CHAPTER II

LITERATURE REVIEW

1. Temozolomide (TMZ) as a novel anticancer agent

1.1 General background

TMZ is a novel anticancer agent, which was synthesised by Professor Malcolm Stevens and colleagues at the University of Aston in Birmingham (Stevens *et al.*, 1984). There has been great interest in this anticancer agent because it has shown a broad-spectrum antitumour activity against murine tumours with schedule dependency (Stevens *et al.*, 1987). Furthermore, TMZ has been shown to demonstrate clinical anticancer activity against malignant melanoma (Newland *et al.,* 1992; Bleehen *et al.,* 1995), mycosis fungosides (Newland *et al.,* 1992), and high-grade gliomas (Newland *et al.,* 1992; Bower *et al.,* 1997) with favourable side effects, and predictable myelosuppression. On the contrary, the predecessor compound, which is mitozolomide, exhibits limited clinical activity, and elicits severe and unpredictable myelosuppression (Newland, 1985).

1.2 Antitumour activity of temozolomide

TMZ displayed pre-clinical antitumour activity against a broad spectrum of murine tumours *in vivo*, including leukaemia, lymphomas, and solid tumours (Stevens, 1987). This activity has been shown to be highly schedule dependence with multiple administrations being more effective than a single bolus dose. Additionally, TMZ is highly effective in the treatment of subcutaneous and intracereberal human brain tumour xenografts, and metastatic lung carcinoma in mice (Plowman, 1994; Tentori *et al.,* 1995). *In vitro* studies of TMZ antitumour activity have demonstrated

activity against a large variety of human tumours such as brain cancer, ovarian cancer, and melanoma, including some tumours usually resistant to chemotherapy with conventional drugs (dacarbazine, carmustine, cisplatin, doxorubicin, 5-fluorouracil, etoposide, and vinblastine) (Raymond *et al.,* 1997).

According to the promising antitumour activity of TMZ in pre-clinical studies, clinical trials of this anticancer agent have been carried out.

TMZ was initially tested in phase I clinical studies. Its potential activity has been seen in malignant melanoma, mycosis fungosides, and high-grade gliomas (Newland *et al.,* 1992). Moreover, therapeutic potential has also been discovered against leukemia, and breast cancer (Rathbone, 1999). Phase II and III clinical studies of TMZ are concentrating on its effectiveness in the treatment of melanoma and brain tumours (Wang and Stevens, 1997).

TMZ has been approved by European Medicine Authority as an anti-brain cancer drug in 1999. However, its activity against skin cancer has not yet been fully established in UK and US.

1.3 Temozolomide as a new anti-skin cancer drug

Phase II trials of TMZ have confirmed that it has significant activity in patients with metastatic melanoma (Bleehen *et al.*, 1995). Recently, a phase III study of TMZ in the treatment of patients with advanced metastatic malignant melanoma indicated that the efficacy of TMZ is equal to dacarbazine, which is an oral alternative

for the patients with advanced metastatic melanoma (Middleton *et al.,* 2000). However, dacarbazine presents severe cumulative bone marrow toxicity (Stevens, *et al.,* 1987).

The schedule used to administer TMZ in clinical studies was 150 mg/m^2 given orally for five consecutive days in the first course. Courses were repeated every 4 weeks and if no myelosuppression (*i.e.* leukopenia, lymphopenia) was detected after the initial course, the subsequent doses were escalated to 200 mg/m^2 (Newland *et al.,* 1992; Bleehen *et al.,* 1995; Middleton *et al.,* 2000). The symptomatic toxicity from TMZ was mainly nausea and vomiting. Alopecia, rash, and constipation could also be found to a lesser degree. Finally, the major toxicity reaction was leukopenia (Newland *et al.,* 1992; Bleehen *et al.,* 1995; Middleton *et al.,* 2000).

1.4 Pharmacokinetics

Pharmacokinetic studies of TMZ in mice showed a rapid absorption phase, elimination half-lives of 1.13 h (i.p.) and 1.29 h (p.o.), and approximately 100% bioavailability after one oral administration (Stevens, *et al.,* 1987). These studies were repeated in a phase I clinical trial, and it was found that TMZ is rapidly absorbed, with maximum plasma concentrations being obtained in 0.7 h, a distribution half life of 1.8 h, and a good bioavailability after post dosing (i.v.) (Newland *et al.,* 1992). Like its predecessor mitozolomide, TMZ demonstrates good tissue distribution *via* kidney, lung, liver, and can traverse the blood-brain barrier (Brindley *et al.*, 1986; Newland *et al.,* 1997).

During preliminary elimination studies performed in mice (Tsang *et al.,* 1990), it was found that renal excretion was the predominant route of elimination.

TMZ and its metabolite (3-methyl-2,3-dihydro-4-oxoimidazo[5,1-d] tetrazine-8-carboxylic acid), the carboxylic acid of TMZ, were found in human urine (Tsang *et al.,* 1990). This metabolite exhibits cytotoxicity against lymphoma cells equal to TMZ.

1.5 Pharmacology

Unlike dacarbazine and mitozolomide, which require metabolic activation, TMZ can degrade in physiological fluid to generate the cytotoxic methylating species, 5(3-methyl-1-triazeno) imidazole-4-carboxamide (MTIC) (Tsang *et al.,* 1991).

The antitumour activity of TMZ is largely attributed to the methylation of DNA bases forming methyl addition products at N^7-guanine, N^3-adenine, and O^6-guanine leading to DNA strand breakage and cell death (Bull and Tisdale, 1987; Wedge *et al.,* 1997). Although a small percentage of methylation adducts at O^6-position, there is increasing evidence that O^6-alkylguanine is a major cytotoxic lesion (Baer *et al.*, 1993). This finding is supported by several experiments correlating with O^6-alkylguanine-DNA-alkyltransferase (ATase), which is a cytoprotective DNA repair protein, providing protection against the toxic, mutagenic, and carcinogenic effects of alkyl agents. Methyl groups adduct at O^6-guanine in DNA are repaired by ATase, which principally removes these groups from DNA. This reaction results in an irreversible inactivation of this repair protein. Therefore, when lesion repair has occurred, cells are depleted of ATase until the synthesis of new enzyme molecules takes place (Lacal *et al.*, 1996). Obviously, the depletion of ATase by pre-treatment with O^6-benzylguanine, which is a potent ATase inactivating agent, greatly improved

the cytotoxicity of TMZ (Wedge *et al.*, 1996; Dolan, 1997). In addition, it has been shown that a number of human tumours are inherently resistant to the cytotoxic effects of alkylating agents due to higher levels of ATase expression (Chinnasamy *et al.*, 1997).

Poly (ADP-ribose) polymerase (PADPRP) is also secondarily indicated in DNA-repair mechanisms. This can be explained by pre-clinical studies, which indicate that the combined treatment of leukaemia cells with TMZ and a PADPRP inhibitor greatly potentiates the cytotoxicity of this anticancer drug (Boulton *et al.*, 1995). Moreover, Friedman and colleagues (1997) reported that the development of methylating agent resistance is caused by the deficiency of mismatch repair mechanisms. This result suggests that the cytotoxicity of TMZ is also dependent on a function of the DNA mismatch repair pathway.

Whilst the biological effects of TMZ have been widely studied, the precise chemical mechanism of its activity remains less clear.

1.6 *In vitro* studies of the mechanism of action of temozolomide

In vitro investigations into the mechanism of action of TMZ also confirm that TMZ behaves as a prodrug, generating active metabolite, which transfers a methyl group to a nucleophile.

Decomposition of TMZ was studied in an aqueous system, deuteriated phosphate buffer solution, pH 7.4 (Stevens *et al.*, 1984; Wheelhouse and Stevens, 1992; Wheelhouse and Stevens, 1993). These studies indicate that the antitumour

prodrug temozolomide undergoes ring opening in aqueous solutions to regenerate the reactive species (MTIC). Presumably, MTIC does not react directly with DNA, but highly reactive methyl-diazonium species, which are unimolecular fragments of MTIC, transfer the methyl group to the bionucleophile on DNA.

The mechanism of degradation of temozolomide in aqueous solution is shown in figure 1. The rate-limiting step is the base-catalysed addition of water to form a tetrahedral intermediate, which collapses with breakdown of the tetrazinone ring followed by spontaneous decarboxylation. The further reaction of MTIC requires acid catalysis, and follows by the fragmentation of the triazine to form 5-aminoimidazole-4-carboxamide (AIC) and methyldiazonium ion. The final step is the reaction of methyldiazonium with a nucleophile, which may be water or the components of the buffer in the solution. The temozolomide molecule can therefore be considered, as a prodrug exquisitely developed to deliver a methylating fragment to bionucleophiles (DNA bases).

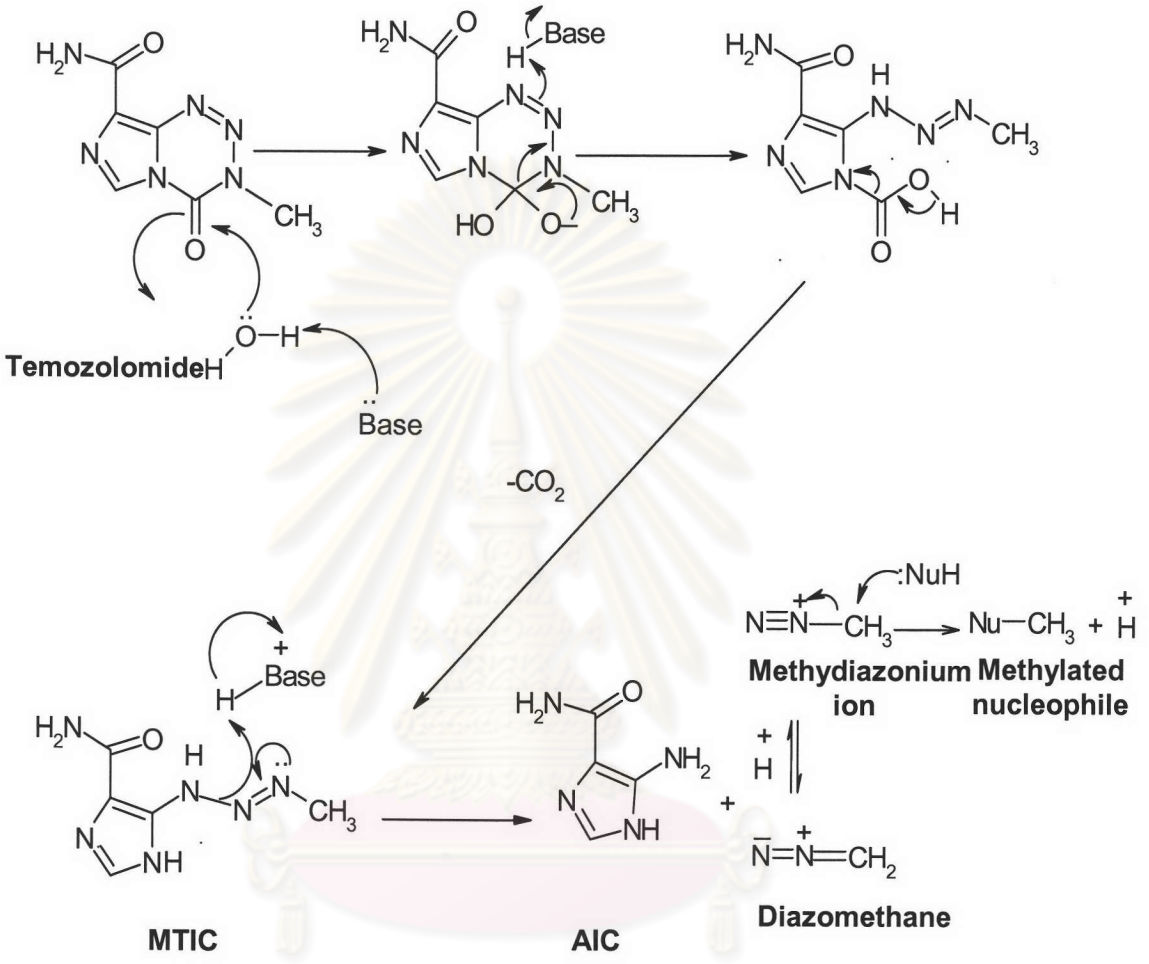


Figure 1. Scheme of decomposition mechanism of TMZ in aqueous solution (pH 7.4)

1.7 The synthesis of temozolomide

The original synthesis of TMZ was from the reaction of 5-diazoimidazole-4-carboxamide with methyl isocyanate (Stevens *et al.,* 1984). This reaction is based on the general synthesis of azolotetrazinones.

