CHAPTER I

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are the main pharmacological agents used in the management of acute painful conditions. They are also frequently used as the first-line therapy in major arthritis diseases of osteoarthritis and rheumatoid arthritis (Blagbrough et al., 1992).

Diclofenac is a potent non-steroidal anti-inflammatory drugs. It has been proven as a safe and efficacious drug in the treatment of a variety of inflammatory and rheumatic disorders (Jayanthi and Udupa, 1992; Ho et al., 1993). It is employed chiefly in oral formulations, and to some extent, also in formulations for intramuscular injection. It has been established that this drug is completely absorbed in the gastro-intestinal tract (GIT) and it is extensively metabolized in the liver and mainly excreted in urine. For topical formulations, the serum levels and bioavailability following transdermal application compared to the oral route are observed and it is found to be as effective as an oral formulations at the equivalent dose (Jayanthi and Udupa, 1993, 1994). Reiss et al. (1986) found that the transdermal release of diclofenac is superior to oral administration is cases where well defined areas near the body surface need to be treated. It is a potential drug candidate for transdermal delivery due to its GIT irritancy, smaller dose used, significant first pass metabolism and short half-life.

In this study, the development of transdermal drug delivery system to increase the duration of action and the postulate release and permeation kinetics

of diclofenac diethylamine was evaluated. Shed snake skin of *Elaphe obsoleta* (Black rat snake) has been used as a model membrane for the *in-vitro* study, since there are similarities between human skin and the shed snake skin of this species in terms of structure/composition, permeability of several compounds, and the functional group contribution to the permeability. It is also easily obtained, stored, and used (Bhattachar et al., 1992; Harada et al., 1992). For the *in-vivo* study, the anti-inflammatory effect of the transdermal patch has been evaluated using carrageenan-induced paw edema in rat model.

Objectives of the study

1. To develop the diclofenac diethylamine sustained-release transdermal delivery system.

2. To study the release and permeation kinetics of diclofenac diethylamine from the transdermal patch by *in-vitro* skin permeation method.

3. To investigate the permeation of diclofenac diethylamine from the transdermal patch through the rat skin, compared with the commercial Voltaren® emulgel.

Osteoarthritis and Rheumatoid Arthritis

Arthritis is one of the most troublesome diseases which affect the significant prevalence and debilitating chronic symptoms in elderly patients. The two most frequently found arthritis disorders are osteoarthritis and rheumatoid arthritis.

1. Osteoarthritis

Osteoarthritis, one of the most common disorders and recognized as a degenerative joint disease (DJD), is a disease characterized by progressive pain, stiffness, limitation of motion, and deformity of joints. Some surveys have estimated that as many as 85% of the people ranging in age from 70 and 79 years have developed some form of osteoarthritis (Pugh, 1989). The disease involved the most common joints such as the knees, the hips, the cervical and lumbar spine, and the first metatarsophalangeal joints.

2. Rheumatoid arthritis

Rheumatoid arthritis (RA), a chronic systemic inflammation disease, is less common than DJD, and its incidence is estimated to be only 1% (Pugh, 1989). It affects people of all ages and is about two times more prevalent in women than men. The exact cause of RA is unknown, but it is widely speculated to be an autoimmune phenomenon. The joints of the hands, wrists, and feet are usually affected first. Diarthrodial joints (freely movable joints with synovials lining) such as elbows, knees, shoulders, ankles, and hip may also be affected. RA is characterized by inflammation, frequently resulting in extensive joint destruction. Laboratory, radiographic and clinical features

may also differ between RA and DJD. Symptom criterion for RA are as follows (Kennedy and Krawczeniuk, 1993).

a) Morning stiffness in and around a joint lasting an hour or longer,
b) Swelling of specific joints of the wrist or fingers,
c) Swelling of the soft tissue around three of more joints,
d) Symmetric swelling of one joint area,
e) Rheumatoid nodule,
f) Rheumatoid factor by method positive in less than 5% of normal population, and
g) Radiograph changes on wrist or hands; erosions or juxta-articular osteoporosis.

3. Therapeutic Approach

There are many factors affect to the therapeutic method, including type of arthritis, the severity of the disease, and the patient's medication preferences. However, common therapeutic goals for all arthritis patients are as follows: 1) relieve pain, 2) improve movement, 3) decrease inflammation, and 4) slow or stop disease progression (primarily in RA) (Kennedy and Krawczeniuk, 1993). The measurements of pain and stiffness, grip strength, walking speed, degree of morning stiffness, and adverse reactions to therapy are determined, consequently, to determine the therapy effectivity.

3.1 Non-pharmacological Therapy

Non-drug measures are the foundation of a successful treatment plan. These measures include physical therapy, patient education, diet instruction, weight reduction, and psychological support. These strategies are often used in

conjunction with pharmacological therapy but can also be quite effective when used alone.

3.2 Pharmacological Therapy

Over-the-counter oral or topical analgesics may be employed in an initial pharmaceutical care plan. The pharmacist must consider the advantages and disadvantages of different anti-inflammatory agents before making a recommendation, to ensure maximum pain relief and patient compliance. Systemic medication offer symptomatic relief of pain and inflammation but may cause adverse reactions, drug interactions, and may have an effect on concomitant diseases.

Aspirin and other salicylates, and nonsteroidal anti-inflammatory agents (NSAIDs) are the major therapeutic agents used to treat arthritis. These agents exert their pharmacological actions by inhibiting the enzyme cyclooxygenase, which results in a decreased biosynthesis of prostaglandins. Prostaglandins which are produced in response to trauma, inflammation, or noxious stimuli, are responsible for sensitizing pain receptors. Aspirin possesses analgesic activity at lower dose ranges and produce anti-inflammatory effects at higher doses. Acetaminophen, however, has no significant anti-inflammatory activity and is thus used only in combination with an anti-inflammatory agent.

The NSAIDs group is the result of major research efforts by the pharmaceutical industry to identify an agent superior to aspirin. Each agent has been tested against aspirin and found to be at least as effective in the anti-inflammatory, analgesic, and antipyretic properties, as aspirin. Advantages of NSAIDs over aspirin are fewer gastro-intestinal problems and less frequent

administrations, which may increase the patient compliance. These agents are considerably more expensive than aspirin; therefore, NSAIDs are used for patients unresponsive who can no longer tolerate aspirin.

NSAIDs have a proposed mechanism of action similar to that of aspirin: inhibition of prostaglandin synthesis. Person to person responses may vary. If one compound is ineffective, another compound can be used instead.

