CHAPTER I

INTRODUCTION

For the management of heart disease, hypertension and angina pectoris, a new class of drugs, calcium channel blocking agents with a variety of potent cardiovascular effects, have become important chemicals available. These agents have been found to be directly potent coronary vasodilation with additional effect including depression of myocardial contractility. They have been shown to offer potential prophylactic benefit in angina pectoris and hypertension (Comess and Fenster, 1982; Temkin, 1982).

Nifedipine (NFP) is one of the most potent calcium channel blocking agents. It is also the most potent vasodilatory agent. Its major effects are to vast reduce total peripheral resistance to increase the heart rate and the cardiac output, and also to increase myocardial oxygen demand. It is a safe and effective drug in the long term treatment and prophylaxis of hypertension and angina pectoris (Comess and Fenster, 1982; Ebner and Daniel, 1983; Muller and Chahine, 1983; Olgilvie, 1983; Temkin, 1982). However, the absorption of NFP is inferior when administered orally in a solid dosage form, due to its poor water solubility (Kohri, Mori, Miyazaki, and Arita, 1986). NFP is rapidly and completely metabolized on first

pass through the liver into its inactive pyridine metabolites, its bioavailability is rather low, only about 50% of the oral dose. Its duration of action is very short that its biological half life is in the range of 2-5 hours (Comess and Fenster, 1982; Foster, Hamann, Richard, Bryant, Graves and McAllister, 1983; Reynolds, 1982). These properties create problems in the treatment of hypertension. Thus, it is necessary to administer NFP frequently in order to maintain a constant and effective drug plasma concentration. The usual dose of commercially available NFP is 10 mg three or four times daily and the maximal dose is 120 mg daily. (Comess and Fenster, 1982; Mcevoy, 1987). The too frequent oral dose is the cause of inconvinience and noncompliance of patients. The dosage form development of NFP is therefore important in order to improve the bioavailability and prolong the action of NFP with the purpose of reducing the frequency of drug administration and the incidence and intensity of side effect for the long term treatment of hypertension and angina pectoris.

Because of the advantage of transdermal drug delivery system (TDDs) such as the induction of the drug into the systemic circulation without an initial first pass through the liver and elimination of the peaks and trough in drug plasma concentrations, the efficacy of NFP may be improved. In addition, TDDs is so easy to administer, simply directly applied the dosage form with a

premeasured amount of drug on the skin, the drug would be remained controlled to be released with a expected peroid of time, over 12 hours, thus the frequency of drug administration could be disminished.

In order to overcome the aforementioned problems of oral dosage form of NFP, this research study is aimed to develop the preparation of NFP as TDDs using different gelling agents as rate controlling matrices.

Objectives of the study

1. To design and develop a controlled release transdermal drug delivery system of nifedipine using hydrophilic and hydrophobic gelling agents (poloxamer and Aerosil) as matrices.

2. To study the effects of different types and concentrations of gelling agents on the physical properties of dosage form.

3. To study the effect of additives e.g. organic modifiers, co-solvent and thickenig agent on the physical properties and the permeation rate of nifedipine dosage form.

4. To investigate and compare the permeation rate and mechanism of nifedipine which influence by different gelling agent, from the saturated solution of nifedipine and the designed dosage forms by *in-vitro* skin-permeation study.

General Information of Transdermal Drug Delivery System

Transdermal drug delivery system (TDDs) is a system for introduction of drug into the systemic circulation via the skin in order to prevention an initial first pass effect, gastrointestinal incompatibility and elimination of the peaks and trough in drug plasma concentration associated with use of convenient dosage forms. Many of TDDs have been successfully developed to control the rate of drug delivery in order to prolong the duration of therapeutic action and to target the delivery of drug to a certain point in the body (Chien, 1982; Chien, 1983; Chien, 1984; Shaw and Dohner, 1985; Guy and Hagraft 1989). The major factor to be considered in development of a TDDs is to know the variability in skin permeability to drug within and between patients, however, the dosage form can be designed such that despite the variability in skin permeability. The drug is delivered to the circulation at a controlled rate, within the limits of the therapeutic window in plasma concentration (Shaw and Dohner, 1985; Chien, 1983).

The transdermal route of administration can not be employed for a large number of drugs. In general, TDDs is suitable only for drugs for which the daily dose is of the order of a few milligrams (Guy and Hadgraft, 1985).

A well-designed TDDs is expected to provided most of the benefits outlined as follow :

a. Bypass hepatic "first pass" metabolism and gastrointestinal incompatibility of drug.

b. Minimize inter- and intra-patient variations.

c. Maintain steady state drug concentration.

d. Provide predictable, extented duration of drug action.

e. Enhance therapeutic efficacy.

f. Reduce frequency of drug dosing.

g. Improve patient compliance.

A general transdermal device is illustrated in Figure 1. Transdermal dosage forms require specialised material which can be devided in five components as following :

A. Backing substrate : It is an impermeable membrane or film as backing support of the system, must be impermeable to the drug and additive, if used, and as result, it is usually impermeable to water vapor i.e. occlusion. It is need which has good flexibility,

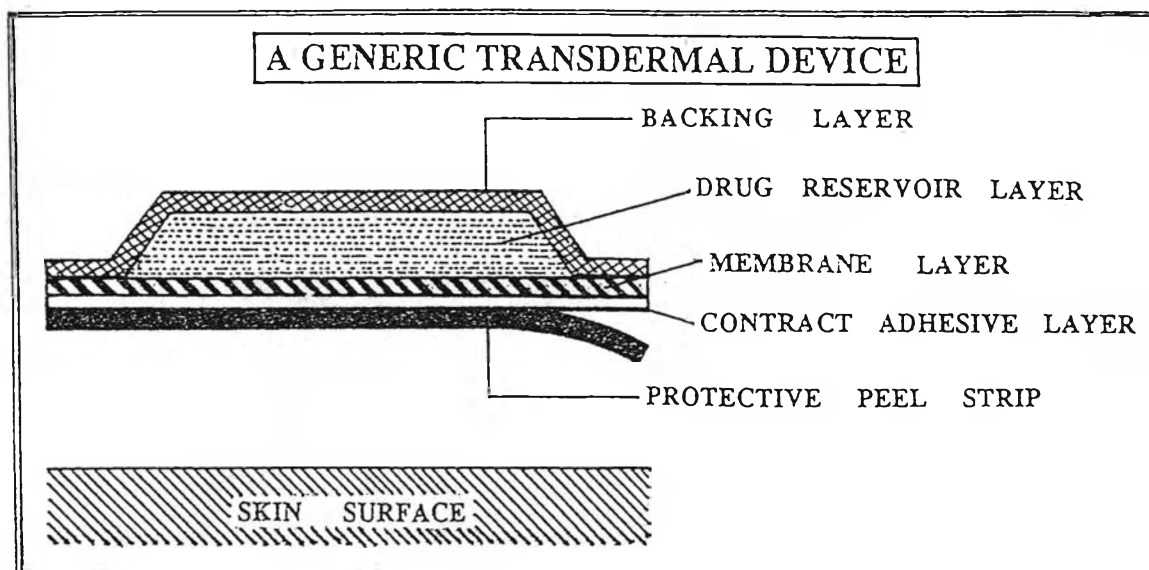


Figure 1 Schematic Illustration of A Generic Transdermal Delivery System.

provides a good bond to the drug reservoir, prevents drug from leaving the dosage form.

B. Drug reservoir : It may be a single or polylayer where the required amount of drug is stored in a stable form. If the adhesive made up this layer, it must possess the desired adhesive and cohesive properties and be compatible with skin.

C. Control membrane : This film controls the rate of drug flux from the dosage forms. TDDs can be produced with microporous membranes which act to control drug flux by the size and tortuosity of pore in the membrane. TDDs can also produced with dense polymeric membrane, through which drug permeates by a dissolution and diffusion mechanism.

D. Contact adhesive layer. This layer is applied to provide an intimate contract to skin surface that materials used here must provide desired adhesive and cohesive properties and must be skin compatible. The adhesive must then hold the device securely in place for long period of time, yet remove must not so painful so as to discourage patient from using it. The protective peel strip which over the adhesive must be easily removed and none of the adhesive should stay on the peel strip.

Table 1 Current Commercial Transdermal Drug Delivery Systems and Their Components.