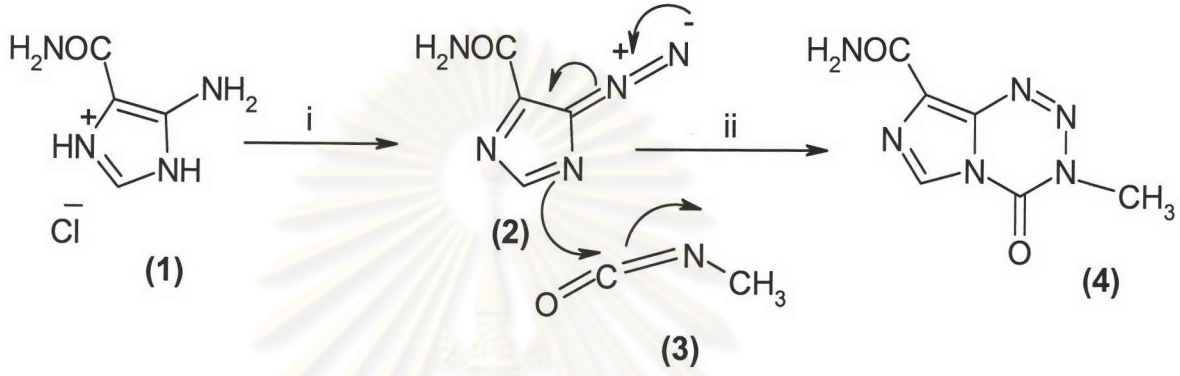


Figure 2. Scheme of the original synthetic route of TMZ; *Reagents and conditions:* i, excess $NaNO_2$, 0-5 °C; ii, EtOAc-DMSO, 25°C

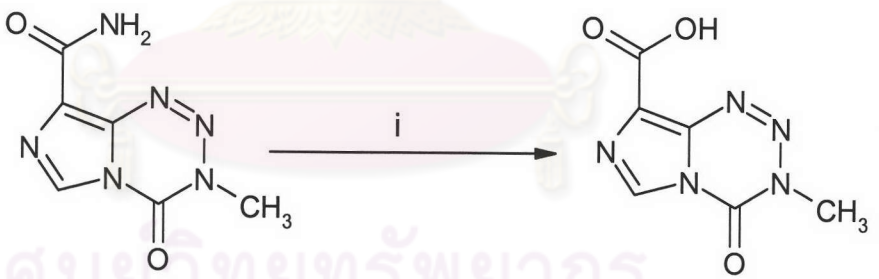
Figure 2 shows the original synthetic route of TMZ. This route started with 5-aminoimidazole-4-carboxamide, commercially available as a hydrochloride salt **(1)**. This was converted to 5-diazoimidazole-4-carboxamide **(2)**, which reacted slowly with methyl isocyanate **(3)** at 25°C to afford the imidazotetrazine **(4)** in high yields.

This first synthetic route has been proven highly versatile in providing access to a wide range of analogues from the various aminoimidazoles and isocyanates.

However, isocyanates can be severely toxic. Consequently, new alternative routes, which do not necessitate the use of such agents, have been developed (Wang, and Stevens, 1997).

1.8 Synthesis of TMZ-HE derivative

Similar to mitozolomide drug, the key intermediate to successfully synthesise ester derivative of TMZ, is *via* the 8-carboxylic acid derivative, which provides a ready handle for the synthesis of a wide range of chemical analogues (Horspool *et al.*, 1990). To synthesise the TMZA the starting compound is TMZ itself, and concentrated sulphuric acid is used for hydrolysis of the carbamoyl group to carboxylic acid (see figure 3). The resulting TMZA from this reaction can then serve as a precursor to synthesise TMZ-HE.

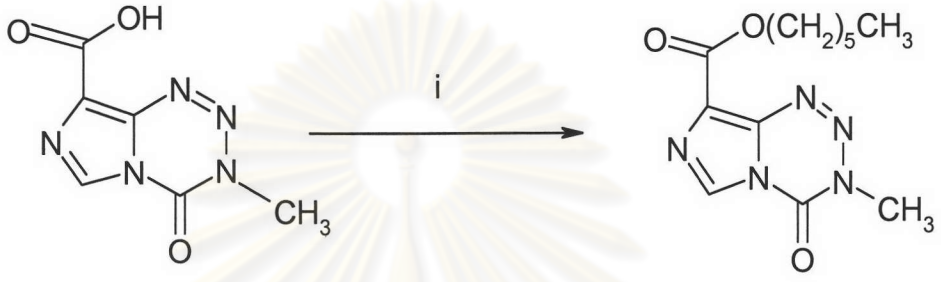


TMZ TMZA

Figure 3. Scheme of the synthesis of TMZA from TMZ free drug; *Reagents and conditions:* **(i)** $NaNO_2$, H_2SO_4 (conc.), distilled water, T° below 15°C

Similar to previous project (Suppasansatorn *et al.*, 2006), to successfully synthesise TMZ-HE products, Pybrop® and DMAP were used as the coupling agents (figure 3) which allowed the reaction to proceed to successfully yield the products.

The optimum conditions were found to be a ratio of alcohol: drug: Pybrop®: DMAP of 2.2: 1: 1: 2. The reaction time for each was around 12 hours at room temperature. TMZ-HE product was purified using siliga gel packed in the column, the percentage yield of TMZ-HE product was obtained around 40-60 %.



TMZA TMZ-HE

Figure 4. Synthetic scheme of TMZ-HE derivatives **(i)** Pybrop®, DMAP, DMF, THF

2. Transdermal delivery of drug

Transdermal drug delivery for systemic pharmacological effects is currently recognised as a viable means to administer therapeutic agents. Transdermal medication confers the following potential benefits (Chien, 1992; Kydonieus, 1987; Ranade and Hollinger, 1996):

1. Bypass of the variation in the absorption and metabolism associated with oral administration

2. Provide a simplified therapeutic regime leading to better patient compliance

3. Permit a rapid termination of medication by simple removal from the skin surface

4. Allow effective use of drugs with short biological half-lives

5. Allow administration of drugs with narrow therapeutic windows

2.1 Anatomy and function of the skin

The goal of transdermal is to optimise the permeation of a drug to pass through the skin at therapeutic levels. Consequently, understanding of the anatomy and functions of the skin is extremely important.

The skin is one of the most extensive and readily accessible organs of the human body. It covers the entire body, has a surface area of 1.5 to 2 square meters, weighs 4 to 5 kg, and accounts for about 7% of total body weight in the average adult (Marieb, 1998). The skin receives about one-third of all blood circulating through the body (Chien, 1992).

The skin is elastic, rugged, and self-regenerating, under normal physiological conditions (Montagna and Parakkal, 1976). It varies in thickness from 1.5 to 4.0 millimetres (mm) or more in different parts of the body (Marieb, 1998). The skin separates the underlying blood circulation network and viable organs from the outside environment.

2.1.1 Functions of the skin

The skin performs a variety of functions that affect body metabolism, and protect the body from external factors such as bacteria, abrasion, temperature, and chemicals. It behaves as a site of temperature regulation, sensation, metabolic function, blood reservoir, and excretion. The main functions of the skin are briefly presented in table 1.

Table 1. The main functions of the skin; Source: Adapted from Barry, (1983).

1. To contain body fluids and tissue
2. To protect the body from potentially harmful external stimuli (the protective or barrier function):(a) microorganism; (b) chemical; (c) radiation; (d) heat; (e) electrical barrier; or (f) mechanical shock
3. To receive external stimuli (the sensory function), *i.e.*, to mediate sensation: (a) tactile (pressure); (b) pain; or (c) heat
4. To regulate body temperature
5. To synthesise and to metabolise compounds
6. To dispose of chemical wastes (glandular secretions)
7. To provide identification by skin variation
8. To attract the opposite sex
9. To regulate blood pressure

2.1.2 Structure of the skin

The skin is a multilayered organ composed of, anatomically, many histological layers. However it is generally described in terms of three tissue layers: the epidermis, the dermis, and the subcutaneous fat tissue (Barry, 1983; Chein, 1992)

(see figure 5). The epidermal layer covers the external surface of the body and the underlying dermis. The subcutaneous tissue is a layer underneath the dermis containing mostly adipose tissue. The epidermis, dermis, and subcutaneous tissue form a functional unit and are called the skin (Christopher *et al.,* 1989).

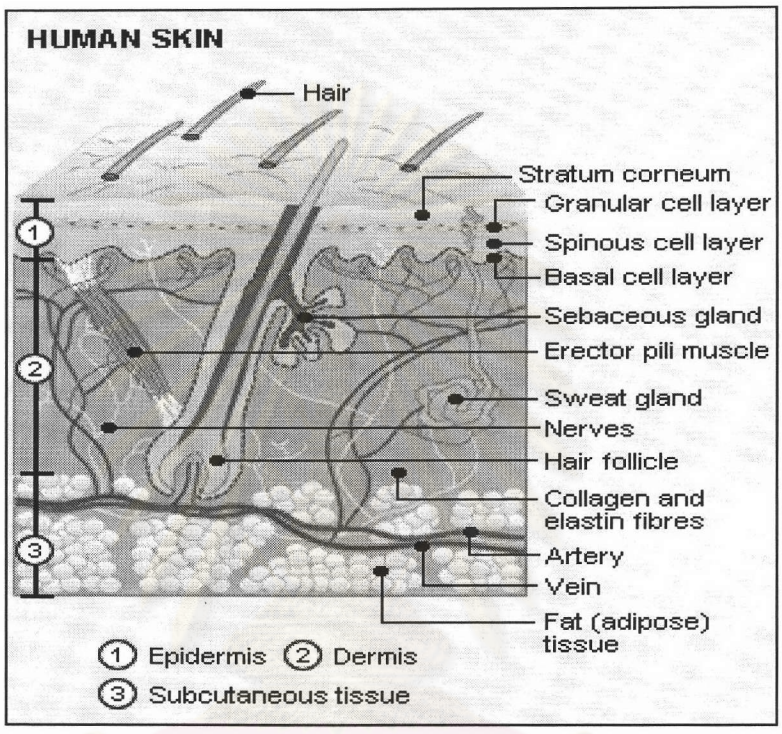


Figure 5. Three-dimensional view of the skin structure; Source: from http://www.mydr.com.au/default.asp (1 May 2006)

2.1.2.1 Epidermis

The outer epidermal layer of the skin is composed of stratified squamous epithelial cells. The epithelial cells are held together mainly by highly convoluted interlocking bridges, which are responsible for the integrity of the skin (Chein, 1992). The epidermis is thickest in the area of palms and soles and

becomes thinner over the ventral surface of the trunk. Structurally, the epidermis consists of four distinct cell types and four or five distinct layers.