Transdermal Drug Delivery Systems

Transdermal drug delivery systems (TDDs) are self-contained, discrete dosage form, which deliver the drug through the skin at a controlled rate to the systemic circulation. The basic compositions of TDDs consists of five compositions as outlined below :

a. Backing membrane : It is an impermeable membrane or film, act as a backing support for the system.
b. Drug reservoir : This may be a single or multilayered part where the required amount of drug is stored in a stable form.
c. Rate controlling polymeric membrane : This can establish and maintain the prescribed rate of drug administration through the operational life of the system.
d. Contact adhesive layer : This component is applied to provide an intimate contact with the skin surface. It should not irritate the skin.
e. Protective peel strip : Protects the TDDs system from the environment before administration.

TDDs offers the following potential advantages over classical drug delivery systems administered via other routes (oral and parenteral).

- ◊ Ease of self-administration
- ◊ Good patient compliance
- ◊ Avoidance of variation in gastrointestinal absorption
- ◊ Bypass of the hepatic first pass metabolism
- ◊ Production of sustained and constant plasma concentrations of drugs
- ◊ Reduction in repeated dosing intervals
- ◊ Reduction of potential adverse side effects
- ◊ Removal of TDDs provokes an immediate decrease of drug plasma levels
- ◊ Substitute for oral or parenteral administration in certain clinical situations (pediatrics, geriatrics, nausea, etc.)
- ◊ Adaptability to drugs with a short half-life
- ◊ Suitable for drugs which produce a therapeutic response at very low plasma concentrations

Several technologies have been successful in developing a device which can control the releasing rate and the skin permeation of drugs. These technologies can be classified into five approaches as outlined.

1. Pressure-Sensitive Adhesive (PSA) Matrix Devices

PSA matrix device is one of the simplest TDDs (Figure 1a). The drug reservoir is also the adhesive. The rate of drug release is defined by W.I. Higuchi and T. Higuchi, which these equations can be used for drug-in-solution or suspension formulation as a matrix device. The PSA can be positioned on the face or the back of the device and extended peripherally. It must fulfill the following requirements : cause no irritation and no sensitization during its period of contact with skin, provide sufficient adhesion to skin during the

dosing interval, and easily removed without leaving an unwashable residue. The most typical PSAs are acrylic, rubber or silicone adhesive. The examples of monolithic PSA, are Frandol® and Nitro-Dur II® (Sugibayashi and Morimoto, 1994).

Madhavan and Hwang (1992) investigated the *in-vitro* release and permeation profiles through excised hairless mouse skin and the anti-inflammatory effect of monolithic-type transdermal flufenamic acid (FA) delivery systems using an acrylate pressure sensitive adhesive (PSA). It was observed that the drug released via a solution-diffusion mechanism (further evaluated for its anti-inflammatory effect by applying the system on carrageenan-induced paw edema in mice) showed a significant inhibition of swelling as compared to the placebo system.

2. Membrane System

In this system, the drug reservoir is totally encapsulated in a shallow compartment molded from a drug impermeable metallic plastic laminate and a rate-controlling polymeric membrane (Figure 1b). The drug molecules are permitted to release only through the rate-controlling membrane. In the drug reservoir compartment, the drug solids are dispersed in a solid polymer matrix or suspended in an unleachable, viscous liquid medium to form a paste-like suspension or dissolved in a releasable solvent to form a drug solution. The rate-limiting membrane can be either a microporous (eg. polyethylene film) or a nonporous polymeric membrane with a defined drug permeability property (eg. ethylene-vinyl acetate copolymer). On the external surface of the polymeric membrane, a thin layer of drug-compatible, hypoallergenic adhesive polymer

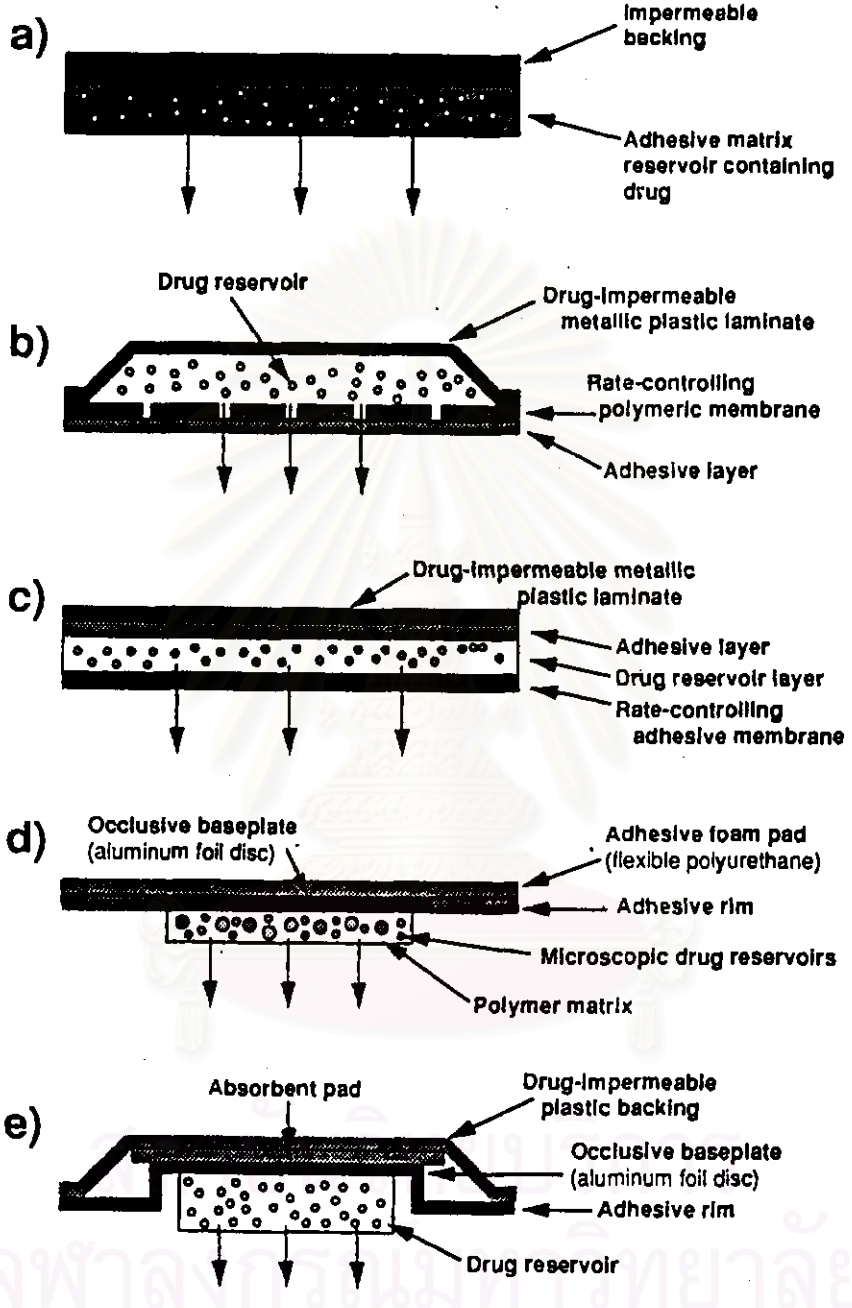

Figure 1 The cross-sectional view of several TDDs : (a) PSA matrix device; (b) membrane-moderated TDDs; (c) adhesive-controlled TDDs; (d) microreservoir-type TDDs; (e) matrix dispersion-type TDDs (Sugibayashi and Morimoto, 1994).