Drug	Manufacture	Trade name	Type of device	Backing layer	Drug reservoir or matrix materials	Membrane material	Adhesive	Peel strip	Package
Niroglycerin	Alza/Ciba-Geigy	Transderm Nitro	Reservoir	Flesh-colored polyfoil (?)	Silicone oil-nitroglycerin	Poly(ethylene-vinyl acetate)		Fluorocarbon polyester film	Foil
Nitroglycerin	Key Pharmaceuticals	Nitro-Dur I	Monolithic	Foil-paperred combination	Plasticized poly(vinyl pyrrolidone-poly-(vinyl alcohol)			Paper foil combination	Paper package (?)
Nitroglycerin	Key Pharmaceuticals	Nitro-Dur II	Monolithic					Fluorocarbon-polyester film (?)	Foil
Nitroglycerin	Pharma Schwarz	Deponit	Mixed-monolithic-reservoir	Flesh-colored polyfoil drug mixture	Layered isobutylene adhesive and		Polyisobutylene	Silicone foil	Foil
Nitroglycerin	Searle	Nitrodisc	Monolithic	Foil-polyethylene combination	Cross-linked silicone rubber matrix			Paper foil combination	
Nitroglycerin	Health Chem Bolar		Monolithic	Poly(vinyl chloride)	Plasticized Poly-(vinyl chloride)		Acrylic		Paper packet
Isosorbide dinitrate	Nitro Eelctric Co.	Frandol Tape	Monolithic	Clear polyester (?) polyester	Adhesive and drug mixture			Siliconized paper	Foil
Scopolamine	Alza/Ciba-Geigy	Transderm-Scope	Reservoir	Flesh-colored aluminized polyester	Mineral oil and polyisobutylene	Mineral oil-impregnated microporous polypropylene	Polyisobutylene	Flurorcarbon-polyester film	Foil
Clonidine	Alza/Boehringer Ingeiheim	Catapress-TTS	Reservoir	Flesh-colored polyester	Mineral oil-polyisobutylene-colloidal silica		Polyisobutylene	Polyester film (?)	Foil
Estradiol	Alza/Ciba-Geigy	Estraderm	Reservoir	Clear polyester-polyethylene composite	Ethanol, estradiol	Poly(ethylene-vinyl acetate)	Polyisobutylene		Foil

(?) Precise information not available.

E. Protective peel strip. The peel strip used to protect the TDDs from the enviroment until use. It prevents the loss of drug that has migrated into the adhesive layer during storage, and protects the finish device against contamination.

In general, the devices of TDDs can be devided into three general types (Baker and Heller, 1987), e.g. adhesive device, monolithic device and reservoir device. The available TDDs in market today is shown in Table 1. In the present, several technological approaches have been successfully developed to provide rate-control over the release and the transdermal permeation of drugs. Thus, TDDs can be divided by technological approaches into four systems as follow (Chien, 1987A; Chien, 1987B; Chien, 1985).

A. Membrane-Moderated Systems In this system, the drug reservoir is prepared by dispersing or suspending solid drug in a solid polymeric matrix or in a unleachable, viscous medium, then to totally encapsulated in a shallow consisting of a rate-controlling polymeric membrane surface. The drug molecule are permitted to release only through the rate controlling polymeric membrane which can be either a microporous or non-porous membrane. The release rate of drug can be tailored by varying the polymer composition, permeability coefficient and/or thickness of the polymeric membrane and adhesive.

The release of drug from this system is theoretically constant and is controlled by diffusion process, The intrisic rate for a microporous-membrane can be defined as follows :

$$Q/t = \frac{(K_{m/r}K_{a/m}D_mD_a)}{(K_{m/r}D_md_d + K_{a/m}D_ad_m)} . C_R \quad \text{(Eq. 1)}$$

where Q/t is the rate of drug release at steady state; C_R is the drug concentration in the reservoir; $K_{m/r}$ and $K_{a/m}$ are, respectively, the partition coefficients for the interfacial partitioning of drug molecules from the reservoir to the membrane and from the membrane to the aqueous diffusion layer; D_m and D_a are, respectively, the diffusion coefficients in the rate controlling membrane with a thickness of d_m and in the aqueous diffusion layer with a thickness of d_d. TDDs which have been materialized from this technology are best exemplified by the development and marketing of nitroglycerin releasing transdermal therapeutic system (TTS) (Transderm-Nitro System/Ciba) and of scopolamine-releasing TTS (Transderm-Scop System/Ciba)(Good, 1983; Chien, 1987).

B. Adhesive Diffusion-Controlled Systems In this system the drug reservoir is formulated by directly dispersing the drug in an adhesive polymer and then spreading the medicated adhesive onto a flat sheet of backing to form a thin drug reservoir layer. On the top

of the drug reservoir layer, layers of non-medicated, rate-controlling adhesive polymer of constant thickness are applied to produce the TDDs. The rate of drug release is defined by

$$dQ/dt = \frac{K_{a/r} \cdot D_a \cdot C_R}{d_a} \qquad \text{(Eq. 2)}$$

where $K_{a/r}$ is the partition coefficient for the interfacial partitioning of drug from the reservoir layer to the adhesive layer, others is deseribed before.

C. <u>Matric Dispersion-Type Systems</u>. In this type, the drug is formed homogeneous by dispersing the drug solids in a hydrophilic or lipophilic polymer matrix and then is molded into medicated disc with a defined surface area and controlled thickness. The disc is then glued onto an occlusive baseplate in a compartment fabricated form backing and the adhesive polymer is spreaded along the circumference to form a strip of adhesive rim around the medicated disc. The release of drug from this type at steady state, is not constant controlled by diffusion from a matrix and defined as follows (Chien, 1982).

$$Q/t^{1/2} = [(2A - C_p)\ C_pD_p]^{1/2} \qquad \text{(Eq. 3)}$$

for $A >> C_p$ equation (3) is reduced as follow

$$Q/t^{1/2} = [2A\ C_pD_p]^{1/2} \quad \text{(Eq. 4)}$$

where A is the initial drug loading dose dispersed in the polymer matrix; C_p and D_p are the solubility and diffusivity of the drug in the polymer, respectively. In view of the fact that only the drug species dissolved in the polymer can release, hence, C_p is practically equal to C_R. This type of TDDs is exemplified by Nitro-Dur system/Key nitroglycerin releasing TTS which be approved by FDA for once-a-day medication of angina pectoris (Keith, 1983).

D. <u>Microreservoir system</u> In this type, solid particle of drug is suspended in an aqueous solution of water-miscible polymer, forming a homogenous dispersion of millions of discrete, unleachable, microscopic drug reservoir in a polymer matrix. The microdispersion is accomplished using a high energy dispersion technique (Chien, 1977; Chien, 1985). The device can be coated with an additional layer of biocompatible polymer, if necessary, to modify the mechanism and the rate of release. The rate of drug release (dQ/dt) can be define by the following equation :

$$dQ/dt = \frac{D_pD_s\alpha' K_p}{D_p\delta_d + D_s\delta_p\ 'K_p}\left[\beta S_p - \frac{D_1S_1(1-\beta)}{\delta_1}\right](1/k_1+1/k_m)$$

(Eg. 5)

where $\alpha' = d'/\beta'$, d' is the ratio of the drug concentration in the bulk of elution solution over the drug solubility in the same medium and β' is the ratio of the drug concentration at the outer edge of the polymer coating membrane over the drug solubility in the same polymer composition; K_l, K_m and K_p are the partition coefficients for the interfacial partitioning of the drug from the liquid compartment to the polymer matrix, from the polymer matrix to the polymer coating membrane, and from the polymer coating membrane to the elution solution (or skin), respectively; S_l and S_p are the solubilities of the drug in the liquid compartment and in the polymer matrix, respectively; d_l, d_p and d_d are the thickness of the liquid layer surrounding the drug particles, the polymer coating membrane around the polymer matrix, and the hydrodynamic diffusion layer surrounding the polymer coating membrane, respectively; β is the ratio of the drug concentration at the inner edge of the interfacial barrier over the drug solubility in the polymermatrix.

The release of drugs from this type can follow either a partition-control or matrix diffusion-control process depending upon the magnitude of S_l and S_p. Therefore, either a release profile of Q vs t or Q vs $t^{1/2}$ may be resulted (Chien, 1984).

In consideration of these technologies and the requirements to control the release rate of NFP and TDDs formulation, the matrix dispersion-type system is interested. It is just that because the drug reservoir of this type is easily prepared by dispersing drug into polymer matrix or gel matrix to form medicated disc with a defined surface area and control thickness. The factor effected the release of drug from the matrix dispersion-type system are the solubility and the diffusivity of drug in polymer that depend upon the used type polymer. Moreover, in large-scale produced the matrix diffusion controlled TDDs is easily production with least expense comparing to the other systems (Monkhous and Hug, 1988).

Therefore, for this research study, the selected TDDs for the development of NFP dosage form is the matrix dispersion system.

1. Nifedipine

1.1 Formula (United State Pharmacopoeia Convention, 1990; Budavari, 1980; US patent, 1969).

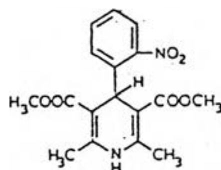


Figure 2 Structure formula of Nifedipine

Chemical name : Dimethyl 1,4-dihydro-2,6-dimethyl-(o-nitrophenyl)-3,5-pyridine dicarboxylate

Empirical formula : $C_{17}H_{18}N_2O_6$

Molecular weight : 346.34

1.2 Physicochemical properties :

NFP is a yellow crystalline, odorless and tasteless powder. Its melting point ranges between 171° and 175°C (United State Pharmacopoeia Convention, 1990; Reynolds, 1989).

Solubility : NFP is practically insoluble in water, soluble in acetone and chloroform, and slightly soluble in ethanol and methanol (Reynolds, 1989; Budavari, 1980; Her Majesty's stationery office,1988; Mcevoy, 1987).