I. Cells of the epidermis; The cells populating the epidermis include keratinocytes (corneocytes), melanocytes, Merkel cells, and Langerhans' cells. Most epidermal cells are keratinocytes (Marieb, 1998). (see figure 6)

(a) Keratinocytes; The principal role of keratinocytes is to produce keratin, the fibrous protein that provides the protective properties of the epidermis. Intercellular junctions, in which desmosomes are the most prominent, interconnect either adjacent keratinocytes or Merkel cells and keratinocytes. The keratinocytes originate by mitotic division in the deepest layer of epidermis and migrate to the outermost layer of the epidermis. Both cell production and formation are accelerated in body areas regularly subjected to friction, such as the hand and feet.

(b) Melanocytes; Melanocytes are found in the deepest layer of epidermis (see figure 7) and are the special epidermal cells which synthesise and distribute the pigment melanin to the keratinocytes. The melanin granules accumulate on the superficial side of the keratinocyte nucleus forming a pigment shield that protects the nucleus from the damaging effects of ultraviolet (UV) radiation in sunlight.

(c) Langerhans' cells; Langerhans' cells arise from bone marrow and migrate to the epidermis. These cells are potent stimulators that help to activate the immune system when foreign substances or antigens are present (Christopher *et al.*, 1989).

(d) Merkel cells; Merkel cells are presented in small numbers at the epidermo-dermal junction. Each Merkel cell is intimately associated with a sensory nerve ending (see figure 7). The combination, called a merkel disc, functions as a sensory receptor (Maribe, 1998).

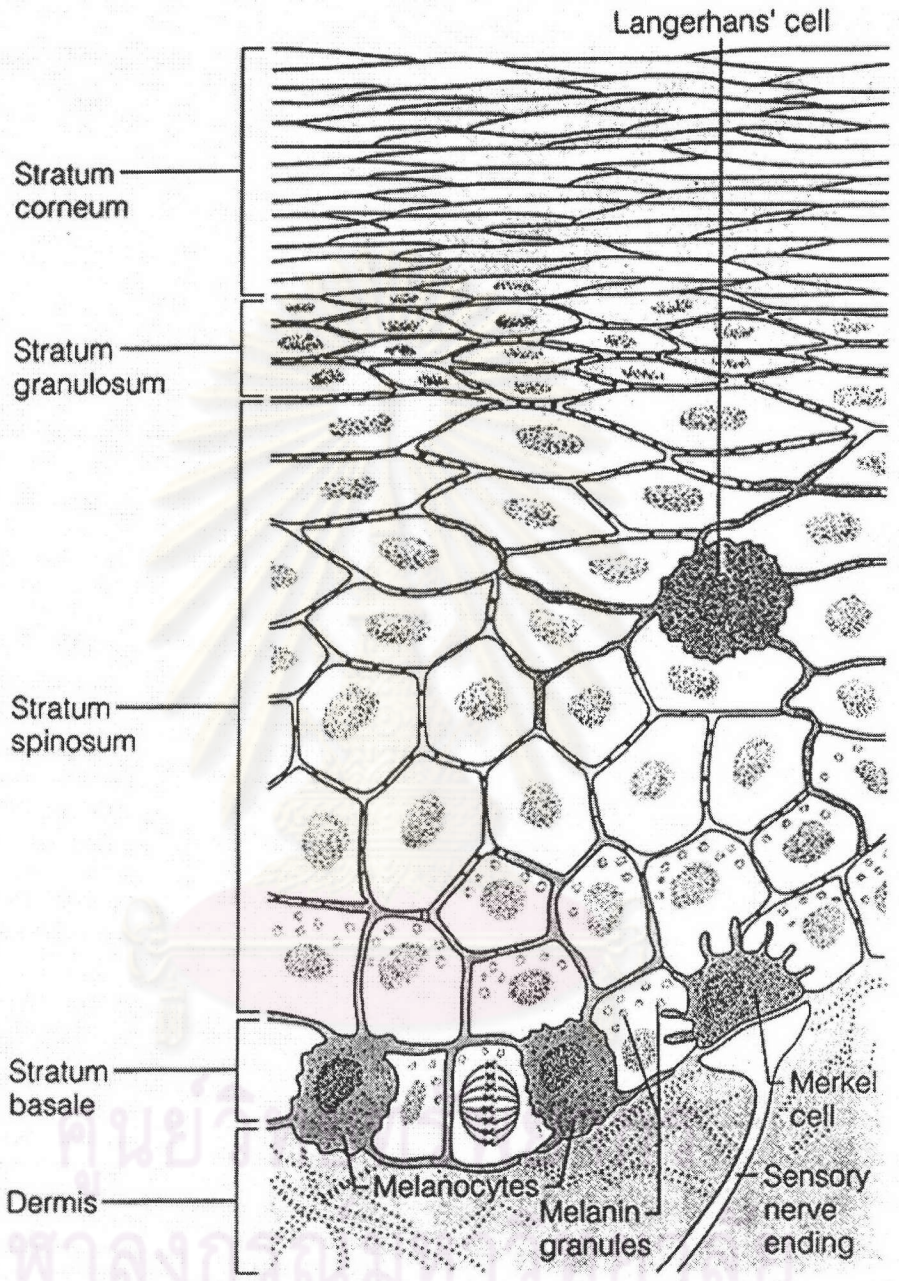


Figure 6. Cells of the epidermis; Redrawn from Marieb, 1998.

II. Layers of the epidermis; In thick skin, which covers the palm, fingertips, and soles of the feet, the epidermis consists of five layers (see figure 7). These layers are the stratum basale, stratum spinosum, stratum granulosum, stratum lucidum, and stratum corneum. However, in thin skin, which covers the rest of the body, the stratum lucidum is absent and the other four layers are thinner.

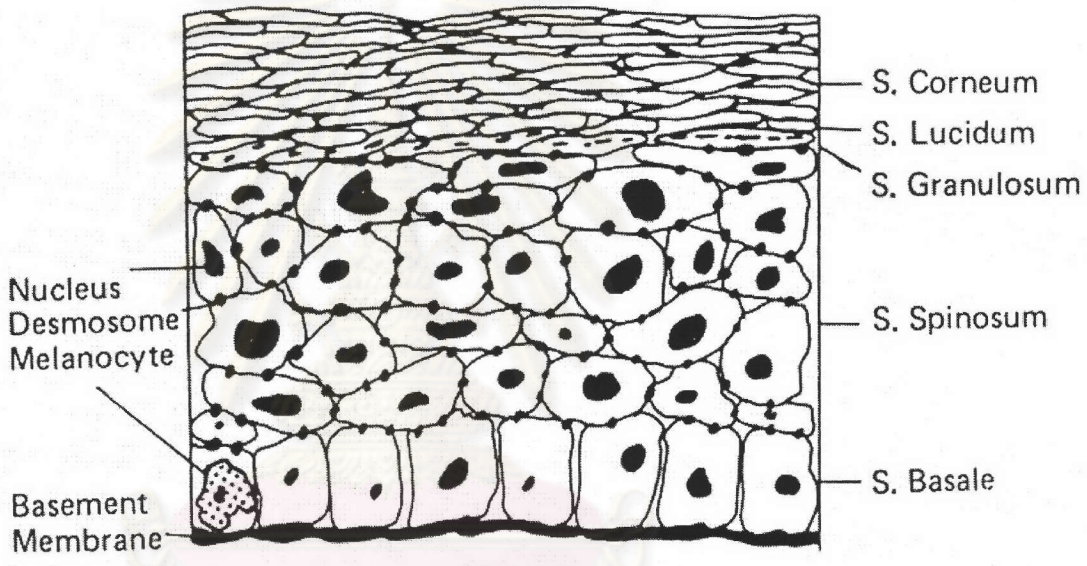


Figure 7. The sublayers of epidermis; Redrawn from Barry, 1983.

(a) Stratum basale (basal layer); The stratum basale is the deepest epidermal layer. For the most part, it comprises a single row of cells representing the youngest keratinocytes, which are cuboidal or low columnar in shape. Mitosis of the basal cells constantly renews the epidermis. In healthy epidermis, the rate of new cell production equals to the rate of cell loss at the skin surface, and the normal turnover time for replenishment is, on average, 28 days (Barry, 1983). The epidermis thus remains constant in thickness. During the normal life cycle of epidermal cells, the cells

progress upwards, become round (stratum spinosum), and then distinctly flattened (stratum corneum) above the basal layer.

(b) Stratum spinosum (Prickly layer); The spinous layer of cells undergoes a rapid differentiation. The keratinocytes in this layer are round or polyhedral in shape. Langerhans' cells and melanin granules are most abundant in this epidermal layer.

(c) Stratum granulosum (Granular layer); The thin stratum granulosum consists of three or five cell layers. The keratinocytes in this layer flatten their nuclei and organelles begin to disintegrate, and they accumulate keratohyalin granules and lamellar granules.

(d) Stratum lucidum (Clear layer); The stratum lucidum appears as a thin translucent layer above the stratum granulosum. It comprises of a few rows of clear, flattened, dead keratinocytes with indistinct boundaries. This layer is present only in thick skin.

(e) Stratum Corneum (SC) (Horny layer); The outermost stratum corneum is a broad zone, twenty to thirty layers thick. It consists of dead, compacted, flattened, dehydrated keratinised cells, and accounts for up to three-quarters of the epidermal thickness (Chien, 1992). It is now accepted that this layer serves as the most effective skin barrier for the penetration of most compounds.

2.1.2.2 Dermis

The dermis is a strong, flexible, connective tissue layer, which constitutes the majority of the mass of the skin (Bissett, 1987). It is made up of a dense network of fibrous protein, which are collagen, elastin, and reticular fibres (Chein, 1992; Marieb, 1998).

The dermis is richly supplied with nerve fibres, blood vessels and lymphatic vessels. The blood supply in this tissue layer is important in the systemic absorption of substances applied to the skin. The major portions of hair follicles, as well as sweat glands, are derived from epidermal tissue but reside in the dermis.

The dermis has two major layers: the papillary and reticular layers (Barry, 1983; Marieb, 1998). The thin superficial papillary layer is heavily invested with blood vessels. The deeper reticular layer, accounting for about 80% of the dermis, is mostly made up of collagen fibre (Bissett, 1987).

2.1.2.3 Subcutaneous fat tissue

This is the sheet of fat, which is known as the superficial fascia, attaching the dermis to the underlying structure. Its thickness varies with the age, sex, and nutritional status of individual. The subcutaneous fat provides flexible linkages between the underlying structures and the superficial skin layers leading to development of its thermal barrier and mechanical cushion properties.

2.2 Fundamentals of skin permeation

The skin was generally regarded in the scientific literature as an impermeable barrier. It is now more accepted that the skin is also particularly effective as a selective barrier to the penetration of a diverse range of substances. However, the skin is normally more permeable to lipid-soluble substances than to water-soluble compounds (Chein, 1992; Barry, 1983).