may be applied to achieve an intimate contact of the transdermal patch with the skin. The rate of drug release from this type of transdermal drug delivery system, can be tailored by altering the polymer composition, permeability coefficient, and thickness of the rate-limiting membrane and adhesive. Several TDDs have been successfully developed from this technology and are best examplified by Transderm-Scop®, Transderm-Nitro®, Estraderm®, and Catapress®-TTS (Shaw and Dohner, 1985; Sugibayashi and Morimoto, 1994).

The selected type of diclofenac diethylamine TDDs in this research study is the membrane-moderated type. Many studies have reported the attempt to prepare this transdermal dosage form.

Dehghan, Parakh, and Deshpande (1993) have formulated and evaluated the polymeric matrices and membrane systems for transdermal drug delivery devices using ephedrine hydrochloride as a model drug. Polymers such as ethyl cellulose, polyethylene glycol 6000, polyvinyl pyrrolidone, hydroxypropyl methylcellulose, Eudragit RLPM, and Eudragit RSPM were employed, and plasticizers such as glycerol and dibutylphthalate were incoporated. Polymeric films were evaluated for mechanical and physical properties. Drug containing films were evaluated for thickness uniformity, weight variation, area variation, and drug content uniformity. The matrices and membrane systems were studied for in-vitro drug release. The results indicate that the formulations studied appear promising.

Ogiso et al. (1993) have formulated a transdermal dosage form of terodiline, an anticholinergic and calcium antagonist, and evaluated the *in-vitro* and *in-vivo* absorption through rat skin. It penetrated rapidly from the gel formulation through the skin without any enhancers. When applied to rat

abdominal skin, high plasma concentrations of terodiline were observed for upto 24 hours, thereby resulting in a high bioavailability which equivalent to that of an intravenous injection.

3. Adhesive Membrane System

This type of drug delivery system is a simplified version of the membrane-moderated drug delivery system just described in the second approach. Instead of completely encapsulating the drug reservoir in a compartment fabricated from a drug-impermeable metallic plastic backing, the drug reservoir is formulated by directly dispersing the drug in an adhesive polymer and then spreading the medicated adhesive by solvent casting or heat molding onto a flat sheet of drug-impermeable metallic plastic backing to form a thin drug reservoir layer (Figure 1c). On the top of drug reservoir layer, layers of non medicated, rate-controlling adhesive polymer of constant thickness are spread to produce an adhesive diffusion-controlled drug delivery system. The rate of drug release generally obeys Fick's law. Drug release from the Deponit® system composed of several pressure-sensitive adhesive (PSA) layers, and is controlled by different diffusivities of the layers (Crabbe, Wordell, and Leigh, 1987; Sugibayashi and Morimoto, 1994).

4. Microreservoir System

Micoencapsulating agents are one of the most important components in this system, and several hydrophilic and hydrophobic polymers are available for this purpose. Different polymer and polymeric materials can be used to produce microcapsules and macro capsules in reservoir devices, such as hollow fibers, porous polymer sheet, and foam (as the wall of a capsule). This type of drug delivery system can be considered as the hybrid of reservoir and matrix

dispersion-type drug delivery systems. In this strategy, the drug reservoir is formed by suspending the drug solids in an aqueous solution of water-soluble liquid polymer. The drug suspension is then dispersed homogeneously in a lipophilic polymer, by high-shear mechanical force, to form thousands of unleachable, microscopic spheres of drug reservoirs. This thermodynamically unstable suspension is stabilized by immediately cross-linking polymer chain in situ, which produces a medicated polymer disc with a constant surface area and a fixed thickness. A transdermal therapeutic system thus is formed with the medicated disc at the center and surrounded by an adhesive rim (Figure 1d). This technology has been utilized in the development of Nitrodisc® (Crabbe, Wordell, and Leigh, 1987; Sugibayashi and Morimoto, 1994). Release of a drug from microreservoir-type TDDs can follow either a partition-control or a matrix diffusion-control depending upon the relative magnitude of solubility of the drug in the liquid compartment and in the polymer matrix.

5. Polymeric Matrices

The simplest and least expensive way to control the release of drug is by using a polymeric matrices. The drug reservoir is formed by homogeneously dispersing the drug solids in a hydrophilic or lipophilic polymer matrix. The medicated polymer is then molded into a disc with defined surface area and precise thickness. This drug reservoir polymer disc, is glued onto an occlusive base plate fabricated from a drug-impermeable plastic backing (Figure 1d). Instead of spreading the adhesive polymer onto the surface of the medicated disc as discussed earlier in the first two systems, the adhesive polymer is applied along the circumference to form a strip of adhesive rim around the medicated disc. This type of TDDs is exemplified by the Nitro-Dur® (Crabbe, Wordell, and Leigh, 1987; Sugibayashi and Morimoto, 1994).

Indomethacin polymeric matrices containing 1, 2, and 4% of drug were formulated and evaluate by Lai, Chiang, and Wu (1987). The results indicated the Higuchi's model following the release studies. The effective diffusivity of indomethacin (D_m) was $1.32x10^{-7}\pm7.4\%$ cm^2/sec, whereas the amount released in 24 hours from 10 cm^2 matrices with three different concentrations of indomethacin, were 12.0, 18.6, and 25.0 mg, respectively.