Stability : NFP is very sensitive to light, especially in the form of solution. Nifedipine undergoes photochemical oxidation when exposed to light. Depending on the source of irridation, nitro phenylpyridine product is formed by exposing to ultraviolet light and the formation of nitrosophenylpyridine is dued to day light irradiation (Majeed, Murray, Newton, Othman, and Al-turk, 1987; Pietta, Rava and Biondi, 1981; United State Pharmacopoeia Convention, 1990).

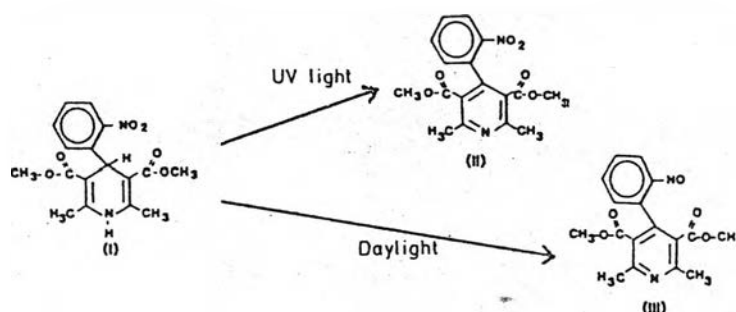


Figure 3 Photo-oxidation of Nifedipine under Ultraviolet and Daylight Exposure

Majeed, et al., (1987) studied the reaction kinetics and investigated the effects of concentration, light intensity and pH on the stability of NFP. It was found that the rate of photodecomposition of NFP was concentration-independent and the rate of reaction decreased exponentially as the light intensity decreased. The maximum rate was found at pH 2 and the rate decreased to minimum when pH increased up to 5. Thus, it is necessary that the whole experiment of NFP has to be performed in the dark room or under golden fluorescence

and all glasswares have to avoid directly contact with the light by well wrapped with aluminum foil.

Storage : NFP must be stored in tight, light-resistant container at a temperature of 15 - 25°C (Reynold, 1989).

1.3 Pharmacology :

NFP is a calcium-channel blocking agent with potent electrophysiologic and hemodynamic effects. It is a potent dilator of coronary and other systemic arteries. It reduced peripheral resistance, blood pressure and afterload, along with a negative ionotropic effect produces a marked decrease in myocardial oxygen requirement (Comess and Fenster, 1982; Ebner and Daniel, 1983; Reynolds, 1989). The mechanism of action of NFP is to reduce total calcium conductance of a cell without altering the rate of activation or recovery (Comess and Fenster, 1982; Ebner and Daniel, 1983; Ogilvie, 1983; Temkin, 1982). The lowering of the peripheral vascular resistance as the result of vasodilation and afterload reduction, increase perfusion, particularly in the extramural vascular regions which is especially subceptible to arteriosclerosis (Comber, et al., 1982; Temkin, 1982; Ebner and Daniel, 1983; Kleinboesem, et al., 1984; Ogilvie, 1983).

The LD_{50} of NFP in mice, rat, by orally, was 494, 1022 mg/Kg of animal weight, respectively and 4.2, 15.5 mg/Kg by intravenous administered, respectively (US patent, 1969; Budvari, 1989).

1.4 Pharmacokinetics :

NFP, a poorly water-soluble drug, has low bioavailability (Sugimoto, Kuchiki, Hakagawa, Tohgo, Kondo, Iwane and Takahashi, 1980). Its bioavailability after oral administration is only about 50% and biological half life is rather short (Kleinboesem, et al., 1984; Sugimoto, et al., 1980; Kuri, Miyaraki, Arita, Shimono, Nomura, and Yasuda, 1987; Reynolds, 1989). However, more than 90% of the oral administered, NFP liquid filled capsule, was rapidly and almost completly absorbed from gastrointestinal tract but only about 65 - 75% of an oral dose reached systemic circulation as unchanged drug. Fooster, et al., (1983) studied the absorption of NFP in the 12 normal subjects and reported that following a single oral dose of 10 mg of NFP, a mean peak plasma concentration of 0.07 ug/ml was attained with in 0.6 hours. The similar result was obtained by to Banzet (1983) in which a mean peak plasma concentration of NFP, following a single oral dose of 20, 40 and 60 mg to 8 hypertensive subjects were 0.06, 0.11 and 0.17 mg/ml at 1.6, 2.1 and 1.8 hours, repectively.

The low bioavailability of NFP might due to its metabolism, following administration by mouth. NFP is rapidly and completely metabolized on first pass through the liver to 3 pharmacologically inactive metabolites and the rate of metabolsm is apparently dependent upon the dosage form in which it is administered (Reynolds, 1989). Approximately 70 - 80% of an oral dose is excreted via urine as inactive metabolites, and only 0.1% unchanged NFP (Kondo, Kuchiki, Yamamoto, Akimoto, Takahashi, Awata, Sugimoto, 1980).

Kleinboesem, et al., (1984) indicated the minimal effective concentration of NFP in changing on diastolic blood pressure was about 15 ng/ml and the lowest therapeutically effective, which was reported by Aoki, Sato, Kawaguchi and Yamamoto (1982), might be in the range from 20 to 30 ng/ml.

1.5 Therapeutic Use

NFP is used in the treatment and prophylaxis of angina pectoris particularly when a vasospastics element is present, and in the treatment of hypertension and Raynaud´s syndrome (Comess, et al., 1982; Temkin, 1982; Ogilvie, 1983; Reynolds, 1989).

1.6 Adverse effects :

Most of the common adverse reactions of NFP result from the vasodilation of vascular smooth muscle and the occurance of dizziness, lightheadedness, giddiness, flushing or heat sensation and headache are up to 25% of patients. Serious adverse reactions such as hypotension, weakness, peripheral edema, palpitation, which required the discontinuance of NFP therapy or the dosage adjustment are relatively rare. The incidence and severity are generally dose related and occasionally may be obviated by a reduction in dosage (Comess, Fenster, 1982; Temkin, 1982; United Pharmacopeia Convention, 1990; Reynolds, 1989).

1.7 Administration :

Presently, NFP is administered orally, sublingually or intrabuccally, and injection. For sublingual or intrabuccal administration, the liquid-filled capsule must be punctured, chewed and/or squeezed to express the liquid into the mouth (United Stated Pharmacopeia Convention, 1990).

1.8 Dosage :

The usual initial adult dose of NFP is 10 mg three or four times daily. The dose is titrated upwards until symptoms are controlled or adverse effects occur. The dosage should be gradually reduced to the lowest level that will maintain relief of symptoms (Comess and Fenster, 1982; United Pharmacopeia Convention, 1990). The usual adult maintainance dosage is 10 - 20 mg three time daily, or 20 - 30 mg three or fours time daily in such patients. The usual maximum dose is 120 mg daily. The dose should generally not exceed 180 mg daily, since the safety and efficacy of higher dosages have not been established.

1.9 Preparations in Thailand

Presently, the NFP preparations which are available in Thailand as shown in Table 2, are in the dosage forms of soft gelatin liquid-filled capsule, and retard tablet. A 5 and 10 mg capsule under the trade names of Adalat, Avenol, Calcegard, Nelapine and a 20 mg tablet under the trade name as Adalat and Calcegard are commercially available.

TABLE 2 Current Commercial Nifedipine Dosage Forms Available in Thailand (From TIMS vol.20 No.3 1991)

Trade Name	Dosage Forms	Manufacture/Distributor
Adalat	Capsule 5 mg, 10 mg	Bayer/Bayer(Thailand)
Adalat	Retard Tablet 20 mg	Bayer/Bayer(Thailand)
Apo-Nifed	Capsule 10 mg	Aptex/Bara Winsor
Calcigard	Capsule 5 mg, 10 mg	Torrent/Union medical
Calcigard	Retard Tablet 20 mg	Torrent/Union medical
Coracten	Spanule 20 mg	SK & Beecham/Diethelm
Fenamon	Capsule 10 mg	Medochemie/Medochemie
Nelapine	Capsule 5 mg, 10 mg	Berlin/Berlin
Nifecard	Tablet 10 mg	Lek Pharm & Chem Work/Rx
Nifecard	Retard Tablet 20 mg	Lek Pharm & Chem Work/Rx
Nifelat	Tablet 10 mg	Remedica/Pharmaland
Nifecard	Capsule 10 mg	Ranbaxy/Ranbaxy (Thailand)

1.10 Development of nifedipine preparations

There many investigation which purposed to develop prolonged dosage forms of NFP in order to improve bioavailability, to reduce the frequency of drug administration and to reduce the incidence and intensity of side effect which depended upon the maximum peak concentration of NFP.

Sugimoto and et al., developed the preparation of nifedipine by using coprecipitation and solid surface dispersion techniques. They prepared fine granules by coating an inert core, crystalline lactose, with dispersion system of NFP and water-soluble polymer, polyvinylpyrolidone (PVP), hydroxypropyl methylcellulose (HPMC). Their results indicated that HPMC might be useful to improve the bioavailability and stability of NFP, a poorly water-solüble drug (Sugimoto, Kuchiki, Nakaganu, Tohgo, Kondo, Iwane and Takahashi, 1980; Sugimoto, Sasaki, Kuchiki, Ishihara, and Nakagawa, 1982).

