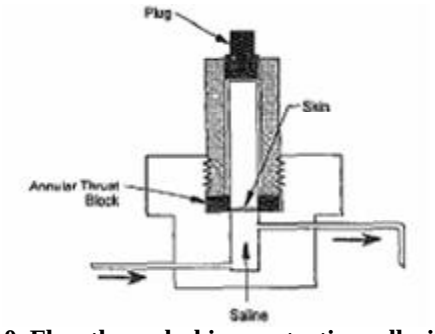

Figure 10: Flow through skin penetration cell with active surface area of 0.2cm^2,the flow rate of the receptor solution (isotonic saline,38c)was 10-20 ml/h.redrawn from Marzulli[42].

for homogeneous dispersion, both cell designs gave nearly instantaneous mixing(< 30 s).

Flow through systems offer an alternative to sampling ports; by replacing the entire contents of the receptor chamber on a continuous basis, sink conditions are more easily maintained. As a result, flow-through cell design coupled with a vertical chamber represents conditions similar to those encountered in vivo. One of earliest flow-through cells was that of Marzulli[42] (see Figure 11). The cylindrical design may not have optimum hydrodynamics: the inlet to the receptor solution is not directed toward or at the membrane. Removal of the permeant may not be complete in the Marzulli de-

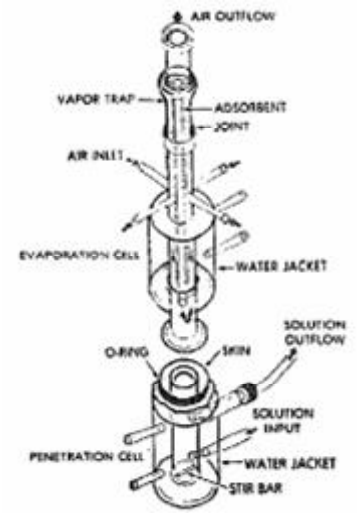

Figure 11: *In vitro* skin penetration evaporation cell from Hawkims and Reifenrath

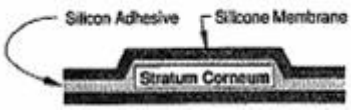

Figure 12: Stratum corneum silicone membrane sandwich. The stratum corneum is held in place with a silicone adhesive.Redrawn from Tiemessen et al.

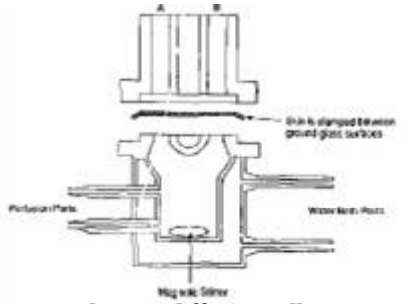

Figure 13: Ionto phoretic diffusion cell constructed of glass effective surface area is 0.8 cm2 with a donor phase volume of about 0.5cm3. receptor phase volume is approximately 7 cm3. A and B are the electrode chambers . Redrawn from Glikfeld et al.

sign. An obvious improvement in the design would be introduction of a stirrer. At the same time, stagnant diffusion layers are probably only of major concern when using relatively lipophilic permeants.

A relatively complicated permeation cell for assessing penetration-evaporation was designed with a flow-through system and a magnetic stir bar (see Figure 12). The receptor chamber (thermostatically jacketed) is stirred with a magnetic stir bar. The donor chamber was designed to control evaporation from the surface of the skin by forced, warm air ventilation.

A problem with many Row-through systems is the entrapment of air bubbles under the surface of the skin. These bubbles can reduce the diffusional area substantially and should therefore be minimized by degassing of solvents or the use of bubble traps. Another problem associated with the use of flow through Systems is absorption of drugs, in particular hydrophobic drugs, into plastic tubing used to coIlect the eftIuent from the receptor chamber. Teflon used in magnetic stirrer could also absorb hydrophobic solutes as well.

Often, biological membranes are fragile and therefore difficult to handle. In particular, measuring the permeability of sheets of stratum corneum can be a formidable task. Maintaining a specific water content in the Stratum corneum can also be a demanding prospect. While support structures have been used to maintain the integrity of the stratum corneum (see for iostance Figures 4 and 8), a new technique has been developed recently to control the integrity and water content of stratum corneum Sheets (see Figure 13). It is possible to control the humidity such that the Stratum corneum inside the sandwich adopts a water content which is in thermodynamic equilibrium with salt solutions outside the membrane. A minor drawback of this system is the need to determine not only the permeability of stratum comeum, hut also that of the silicone sandwich.