***In-vitro* Study of TDDs**

Drug absorption by the skin can be measured directly in human or animals by analyzing the drug concentration profile in the blood or in the urine following topical administration. However, during the development of TDDs, a quantitative assessment of the mechanisms and rates of transdermal permeation of drug can be achieved by analyzing the drug permeation profile through an excised skin mounted on the diffusion cell. Any unwanted complications from the specific pharmacokinetic rate processes, such as distribution, metabolism, and excretion of drug in the body can be eliminated (Keshary and Chien, 1984). The aim of the *in-vitro* experiment in TDDs is to understand and/or predict the delivery and penetration of drug molecules from the delivery device into the body via the skin of a living animal (Gummer, 1989). The i*n-vitro* experiment is useful in an evaluation of a dosage form because it is cost efficient and is able to test a large number of formulations in a relatively short time. In addition, the *in-vitro* studies can be used to screen for candidate formulations as well as test the effects of various ingredients on skin permeation.

The general major assumptions which may be made when conducting *in-vitro* experiments, are as follow :

a. the stratum corneum is the rate-limiting barrier to permeation,
b. the skin's barrier properties are not compromised by the removal from living organism, and
c. the possibility of metabolism with the skin is ignored (Zatz, 1990).

1. Diffusion Cell

According to the assumption that the drug release profiles obtained from the *in-vitro* study is similar to that obtained from the *in-vivo* study, a diffusion system was designed to mimic an *in-vivo* situation. Usually, the system of diffusion study consists of the following compositions:

* skin of a membrane as a barrier of the drug diffusion,
* diffusion cell with two compartments : donor and receptor,
* water circulating system for controlling the temperature of the whole system at 37±1°C, and
* stirring system for ensuring homogeneity of drug concentration in the medium solution.

Diffusion cell is the only composition which was modified to achieve the convenience and suitable pattern for each diffusion system proposed. There are various diffusion cells used for the *in-vitro* studies. An early model of *in-vitro* diffusion cell has been designed to study the routes of skin penetration since 1965 by Scheuline (Chien and Valia, 1984). Later, several *in-vitro* diffusion cells have been designed to achieve both objectives, ease of operation and quantitative improvement.

Franz-diffusion cell, a finite-dosing upright type, one of the most frequently used *in-vitro* techniques for skin permeation studies, was designed

and developed by Franz (1975). Franz diffusion cell, a commercial model, has been marketed and extensively used for skin permeation studies over the years to assist the development and the evaluation of a controlled-release transdermal therapeutic system. Schematic illustration of the commercially available finite-dosing Franz diffusion cell assembly is shown in Figure 2. Each of the diffusion cells consist of two compartments; a donor compartment, which is exposed to an ambient condition, and a receptor compartment which is maintained at 37±1°C by a circulating water jacket. The solution hydrodynamics in the receptor compartment is kept constant by a tiny rod-shaped magnetic stirrer rotating at 600 rpm by a synchronous motor mounted underneath the cell mounting block.

Keshary and Chien (1984) have designed a new finite-dosing diffusion cell for *in-vitro* skin permeation studies, which is illustrated side-by-side with a unit of the commercially available Franz diffusion cell in Figure 2. This cell improves the temperature control on the skin surface and in the receptor solution as well as enhance the efficiency of solution mixing and the distribution of drug solute following skin permeation. It could be attributed to the combine effect of the thickness reduction of the hydrodynamic boundary layer and better control of the temperature in the diffusion path. So, the skin permeation rate profiles could be visualized with minimal effect from the mass transfer process.

Chien and Valia (1984) have designed the horizontal diffusion cell by aiming to minimize the potential inefficiencies observed in the Franz diffusion cell (Figure 3). It is composed of a skin permeation cell and a magnetic driving unit, and two half-cells in mirror image of each others. Each of the half-cells contains a solution chamber within a stirring platform which will rotate the

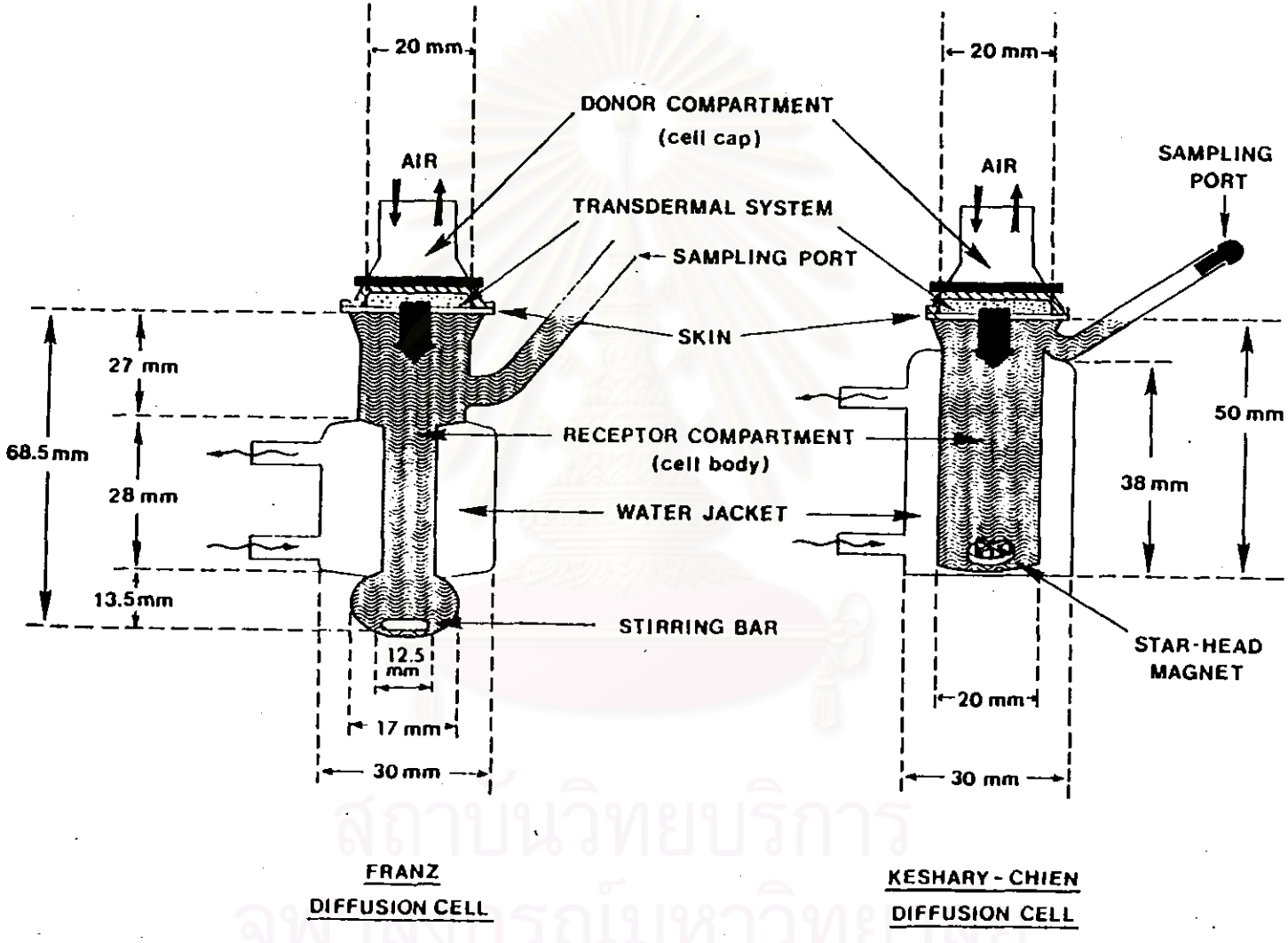

Figure 2 Diagrammatic illustration and comparison of Franz and Keshary-Chien diffusion cells.