The studies in sustained release dosage forms of NFP appeared as granule, suppositories were developed by using solid dispersion and conventional techniques, and a nasal absorption dosage form using gelling agents as drug carrier.

Kohri, et al., (1986, 1987) prepared two kinds of NFP sustained granules. One was pH dependent released granules composed of NFP, hydroxypropyl methyl cellulose phthalate (HPMCP), ethylcellulose (EC) and microcrystalline cellulose. The other was pH-independent released granules composed of NFP, HPMC, EC and corn starch. *In-vitro* studies indicated that the release rate of NFP was decreased with increasing EC. The release pattern of NFP from these sustained granules was first order kinetics, a diffusion-controlled process. *In-vivo* studies in rabbit revealed that the release of NFP from these granules could sustain upto 8 hours. The plasma profile of NFP from the pH-independent released granules was superior to that of the pH dependent released granules, with respect to prolong action of the effective drug concentration in plasma. From clinical study in healthy subjects, the plasma drug concentration which occurred by oral pH-independent release granules was detected over 2 - 12 hours period. Twice-daily dosage of 20 mg pH-independent release granules was sufficient for therapeutic effectiveness.

To prolong absorption of nifedipine, an enteric dosage form was performed by Hasegawa, et al., (1985). The NFP sustained released granule was prepared by spraying the enteric coating agent on an inert core material. HPMCP and Eudragit L, enteric coating agents, were used as carriers of the solid dispersions. These

granules resulted in prolonged absorption of NFP with good bioavailability. Plasma drug concentration was obtained in a period of 8 hours.

The double layer suppositories were performed by using solid dispersion technique by Umeda, et al., (1985). Suppositories base was composed of polyethylene glycol 4000 (PEG 4000), as a water soluble carrier, and cellulose acetate phthalate (CAP), as a poorly water soluble carrier. *In-vivo* studies in rabbit indicated that CAP-PEG matrix suppositories could enhance the bioavailability of NFP and gave a sustain-release characteristic without causing an excessively high peak plasma level in plasma. Ohnishi, et al., (1987) reported the pharmacokinetics of rectal administration of this suppository to healthy volunteers. They indicated that the double layer suppository was able to maintain a therapeutically effective level of NFP from 30 minutes to 10 hours and the pharmacokinetics followed a one-compartment model with first-order release and absorption steps.

Nasal absorption of NFP from gel preparation was developed by Morimota, Tabata and Morisaka, (1987) with using PEG 400 and carbopol 941 as drug carrier. *In vivo* studies with rat model appeared that carbopol-PEG gel, containing 50% w/v PEG 400, performed a relatively high plasma NFP concentration, 0.4 - 2.2 ug/ml, and a prolonged action of 6 hours.

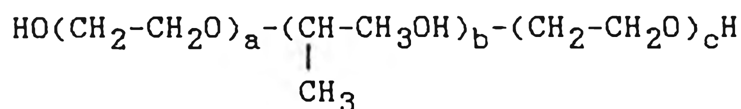
However, all aforementioned preparations could not avoid some disadvantages, granules forms could not avoid the hepatic first pass elemination, suppositories dosage forms was not suitable for all patients especially with serious diarrhea, and the nasal absorption dosage form might be inconvenient, it might disturb respiration. Transdermal drug delivery system (TDDs), the dosage form which provided advantage including the induction of drug into the systemic circulation without an initial first pass through liver. In addition, to used TDDs, could reduce the frequency of drug administration that made patient compliance. Thus, TDDs was become the interested area for this research study to improve bioavailability of NFP.

2 Poloxamer

2.1 Chemical name : polyethylene polypropylene glycol, polymer

2.2 Trade name : Pluronic, Lutral

2.3 Empirical formula :



$$HO(CH_2\text{-}CH_2O)_a\text{-}(\underset{\displaystyle CH_3}{\underset{|}{C}}H\text{-}CH_3OH)_b\text{-}(CH_2\text{-}CH_2O)_cH$$

Figure 4 Structure formula of poloxamer : Pluronic (F-127 a,c = 35, b = 30)

2.4 Characteristics :

Poloxamer is a series of nonionic polyoxyethylene-polyoxypropylene block copolymers. The structural formula is shown in Figure 4, when b is at least 15, a and c are statistically equivalent. $(CH_2\text{-}CHO)_{a+c}$ is varied from 20 to 90% by weight. Its molecular weight ranges from 1,000 to greater than 16,000 (BASF, 1987; Budavari, 1989). The other name of poloxamer is Pluronic, Lutral.

Poloxamers are manufactured by condensation of propylene glycol with propylene oxide, followed by condensation of ethylene oxide onto both ends of the poly (oxypropylene) base (American Pharmaceutical Association, 1986). Polyoxypropylene segment is hydrophobic while the polyoxyethylene segment is hydrophilic. The alteration of both hydrophobe and hydrophile may change in surfactant functions and physical properties (BASF, 1987).

2.5 Physicochemical properties :

Available grades of poloxamers are varied from liquid through pastes to solid waxy flakes which are practically tasteless and odorless. The properties of poloxamers range from hydrophobic liquid which is almost insoluble in water to solid which is very soluble in water and give high HLB value (BASF, 1987). Poloxamers are more

soluble in cold water than hot water. In general, they are soluble in aromatic solvent such as benzene, toluene and xylene, chlorinated solvents, acetone, alcohol, propylene or hexylene glycol, cyclohexanone. They are insoluble in ethylene glycol, kerasene, mineral oil. Most of them are physically stable, stable to acids, alkalies and metallic ions. They have low acute oral toxicity and low potential to cause irritation or sensitization.

2.6 Use :

Poloxamers can be used as food additives, defoaming agents, antistatic agents, wetting agents, detergents, dispersing agents, emulsifying agents, solubilizing agents, thickening agents, gelling agents and dissolution controlling agents (BASF, 1987; Budavari, 1989).

A distinguishing property of poloxamer is that it can be liquified by merely lowering the temperature without coconmitant loss of integrity and then reversed to its original consistency. Therefore, it is called "reversible gel". The more advantage of a reversible gel than a nonreversible gel is that air bubble which may have been accidentally incorporated during the processing could be eliminated.

Pluronic F is a series of poloxamer that is manufactured by BASF Wyandotte, USA. Their properties, generally, are similar to the properties of poloxamer, shown in Table 3. The molecular weight of Pluronic F ranges form 4,700 to 12,600. They compose hydrophilic segment over 70% by weight which make them as solid. All of them are very soluble in cold water and form gel at high temperature with a sufficient concentration.

Pluronic F series consist of F-38, F-68, F-77, F-87, F-88, F-98, F-108 and F-127. Pluronic F-127 is the most efficient gellant in the Pluronic F series due to a high level of hydrophilic segment (BASF, 1987).

Because of the reverse thermal gelation properties of Pluronic, Schmolka have created "cold method" used for preparing gel-matrix (Schmolka, 1977). An appropiate amount of Pluronic is gradually added to cold distilled-deionized water, 5-10°C, under constant agitation to complete hydration. The obtained dispersion is stored overnight in a refrigerator to ensure complete dissolution and release air bubble which accidentally incorporated during processing. Eventually, a clear and viscous solution is formed. By leaving the poloxamer solution at high temperature, the gel is formed. Pluronic F gel consists of large populations of micelles, forming an apparent viscous isotropic liquid crystal (Chen-Chow and Frank, 1981; Rossing and Attwood, 1983).

Table 3 Physicochemical Characteristics of Pluronic F (from BASF)

Pluronic	F-38	F-68	F-77	F-87	F-88	F-98	F-108	F-127
Average molecular weight	4700	8400	6600	7700	11400	13000	14600	12600
POE : POP ratio*	80:20	80:20	70:30	70:30	80:20	80:20	80:20	70:30
Hydrophobe (POP) weight	950	1750	1950	2250	2250	2750	3250	3850
Melting point (^{o}C)	48	52	48	49	54	58	57	56
Physical form at $20^{o}C$	solid	solid	solid	solid	solid	solid	solid	solid
Viscosity**	260	1000	480	700	2300	2700	2800	3100
Surface tenison at $25^{o}C$***	52	50	47	44	48	43	41	41
HLB	> 24	> 24	> 24	> 24	> 24	> 24	> 24	18-23

*POE : POP = Polyoxyethylene : Polyoxypropylene

**by Brookfield, cps., liquid at 25oC, paste at 60oC, solid at 77oC

***dynes/cm

The gel formation of Pluronic F is due to hydrogen bonding. In aqueous solution Pluronic F is surrounding by a hydration layer or sheath of water molecules. Extensive polymer-solvent interactions cause the Pluronic F to become extension and the entanglement of the long thread like molecule leads to an increasing viscosity. At sufficiently high polymer concentration, poloxamer-water mixtures will be gelled. Pluronic F gel, reverse thermal gel, will increase in macroscopic viscosity in order of increasing in temperature, since the desolvation of Pluronic F and enhancement of polymer entanglement. Intermolecular hydrogen bonding may also promote gelling, so, if the number of interactions with neighboring chain increases, by increasing polymer concentration, the viscosity of gel will be increased (Fox, 1984; Miller and Drabik, 1984).

