CHAPTER I

INTRODUCTION

Most recently, there is an increasing recognition that the skin can also serve as the port of administration for systemically active drugs. In this case, the drug which is applied topically will be absorbed first into the blood circulation and then be transported to target tissues, to achieve its therapeutic purposes. The ideal way to determine the transdermal delivery potential of a compound in man is to do the actual study in man. Mechanisms and parameters of transdermal delivery elucidated in-vivo with human skin are most relevant to the clinical situation (Feldmann and Maibach, 1967). However, there are several reservations to this : there is a wide biological variability; the procedures are expensive, time-consuming and must meet with ethical approval. Compounds with known toxicity cannot be tested in this way.

Because of the problems encountered with in-vivo procedures, a reliable in-vitro methodology is required. The in-vitro techniques currently employed involve determination of the transport of chemical across excised skin of either human or animal origin, or across artificial model membranes (Bronaugh and Maibach, 1985). The use of excised skin is subject to species and interindividual variation. Furthermore, sources of excised skin are limited. Artificial model membrane systems offer an alternative to the excised skin. These models have several advantages over biological system; they are much more reproducible, easily manufactured; and the membrane composition is easily manipulated.

Diffusion through the outermost skin layer, the stratum corneum, is accepted as the rate-limiting step in the percutaneous transport of most substances (Scheuplein, 1965). Therefore, the artificial membrane should mimic the barrier properties of human stratum corneum in order to model the skin successfully. The use of animal skin as a human model has been considered by several investigators (Bartek, La Budde and Maibach, 1972; Idson, 1975; Wester and Maibach, 1975; Galey, Lonsdale and Nacht, 1976; Wolejsza and Verar, 1979). It was generally concluded that the skin of the miniature swine most closely resembles the permeability characteristics of human skin both in-vitro and in-vivo (Wester and Maibach, 1987).

Synthetic model membranes have been used to study drug diffusion kinetics. Early studies using polydimethylsiloxane (PDMS) membrane, silastic®, were conducted by Garett and Chemburkar, 1968. PDMS is an isotropic, non-porous, hydrophobic polymer which provides an excellent non-polar barrier for diffusion studies.

Piroxicam is one of the most potent non-steroidal, anti-inflammatory drugs. Piroxicam is well absorbed following the oral administration. However, its use has been associated with a number of gastro-intestinal disorders. To overcome these side effects, it was proposed to develop various topical dosage forms of the drug. Babar et al., 1990 reported that general rank order for the in-vitro piroxicam release rate from all the dermatological bases evaluated was : gel base > hydrophilic base > emulsion base.

Objectives

1. To compare the permeation rate (flux) of piroxicam from gel preparations through polydimethylsiloxane membrane and through pig skin to investigate the feasibility of using PDMS membrane in in-vitro permeability studies.

2. To investigate the effect of different types of gelling agents on membrane permeability.

3. To determine the effect of some additives on membrane permeability.

Skin Permeability.

1. Skin Structure.

With the thickness of only a fraction of a millimeter, the skin separates the underlying blood circulation network from the outside environment and serves as a barrier against physical, chemical and microbial attacks, acts as a thermostat in maintaining body temperature, plays a role in the regulation of blood pressure, and protects against the penetration of ultraviolet rays.

The skin is a multilayered organ composed of many histological layers (Figure 1) (Zanowiak and Jacobs, 1982). Macroscopically, two distinct layers are apparent, the outer epidermis and the inner dermis (Walters, quoted in Hadgraft, 1989). The dermis contains blood vessels, lymphatics and nerve endings, and thus provide physiological support for the epidermis. Because the blood vessels approach the interface of the two layers very closely, the dermis cannot be considered a significant barrier in-vivo. The epidermis comprises the viable epidermis and the stratum corneum. The viable epidermis is a layer of cells that undergo continuous differentiation to produce the stratum corneum, which is the outermost layer of the skin. The viable epidermis may be regarded as an aqueous gel and does not present a significant barrier to penetration in most circumstances. If the stratum corneum is damaged, or if extremely lipophilic drugs are being used, however, the viable epidermis can act as a rate-limiting factor in percutaneous absorption.

The major source of resistance to penetration and permeation of the skin is the stratum corneum. This coherent membrane, which is 15-20 μm thick over much of the human body, primarily consists of blocks of cytoplasmic protein matrices (keratins) embedded in extracellular lipid. The keratin containing cells are arranged in an interlocking structure somewhat akin to bricks and mortar. In human beings the "mortar" consists of a structured complex containing several group of lipids (Table 1). Identification of these lipids by solvent extraction and thin layer chromatography has demonstrated a considerable degree of body region variability. Furthermore, the relative amounts of lipid groups apparently present can be significantly affected by the solvents used for extraction. It is generally agreed, however, that the majority of human stratum corneum lipid consists of ceramides and neutral lipids such as free sterols, free fatty acids and triglycerides. The remainder is made up of phospholipids, glycosphingolipids, and cholesterol sulfate, the latter being of considerable importance to the desquamation process. Despite the very low levels of phospholipid in the skin, there is evidence to show that stratum corneum lipids are capable of forming bilayers, suggesting that the intercellular

