CHAPTER I

INTRODUCTION

1. 1 Ultraviolet (UV) radiation.

Ultraviolet (UV) light is a natural form of electromagnetic radiation emitted from the sun. Ultraviolet radiation (UVR) is more energetic than visible radiation and therefore has a shorter wavelength. UVR is subdivided into three different wavelength bands UVA, UVB and UVC (figure 1). The use of wavelength is a convenient way to classify the rays based on the amount of energy they contain and their effects on biological matter.

Ultraviolet radiation with a wavelength between 100 nm and 280 nm is classified as UVC radiation. Of the three divisions of UVR, UVC has the shortest wavelength and greatest energy. Naturally produced UVC does not reach the earth's surface based on a reaction that occurs in the ozone layer. UVR with a wavelength between 280 nm and 320 nm is classified as UVB radiation. The energy contained within UVB rays is often not sufficient to split an ozone molecule and as a result some will extend down to the earth's surface. The vast majority of UVB radiation however, will be scattered and will not escape the ozone layer. Lastly, UVR with a wavelength between 320 nm and 400 nm is classified as UVA radiation. These rays do not have sufficient energy to break apart the ozone molecules and so they pass through the earth's atmosphere almost unfiltered [1].

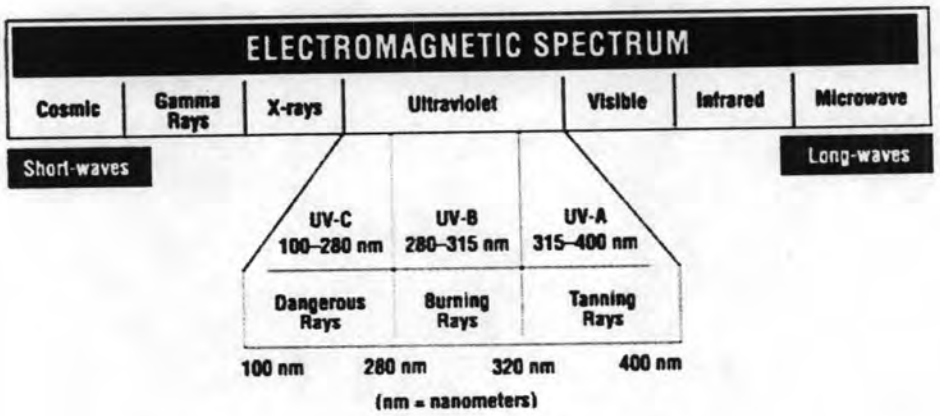

Figure 1.1 Electromagnetic Spectrum [2].

1.1.1 Health effects of ultraviolet (UV) radiation.

UVB light has been related to skin cancers such as melanoma. The radiations ionize DNA molecules in skin cells, causing covalent bonds to form between adjacent thymine bases, producing thymidine dimers, Thymidine dimers do not base pair normally, which can cause distortion of the DNA helix, stalled replication, gaps and misincorporation. These can lead to mutations. which can result in cancerous growths. However UVB has a positive effect in activation vitamin D production in the skin [3].

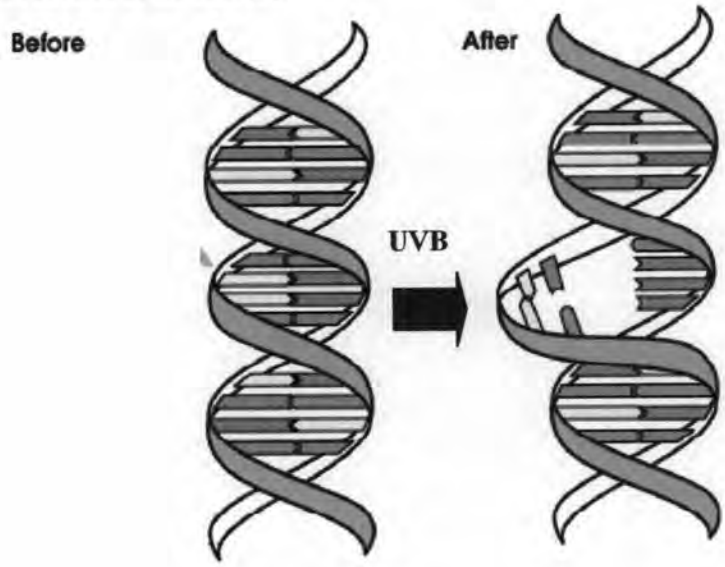

Figure 1.2 DNA UV mutation

Although, UVA is the least harmful, it can contribute to make photoaging [4], DNA damage and skin cancar. UVA has longer wavelength, so it can penetrate most windows. It also penetrates deeper into the skin than UVB light and is thought to be a prime cause of wrinkles.