Pluronic F gel can be modified its consistency to achieve desired strength. Schmolka (1977) reported that the thickening power of poloxamers in water increased as the hydrophobe molecular weight increased and as the ratio of ethylene oxide and propylene oxide increased. The effect of the concentration of poloxamer, organic additive and electrolyte on the rheological behavior of poloxamer vehicle were studied by several researchers such as Chen-Chow and Frank, 1981; Hadgraft and Howard, 1982; Miyazaki, Takeuchi, Yokouchi and Takada, 1984; Miyazaki, Yokouchi, Takamura, Hashiguchi, How and Takada, 1986;

Miller and Drabik, 1984. The results from these studies will aid in identifying vehicles which would be suitable for the development of dosage forms for controlled drug delivery system.

Miller and Drabik (1984) studied on viscosity of poloxamer vehicle as a function of poloxamer concentration. They reported that the system underwent a sol-gel transition and rheological of poloxamer vehicle became non-Newtonian when their concentration or temperature was increased. At a given poloxamer concentration, an increase in degree of poloxamer hydrophilicity caused an increase in apparent viscosity. The dependence of apparent viscosity on concentration of poloxamer was greater for higher molecular weight polymers than low molecular weight polymers.

The effect of organic modifiers such as glycerin and propylene glycol on the viscosity of Pluronic F vehicle at various temperatures was reported. At low temperature and low concentration of organic modifiers, there was a little difference in viscosity, but as the concentration of organic additive increased, the viscosity of the systems had to be increased. At high temperature, the presence of organic modifiers increased poloxamer vehicle consistency which glycerin caused a greater increase in viscosity than propylene glycol. The reason

was enhancing polymer interaction between the poloxamer and hydroxyl group of organic additives.

The presence of strong electrolyte such as sodium chloride also affected the viscosity of poloxamer vehicle as the organic modifiers. The presence of sodium chloride in the formulations, salting out, should increase the viscosity of the poloxamer system, since the gelation temperature or gel point and gel melting point should be lowered. This effectiveness depended on electrolyte concentration.

For the novel dosage forms, it is desirable to release the active ingredients at a controlled rate over a period of time. Poloxamers that form gels can be effective by reducing the mixture's overall rate of dissolution. Pluronic block copolymers with high ethylene oxide content and high molecular weight are most effecitve at reducing the dissolution rate of active ingredient. Thus, Pluronic F has potential application as drug carrier for drug delivery system such as TDDs.

In the recent year, a number of dosage forms containing Pluronic F have been studied and evaluated as a novel dosage form.

Chen-Chow and Frank (1981) studied the *in-vitro* release of lidocaine from Pluronic F-127 gel using a membraneless apparatus. It was reported that the release rate of the drug was inversely proportional to the drug concentration, the concentration of Pluronic F-127 and electrolyte concentration. The drug was released by diffusion through the extramicellar aqueous channels of the gel matrix. Release of the drug was maximal at pH value of the matrix close to its pKa.

The potential use of Pluronic F-127 gel as sustain release depot was evaluated by Hadgraft and Howard (1982). They studied the *in-vitro* release characteristics of barbiturate compound from various Pluronic F-127 gel formulations. Their results were similar to Chen-Chow and Frank's. There was a linear relationship between released amounts of the drugs and square root of time. The apparent diffusion coefficient of the drugs increase in order of decreasing molecular weight in barbiturate compounds. The increasing concentration of Pluronic F-127 in the vehicle made a corresponding decrease in apparent diffusion coefficient of the drugs. They suggested that the mechanism for a reduce release rate might be dued to the reduction size and number of water channels within the gel matrix.

Miyazaki and et.al., (1984) studied on the usefulness of Pluronic F-127 gel as topical drug delivery system of anticancer drug. The investigation was undertaken to determine by means of *in-vitro* experiments the amounts of the drugs, 5-fluorouracil and adriamycin, released from Pluronic F-127 gel matrices. It was found that the Pluronic F-127 gel appeared to have potential application as topical drug delivery system. The apparent released rate of anticancer agents decreased with increasing concentration of Pluronic F-127 in the vehicle. With increasing concentration from 30 to 44%, a corresponding increased in the apparent released rate, although the viscosity of gel matrix increased. They suggested that the reasons for increased release rate might be the decreasing in viscosity of water channels with increasing temperature and since the rate of drug release was determined by the micro-viscosity of the extramicellar fluid of the gel rather than the macro-viscosity.

Pluronic F-127 was evaluated as a vehicle for rectal administration of indomethacin by Miyazaki and et.al., (1986). They studied the effect of the concentration of Pluronic F-127, temperature and drug concentration on the drug released from *in-vitro* released method using a cellulose membrane and evaluated the drug plasma levels from indomethacin-Pluronic F-127 gel compared with commercial suppositories. It was reported

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that the *in-vitro* apparent released rate increased with increasing temperature from 20 to 44°C and increasing in drug concentration. Increasing the concentration of Pluronic F-127 in the vehicle decreased the drug released rate. For comparing different Pluronic F, *in- vitro* studies, the release of indomethacin was the fastest from Pluronic F 68 and the slowest from Pluronic F-127. From *in-vivo* studies, no significant difference in content of bioavailability between the Pluronic F-127 gel and commercial suppositories was found. However, the Pluronic F-127 was supperior to the conventional suppositories, in term of decreasing in the peak plasma concentration and maintainance of indomethacin concentration in plasma.

Miyazaki and et.al., suggested that Pluronic F gel might serve as a rate-controlling barrier and be useful as a vehicle for sustained-released rectal preparation with reduced side effect of the drugs.

The parenteral controlled drug delivery using Pluronic F-127 as vehicle was studied by Johnton and Miller (1989). They investigated the extent to which injectable poloxamer vehicles prolonged the release of a water-soluble compound, inulin, which had short biological half-lives, following intramuscular (I.M.) administration to rats. One physical approach to control the rate of delivery of a water-soluble drug following I.M. administration involved the use of a high-viscosity to

increase formulation viscosity (Thompson, 1960). Poloxamer 407 was formulated with inulin, water soluble agent, at the concentration allowed for the administration of a syrringeable solution followed by gel formation in situ. Plasma clearance of inulin following I.M. administration of the drug-poloxamer was evaluated to interpret the function of poloxamer 407. It was found that the plasma clearance of inulin by glomerular filtration was reduced. So poloxamer 407 could be useful for development prolonged action injection of water-soluble drug.

Falts and Jonhston (1990) studied the potential of poloxamer 407 as a sustained release vehicle for polypeptide drugs. The release profile of the model enzyme, urease, which was formulated in a 20, 30 and 35% w/w poloxamer 407 gel matrix was studied by using a membraneless diffusion system. The release of protein from the gel matrices was relatively constant, zero-order, over 8 hours. The mechanism governing release of protein from the gel matrix was matrix erosion, not diffusion. They suggested that poloxamer 407 might be useful as a vehicle to increase the stability of a protein formulation and sustain the rate of input of a protein into the system circulation following extravascular administration.

The disruption of membrane permeability by function of surface active compounds, cause skin irritation, is probably important for the development transdermal drug delivery system, intramuscular controlled release by using them as vehicles. Jonhston and Miller (1985) studied the toxicity of poloxamer vehicle used as intramuscular vehicle. They investigated for muscle toxicity caused by injection of various poloxamer vehicles, Pluronic F-88, Pluronic F-105, Pluronic P-123 and Pluronic F-127, into the *M.vastus lateralis* of rabbits. The local tissue reaction was grossly scored on a scale of 0 to 5 by the method of Shintani and et.al. (1967). It was reported that the toxicity of poloxamer vehicles was proportional to their lipophilicity, the more lipophilic the poloxamer, the more severe the lession produced following injection. Base on this study, Jonhston and Miller suggested that poloxamer 407 (Pluronic F-127) and poloxamer 238 (Pluronic F-68) might be suitable polymers for incorporation into vehicle for use as injectable gel for prolonged drug delivery.

3. Colloidal Silicon Dioxide

3.1 Chemical name : Silica

3.2 Empirical structure : SiO_2

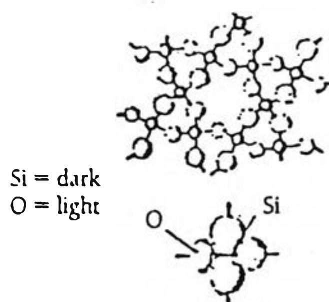

Figure 5 Structure formular of Aerosil

3.4 Molecular weight : 60.08

3.5 Symonyms : Aerosil, Cab-O-sil, Colloidal silica, flumed silica

Colloidal silicon dioxide is an excipient which is used widely in pharmaceutical industry, produced by the vapor phase hydrolysis of silicon compound, such as silicon tetrachloride (American Pharmaceutical Association, 1986). It is submicroscropic, light, loose, bluish-white, odorless, tasteless, non-gritly and amorphous powder in which the diameter of average ranges from 2-10 um (Budavari, 1989).

3.6 Solubility : Colloidal silicon dioxide is practically insoluble in purified water, alcohol, acid except hydrofluoric acid and other organic solvent. It can form colloidal suspension with a wide range of pH depending on its concentration. A 5% aqueous suspension has a pH of 4-8 (Budawari, 1989).