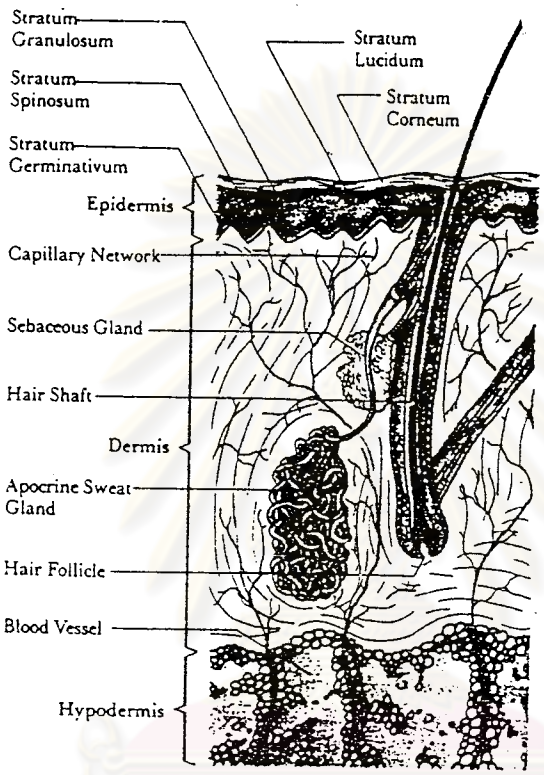

Figure 1 : A cross-section of human skin, showing various skin tissue layers and appendages.

Table 1 : Composition of the horny layer lipids of human abdominal skin.

Polar lipids (4.9 %) :	phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine, sphingomyelin, lysolecithin.
Neutral lipids (74.8 %) :	free sterols (14 %), free fatty acids (19.3 %), triglycerides (25.2 %), sterol and wax esters (5.4 %), squalene (4.8 %), n-alkanes (6.1 %).
Sphingolipids (18.1 %) :	ceramides I (13.8 %), ceramides II (4.3 %), glycosylceramides I and II (traces).
Cholesterol sulphate (1.5 %) :	-

space consists of lamellar liquid crystals (Figure 2) (Loth, 1991). These broad sheets, which contain predominantly saturated lipids, comprise the major epidermal barrier to water and water-soluble permeants.

The "bricks" are the dead, flattened cells of the horny layer, the corneocytes, which contain very little lipid. Their major structural components are aggregates of keratins arranged as bundled of individual keratin filaments. It has been recognized for many years that keratin is not a single substance, but is a complex mixture of proteins whose most importance chemical feature is the pre-ponderance of the sulfur-containing diamino acid cystine. The process of keratinization is complex and results in specific deposition of different keratins at various sites within the corneocyte. The thickened cell envelop, for example, consists of a protein-lipid-carbohydrate mixture in which the protein is rich in disulfide bonds. The majority of amorphous protein within the cell matrix is also rich in disulfide bonds, whereas the fibrous α protein of the filaments does not appear to be so tightly cross-linked. Thus, the strength and durability of the stratum corneum is provided by the amorphous matrix protein surrounding the filaments and the membrane protein surrounding the corneocytes.

2. Routes of Penetration Through Skin.

Based on skin anatomy, several possible routes of drug transport through the skin barrier can be postulated. These are outlined in Table 2 (Scheuplein, 1965; Hadgraft, 1979). These routes of diffusion may be categorized into either transappendageal or transepidermal pathways.

2.1 Transappendageal Pathways.

The stratum corneum is breached by hair follicles and sweat ducts which could provide a shunt-diffusion pathway across the skin. The transfollicular route involves migration through the hair shaft openings which presumably are filled with sebum. This route offers substantially lower diffusional resistance to most drugs than do the other routes but the pathlength is quite long and the density of hair follicles in human skin is quite low. Although the skin appendages offer less diffusional resistance, they occupy only 0.1 % of the area of the skin surface and therefore, contribute relatively little to the total flux after steady-state conditions have been attained. However, the appendages are important in drug absorption just after application to the skin, prior to the establishment of steady-state (Scheuplein, 1967; Touitou and Abed, 1985). During this period most of the molecules penetrating the skin via the shunt pathways. In the case of molecules with very small diffusion coefficients in stratum corneum such as the large