1.2 Sunscreens

Sunscreen has become a primary mean in protecting ultraviolet radiation damaging effects. Sunscreens may be classified according to the machanism of action they offer as either physical blockers and chemical absorbers.

1.2.1 Physical blockers

These are chemicals that reflect or scatter the ultraviolet radiation. They are site on the skin's surface and ideally they should not have the ability to be absorbed. The most well-known

physical blocker is zinc oxide. Other commonly used sunblocks are talc, titanium dioxide, and red vetenary petrolatum. A new physical blocker formulation has been developed that contains micronized titanium dioxide. The advantage of this formulation is that it is almost invisible and still has good UV blocking capabilities [5 -8].

1.2.2 Chemical Absorbers [9].

These chemicals absorb the harmful ultraviolet radiation. Chemical absorbers are classified into either UVA absorbers or UVB absorbers depending upon the type of radiation.

UVA absorbers are chemical that absorb radiation in the 320 to 360 nm regions of the ultraviolet spectrum. UVA absorbers include anthranilates, benzophenones and dibenzoyl methanes.

UVB absorbers generally absorb radiation in the 280 to 320 nm region of the ultraviolet spectrum. UVB absorbers include *p*-aminobenzoic acid derivatives, camphor derivatives, cinnamates, and salicylates.

	Allowable Concentration, %	**Range of Protection**
Chemical Absorbers		
Aminobenzoic acid	Up to 15	UV-B
Cinoxate	Up to 3	UV-B
Homosalate	Up to 15	UV-B
Octocrylene	Up to 10	UV-B
Octyl methoxycinnamate	Up to 7.5	UV-B
Octyl salicylate	Up to 5	UV-B
Padimate O	Up to 8	UV-B
Phenylbenzimidazole sulfonic acid	Up to 4	UV-B
Trolamine salicylate	Up to 12	UV-B
Dioxybenzone	Up to 3	UV-B, UV-A II
Oxybenzone	Up to 6	UV-B, UV-A II
Sulisobenzone	Up to 10	UV-B, UV-A II
Menthyl anthranilate	Up to 5	UV-A II
Avobenzone (Parsol® 1789)	Up to 3	UV-A I
Physical Blockers		
Titanium dioxide	Up to 25	UV-B, UV-AII
Zinc oxide	Up to 25	UV-B, UV-AII, UV-AI

Table 1.1 FDA approved sunscreen ingredients [10].

Since the action of sunscreen is at the skin, transdermal absorption of sunscreens will decrease the efficiency of sunscreen directly. In the past decade there are many studies indicating transdermal absorption of various organic UV filters. For examples, in 1995, Leweke and Lippold showed that isoamyl-4-methoxycinnamate (cinnamate derivatives) could be absorbed through human's skin layers [11].

In 1997, Heyden *et al.* concluded that OMC could penetrate through the skin layer into blood circulation. Such conclusion was drawn from the discovery that OMC could be recovered from milk of human volunteers [12].

In 1999, V.K.Gupta *et al.* studied absorption of sunscreen through Micro-Yucatan Pig skin *in vitro* and evaluate the influence of different formulation by diffusion cell technique. They detected that OMC reached the stratum corneum layer (SC) within an hour and amouts penetrated into viable skin and receptor fluid increased slowly over time [13]. In the same year, G.Potard group studied the quantification of five UV filters (octyl methoxycinnamate, benzophenone-3, benzophenone-4, octyl triazone, octylcrylene and caffeine in various skin layers using diffusion cells. The result showed that benzophenone-3 and caffeine could reach the receptor fluids, indicating very good penetration [14].

In 2000, V. Serveiya *et al.* discovered that oxybenzone could be detected in urine and blood/plasma indicating very good transdermal absorption [15].