2.2.1 The stratum corneum as the primary skin permeation barrier

It is recognised that the various layers of the skin are not equally permeable. Subsequently, it was noted that the epidermis is much less permeable than the dermis. According to numerous experiments, the stratum corneum was approved as the principal barrier, which usually provides the rate-limiting step or the slowest stage in the penetration process in most conditions (Scheuplein, 1965; Williams and Barry, 1991). The best direct evidence that the stratum corneum was essentially a uniformly good permeation barrier came from studies using isotropic tracers (Chein, 1992). The largest amount of isotope was always detected in the outermost layer, and the proportionally decreased toward the dermis. Evidence from controlled stripping experiments has also demonstrated that complete removal of the horny layer by stripping enhances the absorption of many substances in contact with the skin surface (Barry, 1987). Finally, detailed pictures of the stratum corneum, gained from electron microscopy, support the idea that the barrier to penetration consists of the keratin-phospholipid complex in the dead and relatively dry cells of stratum corneum (Idson, 1975). These above observations suggest that the horny layer of the skin greatly impedes the permeation of molecules.

A typical horny cell comprises an amorphous matrix of mainly lipid and nonfibrous protein, in which keratin filaments (60-80 A°) are distributed. Adjacent keratinocytes are interconnected by intercellular lipid. This lipid matrix

behaves as a predominant route for permeation (see section 2.2.3.2 for details). The composition of the stratum corneum is outlined in table 2.

Table 2. Composition of human stratum corneum; from Barry, 1987.

Components	%	Gross biochemical compositions
Cell membranes	5	Lipids and nonfibrous protein
Cell contents	85	Lipid (20%) α-Protein (50%) β-Protein (20%) Nonfibrous protein (10%)
Intercellular materials	10	Lipids and nonfibrous proteins

2.2.2 The phenomenon of percutaneous absorption

The phenomenon of percutaneous absorption can be simply visualised as consisting of a series of steps in sequence (see figure 8). Firstly, a penetrant molecule adsorbs onto the surface layers of stratum corneum. Secondly, it diffuses through stratum corneum, viable epidermis, dermis, and capillary network of blood vessels in the papillary layer of the dermis. Finally, the molecule is taken up into blood circulation for subsequent systemic distribution.

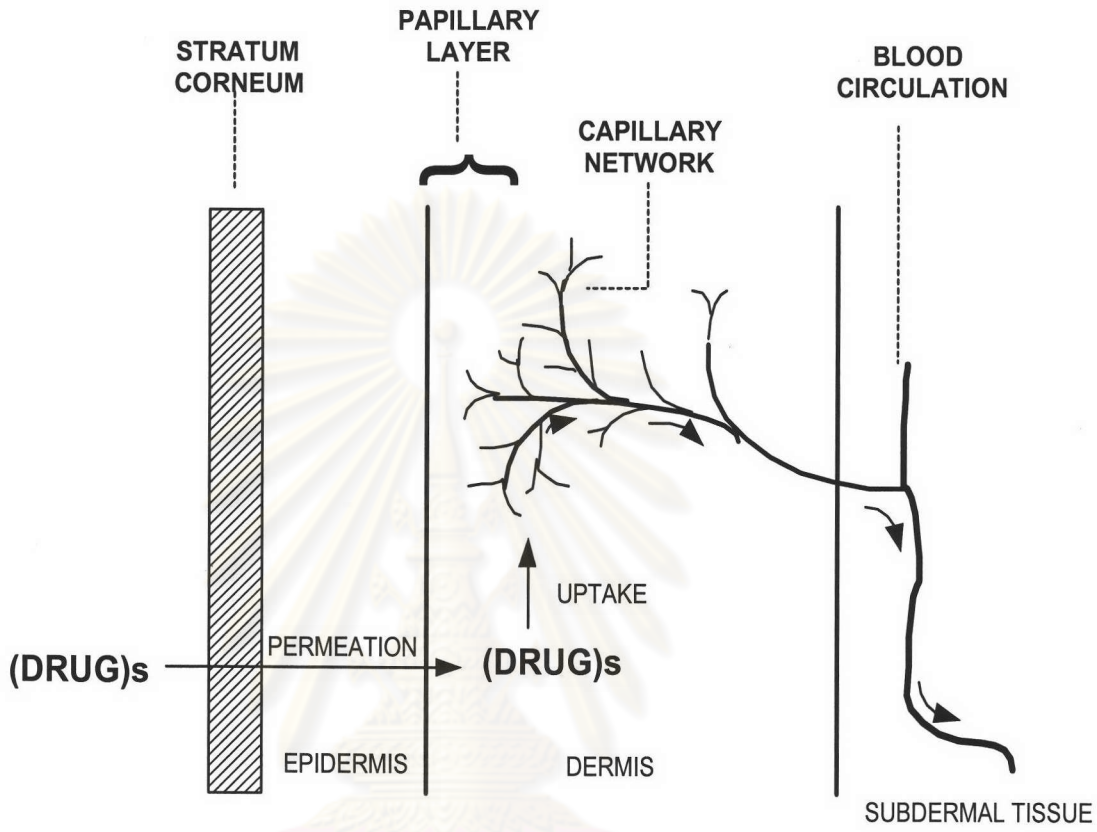


Figure 8. A multilayer skin model showing the sequence of transdermal permeation of drugs for systemic delivery; Redrawn from Chein, 1992.

2.2.3 Transport through the skin

2.2.3.1 Pathways for transport across the skin

The transport mechanisms by which drugs permeate through intact skin are still not fully elucidated despite many years of investigation. However, it has been recognised that there are two majors transport pathways across

the skin that molecules are likely to follow (Barry, 1987) (see figure 9). One involves transport directly through the bulk of the stratum corneum (transepidermal route). Another pathway concerns penetrating across the hair follicles and sweat glands (appendageal route). In fact, absorption through skin appendages is limited because the average human skin surface contains on average, 40-70 hair follicles and 200-250 sweat ducts *per* square centimetre of skin. These skin appendages occupy only 0.1% of the total human skin surface (Barry, 1983; Chein, 1992). Additionally, has been recently shown that the penetration of retinoic acid was greater through the skin of hairless guinea pigs than those with hair, confirming that the structure and composition of the stratum corneum is more important than follicular density for passive permeation. Therefore, skin permeation of most drug molecules is considered as passive diffusion through the intact stratum corneum as a primary process. However, the appendageal route may be important at short diffusion times for ions or large polar molecules (Barry 1983; Barry, 1987).

Passive transport can be defined as the movement of a solute from a high concentration compartment to a low concentration compartment. The driving force for passive diffusion across the membrane is the concentration gradient. The membranes itself do not actively participate in the transfer process. On the contrary, an active process involves active participation by the membrane in the transfer of molecule. Carriers, which may be enzymes or other components of membrane, interact with drug and the drug-carrier complex then move across the membrane. Drugs are finally released from the carrier to the other side of membrane. However, there is no evidence that the active transport is involved in the permeation of any drugs across human skin (Poulsen, 1973).

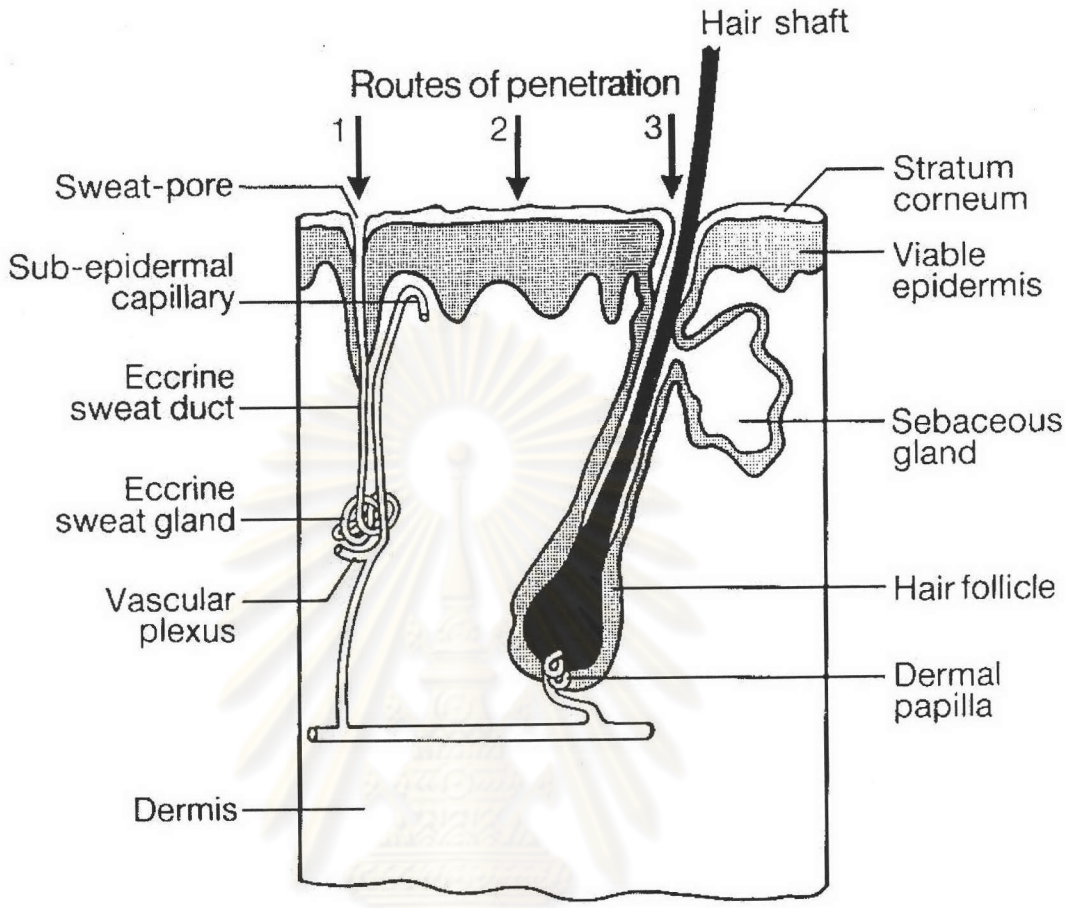


Figure 9. The possible pathways of molecules transport through the skin-across the intact stratum corneum (2) or *via* the hair follicles (3) and sweat glands (1); Redrawn from, Barry, 1987.

2.2.3.2 Pathways for transport across the stratum corneum

As previously described, application of topical or transdermal delivery is limited largely by the stratum corneum barrier properties, which prevent the permeation of most compounds at therapeutic levels. The stratum

corneum is a muticellular membrane, in which intercellular regions are filled with a lipid-rich amorphous material (Matoltsy and Parakkal, 1965) (see figure 10). The intercellular lipid is a layered structure, in which the polar head groups of lipids are gathered in layers with the non-polar chains pointed in the opposite direction (Friberg, 1990). There are two conceivable routes for the drug permeation through this outermost horny layer (Moghimi *et al.*, 1999): the transcellular (*via* the protein-filled cells) and the intercellular (*via* lipid matrix between cells) pathways (see figure 10). The relative contribution of these routes depends on the solubility, partition coefficient and diffusivity of the drug within these protein or lipid phases (Banga, 1998). It was believed that, in hydrated tissue, in which stratum corneum contains more than 40% water, polar molecules may partition and diffuse *via* the hydrated keratin of keratinocytes (Scheuplein, 1972). However, it is now seems more probable that the dominant pathway is the polar region of intercellular lipid, with the lipid chains providing the non-polar route (Katz, 1973; Higuchi, 1977; Barry 1987). Recently, there has been one direct piece of evidence in support of the intercellular pathway as a significant route (Bodde'*et al.*, 1991). A vapour fixation technique with electron microscopy was used to visualise Hg^{2+}permeating through the stratum corneum. It was shown that corneocytes also took up the small amount of Hg^{2+}, and during the washing process, most of the intercellular material was washed away, while the small amount of Hg^{2+}present in the cell remained. This observation suggests that the intercellular lipid pathway is the main route of drug permeation.