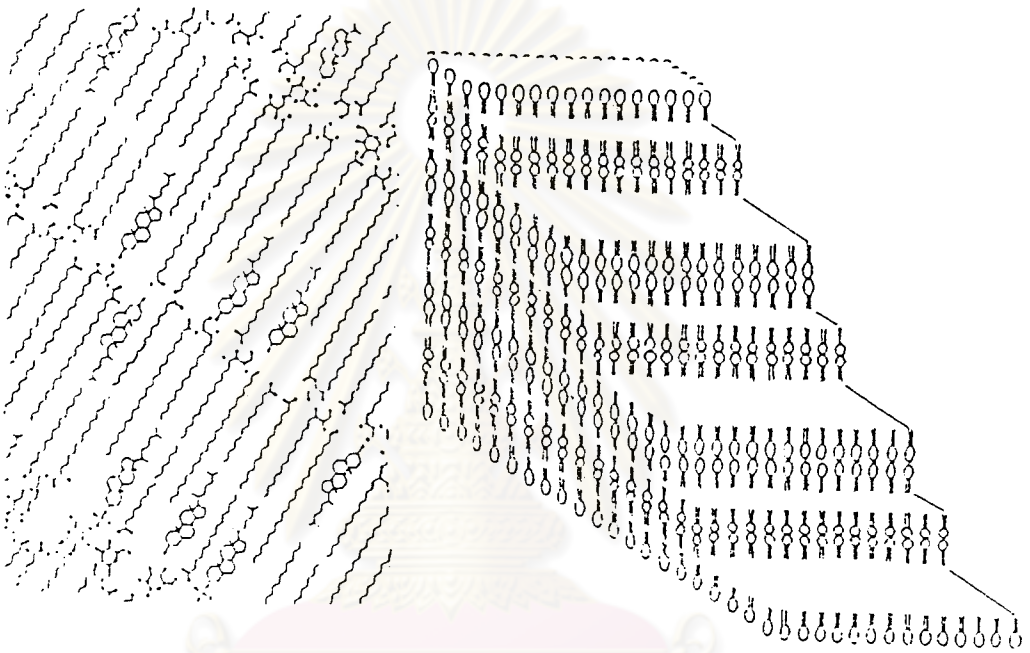

Figure 2 : Scheme of multiple bilayers of the horny layer lipids.

Table 2 : Routes of penetration through human skin.

Route	Relative Surface Area (%)	Diffusional Path Length (μm)	Relative Volume of Stratum Corneum (%)
Transappendageal	0.1	200	0.1
Transepidermal			
- Intercellular	0.7	350	1-10
- Transcellular	99	25	90-99

polar or ionic molecules, the appendages and glands may deliver a significant proportion of drug even after steady-state has been reached (Scheuplein et al., 1969).

2.2 Transepidermal Pathways.

For transepidermal pathways, both the structured lipid environment between the cells and the hydrated protein within the corneocytes play major roles in skin permeability. Figure 3 illustrates two potential routes for drug permeation, the transcellular route and the intercellular route.

The transcellular route, which the drug travels through cells and across them, is the shortest and also the most likely given the relative area presented to a diffusing molecule. Scheuplein, 1967 derived a mathematical model for diffusion through the skin and concluded that the major pathway for penetration of small polar molecules was likely to be transcellular through the stratum corneum.

The intercellular route avoids diffusion through cell contents but is substantially more tortuous. Middleton, 1969 presented evidence that small ions do not penetrate into the cells of the stratum corneum. From this it was reasoned that electrolytes such as sodium and chloride ions must follow the intercellular route when moving through the stratum corneum.

The route through which permeation occurs is largely dependent on the penetrant's physicochemical characteristics, the most important being the relative ability to partition into each skin phase. This has been well demonstrated by the work of Scheuplein, 1973; Flynn et al., 1974; and Behl et al., 1982. Overall, at least for polar drugs, it is likely that the transcellular route provides the main pathway during percutaneous absorption. As penetrants become more non-polar, the intercellular route probably becomes more significant (Barry, 1987).

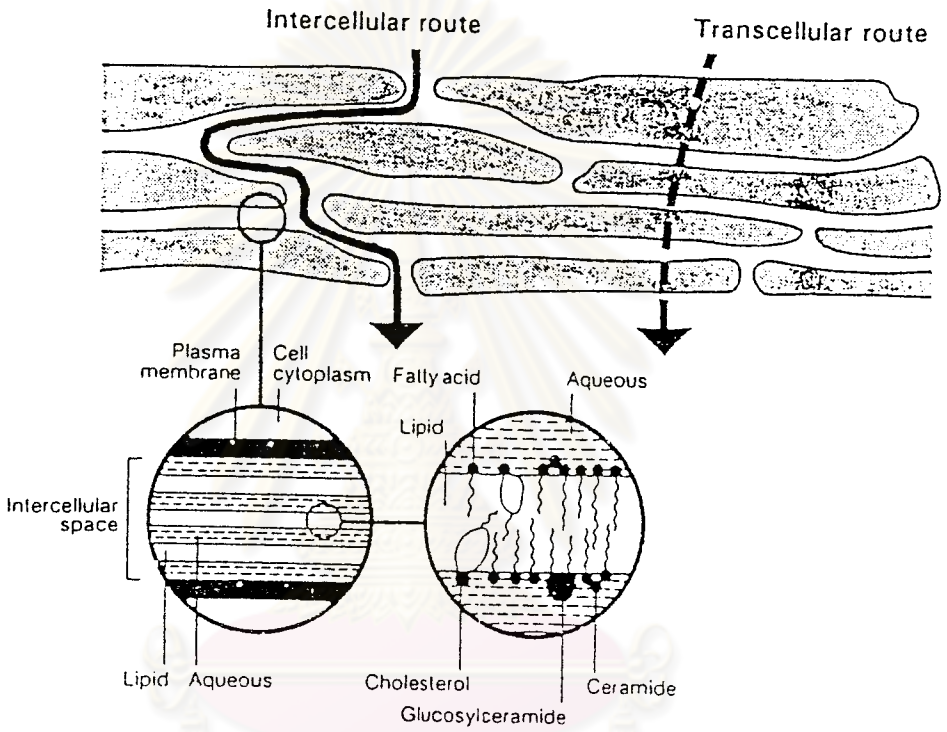

Figure 3 : Suggested routes of drug penetration through human stratum corneum.