In 2003, E. Chatelain *et al.* reported that both OMC and oxybenzone can penetrate upper skin layer through dermis layer [16]. This research indicated that vihicle affected the permeation of UV filters. L'Oreal company studied percutaneous absorption of Mexoryl SX (terephthalylidine dicamphor sulfonic acid) and found that the amouts found in the dermis and receptor fluid,was very small (0.16% of the applied dose) [17].

In 2004, NR. Janjua *et al.* reported that benzophenone-3 (BP-3), octyl methoxycinnamate (OMC) and 3-(4-methylbenzylidene) camphor (4-MBC) were detectable in urine [18].

Similarly, in 2005 Zs.Kertesz *et al.* studied TiO_2-penetration in the epidermis of human skin xenografts by nuclear microprobe and observed TiO_2 in corneocyte layers of stratum corneum [19].

In 2004, F. Menzel *et al.* used high energy ion nanoprobe LIPSION to investigate percutaneous uptake of ultrafine TiO_2 particles and proved that micronised TiO_2 particles from all used formulations could penetrated through intercellular spaces in the stratum corneum into stratum granulosum [20].

In addition to reports on the penetrating of sunscreen, studies on methods to retard such absorption were also carried out by many research groups.

In 2001, Godwin *et al.*determined the influence of Transcutol®CG concentration on the transdermal permeation and skin accumulation of absorbers, oxybenzone and OMC. The results demonstrated that inclusion of Transcutol®CG in sunscreen formulations increased the skin accumulation of the UV absorbers oxybenzone and OMC without a concomitant increase in transdermal permeation [21].

In 2003, G. Yener *et al.*prepared solid lipid microspheres (SLM) of OMC. SLM was used as carriers for OMC in order to decrease the release and the penetration rate of this UV absorber. Incorporation of OMC into SLM also enhanced the photostability of OMC compared to plain absorber in various vehicles. Another important aspect was the effectiveness of OMC in liposphere form which showed nearly the same protection as the free form after exposure to a solar simulator [22].

In 2003, M.M. Jimenez investigated the influence of the nanocapsule carrier (NC) on the *in vitro* absorption of OMC by comparing between oil-in-water and water-in-oil emulsion of free-OMC and OMC entrapped in nanocapsule (OMC-NC). Among these, OMC-NC showed the least penetration [23].

In 2004, S. Simeoni studied influence of cyclodextrins on *in vitro* human skin absorption of butyl-methoxydibenzoylmethane (BMDBM). The results demonstrated that the presence of the excess cyclodextrin decreased drug penetration through the skin [24].

1.3 Physiology of the skin

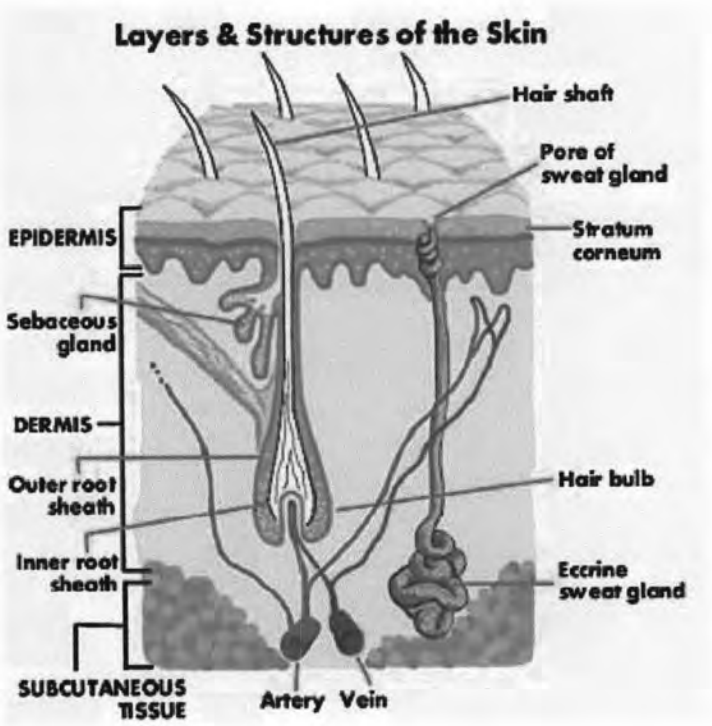

Figure 1.3 Layer and structures of the skin [24].