Permeation into the skin, which is *via* the transepidermal or transfollicular pathway, also depends on numerous factors that may influence the permeability characteristics of each compound. For example, the time scale of

permeation, the physicochemical properties of the penetrant (*e.g.*, pK_a, molecular size, stability, binding affinity, solubility, partition coefficient), integrity and thickness of the stratum corneum, density of sweat glands and follicles, skin hydration, and skin metabolism. Indeed, in order to specify the route of penetration, other existent conditions should be considered. It is probable that the penetrants permeate through a variety of routes mainly depending on time of diffusion, physicochemical properties of the penetrants and skin condition.

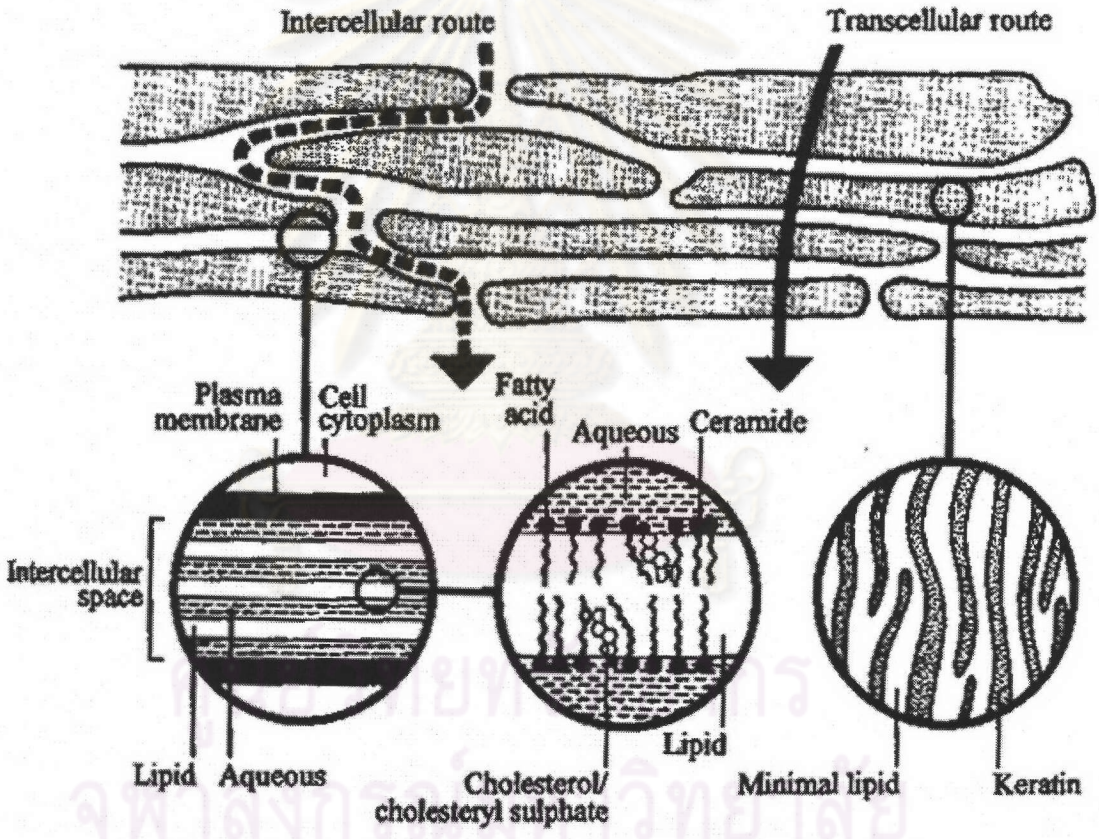


Figure 10. The possible routes for drug transport through the stratum corneum (transcellular or intercellular). Details are shown of the suggested structure of the intercellular lipid and the intracellular protein fibrils with minimal lipid; Redrawn from Barry, 1983.

2.2.4 Factors influencing in percutaneous absorption

The transport of drugs through the skin is complicated, since there are many factors that influence their permeation. However, the concept of percutaneous absorption may be explained by considering a simple diagram, which represents the difficulties and complexity in the permeation process (see figure 11).

The drug particles must first dissolve in the solvent so that the molecules of the drug diffuse within the vehicle to reach the vehicle-stratum corneum interface. The penetrant must partition into the stratum corneum and diffuse within this greatly impermeable barrier. Some drug molecules may interact with a so-called depot site possibly forming a reservoir, demonstrating therapeutic activity for days or even weeks. Free drug eventually diffuses through the horny layer meeting the interfacial barrier of the stratum corneum and viable epidermis, and partitions into viable epidermis. There is a potential problem in that a drug or prodrug designed to partition from a vehicle into the horny layer may have difficulty leaving the stratum corneum to enter the water-rich epidermis. Therefore, the substances which have high affinity for the horny layer and very low water solubility, may not be absorbed percutaneously (Barry, 1987). For highly lipophilic drugs, clearance from viable tissue may replace diffusion through the stratum corneum as the rate-limiting step. Viable epidermis is a living tissue, which may have enzyme activities of 80-90% of those in the liver (Wester and Noonan, 1980; Bronaugh, 1999). Hydrolytic, oxidative, reductive and conjugation reactions all take place. Metabolism in living skin may alter the permeability characteristics of the drugs. For instance, drugs may be activated by enzymes within the skin, generating active or inactive metabolites resulting in altered pharmacological or toxicological activity. The permeation characteristics of these metabolites may be different from parent compounds. It is also probable that the drug

may interact with pharmacological receptors in this viable tissue. After the penetrant partitions into the dermis, additional receptor, metabolic and depot sites may intervene in the progress of drug permeation to blood capillaries, partitioning into the wall, and finally removal by the blood circulation. A portion of the penetrant may even partition into the subcutaneous fat and the underlying muscle to form a further depot.

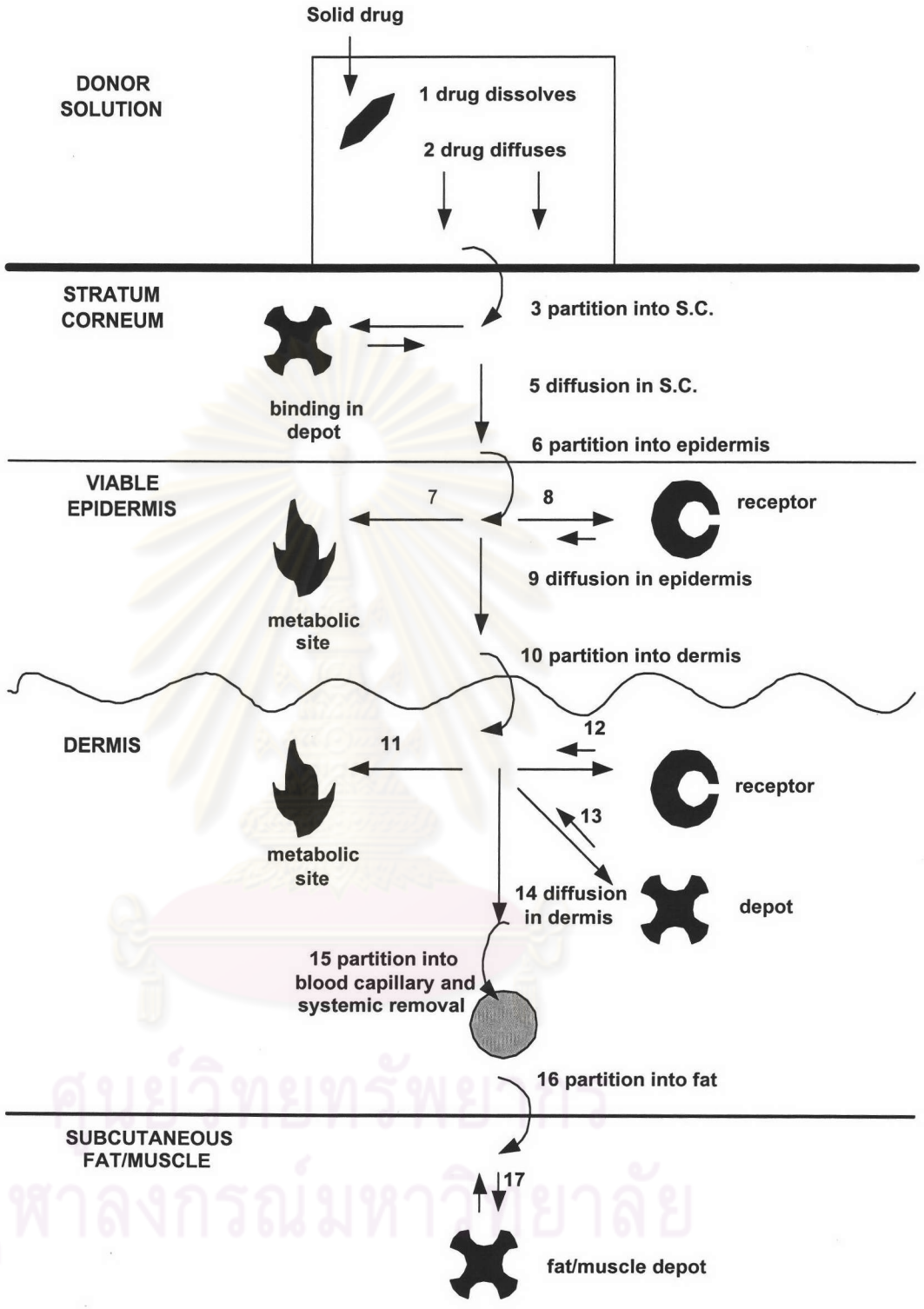


Figure 11. The percutaneous absorption process of drug from solution and some factors which influence the process; Redrawn from Barry, 1983.

2.2.5 Theoretical basis of percutaneous absorption

The process by which a solute (penetrant) moves from a region of high chemical potential (the vehicle) to a region of low chemical potential (the skin) can be referred to passive diffusion (Poulsen, 1973). Percutaneous absorption, whether *via* the intercellular or intracellular route is essential a passive diffusion process. A typical permeation profile of a drug through the skin is presented in figure 12. There is an initial lag phase, which is the period of time that penetrant flux is attaining equilibrium. This period is followed by a linear or steady state phase, which occurs when there is a balance between drug diffusion in and out of the skin membrane.