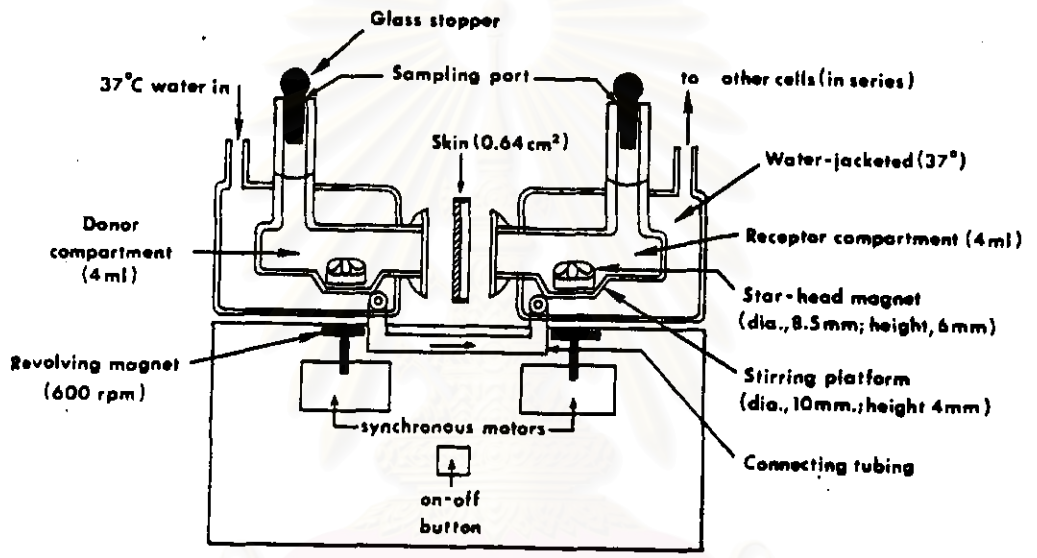

Figure 3 Schematic illustration of Valia-Chien horizontal diffusion cell.

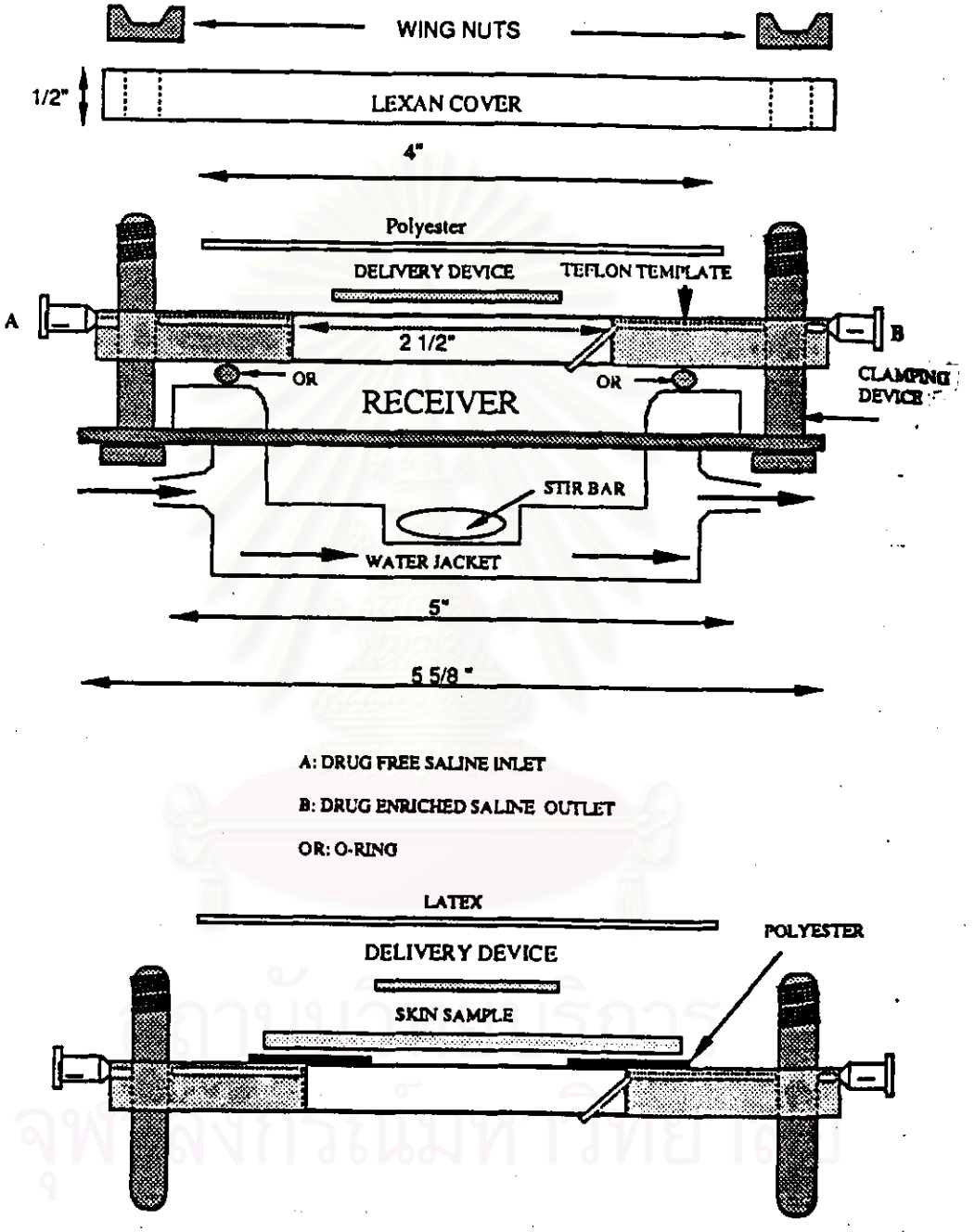

Figure 4 Schematic illustration of patch cell assembly (Mueller, Robert, and Scott, 1990).

magnetic stirrer at a synchronous speed. A sample port on the solution chamber could be tightly closed with glass stopper. Chien and Valia suggested that their diffusion cell showed consistently superior results than the Franz diffusion cell, by comparative studies, in terms of the control of skin surface temperature and the efficiency of solution mixing.