Diffusion cells for intophoresis and phonophoresis

The diffusion celIs described in the previous sections were designed to measure the passive diffusion of drugs. Iontophoresis and phonophoresis are techniques used to increase the transcutaneous flux of drugs. Iontophoresis is defined as the increase in permeation rate of a molecule induced by an applied current through the skin. While the concept is relatively old, it has received considerable attention recently because it appears possible to use iontophoresis to deliver certain macromolecules, viz., peptides and proteins, through skin. phonophoresis is defined as the increase in fate of a solute through skin observed under the influence of art ultrasonic perturbation[43,44].

Diffusion cells used in iontophoresis experiments are simtlar to those used to assess passive diffusion except electrodes have been added, one in the donor chamber and one in the receptor compartment. While this type of apparatus works well experimentally, electrodes can not be placed on opposite sides of the skin

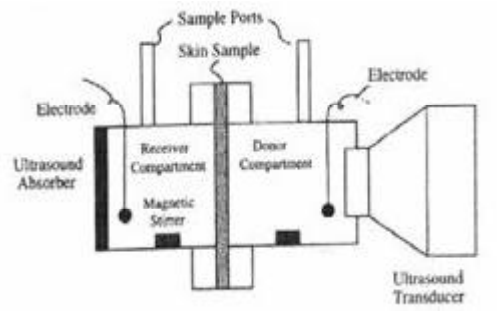

Figure 14: Experimental setup used in iontophoresis experiment donor compartment volume of 8ml.reciver compartment volume is 8.5 ml

in vivo. GlikfeId et al.[45] designed an iontophoretic cell for *in vitro* studies to account for the in vivo situation (see Figure 14). In this design, both electrodes are applied to the same side of the membrane (skin). Such a design should permit better approximation of drug delivery using iontophoresis under in viva conditions. The cell used is based on that of Gummer et al.[46] and hence should exhibit properties similar to those claimed for the diffusion cell shown in figure 10.

Phonophoresis is rarely studied under *in vitro* conditions. Most experiments involve the use of existing commercially available ultrasonic equipment. These systems are too large to use under: *in vitro* conditions, particularly with human skin. Design of an *in vitro* diffusion cell capable of varying experimental parameters important in phonophoretic drug delivery would probably help accelerate research in this area.

Ultrasound (Sonophoresis and Phonophoresis)

Ultrasound involves the use of ultrasonic energy to enhance the transdermal delivery of solutes either simultaneously or via pre-treatment and is frequently referred to as 124 M.B. Brown et al. sonophoresis or phonophoresis. The proposed mechanism behind the increase in skin permeability is attributed to the formation of gaseous cavities within the intercellular lipids on exposure to ultrasound, resulting in disruption of the SC[47]. Ultrasound parameters such as treatment duration, intensity and frequency are all known to affect percutaneous absorption, with the latter being the most important[48]. Although frequencies between 20 kHz - 16 MHz have been reported to enhance skin permeation, frequencies at the lower end of this range (<100 kHz) are believed to have a more significant effect on transdermal drug delivery, with the delivery of macromolecules of molecular weight up to 48 kDa being reported[49]. The SonoPrep®device (Sontra Medical Corporation) uses low-frequency ultrasound (55 kHz) for an average duration of 15 s to enhance skin permeability. This batteryoperated hand-held device consists of a control unit, ultrasonic horn with control panel, a disposable coupling medium cartridge and a return electrode. The ability of the SonoPrep device to reduce the time of onset of action associated with the dermal delivery of local anaesthetic from EMLA cream was recently reported[58]. In the study by Kost et al.[50], skin treatment by ultrasound for an average time of 9 s resulted in the attainment of dermal anaesthesia within 5 min, compared with 60 min required for non-treated skin. The use of other small, lightweight novel ultrasound transducers to enhance the *in vitro* skin transport of insulin has also been reported by a range of workers[51-53].

CONCLUSION

Dermal/transdermal absorption is a multi factorial multi step process, There are many barriers for the transport of drugs through skin which is affected by a number of factors including the animal source and type of skin, physicochemical properties of the tested compound and delivery systems, as well as possible skin pretreatment and environmental factors. To assess the permeation studies the suitable model is important to interpret the data,For a number of tested systems there is a correlation between *in vitro*/in vivo data acquired in animals and in humans. Yet, there are also many examples indicating poor correlation.

In conclusion, *in vitro* permeation experiments and animal models, with all their limitations, provide important tools for screening drug delivery systems, skin permeation enhancers and drug delivery carriers. Also, these tools make it possible to estimate the rank order of percutaneous absorption of a series of molecules.

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