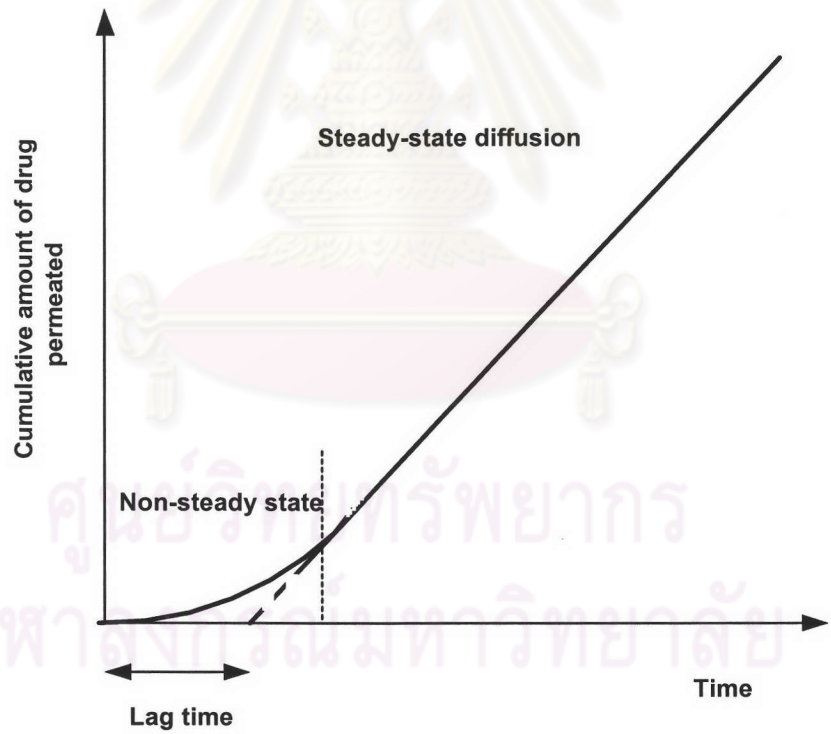


Figure 12. Typical profile for percutaneous drug absorption

At steady state, in which the permeation rate of a substance is directly proportional to the concentration gradient, the passive diffusion of molecules can be expressed by Fick's first law of diffusion as:

$$J = -D\frac{dC}{dx} \qquad (1.1)$$

Where J is the amount of drug penetrating through a unit area of the skin *per* unit time (the flux), D is the diffusion coefficient of drug in the skin, and dC/dx is the concentration gradient across the skin, where C is the concentration and x is the distance. The negative sign signifies that diffusion occurs in the direction of decreasing concentration of penetrant, thus the flux is always a positive quantity.

However, the concentration gradient across the skin cannot be exactly measured under certain conditions. For example, the membrane may exhibit an affinity for the permeant. As a consequence, the drug concentration at the skin surface is not identical to that in the donor solution but is related to the membrane-vehicle partition coefficient. The permeation rate through the skin can thus be approximated by the product of the partition coefficient and the concentration difference across the membrane. The steady state flux, J_s, through the skin barrier is thus given as:

$$J_{ss} = \frac{K.D}{h}.\Delta C_s \qquad (1.2)$$

Where K is the partition coefficient, ΔC_s is the concentration difference, and h is the thickness of the skin. K, D, and h can be combined into a single constant (K_p):

$$J_{ss}=K_p\Delta C_s \qquad (1.3)$$

where K_p is termed the permeability coefficient. Experimentally, if the drug dose in the donor compartment is infinitely large (*i.e.* sink conditions apply) compared to the

amount permeating to the receptor chamber, then ΔC_s can be replaced with C_s, which is the drug concentration in donor compartment. The flux of the penetrant can be calculated from the slope of steady-state diffusion curve. K_p can thus be determined by dividing the steady-state slope by the initial concentration of drug applied to the donor phase. The cumulative amount of drug permeating through the skin (Q_t) is given by:

$$Q_t = \frac{K.D.C_s}{h(t - h^2/6D)} \qquad (1.4)$$

Where C_s is the saturated concentration in donor phase when sink conditions are maintained in the receptor solution. When the steady state line is extrapolated to the time axis (see figure 10), the value of lag time, t_L is obtained by the intercept at $Q = 0$

$$t_L = \frac{h^2}{6D} \qquad (1.5)$$

The intercept, t_L, is the measure of the time that the penetrant takes to achieve a constant concentration gradient across the skin.

However, the equations listed in 1.1 to 1.5 may not be applicable under conditions that are influenced by other factors. For example, the evaporation of donor solution will increase the drug concentration resulting in an increase in the rate of diffusion. Some vehicles such as ethanol can also enhance drug delivery (Barry, 1983).

3. Skin delivery potency of TMZ-HE

There have been many studies on methods to improve topical drug absorption, one of which is the prodrug approach. Exploiting the metabolic capabilities of the skin in the design of prodrugs is an area of interest (Bodor and Sloan, 1983). The drugs can be designed such that they may have more desirable physicochemical properties than the parent compound for skin absorption but may be broken down to the active compound by metabolizing enzyme. Base on the results from resent studies (Suppasansatorn *et al.,* 2006), TMZ-HE exhibited the promising permeability through rat and human skin by means of K_p and J_{ss} values comparing to TMZ parent drug (table 3). TMZ-HE derivative can also be degraded by esterase enzyme within the skin and generate biologically active TMZA. TMZ-HE can thus be recognized as prodrug approach to improve skin delivery of TMZ.

Table 3. The permeability data of TMZ esters

	Silicone membrane		Rat skin		Human skin	
Compound	J_{ss} (±S.D.)[a]	K_p(±S.D.)[b] x10^{-3}	J_{ss}(±S.D.)[a]	K_p(±S.D.)[b] x10^{-3}	J_{ss}(±S.D.)[a]	K_p(±S.D.)[b] x10^{-3}
TMZ	n.d.	n.d.	2.65±1.27	0.17±0.08	5.04±5.10	0.33±0.33
Methyl ester	2.95±0.56	0.21±0.04	2.51±1.08	0.18±0.08	-	-
Ethyl ester	8.28 ±1.04	0.74±0.09	2.50±0.68	0.22±0.06	-	-
Propyl ester	26.00±0.28	3.08±0.03	7.13±3.94	0.85±0.47	-	-
Butyl ester	29.10±4.15	5.24±0.75	11.94±5.24	2.15±0.93	-	-
Hexyl ester	35.14±5.95	22.64±3.83	3.66±0.64	2.36±0.42	2.50±0.81	1.62±0.52
Octyl ester	10.76±3.80	11.01±3.89	1.24±0.46	1.26±0.47	-	-

[a] $nmolcm^{-2}h^{-1}$
[b] cmh^{-1}
n.d. none detected

4 Introduction to microemulsions (ME)

4.1 Definition

Microemulsions are the systems which simply consist of at least, water, surfactant (amphiphile), and oil at appropriate ratio (Peltola *et al.*, 2003; Kawakami *et al.,* 2002; Lawrence and Rees, 2000). It has been increasing much interest in pharmaceutical and cosmetic laboratories as well as among researchers according to its unique characteristics which are thermodynamically stable system, optically isotropic transparency, spontaneous formation and size usually between 10-100 nm (Sintov and Shapiro, 2004). The observed transparency of these systems is due to the fact that the maximum size of the droplets of the dispersed phase is not larger than one-fourth of the wavelength of visible light which is approximately 150 nm. Droplet diameter in stable microemulsions is therefore usually within the range of 10-100 nm (100-1000 A°) (Sintov and Shapiro, 2004).

The word "microemulsion" was first proposed by Jack H. Schulman and coworker in 1959 (although the first paper appeared on this subject back in 1943). It was found that a coarse macroemulsion system containing water, benzene, hexanol, and potassium oleate become transparent when titrated with short chain alcohol (Lindman and Friberg, 1999).

Microemulsions are distinctly different from emulsion in that the former are thermodynamically stable one-phase systems whereas the latter are kinetically stabilized dispersions and will eventually phase separate (Lawrence and Rees, 2000; Malmsten, 1999). Another important difference concerns their appearance; emulsions

are cloudy with droplets size between 1-10 μm (Attwood, 1994) while microemulsions are clear or translucent. In addition, there are crucial differences in their method of preparation, emulsion require a large input of energy (*i.e.* stirring) while microemulsions are not. Due to the larger droplets in emulsion systems, the surface area is generally smaller in emulsions than in microemulsions, and consequently, less surfactant is generally needed to generate an emulsion than a microemulsion system (Malmsten, 2002).

4.2 The Advantages of microemulsions

Naturally, the main advantage of microemulsions compared to emulsions is their thermodynamic stability resulting in ease of preparation and excellent long-term stability. This is due to the very low interfacial tension between oil and water (Trotta, 1999). Furthermore, microemulsions are capable of solubilising large amounts of both water-soluble and oil-soluble drugs, and the microemulsions can be used as sustained release formulations. Moreover, microemulsions have been found to improved the drug bioavailability, for example, in topical administration and in oral administration of peptide and protein drugs, sparingly soluble lipophilic drugs, and drugs labile at the conditions in the stomach. There are also other advantages with microemulsions compared to other drug delivery systems, including ease of filtration (sterilization) and low viscosity (reducing pain on injection) (table 4) (Malmsten, 1999).

Table 4. Pharmaceutical advantages of microemulsions; Source: Adapted from Malmsten, 1999

General advantages
Ease of preparation
Clarity
Stability
Ability to be filtered (for low viscosity microemulsion)
Vehicle for drugs of different lipophilicities in the same system
Low viscosity (no pain on injection)
Specific advantages
Water-in-oil (W/O)
Protection of water-soluble drugs
Sustained release of water-soluble material
Increase bioavailability
Oil-in-water (O/W)
Increased solubility of lipophilic drugs
Sustained release of oil-soluble material
Increased bioavailability
Bicontinuous
Concentrated formulation of both oil- and water-soluble drugs

4.3 Microemulsion formation

There are three approaches which have been used to describe microemulsion formation and stability. These are: (i) interfacial or mixed film theories: (ii) solubilisation theories: (iii) thermodynamic treatments. However, an admittedly simplified thermodynamic rationalization is presented in the following equation (Lawrence and Rees, 2000). The free energy of microemulsion formation can be

considered to depend on the extent to which surfactant lowers, the surface tension of oil and water interfacial and the change in entropy of the systems.

$$\Delta G_f = v\Delta A - T\Delta S \qquad (1.6)$$

Where ΔG_f is the free energy of formation, v is the surface tension of oil-water interface, ΔA is the change in interfacial area on microemulsification, ΔS is the change in entopy, and T is the temperature. An essential requirement for their formation and stability is the attainment of a very low interfacial tension, v. Since the change in ΔA when microemulsions are formed is very large due to the large number of very small droplets formed. Originally worker proposed that in order for a microemulsion to be formed a (transient) negative value of v was required, it is now recognized that while value of v is positive at all times, it is very small and is offset by the entropic component. The dominant favorable entropic contribution is very large dispersion entropy arising from the mixing of one phase in order to form of large numbers of small droplets. However, there are also expected to be favorable entropic contribution arising from other dynamic process such as surfactant diffusion in the interfacial layer and monomer-micelle surfactant exchange. Thus a negative free energy of formation is achieved when large reductions in surface tension are accompanied by significant favorable entropic change. In such case, microemulsification is spontaneous and the resulting dispersion is thermodynamically stable.

4.4 Microemulsion structure

Microemulsion can accommodate a number of different microstrucstures, depending on the nature of surfactants, the system composition, temperature and presence of co-surfactants and co-solutes. Besides microemulsions, structural examinations can reveal the existence of regular emulsion, anisotropic crystalline

hexagonal or cubic phases, and lamellar structures depending on the ratio of components (Malmsten, 2002).