The main layers of the skin are :

1) The stratum corneum

The outermost layer of the skin, the horny layer or stratum corneum, is approximately 10 μm thick when dry but swells to several times this thickness when fully hydrated [26-27]. It contains 10 to 25 layers of parallel to the skin surface lying dead, keratinized cells, called corneocytes. It is flexible but relatively impermeable. The stratum corneum has been identified as the princeple barrier for penetration [26,27]. The barrier nature of the horny layer depends critically on its constituents: 75-80% proteins, 5-15% lipids and 5-10% unidentified material on a dry weight basis. The protein fraction predominantly comprises alpha-keratin (70%),with some beta-keratin (10%) and the cell envelope (5%). The lipid constituents vary with body site (neutral lipids, sphingolipids, polar lipids, cholesterol). Phospholipids are largely absent, a unique feature for a mammalian membrane.

2) The viable epidermis

The viable epidermis is situated beneath the stratum corneum (the horny layer) and varies in thickness from 0.06 mm on the eyelids to 0.8 mm on the palms. It consists of various layers, characterized by different stages of differentiation. Going inwards, the permeant crosses the stratum lucidum, the stratum granulosum (or granular layer), the stratum spinosum (or spinosous layer) and the stratum basale (or basale layer) [26,28]. In the basal layer, mitosis of the cells constantly renews the epidermis and this proliferation compensates the loss of dead horny cells from the skin surface.As the cells produced by the basal layer move outward, they alter morphologically and histochemically, undergoing keratinization to form the outermost layer the stratum corneum [28-29].

3) The dermis

The dermis is 3 to 5 mm thick layer and is composed of a matrix of connective tissue, which contains blood vessels, lymph vessels and nurves. The cutaneous blood supply has an essential function in the regulation of body temparature and provides nutrients and oxygen to the skin, while removing toxins and waste products.Capillaries reach to within 0.2 mm of the skin surface and provide sink conditions for most molecules penetrating the skin barrier. The blood supply thus keeps the dermal concentration of a permeat drug (permeant) very low, and the resulting concentration difference across the epidermis provides the essential driving force for transdermal permeation [26,30].

4) The hypodermis

The hypodermis or subcutaneous fatty tissue merges with the overlying dermis. It supports the dermis and epidermis and serves as a fat storage area. The layer helps to regulate temperature, provides nutritional support and mechanic protection. It carriers the principal blood vessels and nerves to the skin and may contain sensory pressure organs [26,28].

1.4 Permeation pathways

A molecule may use two diffusional rutes to penetrate normal intact human skin: The appendageal route and the epidermal route [26,30,31]. The appendageal route comprises transport via the sweat glands and the hair follicles with their associated sebaceous glands (Figure 1.4). These routes circumvent penetration through the stratum corneum and therefore known as shunt routes. This route is considered to be of minor importance because of their relatively small area, approximately 0.1% of the total skin area. However, recent studies indicate that follicles may

have a greater importance in percutaneous absorption than is generally assumed. The appendageal route may be more important for ions and large polar molecules which hardly permeate the stratum corneum [26].

For drugs which mainly cross the intact horny layer, two potential micro routes of entry exists, the transcellular (or intracellular) and intercellular pathways. The principle pathway taken by a permeant is decided mainly by the partition coefficient (log K). Hydrophilic drugs partition preferentially into the intracellular domains, whereas lipophilic permeants (octanol/water log K >2) travese the stratum corneum via the intercellular route. Most permeants permeate the stratum corneum by both routes. However, the torturous intercellular pathway is widely cosidered to provide the principal route and major barrier to the permeation of most drugs [26].