Mueller, Robert, and Scott (1990) have designed an *in-vitro* diffusion cell that is large enough to accommodate drugs delivery systems up to 20 cm^2, approaches sink conditions for large devices when tested through the skin. The patch cell (Figure 4), is constructed from glass, teflon, and stainless steel. The patch cell consists of a large receiver compartment, with a volume of approximately 200 ml, completely surrounded by a glass water jacket containing inlet and outlet ports which connect to a water bath. The patch cell can accommodate a large variety of device sizes for the *in-vitro* percutaneous absorption studies. When using large patches, the skin is mounted directly on the teflon template with the dermal side in contact with the receiver fluid. When using smaller device sizes, an aperture smaller than that in the teflon templated, it can be punched into the polyester frame. The polyester can then be cemented onto the teflon template, and the skin can be cemented to the polyester. The delivery system can now be placed over the skin and the cell assembly completed as shown.

2. Skin Model

The preparation of skin model to be used in a diffusion cell is an important step for the *in-vitro* percutaneous absorption study. In general, excised human skin is the most accurate, preferred membrane for the *in-vitro* skin permeation study. However, human skin is in short supply and has a

variety of conditions that could induce a high permeability variation between individuals. If one assumes that the *in-vitro* experiment should reflect exactly the *in-vivo* situation, then only human skin can be used (Zatz, 1990).

In-vitro permeation studies can be conducted using animal skin, such as hairless mouse, guinea pig, fuzzy rat, rabbit, monkey, snake, and miniature pig. Throughout the history of TDDs, investigators have been striving to find a predictive correlation between the penetration of molecules through animal and human skin (Harada et al., 1993; Itoh et al., 1990). Although there are a number of similarities between the two systems, but no animal skin could completely mimics the penetration characteristics of a human skin (Gummer, 1989). Due to the fact that most animal skin are more permeable than human skin partly because of a larger number of hair follicles. Furthermore, the use of artificial membrane in transdermal research is limited because they lack keratinized proteins and lipids which are primary components in the stratum corneum of mammalian skins (Itoh et al., 1990).

Stratum corneum is a major contribution to human skin which provides nearly impermeable barrier to the transport of most drugs (Bhatt et al., 1991). It is composed of nonliving cornified tissue that is enriched in its solid composition. The unique barrier properties of the stratum corneum are due chiefly to its lipodal material (Bronaugh and Stewart, 1986).

There are many studies reported about the similarities between human stratum corneum and snake skin, and the use of snake skin in percutaneous absorption study (Bhat et al., 1989; Itoh et al., 1990). Both shed snake skin and human stratum corneum are composed of keratinized proteins and lipids, and have similar water permeation. It has found that snake skin has some features

that make it useful as a model membrane. Since shed skin has no living tissue, it can be stored at a refrigerated temperature for a relatively long period.

The model membrane examined in this study is shed snake skin, which is a nonliving pure stratum corneum with no hair follicles. Human stratum corneum consists of 10-20 layers of an alpha-keratin-rich intracellular layer and a lipid - rich intercellular layer, but shed snake skin consists of three distinctive layers. These are the beta-keratin-rich outermost beta layer, alpha-keratin- and lipid-rich intermediate mesos layer, and alpha-keratin-rich inner most alpha layer. Further, the mesos layer shows three to five layers of multilayer structure with cornified cells surrounded by intercellular lipids, which is similar to human stratum corneum.

Table 1 Comparison of thickness, lipid content, and water evaporation rate between human stratum corneum and shed snake skin (Itoh et al., 1990).

	Human stratum corneum	*Shed snake skin (Elaphe obsoleta)*
Thickness	13-15 μm	10-20 μm
Lipid content	2.0-6.5%	6%
Water evaporation rate	0.1-0.8 mg/cm^2 hr	0.15-0.22 mg/cm^2 hr

Table 1 shows the similarities between the shed snake skin of *Elaphe obsoleta* (black rat snake) and the human stratum corneum in terms of thickness and lipid content. Also, shed snake skin and human strum corneum have similar lipid compositions, that is, neutral lipids are a major lipid component in

both skins and fatty acids, with carbon-chain lengths of C15 and C18 predominant.

Bhatt, Rytting, and Topp (1991) studied the *in-vitro* influence of the penetration enhancers Azone® and lauryl alcohol on the transport of acetaminophen and ibuprofen through shed snake skin (*Elaphe obsoleta*). The addition of either enhancer increased the amount of acetaminophen transported. In contrast, only lauryl alcohol increased the amount of ibuprofen transported, while Azone® provided no significant enhancement. Itoh et al. (1992) have found that the shed snake skin became more permeable after Azone® or lauryl alcohol pretreatment, with a greater permeability if a more hydrophilic and larger-molecular size permeants were used.

Bhattachar et al. (1992) have reported that salicylic acid complex solubility in squalane was less than plain drug, but the solubility of diclofenac and indomethacin complex increased upon complexation with hydrogenated phospholipid (HPL). Salicylic acid and diclofenac permeability coefficients across shed snake skin are not affected by HPL, while, indomethacin-HPL complex permeability coefficients increased.

Selecting model membranes to simulate human skin from the *in-vitro* permeability studies of salicylic acid using cadaver, rodent, and shed snake skin has been examined by Harada et al (1993). Shed snake and hairless rat skin were found to show similar permeability to human breast and thigh skin, where wistar rat and mouse skin showed similar permeability to human cheek, neck, and inguinal skin.

Materials Information

1. Diclofenac

Diclofenac, a phenylacetic acid derivative, is a synthetic, nonsteroidal anti-inflammatory and analgesic compound.

1.1 Physicochemical Properties (Adeyeye and Li, 1990; Riess et al., 1986).

Chemical name : 2-[(2,6-dichlorophenyl) amino] benzeneacetic acid diethylammonium salt

Empirical name : $C_{18}H_{22}Cl_2N_2O_2$

Structural formula :

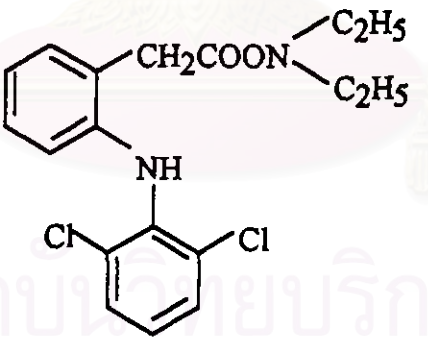

Figure 5 Chemical structure of diclofenac diethylamine.