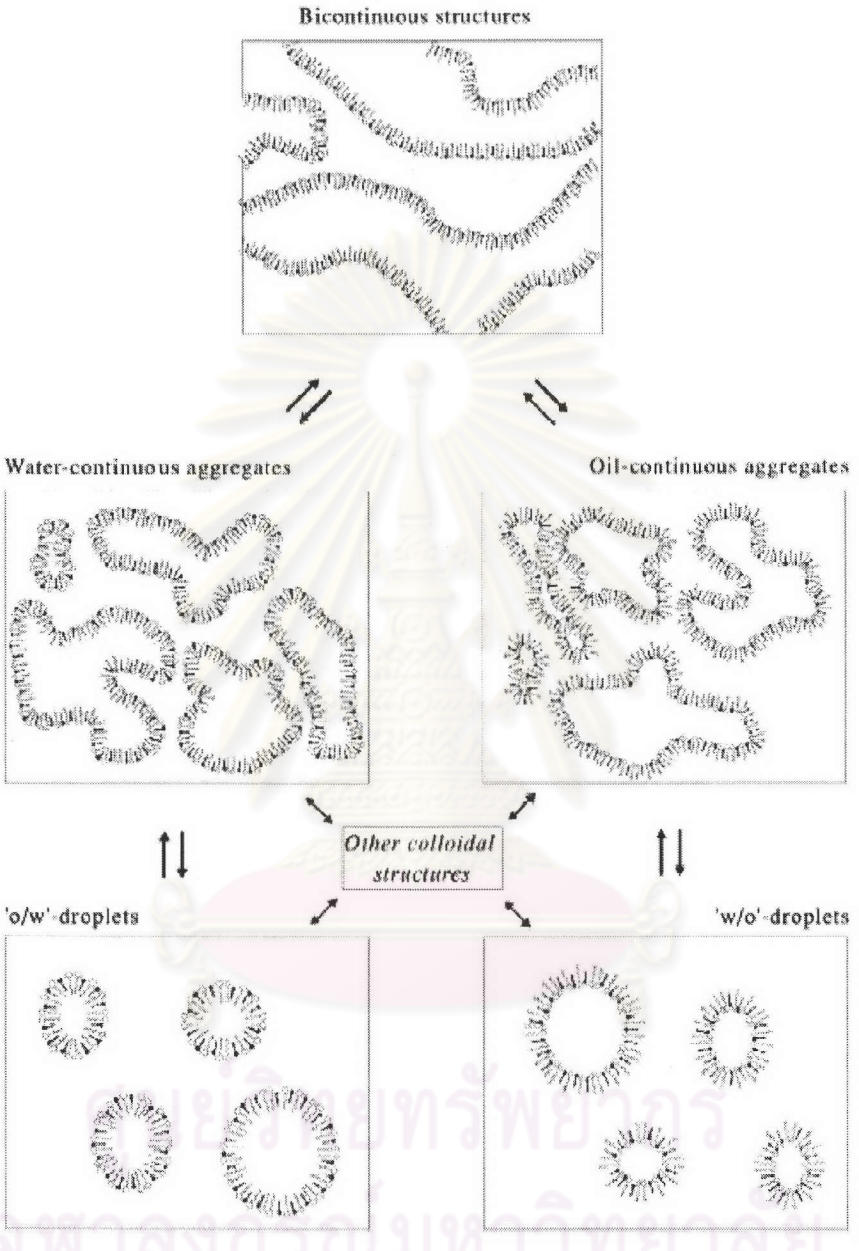


Figure 13. Basic dynamic microemulsion structure formed by oil phase (grey), aqueous phase (white) and surfactant/co-surfactant interfacial film, and plausible between the structure (indicated by arrows) by increase of oil fraction (clockwise from left to right) and water fraction (anti-clockwise from right to left), respectively; Redrawn from Kreilgaard, 2002.

However, the simplest representation of the structure of microemulsion is the droplets model in which microemulsion droplets are surrounded by an interfacial film of both surfactant and co-surfactant molecules (see figure 13) (Attwood, 1994; Lawrence and Rees, 2000; Kreilgaard, 2002).

4.5 Phase studies

The relationship between phase behavior of a mixture and its composition (oil, water and surfactant) can be studies by the aid of phase diagram. The each corner of diagram represents 100 % of such particular components. Composition variables can also be investigated as a function of temperature and pressure (Lawrence and Rees, 2000). In the general case, surfactant, oil, and water are presented in the microemulsion systems and the phase diagram used is generally referred to *ternary phase diagram*. In the case where four or more components are investigated a corner will typically represent a binary mixture of two components such as surfactant/co-surfactant, water/drug or oil/drug.

Microstructural features can also be illustrating by using ternary phase diagram. Transition from w/o to o/w microemulsion may occur *via* a number of different structural states including bicontinuous lamellar and also multiple phase systems (see figure 14).

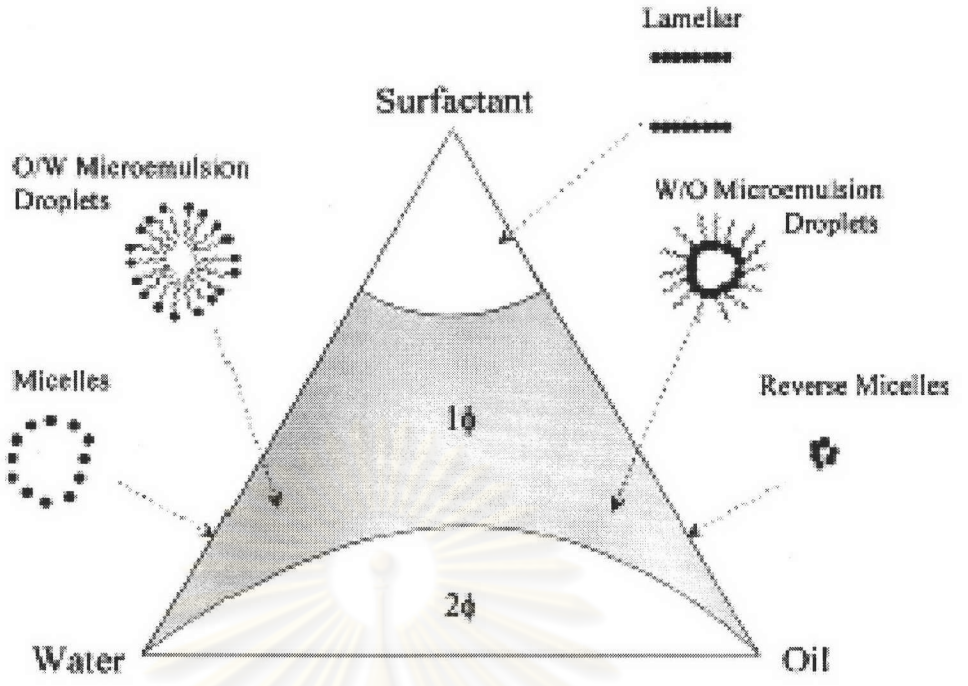


Figure 14. A hypothetical pseudo-ternary phase diagram of an oil/surfactant/water system with emphasis on microemulsion and emulsion phase. Within the phase diagram, existence fields are shown where conventional micelle, reverse micelle or water-in-oil (w/o) microemulsion and oil-in-water (o/w) microemulsion are formed along with the bicontinuous microemulsion. At very low surfactant concentration, two phase systems are observed; Redrawn from Lawrence and Rees, 2000

4.6 Role of surfactant

The types of surfactants used to incorporate the oil into microemulsion systems may be: (1) non-ionic, (2) switterionic, (3) cationic, or (4) anionic surfactants. Combinations of these particular ionic and non-ionic, can be very effective for increasing of microemulsion region. Polyoxyethylene compounds or sugar esters such as sorbitan monooleate (Span 80) are the example for non-ionic surfactants. Phospholopids are a notable example of zwitterionic surfactant that exhibit excellent biocompatible. Lecithin preparations from various sources including soybean and egg are commercial available. Quaternary ammonium alkyl salts from one of the best

known classes of cationic surfactants, with hexadecyltrimethyl ammonium bromide (CTAB), and the twintailed surfactant didodecylammonium bromide (DDAB) amongst the most well known. The most widely studies anionic surfactant is probably sodium bis-2-ethylhexylsulphosuccinate (AOT) which is twin-tailed and is particularly effective stabilizer of o/w microemulsion (Lawrence and Rees, 2000).

The type of associated structures formed in surfactant systems depends on a number of parameters, including (1) surfactant structure, (2) composition of system, (3) presence of salt, oil, and co-solutes, and temperature (Malmsten, 2002).

In order to understand the formation of these structures it is helpful to understand the Critical Packing Parameter (CPP, cpp) (Malmsten, 2002). The CPP can be calculated using the following equation (Lawrence and Rees, 2000):

$$CPP = v / a.l \qquad (1.7)$$

Where v is the partial molar volume of hydrophobic portion of the surfactant, a is the optimal head group area and l is the length of surfactant tail. The latter parameter is often express as l_c that is the critical length of the hydrophobic chain, generally assumed to be 70-80% of its fully extended length (see figure 15). As can be seen in this figure, there is a direct correlation between the value of the cpp and the type of aggregate formed. Starting from lamellar phase, the surfactant molecules occupy a cylindrical space where $v = a.l$ that is cpp = 1. The more the surfactant aggregate curves toward the oil (i.e., the progression lamellar → hexagonal → micellar), the smaller the value of cpp. For the reverse structure, the cpp increases in the order

lamellar → reverse hexagonal→ reverse micellar (Malsten, 2002). The change in microemulsion composition will modify the microenvironment of the surfactant, which will lead to changes in the apparent cpp of the surfactant. For example, increases in ionic strength would be expected to result in a decrease in the effective head group area of ionic surfactants. The present of hydrophilic molecules such as glycerol and sorbitol in aqueous phase will also influence optimal head group area by altering the solubility of the head group in the aqueous phase. Because of these effects, water-soluble hydrophilic materials have been used to aids microemulsion formation (Attwood and Mallon, 1992).

The hydrophilic-lipophilic balance (HLB) can also be a useful guide to surfactant selection. It is generally accepted that low HLB (3-6) surfactants are favored for the formation of w/o microemulsions while surfactants with high HLB (8-18) are preferred for the formation of o/w microemulsions (Lawrence and Rees, 2000).

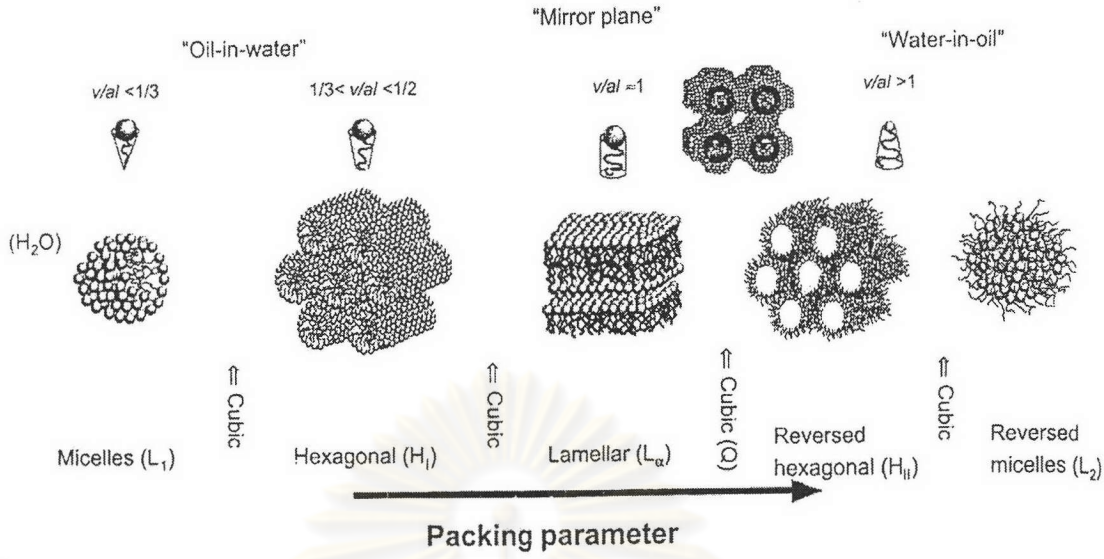


Figure 15. Schematic illustration of association structures formed in surfactant, and the packing of surfactant molecules in different association structure; Redrawn from Malmsten, 2002.