Methods for Measuring Percutaneous Absorption.

1. In-vivo Method.

1.1 Human Studies (Wester and Maibach, 1987).

The in-vivo percutaneous absorption is usually determined by an indirect method of measuring radioactivity in excreta following a topical application of a labeled compound. In human studies, plasma and urine levels of the compounds are extremely low following topical applications, often below their assay detection level.

Another approach taken to determine the in-vivo percutaneous absorption is to determine the loss of material from the surface as it penetrates into the skin. Skin recovery from an ointment or solution application is difficult because the total recovery of the compound from the skin is never assured.

Another in-vivo method of estimating absorption is to use a biological/pharmacological response. There, biological assay is substituted for a chemical assay and absorption is estimated. An obvious disadvantage of the use of biological responses is that they are limited to compounds which elicit responses that can be measured easily and accurately. An example of a biological response would be the vasoconstrictor assay when the blanching effect of one compound is compared to a known compound. This method is more qualitative than quantitative.

Other qualitative methods of estimating the in-vivo percutaneous absorption include whole body autoradiography and fluorescence. The whole-body autoradiography provides an overall picture of the dermal absorption followed by the involvement of other body tissues.

1.2 Animal Studies.

Because of all the problems encountered in in-vivo percutaneous absorption study in man, the use of either in-vivo animals or in-vitro animal skins has been widely practiced. However, no single animal is useful in all circumstances.

Bartek et al., 1972 undertook a comparative study of in-vivo percutaneous absorption in rats, rabbits, miniature swine, and man. Methodology in the animals was similar to that in man. This study showed rabbit skin to be the most permeable to topically applied compounds

(Haloprogin, Acetylcysteine, Cortisone, Caffeine, Butter Yellow, and Testosterone) and was followed closely by rat skin. And it appears that the permeability of the skin of the miniature swine is closer to that of human skin. Clearly, percutaneous absorption in the rabbit and rat would not be predictive of that in man. It is not known if the subtle differences seen between pig and human skins were due to methodology or skin itself. Generally the pig appears to be a good predictor of percutaneous absorption in man.

Bartek and La Budde, 1975 also studied the percutaneous absorption of pesticides in rabbits, pigs, and squirrel monkeys. And they compared the results with the absorption obtained in man. It appeared that the in-vivo percutaneous absorption of pesticides in the rabbits were again much greater than in man, whereas penetration in the pigs and squirrel monkeys were closer to that in man.

2. In-vitro Method.

For many purposes, in-vitro penetration experiments are advantageous. They are preferred for screening a series of compounds or for comparing several formulations of a drug to investigate the one with the most favorable skin penetration characteristics. The in-vitro techniques of percutaneous absorption studies have played an important role in research strategies aimed at developments of conventional topical dosage forms and novel transdermal systems.

2.1 Diffusion Cells Used in the In-vitro Studies.

The use of excised human skin to study the skin penetration of chemicals has been widespread (Bronaugh et al., 1986). The method is to measure the penetration of drugs through excised human skin under conditions that would mimic the biological systems. The investigators use some types of two-compartment diffusion cell between which animal or human skin is mounted. And the diffusion of compounds through the full thickness of skin or stratum corneum sheets into a receptor system is measured.

In general in-vitro percutaneous absorption are conducted on either vertically or horizontally oriented diffusion cells. The receptor compartment would be either static or flow-through cells. For the study of the steady-state diffusion of molecules across a membrane, the vertically oriented diffusion cells is more popular. Vertically oriented diffusion cells lend themselves to mimic the in-vivo situations. The donor compartment of the cells is exposed to ambient temperature. While the receptor compartment would mimic blood circulation system by keeping sink conditions. The temperature

of the receptor phase is easily manipulated. Additionally, the vertical cells lend themselves to ease of dose application.

Vertically oriented diffusion cells are usually based on the Franz cell (Figure 4). The donor portion contains the applied drug and makes contact with the stratum corneum. After passing through the skin, drug molecules enter the receptor solution. In a diffusion cell, precise measurements of permeation under carefully controlled conditions are possible. However, these cells show slow or incomplete stirring of the receptor phase (Gummer et al., 1987). The modified - Franz cells were designed in order to stir the receptor solution quickly and uniformly. One of the modified - Franz cell design is shown diagrammatically in Figure 5. Critical to the cell is the design of the receptor compartment. It is important to maintain a wide diameter of the receptor side in relative to its height in order to achieve rapid and uniform stirring. The degree of mauve coloration from the potassium permanganate crystal was used to qualitatively assess the degree of stirring. Complete colouration within 30 seconds was considered as instantaneous and acceptable stirring (Gummer et al., 1987). The modified - Franz cell design should meet this existing requirement.