3.7 Stability : Colloidal silicon dioxide is hygroscopic, but absorbs large quantity of water without liquefy. It must be stored in a well-closed container.

Application in Pharmaceutical Formulations : Colloidal silicon dioxide is widely use in pharmaceutical industry as hygroscopic materials, absorbent, dispersing agent of liquid in powder or suppositories. It is used as glidant and anti-adherent in tablet and capsule with range in 0.1 - 0.5%. In aerosol, colloidal silicon dioxide promotes particular suspension, eliminates hard setting and minimizes clogging of the spray nozzle. It can be used as viscosity modifier, thixotropic thickening and suspending agent in suspension semisolid and gel preparation in the range of 2-10% (Sherriff and Enever, 1979).

3.8 Commercially Available : There are several grades of commercial silicon dioxide, Cab-O-Sil and Aerosil which produced by vary manufacturing process. However, the modification do not affect the content of

silicon but particle size, surface area and bulk density are affected. Aerosil has a wide range of grades classified by particle size distribution. Aerosil A-200 (Ae-200) one of colloidal silicon dioxide produced by Degussa, Inc. USA, has average particle size of 12 nm, 200 ± 25 m^2/g of BET surface area, 60 g/l of bulk density and < 1.5% of moisture content. It provides 3.6 - 4.3 of pH for 4% aquous suspension.

For using as NFP carrier gel matrix, Ae-200 was designed to use as gelling agent to form hydrophobic gel matrix by increasing viscosity properties at a wide range of concentration (Budavari, 1989; Schmolka, 1984). In general, colloid silicon dioxide gel is gelled with mineral oil, a non-hydrogen bonding liquid, as anhydrous system to provide a hydrophobic transparent gel. The gel formation of colloidal silicon dioxide is due to the ability of very small silica particles to form a network structure throughout the medium by interparticle hydrogen bonding via the silanol groups on the silica surface (Marshall and Rochester, 1975). In addition to these particle interactions, there is possible bonding between the silanol groups and other components that one also capable of hydrogen bonds formation. The degree of increasing viscosity depends on the polarity of liquid in which a polar liquid generally requirs greater concentration than non polar liquid (American Pharmaceutical Association, 1986). Modification of

different consistencies is possible by selecting the disperse media that differ from each other in hydrogen bonding ability.

Jurgen and Becker (1974) evaluated gel forming characteristics of micronized fumed silica gel which used hexadecyl alcohol as liquid vehicle compared to commonly used ointment base. It was found that oleagenous base of colloidal silicon dioxide was very stable in wide range of temperature, 0 - 50°C. The gel had high capacity in water absorption and showed the best release of drug over the common used ointment base.

Ali, Geneidi and Salama (1977) investigated the stability and drug relased from oil-based Aerosil Gel. They used liquid paraffin as a vehicle fluid. It was found that oil-base Aerosil gels provided steady viscosity over a wide range of temperature, 25-60°C and the release rate of benzocaine and salicylic acid was found to be greater from these base. They indicated that the release rate was tend to increase with increasing drug concentration.

The investigation of rheological and drug release properties of fumed silica was done by Sherriff and Enever (1979). The colloidal silica gels were produce with different vehicles, n-dodecane and 1-dodecanol and incorporated with methyl salicylate. They indicated that

the gel which produced from high ability of by drogen bonding, excess of hydroxyl groups, exhibited weaker network structure than gel from less ability of hydrogen bonding and the drug which could form hydrogen bonding might affect the rheology of gel. In addition, they found that the interaction of drug with the silanol groups in the gel which produced from non hydrogen bonding media didnot affect the drug release from the gel.

With the increasing in viscosity properties and the particularly good drug release characteristics from its gel, Aerosil gel was selected to be used as drug carrier for this research study.

4. ***In-vitro* skin permeation study of transdermal drug delivery systems.**

A. *In-vitro* study

General, the evaluation of all topical dosage forms may investigate on permeation of active compound through the skin. During the development TDDs, *in-vitro* experiments is more useful method for evaluation dosage form. It is low cost and high ability to test large number of formulation in a relative short time (Gummer, 1989). In addition, *in-vitro* skin permeation studies are possible to screen candidate formulation as well as test the effect of various ingredients on skin permeation,

therefore, it can be used to identify the rate-limiting skin layer for given compound (Zatz, 1990).

The general major assumption which may be made when conducting *in-vitro* experiments are as follow (Zatz, 1990).

a. the stratum corneum is the rate-limiting barrier to permeation,

b. the skin's barrier properties are not compromised by the removal of skin from the living organism, and

c. the possibility of metabolism with the skin is ignored.

B. **Skin model**

According to skin permeation of several compound, stratum corneum is primary barrier to the absorption of chemicals. It is remarkable that the principal resistance to permeation of most, though not all, substances and should be assumed as the rate-limiting barrier to *in-vitro* permeation.

The stratum corneum, paper-thin outer layer of skin, is generated by the underlying cells of the epidermis. It is a compact almalgam of dried, dead, elongated cells and has a low effective dielectric constant. Within the cells are low molecular weight

hydrophilic substances. The resistance to transport through the horny layer depends on the properties and arrangement of its alternating hydrophilic and hydrophobic layers, as well as thickness, all of which vary from species to species and even from place to place on the same individual. Generalize and rank permeability of body site as genitals > head areas > trunk > limbs (Zatz, 1990). Stratum corneum seems to retain its barrier properties after excision from the host (Bronaugh and Maibach, 1985). The horny layer was thought to be the rate limiting for transport of a compound. The permeation will develop a substantial concentration gradient. Stratum corneum should be able to function in a dual capacity, acting both as a barrier and reservoir, storing drug and chemical for a period of time (Zatz, 1990).

In general, excised human skin is the most valuable, prefered membrane for *in-vitro* skin permeation studies. However, human skin is short supply and has a variety conditions, e.g., from tummy reductions, mastectomies, amputations and cadavers that could make a large variation in permeability between individuals, so that precludes sole use of this membrane. In addition, the decision to use human skin rests on availability and not to search for scientific excellence. If one assumes that the *in-vitro* experimental design should reflect exactly the *in-vivo* situation then only human skin can be used (Zatz, 1990).

When human skin is not available, *in-vitro* permeation studies are possible to work with animal skin, in particular hairless mouse, fuzzy rat, guinea pig, rabbit, miniature pig and monkey. Throughout the history of TDDs, investigator have sought to find a predictive correlation between the penetration of molecule through animal and human skin. Although there exists a number of similaries, there is as yet no animal skin that completely mimics the penetration characteristics of human skin (Gummer, 1989). However, in such field as product developments which compare different formulations containing the same permeate with the greatest or least permeation, *in-vitro* experiments using animal skin may be useful.

There are several studies compared between human and animal skin. For the large animals, they provide many skin samples that can mimimize inter-individual variation in skin structure especially small laboratory animals. They are ease of handling, relative low cost and ready available. Skin from laboratory animals tend to exhibit minimal intra-individual differences because of standardized breeding and freeding (Zalz, 1990).

Bronaugh and Maibach (1985) summarized the comparative studies between human skin and animal skin of several investigators, e.g., Ainsworth; Bartek, Labbudde and Maibach; Bronaugh, Stewart and Congdon. ect. All of

the studies investigated on *in-vitro* permeation experiment and based on skin permeability data. By comparative permeability data from a number of studies and permeability constants which were obtained from human and animal skin, they concluded that human skin is preferable for *in-vitro* studies and the animal model of choice for human skin may depend on the test compound. Pig and monkey skin seem to give permeability values similar to human skin and the skin of hairless mouse, mouse and rat have been found to be more permeable than human skin. These conclusions are similar to Wester and Maibach (1985). They suggested that the perfect comparative studies probably could not be done, since the difference in percutaneous absorption existed between species difference of animal. The absorption in common laboratory animal-skin, rat and rabbit, were higher than that in human while the absorption in the pig and monkey, squarrel and rhesus, appeared more predictive of absorption in human.

With the comparative *in-vitro* studies done with skin from different species, flavorably agree with the *in-vivo* results. Thus, the animal model could predict correlation between the penetration of molecule through animal skin and through human skin. It appeared that the animal model most predictive of percutaneous absorption in man was the pig and monkey.

C. **Apparatus**

The ultimate goal of all investigators in the field of TDDs is to produce an *in-vitro* experimental design that will exactly predict the penetration of the candidate molecule into the human body *in-vivo*. Although there are many investigation conducted drug release rate, it appears that no one apparatus or procedure has yet emerged as the most flavor or to be widely accepted as a quasi-standard for other in the field (Abdou, 1989).