Description : Diclofenac diethylamine occurs as an odorless, white to beige, and hygroscopic powder. It is soluble is methanol, ethanol 95%, chloroform and water.

1.2 Pharmacology (Adeyeye and Li, 1990)

Diclofenac is a potent anti-inflammatory agent. The drug is among the most effective inhibitors of PGs synthetase and has analgesic and anti-pyretic effects.

1.3 Pharmacokinetics

Diclofenac is rapidly and completely absorbed from the GI tract after oral administration and there is a very high first pass metabolism of the drug. While the percutaneous route, such as gel and cream, is absorbed without the first pass metabolism. The drug in form of suppositories, is also absorbed with rapid peak plasma concentration at a rate and level of the same order as oral administration (Nishihata et al., 1988). Diclofenac is extensively bound (>99%) to plasma proteins, and not less than 99-99.4% is bound to serum albumin fraction (Karim, 1993). The half-life of the drug is very short of approximately two hours. Renal excretion is greater than biliary but very little unchanged drug is excreted in the urine.

1.4 Therapeutics Efficacy

Diclofenac has a well proven efficacy in treating rheumatoid arthritis and osteoarthritis including ankylosing. However, as with all NSAIDs, its main disadvantage is associated with gastro-intestinal adverse effects including ulcer, hemorrhage, and perforation (Geis, 1993; Downie, 1993; McKenna, 1993; Zuinen, 1993).

1.5 Preparations

There are many dosage forms of diclofenac formulations in Thailand such as tablets (25mg, 50mg), enteric-coated tablets (25mg, 50mg), sustained-release tablets (100mg), injection (75mg/3ml or 2ml), suppositories (50mg, 100mg), and gels (diclofenac sodium 1%, diclofenac diethylamine 1.16%) eg. Voltaren® emulgel.

1.6 Development of Diclofenac Preparations

Many researchers developed diclofenac sustained release dosage forms in order to improve bioavailability, reduce frequency of drug administration, and reduce the incidence and intensity of side effects.

Nishihata et al. (1987) have found poor penetration of diclofenac through the *in-vitro* rat dorsal skin including subcutaneous tissue. In contrast, the *in-vitro* and the *in-vivo* percutaneous of diclofenac in the aqueous gel form, prepared with phospholipid, were increased. In 1988, Nishihata et al. reported that the addition of 10% w/w ethanol in aqueous solution containing sodium diclofenac apparently increased the percutaneous absorption of diclofenac. Which was shown to be because of an increase in the sodium diclofenac concentration in the applied solution rather than by an increase in dorsal skin permeability to diclofenac. Moreover, they have investigated the plasma diclofenac concentration profiles of sustained release suppositories of sodium diclofenac (SR-Supp) in 4 healthy male humans, and the clinical effect of in 8 patients who were in pain. Plasma diclofenac were found in all 4 subjects at a concentrations of more than 50 ng/ml and were maintained for 8-12 hours. The effect of SR-Supp in alleviating pain was sustained for 10 hours or more. As a

result, the patients can sleep comfortably through the night. Next, Nishihata et al. (1990) investigated the usefulness of water absorbable polymer (Poys® SA-20) for the preparation of sustained release suppository of sodium diclofenac. The *in-vitro* release showed a slow release of the drug, and the *in-vivo* absorption in dogs showed that the transient high plasma diclofenac concentration could be avoided and the effect was prolonged.

Nishihata and Rytting (1991) examined the transport of ketoprofen and sodium diclofenac using rat dorsal skin and shed snake skin as models of the stratum corneum for the evaluation of transdermal formulation. The aqueous-lipid vehicle formulations prepared with hydrogenated soya phospholipid increased the transport of both NSAIDs, and the addition of tetradecanol to the formulation increased the transport more markedly.

Reiss et al. 1986 reported the percutaneous absorption of diclofenac diethylammonium 1.16 % (w/w) in a combination of emulsion cream and gel (Voltaren® emulgel), as well as of diclofenac sodium 1% (w/w) in a cream formulation (Voltaren cream) in guinea-pig, rabbit, and man. Percutaneous absorption in guinea-pig of diclofenac sodium was 3-6 % of the dose when the cream formulation was applied under an occlusive dressing and left in place for 6 h. When applied topically to the knee joints of rabbits, diclofenac penetrated into the patellar ligament, the fatty tissue, and the synovial fluid. In man percutaneous absorption was as high as 6% of the dose when the emulgel formulation (5 mg/cm^2) was applied to non-occluded skin and left in place for 12 h. After the emulgel formulation had been applied to the hands of 8 arthritic patients for 3 days, diclofenac was found to be present the concentration ranging from lower to the highest in the plasma, the synovial fluid, and the synovial tissue, respectively.

Tomida et al. (1987) studied the release of diclofenac and hydrocortisone from the aqueous gels of Pluronic F-127 (PF-127) in an *in-vitro* membraneless release model. It has been found that the release rate decreased with increasing PF-127 concentration, but increased with increasing temperature. A linear relationship was obtained between the apparent release rate and the initial drug concentration The release of diclofenac was largely dependent upon the gel pH and was maximal at pH approximately 7. Furthermore, among the various factors affecting drug release, pH in gel formulations appeared to be very important when the formulated drug is a weak acid or base.

Nozawa, Suzuki, and Sato (1989) investigated a transdermal therapeutic system (TTS) with thermo-responsive membrane incorporated as a rate controlling membrane. It releases NSAIDs (indomethacin, ketoprofen) and antipyretic drugs (acetaminophen, ethenamide) in response to temperature changes between 32 and 38°C in the *in-vitro* experiments. Then, the *in-vivo* experiments were conducted in a rabbit model.

Schapira, Linn, and Scharf (1991) have found statistically significance of clinical improvement in patients with lateral epicondylitis of the elbow, which was treated with diclofenac diethylamine gel as compared with the control group.

A transdermal drug delivery system of diclofenac based on polymeric pseudolatex dispersion was developed for prolonged and controlled release of diclofenac by Vyas, Gogoi, and Jain (1991). The designed system exhibit a linear relationship between drug release (Q) versus square root of time ($\sqrt{t}$). The *in-vitro* anti-inflammatory activity and the *in-vivo* evaluation indicated that the system could maintained a constant and effective plasma level for 24 hours.