2.2 Animal Skins Used in the In-vitro Studies.

The percutaneous absorption can also be determined using the in-vitro techniques. The in-vitro permeabilities through animal skins had been determined by several investigators (Mc Gresh, 1965; Tregear, 1966; Marzulli et al., 1969). Generally, the studies have shown that the skin of common laboratory animals (rabbit, rat and guinea pig) are more permeable than the skin of man. On the other hand, the permeabilities through pig and monkey skins are closer to those through human skin (Wester and Maibach, 1987). Not surprisingly this general ranking is in close agreement with the in-vivo data discussed earlier.

Campbell et al., 1976 investigated the in-vitro permeation of scopolamine through rat, rabbit and human skins. The results indicated that the human skin was the least permeable of the three species tested. And the relative order of the permeabilities through rat and rabbit skins depended both on their skin location and on the method used to remove their hair.

In those instances in which the in-vivo and in-vitro penetrations have been compared experimentally under similar conditions, remarkable similarities have been found. Franz, 1975 studied the permeation of twelve organic compounds through excised human skin. The drugs were dissolved in acetone and pipetted onto the epidermal surface in a manner

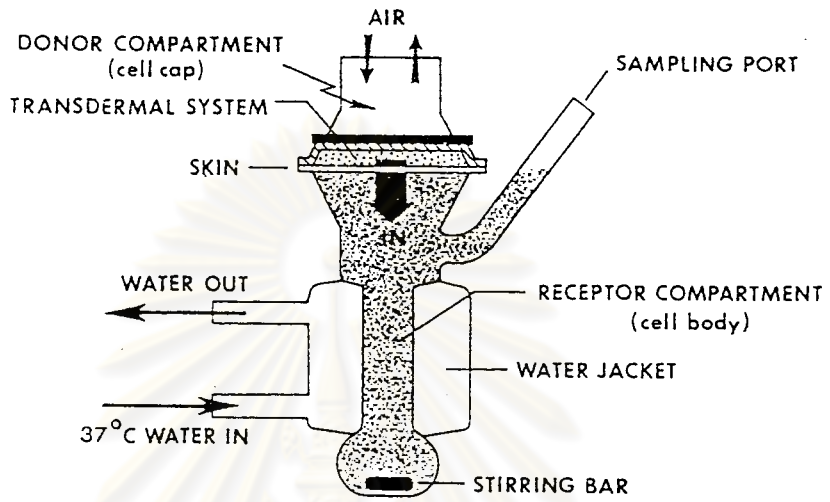

Figure 4 : Diagrammatic illustration of the Franz diffusion cell.

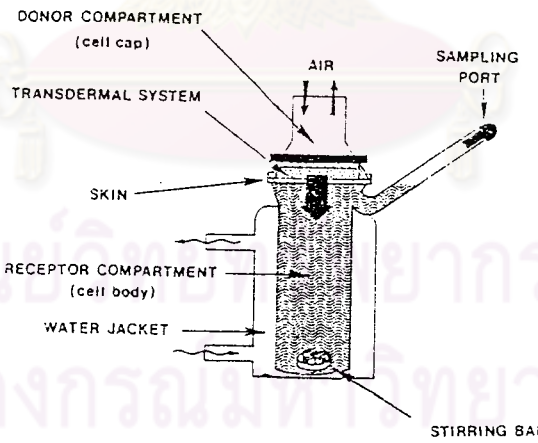

Figure 5 : Diagrammatic illustration of the modified Franz diffusion cell.

similar to that employed in a previous in-vivo study (Feldmann and Maibach, 1970). Care was taken to ensure that his in-vitro conditions closely followed those of the in-vivo studies. There was a definite correlation between the percentage of penetrations as a function of time obtained in the two studies. Scheuplein and Ross, 1974 used a diffusion cell to follow the penetration of cortisone through epidermis. Their results correlated well with in-vivo data (Feldman and Maibach, 1969) for the same drugs applied in the same solvents. Chowhan et al., 1978 found reasonable agreement between the in-vitro and in-vivo values for percutaneous absorption of naproxen in rats. Hawkins and Reifenrath, 1984 demonstrated a statistically significant correlation between percutaneuous absorptions of ten compounds through pig skins and through in-vivo human skins. In their further study (Hawkins and Reifenrath, 1986) using another eleven compounds, they also found good correlations between diffusions through in-vitro pig skins and diffusions through in-vivo human skins.

When the percutaneous absorptions through different species are compared, it becomes obvious that differences do exist. Some of these differences are due to the species themselves and some of the differences are due to techniques used in the study.

In conclusion, pig and monkey appear to be good species for comparing both in-vivo and in-vitro permeabilities to those of human beings.

2.3 Artificial Membranes Used in the In-vitro Studies.

Since the animal skin model is far from convenient and it is subject to considerable biologic variation. A synthetic membrane that could mimic the permeability characteristics of human skin would provide a convenient, accessible, and reproducible experimental model without the inconveniences and limitations of the animal ones.

Synthetic membranes usually used to study drug diffusion kinetics is polydimethylsiloxane (PDMS). PDMS is an isotropic, non-porous, hydrophobic polymer which provides an excellent non-polar barrier for diffusional studies. It also has high specific diffusivity, little or no tendency to imbibe polar solvents, especially water, and good biocompatability (Flynn and Yalkowsky, 1972).