Flynn (1983) classified *in-vitro* techniques into three main categories as follow.

a. *In-vitro* experiment : This is a classical permeation study in which excised human or animal skin is used as a membrane. The excised human or animal is used to mount in open diffusion cells to determine the concurrent transport of the molecule from the topical dosage forms. This technique has been the mainstay to work aimed at solving the mechanism of percutaneous absorption. By obtaining permeability data in a given medium for chemicals with systemic variation in structure, it becames possible to infer the essentials of membrane mechanisms from the chemical structure-permeability coefficient patterns.

b. Finite dose technique : In this procedure the topical preparation is applied lightly to the skin in a manner similar to the actual way which is used with the patient. This type of diffusion cell works well in formulation design and for optimization of vehicle properties prior to chemical effectiveness studies.

c. Drug release studies in the presence and absence of synthetic membranes : In this procedure a synthetic membrane is used to separate the donor and receptor compartments, the dialysis technique, or where no membrane is utilized that the topical preparation is kept in direct contact with a solvent acting as a sink. The valuable of this technique is that it can be used to identify the crossover point of thermodynamic and kinetic control of drug release that is very useful information in formulation design. However, it has some disadvantages that it does not reflect closely the clinical setting. In addition, the interface between the drug system and a collection medium bear no similarity to the skin model. However, this technique is simplicity and economic advantage, so it has been gaining wide acceptability. For absence of synthetic membrane model, water or an organic solvent, usually isopropyl myristate is acted as a receptor phase sink and the various types of semipermeable membrane such as dialysis filter papers, tubing or synthetic membranes filter, are used to mimic the function

of the horny layer as a barrier between the donor and receptor phase.

In the USP, apparatus for the determination of release rate from TDDs was classified in three different types (Abdou, 1989 : United States Pharmacopeia convention, 1990).

The first type is a modified USP apparatus I, where the basket is replaced with a hollow stainless steel cylinder. TDDs is attached to the surface of the cylinder and immersed in the dissolution medium.

The second type is the USP apparatus II, paddle method, where an appropriate disk- or cell-TDDs attached at the bottom of dissolution vessel containing the dissolution vessel containing the dissolution medium.

The third type, a reciprocating or rotating disk, where the TDDs is attached to the bottom of the disk, which is placed in a 25 x 150 mm testube containing the dissolution medium.

However, there are several variations occurred from the official system used for evaluation of TDDs. The major variation is nonuniform flow pattern in dissolution medium, e.g., a modified apparatus II, the paddle must be replaced with a Teflon disk having a rubber O-ring

connected at the end of the shaft and slightly above the disk of TDDs to provide the necessary agitation of the solution medium. It is imperative to maintain a uniform flow pattern which precisely defined hydrodynamics conditions in order to realiable and reproducible results.

According to the assumption that the release of drug obtained from the *in-vitro* experiment is similar to drug released from *in-vivo* study, the system of diffusion study is aimed to provide a mimic *in-vivo* situation. Finite dose diffusion system is the only composition which has been designed to close to *in-vivo* studies. Its results are easily and rapidly manipulated and achieved the suitable pattern for each diffusion system purposed. In general *in-vitro* transdermal delivery experiments are conducted on either vertically or horizontally arranged diffusion cells (Figure 6). For the production, it is important to appreciate the need to control the experimental conditions or variables which can affect the apparent kinetics of penetration that may lead to errorneous *in-vivo* predictions (Gummer, 1989). The major variables which should be concerned in design characteristics of diffusion cell are temperature control and the condition of hydrodynamics, stirring rate, in the receptor compartment.

Because of the rate of diffusion will inerease with increasing temperature (Guy and Hadgraft, 1980). In addition, the attention to human body temperature, the skin surface temperature ($32 \pm 1^{o}C$) and the body core temperature ($37^{o}C$). The receptor compartment, as with the donor compartment, is essential to be designed in order to achieve and maintain the temperature mimic the target body temperature and to avoid change in ambient conditions at least 24 hours. Typically skin diffusion experiment are conducted at $35-37^{o}C$ (Gummer, 1980).

As mentioned, the diffusion of the molecule under study through the vehicle may act as a rate-limiting step. The stirring of the vehicle would enhance homogeneity of the formulation and hence presentation of the candidate molecule to the skin surface. In addition, the stagnant or boundary layer is known to have a predictable effect in control partitioning experiment. Thus, good designation in optimum stirring rate can be improved the flow pattern of medium solution, the hydrodynamics of medium solution and mixing efficiency which provide homogeneity of the molecule. It is necessity to design the stirring rate of the experiment to provide adequate stirring in order to provide the true release rate or the permeation rate (Tojo, 1985). Gummer, Hinz and Maibach (1987) have designed a simple test to establish both rapidity and degree of stirring within any diffusion cell. The optimum and adequate stirring rate

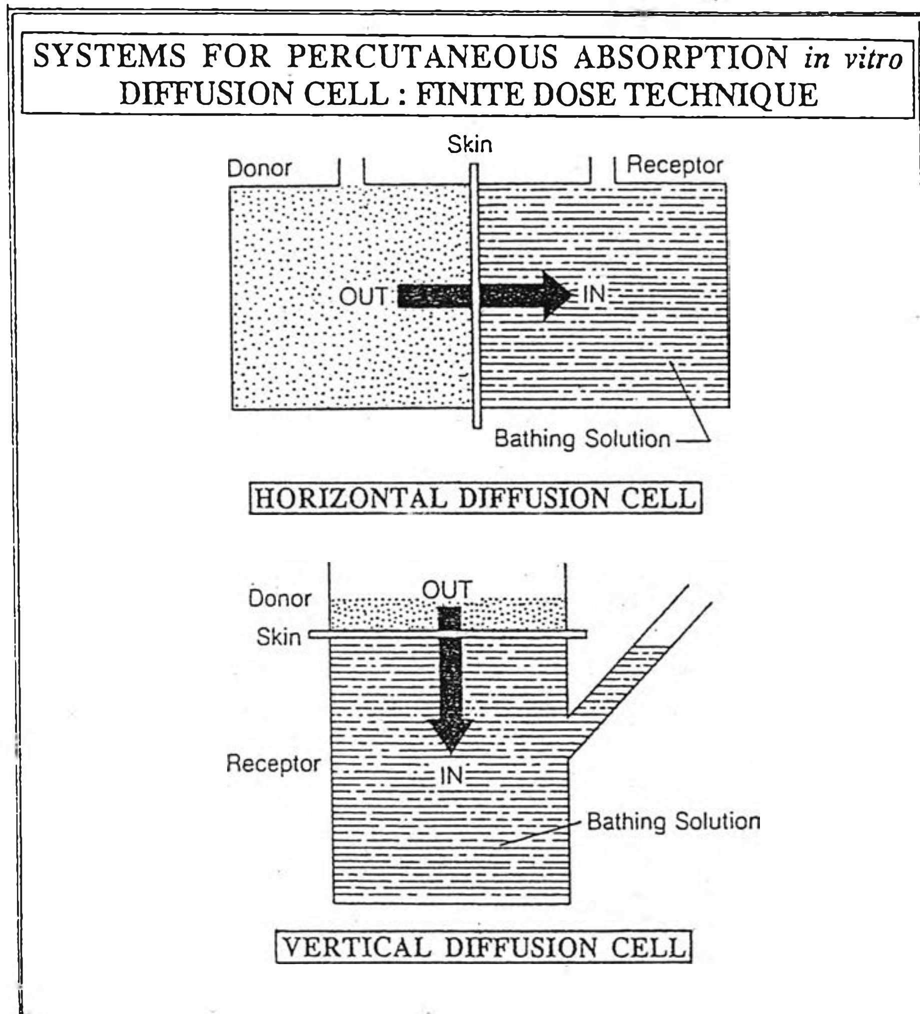

Figure 6 Schematic Illustratio of Horizontal and Vertical Diffusion Cells.

should be achieved, it is degree of stirring which can disperse of mauve coloration from the permaganate crystal added to receptor fluid within 30 seconds.

In-vitro diffusion cell, early model, have been designed to study the routes of skin penetration since 1965 by Scheulein (Chien and Valia, 1984). Later, several *in-vitro* diffusion cells have been designed to achieve both objectives, ease of operation and quantitative improvement, e.g., the diffusion cells which were designed by Menczel and Maibach (1970), by Michaels, Chandrasekaran and Shaw (1975), and by Dunheim, Flynn, Highuchi and Behl (1980). However, many of these cells are subject to flaws in both design and production. The deficiencies of these cells are as follow.

a. The set up of temperature control could not simulate exactly the clinical setting. Since both the donor and receptor solution are maintained at the same temperature by either the whole in a temperature regulated water bath or retaining it in thermally controlled oven.

b. Loss of medium solution that may affect the concentration of penetrant. This event due to the solution in the donor and receptor compartments is constantly exposed to the atmosphere through the openings for stirring and sampling.

These deficiencies need to be corrected for the long-term skin permeation studies of TDDs.

Franz-diffusion cell, an 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 7. Each of the diffusion cells in a cell mounting block and consists of two compartments; a donor compartment, which is exposed to an ambient condition, and a receptor compartment which is maintained at 37°C by circulating a thermostated water through the water jacket. The solution hydrodynamics in the receptor compartment is kept at constant by a tiny rod-shaped magnet rotating at 600 rpm by a synchronous motor mounted underneath the cell mounting block. It was designed to simulate the clinical conditions.