4.7 Choice of microemulsion components

The choice of components for pharmaceutical microemulsions is often a balance between compounds, which are able to form microemulsions, are nontoxic and are able to fulfill the requirements of a good vehicle for optimum dermal absorption, i.e., high solubility of drug interest and a high thermodynamic activity of the drug. As the level of surfactants and oil phase in microemulsions typically is relatively high, it is thus important to consider the irritancy of these compounds (Kreilgaard, 2002). Moreover, the vast majority of phase behavior studies have been carried out using surfactants and oils, which do not have regulatory approval for use in pharmaceutical products (Lawrence and Rees, 2000).

In microemulsion systems, the main concern regarding the toxicity has to do with the co-surfactants used. For example, the majority of the work on the

pharmaceutical application of microemulsions has involved the use of short- or medium- chain alcohols which there are significant toxicity and irritancy issue with these agents (Lawrence and Rees, 2000; Malmsten, 1999).

Lecithins and triglycerides from natural source have been accepted to use in microemulsions as non-toxic surfactants and oils respectively. Non-ionic surfactants can also be useful alternatives to naturally occurring surfactants, and polyoxyethylene sorbitan n-acyl esters (Tweens), for example, have been reported to have minimal toxicity (Lawrence and Rees, 2000; Attwood, 1994). Moreover, many non-ionic surfactants have the advantage over the charge surfactants in that they can form microemulsions even without co-surfactants. Plurol isostearique, have recently been introduced into topical microemulsion formulations, and have been demonstrated to provide extensive region of existences with various surfactant and oils (Kreilgaard, 2002).

4.8 Preparation of microemulsions

Microemulsions can be simply prepared by blending oil, water, surfactant and co-surfactant with mild agitation. The usual method of preparing microemulsion is to dissolve the surfactant in the oil and then add the water to the solution of oil and surfactant with gentle shaking. The microemulsions rapidly become first translucent and then optimally clear after few second. The order of mixing the components is generally considered not to be critical since microemulsions form spontaneously. Although microemulsification is spontaneous process, the driving forces are small and the time taken for these systems to reach an equilibrium interfacial tension may be long (Swarbrick and Boyland, 1994).

4.9 Characterization of microemulsions

There are many different techniques used to characterize microemulsion systems, but generally more than one method to fully evaluate microemulsion formulations. The isotropic nature of microemulsions and their optical clarity make their study by cross polarization microscopy techniques straightforward, particularly in comparison to conventional macroemulsion. Transmission electron microscopy (TEM) has been used to study size and shape of microemulsion. Freeze-fracture electron microscopy (FFEM) has also been used to study microemulsion structure, however, extremely rapid cooling of the sample is required in order to maintain structure and minimize the possibility of artifacts. FFEM technique has been shown to be effective in studying bicontinuous microemulsion (Burauer *et al.,* 2003).

Pulse field gradient NMR for example has been used extensively to measure self-diffusion coefficients of the various components and yields information on mobility and microenvironment. NMR self-diffusion allows straightforward determination of whether the microemulsion consists of oil droplets in aqueous phase or water droplets in an oil phase or bicontinuous structure (Malmstem, 2002). Scattering techniques have also been invaluable in elucidating microemulsion structure and methods employed include dynamic light scattering, small angle neutron scattering (SANS) and small-angle X-ray scattering (SAXS) (Lawrence and Rees, 2000). Parameters which may be determined with these techniques include, *i.e.,*

(o/w) and/or (w/o) Size of micelles

Size of micellar core

Thickness of palisade layer

	Micellar aggregation number
	Micellar water content (oil content)
	Micellar shape
Bicontinuous	Correlation length

At the macroscopic level viscosity, conductivity and dielectric method provide useful information. Viscosity measurement for example, can indicate the presence of rod-like or worm-like reverse micelle.

4.10 Microemulsions as drug delivery systems

Regarding to the advantages of microemulsions, as state above, these systems are therefore recently candidates as drug delivery vehicles because of their improved drug solubilisation with different physico-chemical properties (various hydrophilic and lipophilic properties), thermodynamic stability and ease of preparation (Junping *et al.*, 2003; Lawrence and Rees, 2000; Bonina *et al.*, 1995; Bhatnagar and Vyas, 1993)

The dispersed phase, lipophilic or hydrophilic, can behave as a potential reservoir of lipophilic or hydrophilic drugs, respectively. Hence, a lipophilic, water-insoluble drug is preferable to be incorporated in dispersed oil phase of an o/w microemulsion as the release of hydrophobic drugs is slow while a hydrophilic material would be most likely to be incorporated into the dispersed aqueous phase of a w/o microemulsion as the situation is opposite (Malmsten, 2002). For balanced microemulsions, relatively fast diffusion and release occur for both water-soluble and oil-soluble drugs due to the bicontinuous nature of microemulsion structure (Malmsten, 1999). The drug will be partitioning between dispersed and continuous

phase, and when the system comes into contact with semipermeable membrane, with skin or mucous membrane, the drug can be transported through the barriers. Controlled release of drug may be obtained, depending on the volume of dispersed phase, the partition of the drug among interphase and continuous and dispersed phase, and the transport rate of drug.

4.11 Pharmaceutical applications of microemulsions

Microemulsions have used primarily in topical and oral administrations, but also some extent in other applications, such as buccal, ocular, and nasal drug delivery. On the other hand, the use of microemulsions in parenteral administration is much less explored due to issues relating to the stability of the system on dilution after intravenous administration, and toxicity issues relating to the use of formulations containing high amount of surfactants in this delivery route (Malmsten, 2002).

4.12 Microemulsion as skin delivery systems

In recent years, many researchers have focused their attention on skin as a target site for the application of drugs. Drugs administered by conventional means, notably oral administration, might often have harmful side effects and are sometimes ineffective (Bonina, 1995; Trotta, 1999; Malmsten 1999). Skin delivery may provide an improved approach. However, drug permeation through the skin is greatly limited by the stratum corneum, the outermost layer of the skin which is composed of keratin-rich dead cells embedded in a lipid matrix (Delgado-charro and Guy, 2001).

Numerous papers have demonstrated that microemulsion formulations designed for topical or transdermal application may enhance permeation of drug molecules through the skin (see tables 4 and 5). These systems can increase topical or transdermal delivery of a compound by different mechanisms. Firstly, a large amount of drug can be introduced in the formulation due to the high solubilisation power. Secondly, an increase in the transdermal flux can be expected in that the thermodynamic activity of the drug in the microemulsion can be modified to favor partitioning into the stratum corneum. Thirdly, the surfactants in the microemulsion may reduce the diffusion

n barrier of the stratum corneum (Delgado-charro *et al.,* 1997). Moreover, in some cases, oil phase itself acts as a penetration enhancers to facilitate transdermal drug delivery (Lawrence and Rees, 2000).

Recently, Kreilgaard has been reviewed the cutaneous drug delivery studies with microemulsion *in vitro* and *in vivo* (see tables 5 and 6).

Table 5. Overview of cutaneous drug delivery with microemulsions *in vitro* from Kreilgaard, 2002.

Drug	Microemulsion			Membrane/skin
	Oil phase	Surfactants	Aqueous phase	
[^{3}H] H_2O	Octanol	Dioctyl Na Sulphosuccinate	Water	Human
8-Methosalen	IPM	Tween 80, Span 80 1,2 octanediol	Water	Pig
Apomorphine Hydrochloride	IPM, Decanol	Epicuron 200 1,2 propanediol	Water	Mouse
Diclofenac	IPP	Lecithin	Water	Human
	Diclofenac diethylamine	Lecithin	Water	Human
Diphenhydramine Hydrochloride	IPM	Tween 80, Span 20	Water	Human
Felodipine	IPM, benzyl Alcohol	Tween 20, taurodeoxycholate	Water, Transcutol, Carbopol	Mouse
Glucose	Octanol	Dioctyl Na, sulphosuccinate	Water	Human
Hematoporphyrin	IPM, decanol, Hexadecanol, Oleic acid, monoolein	Lecithin, sodium monohexylphosphate, benzyl alcohol	Water, PG	Mouse
Indomethacin	IPP	Lecithin	Water	Human
Ketoprofen	Triacetin, Myvacet, Oleic acid	Labrasol, Cremophor RH	Water	Rat
	Mygliol 812,	Epikuron 200 n-butanol	Water	Human
Lidocaine	Isostearylic, Isostearate	Labrasol, isostearique	Water	Rat
Metrotrexate	Decanol	Lecithin, Benzyl alcohol, Labrasol/Plurol	Water, PG	Mouse
	Ethyl oleate	Isostearique	145 mN NaCl (pH 7.4)	Pig
	IPM	Tween 80, Span 80 1,2 octanediol	Water	Pig
Nifedipine	Benzyl alcohol	Tween 20 taurodeoxycholate	Water	Pig
Prilocaine Hydrochloride	Isostearylic, Isostearate	Labrasol, Plurol Isostearique	Water	Rat
Propanolol	IPM	Polysorbate 80	Water	Artificial
Prostaglandin E_1	Oleic acid	Labrasol, Plurol Oleique	Water	Mouse
Sodium salicylate	IPM	Tween 21/81/85, Bis-2-(ethylhexyl)	Water, gelatin	Pig
Sucrose	Ethyl oleate	Labrasol, Plurol	154 mN	Mouse

Table 6. Overview of cutaneous drug delivery with microemulsions *in vivo* from Kreilgaard, 2002.

Drug	Microemulsion			Membrane/skin
	Oil phase	Surfactants	Aqueous phase	
Bupranolol	IPP	Tween 85	-[a]	Rabbit
	IPP	Targat, Glycerol monooleate	-[a]	Rabbit
	IPP	Tween 85 poloxamer	-[a]	Rabbit
Carazolol	IPP	Tween 85	-[a]	Rabbit
Hydrocortisone	IPM	Sucrose laurate, PG	Water	Human
	IPM	Targat, Plurololeat	Water	Rat
Lidocaine	Isostearylic Isostearate	Labrasol, Plurol Isostearique	Water	Rat
	Isostearylic Isostearate	Labrasol, Plurol Isostearique	Water	Human
Methyl nicotinate	IPP	Lecithin	Water	Human
Piroxicam	IPM	Hexadecyltrimethyl--ammonium chloride	Phosphate Buffer (pH 5.5)	Rat
Prilocaine	Isostearylic Isostearate	Labrasol, Plurol Isostearique	Water	Rat
Timolol	IPP	Tween 85	-[a]	Rabbit

[a] Supersaturated vehicle with water uptake during the study.