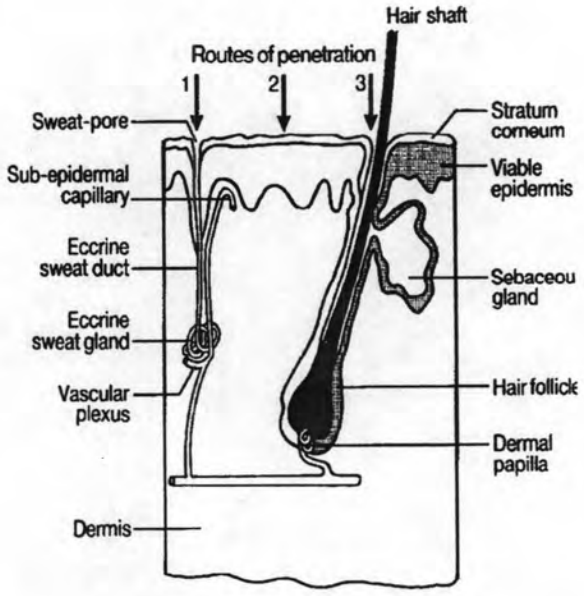

Figure 1.4 Possible routes for drug entry through the skin [31].

1.5 Diffusion cells for measuring *in vitro* permeation.

The most common technique used to gather transdermal penetration of a substance involves diffusion cells. Diffusion cells generally comprise two compartments, one containing the active component (donor vihicle) and the other containing a receptor solution, separated by a piece of excised skin or other membrane. Although many variations of diffusion cells exist, there are two basic designs: the static, or nonflowing cell, and the flow-through cell [32-36].

The Franz diffusion cell is one of the most widely used static designs for studying *in vitro* permeation. This cell has a static receptor solution reservior with a side-arm sampling port (Figure 1.5). The skin is positioned between the two cell halves of a glass chamber. The two compartments are held together with a clamp. A thermal jacket is positioned around the receptor compartment and is heat with an external circulating bath. During the cause of an experiment, small volumes are withdrawn from the stirred receptor solution for analysis and the receptor compartment is refilled with receptor solution to keep the volume of solution constant during the experiment.

In contrast to the sampling from static cells, the flow-through cells can provide automatic replacement of the receptor solution. As a result, the flow-through cell represents conditions more similar to those encountered in vivo because the entire contents of the receptor compartment are replaced on a continuous basis [32-36].

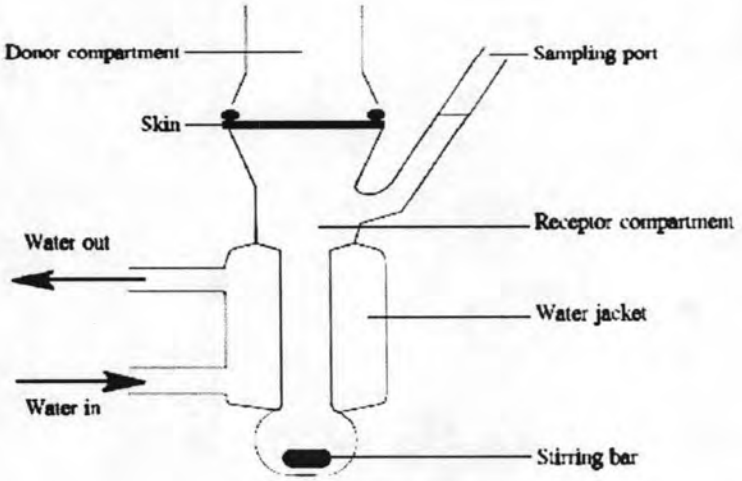

Figure 1.5 Franz diffusion cell.

By using diffusion cells, the conditions for drug delivery should be controlled because drug permeation may very with the skin or membrane used, and with the composition of the donor and the receptor solution, respectively.

Membrane selection

Clearly, the most approprate membrane for permeation studies is human skin [36-39] However, *in vitro* permeation experiments with human skin are difficult to conduct due to the scarcity of human skin and controlling the gender, race, anatomical site, age and skin condition of the donor. Therefore, most *in vitro* permeation studies use animal skin.

A large number of different animalskins have been tested as possible models for human skin, such as the hairless mouse (*Mus musculus.*), rabbit, guinea pig, rat, pig or shed snake skin. The criterion for selection should be the correlation between permeation rates using animal skin snd human skin. Knowledge of the anatomical and histological properties of animal skin may be helpful to interpret the permeation data [36].

Another alternative for human skin is the use of the synthetic membranes such as Silastic®(polydimethyl siloxane), cellulose acetate or polyurethane. Due to the complexities of skin, permeailities cannot be predicted adequately from the synthetic membrane data. Therefore, whenever possible human skin should be used in preference to animal skin or artificial membranes.