Nevertheless, there are several deficiencies in the Franz diffusion design. It is shown incomplete stirring of the receptor phase that could not achieve the solution hydrodynamics, mixing efficiency. It is sensitive to any variations in the atmospheric temperature

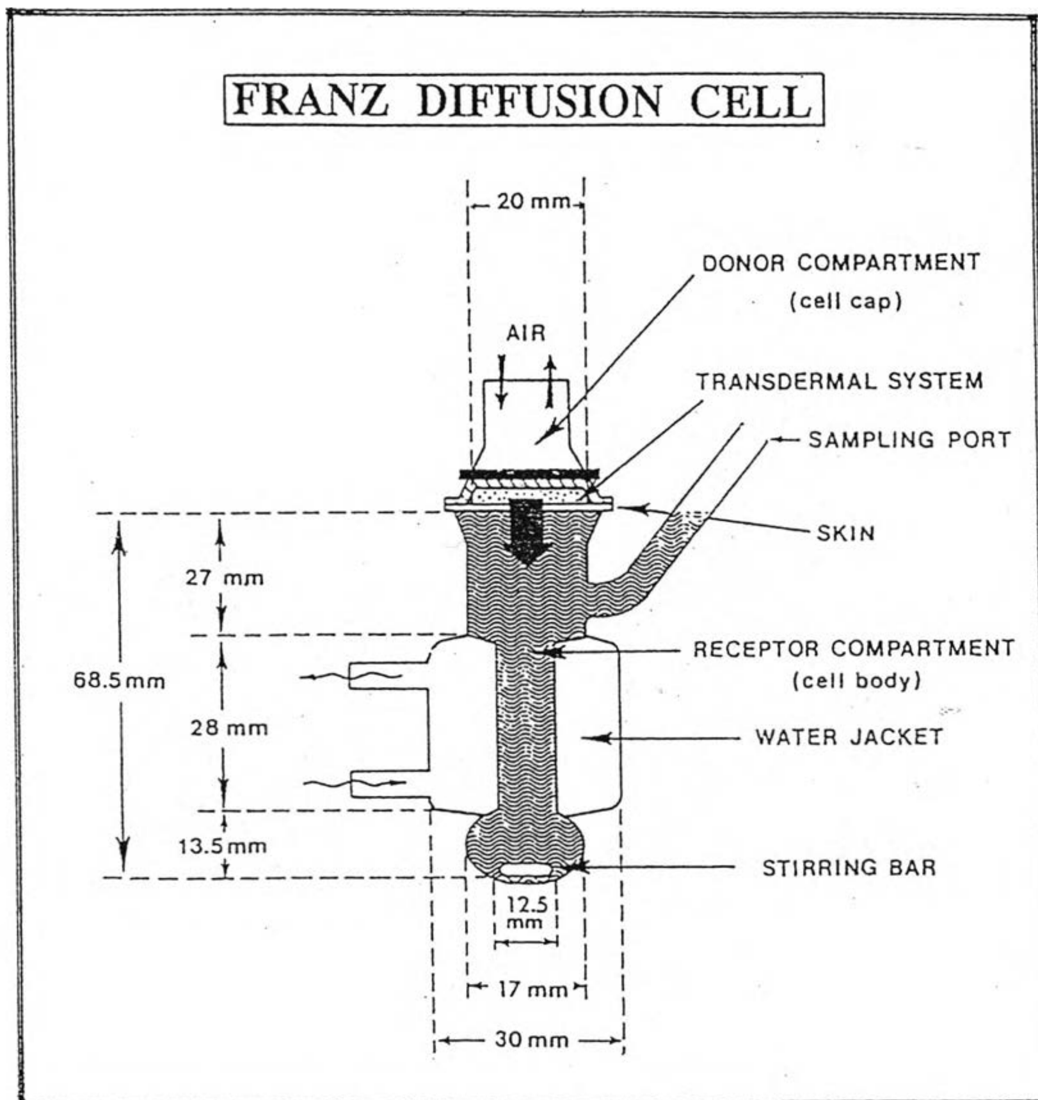

Figure 7 Schematic Illustration of Franz Vertical Diffusion Cells.

that insufficiency for controlling temperature which required in the quantitative evaluation. With the result of comparison studies, several investigators have been designed a new finite-dosing skin permeation system for improving to overcome inherent deficiencies of the Franz diffusion cell (Keshary and Chien, 1984).

Chien and Valia (1984) have designed the horizontal arrange diffusion cell with aiming to minimize the potential deficiencies which observed in the Franz diffusion cell. This cell called Chien-Valia side by side cell (Figure 8). It is composed of a skin permeation cell and a magnetic driving unit, where consists of two half-cells in mirror image. Each of the half-cells contains a solution chamber within a stirring platform to rotate at a synchronous speed. A sample port on solution chamber could be tighly close with glass stopper. Chien and Valia suggested that their diffusion cell showed consistently superior than the Franz diffusion cell, by comparative studies, in terms of the control of skin surface temperature and the efficiency of solution mixing. This horizontal diffusion cell is capable of simulating a clinical setting by maintaining the receptor solution at body temperature, while varying the temperature on skin surface.

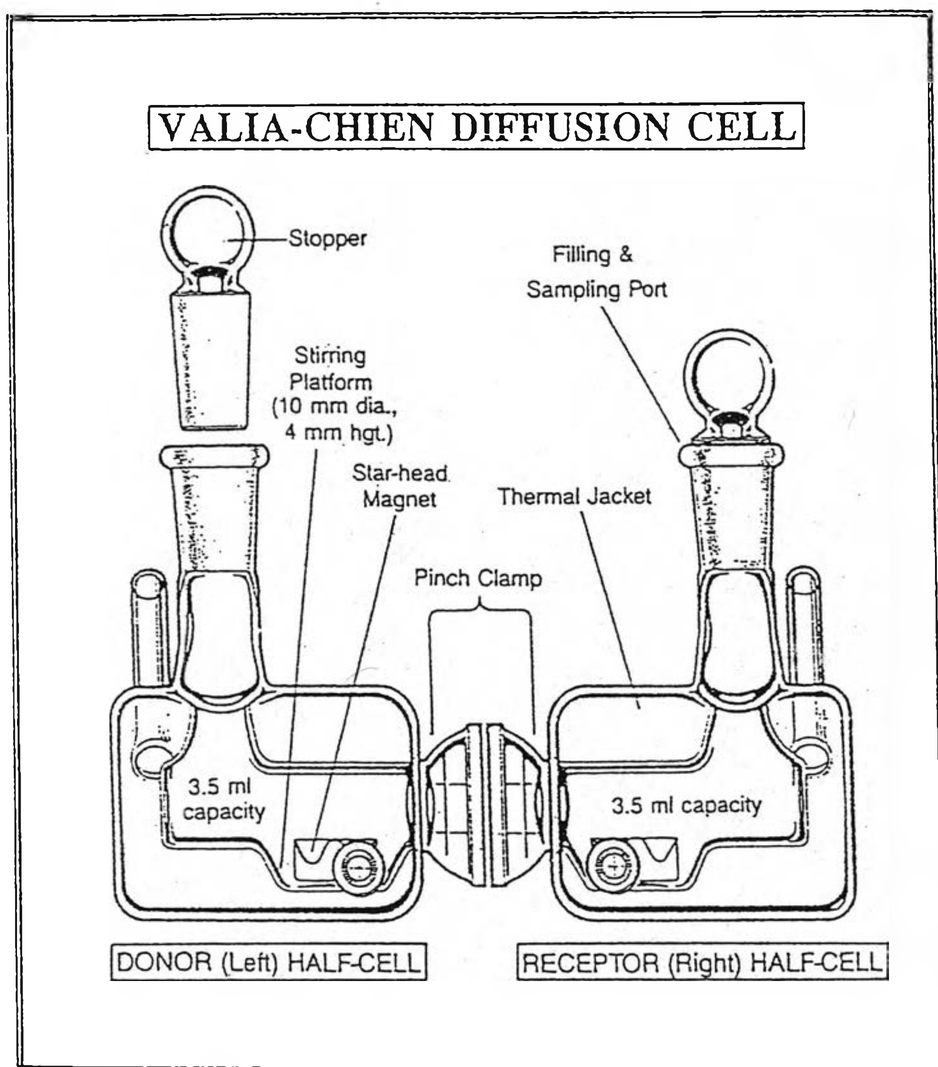

Figure 8 Schematic Illustration of Valia-Chien Horizontal Diffusion Cells.

Other upright finite-dosing diffusion cell was designed by Keshary and Chien (Keshary and Chien, 1984). Their diffusion cell, Keshary-Chien diffusion cell, was comparative studied with the Franz diffusion cell, which the results indicated that this cell could achieve and maintain the target body temperature on the skin surface and in the receptor solution and could improve solution mixing efficiency. Keshary-Chien diffusion cell was designed to enhanced the mixing efficiency by reducing the height of receptor compartment while the inner diameter was widened and the stirring bar was replaced with a star-head magnet. In the meantime, the water jacket compartment was extended to envelope a greater surface area of the receptor. The dateched glass stopper was designed to prevent any possible evaporation of the elution solution from the sampling port of receptor compartment. Keshary and Chien suggested that the improvement of solution efficiency could provide the drug distribution and concentration homogenized within a short duration and could be attributed to the combine effect of the reducing thickness of hydrodynamic boundary layer and the better control of the temperature in the diffusion path, so the skin permeation rate profiles could be realized with minimal effect from the mass transfer process.