Early studies using PDMS (Silastic®) were conducted by Garrett and Chemburkar, 1968 who demonstrated facile permeability of several drug species. Yeung et al., 1987 reviewed the study of steady-state flux and permeability coefficient of a saturated solution of salicylic acid in 50 %

ethanol/water. Of the various synthetic membranes tested (cellulose acetate, diaflo®, silastic®, trilaminar cellulose acetate, trilaminar silastic®, and multimembrane system), no single polymeric membrane was found to mimic the permeability of human skin. However, the silastic® sheeting was found to yield flux and permeability coefficient which are closer to that of excised human skin than the other membranes. A study by Barry et al., 1985 demonstrated that the diffusion of benzyl alcohol through 0.127 mm thick silastic® membrane was about 13 times faster than that through excised human skin.

Theory of Diffusion.

Percutaneous absorption is usually purely a passive process (Tregear, 1966). The basic laws of passive diffusion can be used to describe transdermal absorption. The drug diffusion may be expressed by Fick's first and second laws. Fick's first law states that the rate of transport of a diffusing substance through a unit area of a membrane is proportional to the concentration gradient. It is mathematically written as

$$J = -D\frac{dc}{dx} \qquad \text{eq. 1}$$

where J is the one-dimensional flux across the plane at right angles, D is the diffusion coefficient for the diffusant, and dc/dx is the concentration gradient across the plane. The negative sign in the equation denotes that the diffusional flux proceeds from regions of higher to regions of lower concentration. The diffusion coefficient is the proportional constant for the equation. It represents the amount of permeant that across a plane of unit area at right angles to the direction of diffusion in unit time and with a unit concentration gradient. The diffusion coefficient is concentration sensitive as well as being affected by temperature, solvent properties such as viscosity, permeant properties such as molecular weight, and the nature of the barrier. In addition, the value of D for a system may be time-dependent. However, if the concentration of the solution is low, D can be considered to be constant and independent of composition.

Fick's second law is derived from Fick's first law when D is assumed to be independent of the distance, x, between two planes perpendicular to the vector of diffusive flow :

$$\frac{dc}{dt} = D\frac{d^2c}{dx^2} \qquad \text{eq. 2}$$

where the rate of change of permeant concentration in a given volume element, dc/dt, is proportional to the derivative of the concentration gradient, $d^2c/d\,x^2$. This expression represents the diffusion in one-dimension only. Fick's second law is identical with the first except that it represents an expression in terms of concentrations which are experimentally measurable instead of molecular flux which must be determined indirectly. While the first law can only be directly applied to diffusion in steady-state, the second law describes nonsteady-state diffusion as well.

Daynes, 1920 provided a solution of Fick's second law of which assumptions are these : i) the membrane is initially at zero concentration, ii) the concentration gradient is maintained constant, and iii) the diffusant concentration in the receptor compartment is maintained at less than or equal to 5 % of the donor concentration (or a sink condition) during the diffusion experiment. His solution was later generalized by Barrer, 1939 :

$$Q = \frac{DACo}{h}\left(t - \frac{h^2}{6D}\right) \qquad \text{eq. 3}$$

where, Q is the cumulative mass of diffusant which passes through a membrane of area, A, and of thickness, h in time, t. D is the diffusion coefficient of the diffusant within the membrane. Co represents the concentration of permeant in the membrane surface exposed to the donor solution. From a practical standpoint, the donor concentration, Cd, is measured and is related to Co by the distribution or partition coefficient, K, when an equilibrium between the membrane and the phase contiguous to it is established :

$$Co = KCd \qquad \text{eq. 4}$$

Substitution of equation (4) into equation (3) and differentiating with respect to time yields an equation of the steady-state flux, Jss, through the membrane :

$$Jss = \frac{dQ/dt}{A} = \frac{DKCd}{h} \qquad \text{eq. 5}$$

This relationship is used for the calculation of the steady-state flux in a diffusion experiment. The concentration of permeant in the receiving compartment is measured at various time. The concentration is converted to the cumulative amount of diffusing penetrant (Q) by multiplying by the volume of receiving solution. The amount of drug penetrating is quite small at first. This is due to the fact that the diffusional resistance of the membrane limits the number of molecules that can traverse the membrane in a short time. The rate of drug penetration increase gradually approaching a limit constant value (steady-state). The steady-state flux (Jss) is obtained from the slope of a linear portion of a plot of the cumulative amount crossing the membrane versus time. A typical plot is presented in Figure 6.