Receptor solution

The receptor solution used in diffusion cells should not only act as acceptor for permeating drug but should provide the water, biochemicals and ions needed for the skin membrane to function in the permeation experiment at the proper pH and osmotic strength [39-40]. This is physiological saline, Ringer's solution, or some other physiological relevant solution. Other important factors of the receptor solution are temparature, solubility and stirring [37,40].

Control of the receptor solution temparature is important to minimize variations in experimental conditions. The temparature should be kept at normal physiological conditions, since temparature elevation may lead to increased hydration of the skin. It is known that a rise of $10^{\circ}C$ in temparature can produce a 2-to 3-fold increase in permeation [32].

Solubility and stirring are important to allow the permeant to be taken up and transported away from the skin after it has passed through, avoiding a concentration build-up within or below the skin. Stirring of the receptor solution is also important to provide a homogenous receptor solution [32,37]. The concentration of the permeant in the receptor solution should remain low

(less than 10%) compared with its solubility in the solution [40]. If the permeant is relatively insoluble, solubility enhancing components can be added to the receptor solution, however their effects on the skin must be considered.

1.6 Suction blister technique.

The suction blister method was developed to separate the epidermis from the dermis by U.Kiistala in the1968's [41]. Over the decades, new applications of this method have enabled assessment of drug penetrate in human skin *in vivo*, determination of pharmacological agents and cytokines in skin and measurement of the rate of the suction blister wound [42].

To produce blisters, a suction cup with holes is placed on the skin. After that, 150-250 mmHg negative pressure inside the cup is created by a pump. Hole size can vary from 3 to 10 mm. The total diameter of the cup can vary from 20 to 50 mm. The suction results in the separate of the epidermis from the dermis at the level of lamina lucida in the basement membrane. the basement membrane forms the blister floor and epidermis form the blister roof. The blister cavity is filled with tissue fluid. The blister fluid represents interstitial fluid, although a small portion of proteins is diffused from serum [41,43].

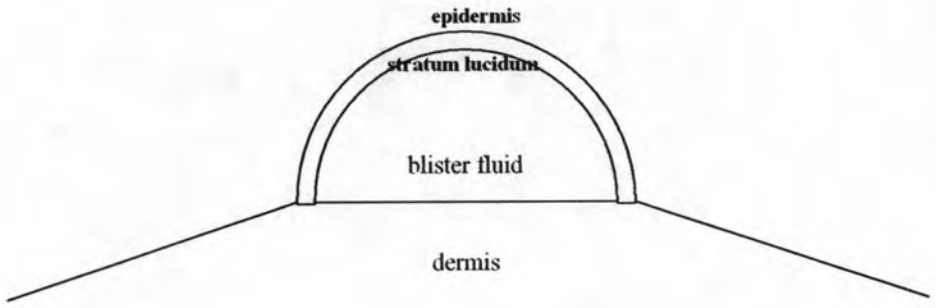

Figure 1.6 The suction blister.

Many research groups used this technique for determination transdermal drug penetration or pharmacological agents and cytokines in skin.

In 1980, J. Marja *et al.* determinated 8-methoxyphsoralen in suction blister fluid and serum [44].

In 1993, C. Surber *et al.* used the suction blister technique to quantitate acitretin in the skin of healthy human volunteers [45].

In 1999, G. Ronald *et al.* studied the dynamics of inflammation by suction blister, i.e., the relationship between psychological stress and the secretion of proinflammatory cytokines at an actual wound site were determined [46].

In 2002, L. Tapani *et al.* investigated radiation reaction in human skin using suction blister technique [47].

In 2006, S. Nanda *et al.* used the suction blister epidermal grafting technique for the treatment of eyelid vitiligo [48].

1.7 Objective of the work

As mentioned earlier that concerns on UV filter penetration have lead to a need to perform transdermal test on newly developed UV filters, in this study, therefore, transdermal absorption tests of various UV filters across baby mice skin (*Mus musculus* Linn.) were performed. In addition, transepidermal absorption in human volunteers using Suction Blister technique were investigated. The results from the two techniques were then compared.