Singh, Pandey, and Udupa (1992) prepared and evaluated transdermal films of flurbiprofen and diclofenac sodium using HPMC and Beta Cyclodextrin, for physicochemical characteristics, release pattern, Pharmacodynamic activity, and Bioavailability. It was reported that beta cyclodextrin could not enhance the transdermal permeability of either of the drugs.

Obata et al. (1993) investigated the effect of ethanol on the skin permeation of diclofenac using excised hairless rat abdominal skin *in-vitro*. The steady-state flux of diclofenac increased with increase in the pH of diclofenac-suspended donor solution, but the steady-state permeability coefficient (P) of diclofenac was inversely proportional to the change in pH of the donor solution. These phenomenons demonstrated a close correspondence with enhancement in the solubility of diclofenac in the donor solution, but the pattern of skin permeation of diclofenac apparently obeyed the pH-partition theory.

Jayanthi and Udupa (1993) evaluated permeation flux of an aqueous cream formulation of diclofenac sodium across freshly excised mouse skin, using carrageenan-induced paw edema and cotton pellet-induced granuloma models in rats an found as effective as the marketed gel and oral administration at the equivalent dose of 25 mg/kg body weight.

Ho et al. (1994) studied the influence of mixture of water, alcohol, and propylene glycol on the *in-vitro* percutaneous penetration of diclofenac sodium from Carbomer 940 gel system via synthetic membrane Durapore and hairless mouse skin using a simplex lattice experimental design. The penetration through synthetic membrane was described by the Higuchi model. It appeared

to be a membrane-controlled mechanism when using hairless mouse skin as the barrier.

2. Poloxamer

$$HO(C_2H_4O)_a(C_3H_6O)_b(C_2H_4O)_aH$$

Figure 6 Structure formula of poloxamer, for poloxamer F-127 : a =35, b =30.

Poloxamer is nonionic polyoxyethylene-polyoxypropylene glycol copolymer which has available grades vary from liquids through pastes to flakes or powders. The general formula is as above, in which a is 2 to 130 and b is 15 to 67.

Poloxamer F-127 is white-colored, waxy, free-flowing prilled granules, practically tasteless, and odorless. It is freely soluble in water, isopropyl alcohol, and ethyl alcohol (95%) but insoluble in propylene glycol. Poloxamers are stable materials which hygroscopic only at greater than 80% relative humidity. Aqueous solutions are stable in the presence of acids, alkalis, and metal ions. It is used as wetting, solubilizing, emulsifying, foaming, and gel-forming agent. *Pluronic* is the trade name used in the USA by BASF Corporation for pharmaceutical and industrial grade poloxamers, whilst *Lutrol* is the trade name used in the UK and Europe for the pharmaceutical grade material.

3. Hydroxypropyl Methylcellulose

Hydroxyproply methylcellulose (HPMC) is a mixed ether of cellulose containing a variable proportion of methoxyl and 2-hydroxypropoxyl groups. The chemical structure is shown as follow ,where R is H, CH3 or $[CH_3CH(OH)CH_2]$.

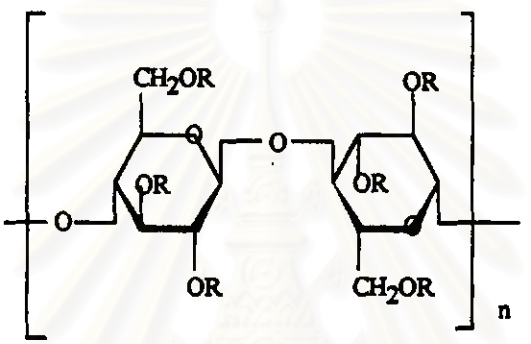

Figure 7 Chemical structure of HPMC.

HPMC is classified according to the number of substituent groups, composition and viscosity. It is an odorless, tasteless, white or creamy-white colored hygroscopic fibrous or granular powder which can be used as film former, protective colloid, emulsifier, stabilizing, thickening, and suspending agent. High viscosity grades are used to retard the release of water soluble drugs. It is soluble in cold water, forming a viscous colloidal solution; insoluble in hot water, dehydrated alcohol, ethanol (95%), acetone, chloroform, and ether, but soluble in mixtures of ethanol and dichloromethane, and mixtures of methanol and dichloromethane. Solutions are stable at pH 3-11. It is incompatible with oxidizing materials.

In this study, HPMC or Methocel® E50 which has normal viscosity of 2% aqueous solution at 20°C, of 50 cps, was used.

4. Sodium Carboxymethylcellulose

Sodium carboxymethylcellulose (CMC) is the sodium salt of a polycarboxymethyl ether of cellulose. The chemical structure is shown as follow, with a degree of substitution (DS) of 1.0.

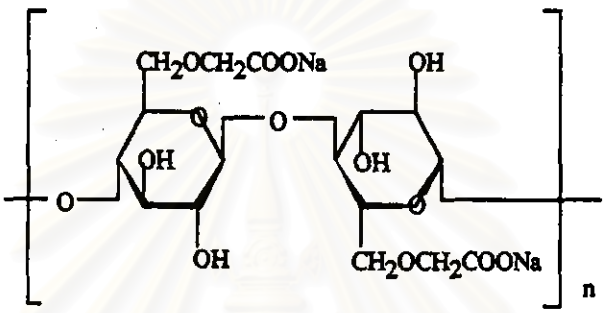

Figure 8 Chemical structure of CMC.

CMC is white to almost white colored, odorless, hygroscopic powder or granular material having a faint paper-like taste. It is soluble in water at all temperature, giving a clear solution; practically insoluble in most organic solvents. The viscosity of 1%w/v high viscosity CMC is 1500-3000 cP. Aqueous solution of CMC exhibit pseudoplastic flow behavior. CMC exhibit maximum viscosity and stability at pH 7-9 and is incompatible with soluble salts of iron and some other metals, such as aluminum, mercury, and zinc. CMC is used as suspending and/or viscosity-increasing agent, coating agent, disintegrant, thickener, and suspension stabilizer.

5. Sodium Alginate

Sodium alginate consists chiefly of the sodium salt of alginic acid. It occurs as an odorless and tasteless, white to pale yellowish-brown colored hygroscopic powder. It is practically insoluble in ethanol, ether, and other

organic solvents, but slowly soluble in water, forming a viscous colloidal solution. Aqueous solutions of sodium alginate are most stable between pH 4-10. Incompatibilities have been observed with acridine derivatives, crystal violet, phenylmercuric acetate and nitrate, and calcium salts. It is widely used as a thickening, suspending, and stabilizing agent.