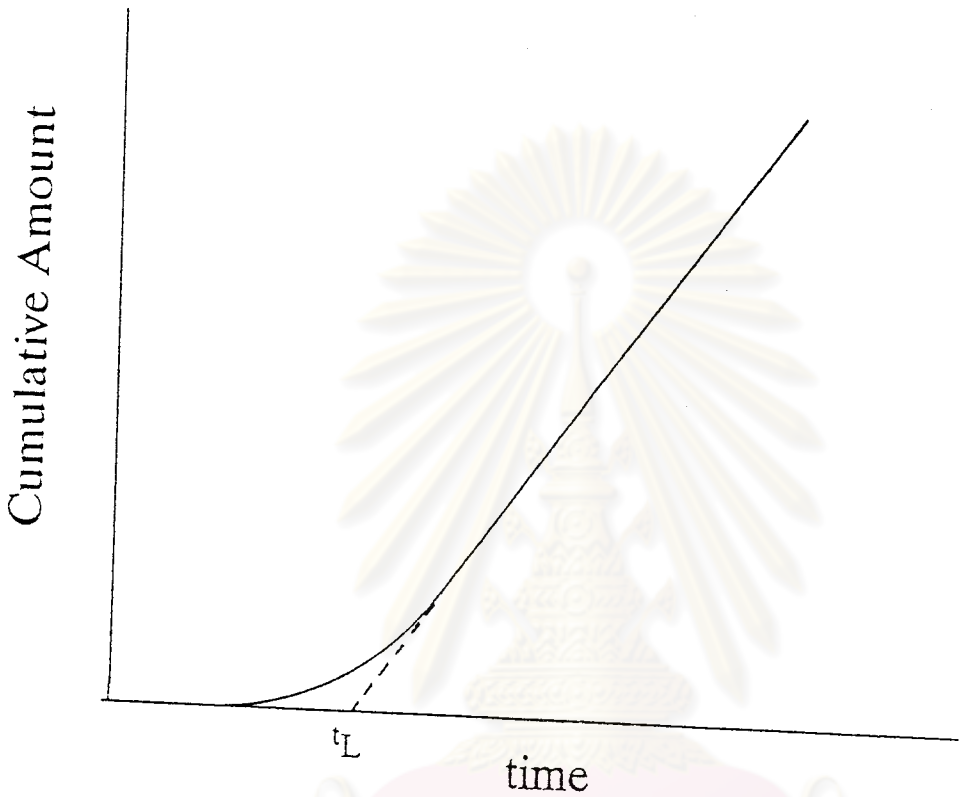

Figure 6 : Typical cumulative amount versus time profile.

If the steady-state portion of such a plot is extrapolated to the time axis (Q = 0), a time value is obtained :

$$t_L = \frac{h^2}{6\,D} \qquad \text{eq. 6}$$

where h is the membrane thickness. This intercept is called the lag time (t_L) and it provides a measure of the time required to establish steady-state. It is believed that steady-state is achieved within 1 % error when time equals 2.7 times lag time (Crank, 1975).

Factors Affecting Percutaneous Absorption.

Factors that determine skin absorption kinetics can be divided into three groups : physiological, drug, and formulation factors.

1. Physiological Factors.

1.1 Skin Hydration.

Hydration can result from water diffusing from underlying epidermal layers or from perspiration accumulating after application of an occlusive vehicle or covering of the skin surface. Under occlusive conditions, the stratum corneum is changed from a tissue that normally contains very little water (5-15 %) to one that may contain as much as 50 % water. Permeability may increase four to five fold as a result of skin hydration. Barry, 1987 suggested that the mechanism of hydration was to increase in fluidity of the bilayer lipid and keratin in the stratum corneum.

1.2 Regional Skin Sites.

There are many variations in absorption of permeant from one site to another due to the thickness and nature of stratum corneum. In different normal individuals, there are wide variations in the absorption rate of a given substance through the same skin site.

1.3 Species Variation.

Human and animal skins display wide differences in physical and structural characteristics which obviously affect the penetration pathways and the penetration resistance of skin (Marzulli, 1969). Among species, there are differences in tissue thickness, desquamation rate, hair density, gland density, lipid composition, etc. There is no perfect substitute for human skin in modeling its properties. However, pig skin is remarkably similar to human skin (Bissett and McBride, 1983). It can be used to model many human skin properties. The skin of rabbits, rats, and mice which have been used frequently in work on percutaneous absorption were more permeable than the skin of man. Skin from pig and monkey were more generally approximate the permeability of human skin (Tregear, 1966; Marzulli et al., 1969; Bartek et al., 1972; Bartek and La Budde, 1975).

1.4 Skin Age.

The relationship of age to skin permeability has been rarely investigated. Generally, fetal and infant skin appear more permeable than adult skin (Idson, 1975).

1.5 Skin Integrity.

Injurious agents which cause damage to the stratum corneum, allowing much faster transport of drug. Experimentally, salicylic acid from aqueous solution was shown to penetrate through damaged skin about ten times faster than through intact skin (Washitake et al., 1973).

2. Drug Factors.

2.1 Molecular Size.

An inverse relationship appears to exist between absorption rate and molecular weight of substances. Small molecules penetrate more rapidly than large molecules. But within a narrow range of molecular size, there is little correlation between size and penetration rate. Diffusion constants through the hydrated stratum corneum for many low molecular weight compounds were approximately the same (Scheuplein, 1969).

2.2 Partition Coefficient.

There are two partitioning processes in percutaneous absorption. First, drug partitioning from the vehicle into the stratum corneum and second, drug partitioning from the stratum corneum into the viable epidermis. To yield high partition characteristics, the drug candidate must favor the stratum corneum over the vehicle and the relative affinity of the drug for stratum corneum and viable tissues must be reasonably balanced. Penetration results for a series of alkanols in aqueous solution passing through excised human skin illustrated the importance of partition coefficient (Scheuplein, 1976). The permeability coefficient increased strongly as the partition coefficient increased.

3. Formulation Factors.

3.1 Drug Concentrations.

The amount of drug percutaneously absorbed per unit surface area per time interval increases as the concentration of the drug in the vehicle is increased. Zatz, 1985 studied the effect of applied volume on the depletion of percutaneous absorption. He found that the decrease in donor concentration along the penetration process leaded to a depletion on percutaneous absorption.

3.2 pH.

Preparations applied to the skin usually have moderate pH values, about 4-9, to avoid damage to the tissues. Skin penetration of nonionic compounds is unaffected by pH within this range. However, the skin penetration of ionizable drugs depends on vehicle pH because the relative proportions of charged and uncharged species are different. Ionized form of a substance has been known to have unfavorable free energy to transfer into lipid phase, thus most of the drugs that can partition is the nonionized form (Touitou and Abed, 1985; Roy and Flynn, 1990). Michaels et al., 1975 found that at a fixed pH the permeability constants of unionized forms of drugs (ephedrine, scopalamine and chlorpheniramine) were considerably greater than those of ionized forms.

3.3 Solvents.

Alteration of solubility characteristics provides one of the most powerful ways to the formulator for selecting a vehicle to control skin absorption. The skin/vehicle partition coefficient cannot be raised by increasing the solubility of drugs in skin since this is essentially a fixed quantity. However, changes in the affinity of drug for the vehicle (vehicle solubility) can influence the partitioning process by controlling the concentration gradient across the stratum corneum. Vehicles that have too high affinity for the drug will retard penetration. In other words, an increase in drug solubility leads to a decrease in the percutaneous absorption rate if the drug concentration in the solution is kept constant. For example, the steady-state penetration flux of diflorasone diacetate through hairless mouse skin from mineral oil/polyoxypropylene 15 stearyl ether solutions was progressively reduced in the presence of higher quantities of the latter which acts as a cosolvent for the drug (Turi, 1979). Similarly, the penetration of butyl paraben through guinea pig skin from aqueous solutions was reduced by the addition of two cosolvents, polyethylene glycol 400 and propylene glycol (Komatsu and Suzuki, 1979).

In addition, some solvents may interact with the barrier membrane to alter skin resistance to diffusion. There is usually an increase in the effective diffusion coefficient through the stratum corneum in such cases. Other possible solvent effects are alteration of skin hydration or drug partitioning due to solvent penetration into the skin as discussed above.

3.4 Surfactants.

Surfactants are used as solubilizers, wetting agents, and emulsifiers in many preparations applied to the skin. Results obtained in a study of naproxen flux through excised human abdominal skin showed that anionic surfactants enhanced percutaneous absorption, cationic surfactants retarded absorption and nonionic agents had little effect (Chowhan, 1978). Although ionic surfactants can penetrate and interact with skin, they are irritating.

Nonionic surfactants are often used in pharmaceutical products applied to the skin because they are less irritating than ionic surfactants. The mode of action of nonionic surfactants appears to be linked to their ability to increase membrane fluidity and their capacity to solubilize and extract membrane components. Although there are many different types of nonionic surfactants, the majority of studies concerning their effects on biological systems are limited to four principal series. These include the polysorbates, polyethoxylated alkyl ethers and esters, polyethoxylated alkyl phenols, and poloxamers.

The polysorbates have been shown to enhance, retard, or have no effect on skin permeability rates (Mezei and Ryan, 1972; Sarpotdar and Zatz, 1986a). An example is that polysorbates 20, 40, 60 and 80 increased the fluxes of hydrocortisone and lidocaine across hairless mouse skin (Sarpotdar and Zatz, 1986b).

Polyethoxylated alkyl ethers and esters have been shown to enhance the percutaneous absorptions of flufenamic acid (Hwang, 1983), methyl nicotinate (Walters and Olejnik, 1983), nicotinic acid (Walters et al., 1984), and naloxone (Aungst, 1986). The magnitude of penetration enhancement depends on the length of both alkyl and polyoxyethylene chain. The surfactants with an alkyl chain length of twelve carbon atoms linked to an ethylene oxide chain of 10-14 units have been found to be most effective (Walters and Olejnik, 1983).

Polyethoxylated alkyl phenols and poloxamers appear to be minimally effective as skin penetration enhancers (Aungst, 1986; Walters, Walker and Olejnik, 1988).

3.5 Penetration Enhancers.

Penetration enhancers are substances that reduce skin resistance to diffusing molecules. Various agents have been reported as penetration enhancers such as hydrophilic solvents, propylene glycol and surfactants. However, the most effective accelerants are aprotic materials such as urea, dimethylsulfoxide, dimethylformamide, and dimethylacetamide. Probably the most well-known and most widely studied enhancer is dimethylsulfoxide (DMSO). But DMSO is not approved for use in topical formulations, largely because of the possibility of toxicity. There is also a problem due to the garlic-like odor of this compound.

Most recently, laurocapram (Azone®) has been shown to be a very effective enhancer of skin permeability. Contrary to other enhancers, azone appears to exert its penetration enhancing effects at a much lower concentration (Stoughton, 1983). It also does not appear to have many of the side effects associated with other enhancers.