CHAPTER I

INTRODUCTION

Nifedipine is one of the most potent calcium channel blocking agent for the treatment and prophylaxis of angina pectoris and hypertension. When it is administered orally as a capsule form, only about 65-75% of the dose reaches the systemic circulation as unchanged drug since nifedipine is rapidly metabolized on first pass through the liver to inactive metabolites (1,2). The biological half life of nifedipine is in the range of 2-5 hours (3). Not only the lack of efficacy but also the adverse effect of nifedipine usually associated with too low or too high, respectively of drug concentration in the blood. So, the often administration of drug is necessary to obtain a constant and effective drug concentration in the blood. Presently, nifedipine is commercially available as capsule with the initial adult dose of 10 mg three times daily. The total dose should not exceed 180 mg per day (4). Such administration causes inconvenience and complaint to the patient.

In the mean time various sustained release preparations of nifedipine have been developed with the purpose of reducing the frequency of drug administration and the incidence and intensity of side effect. The sustained release preparations appear as a granule form (5,6,7), a suppository (8,9) and a nasal absorbed aqueous gel (10). Since all of the aforementioned preparations still conserve some disadvantages such as : the first pass effect cannot be avoided by using the granule form; the suppository is unsuitable for patients with serious diarrhea; the nasal absorbed aqueous gel may still be inconvenienct or disturb the respiration. Therefore, the other

routes of administration are interesting.

The recent development in transdermal drug delivery system using skin as a route of drug administration become the interested area for this study. By this route, the first pass effect of drug can be avoided so the efficacy of drug is increased. Transdermal drug delivery system (TDDS) is conveniently administered by applying the dosage form directly on thr skin for once a day or two day once depending upon the therapeutic drug level needed.

Although the transdermal drug delivery system has been utilized as a dosage form preparation for many kinds of drug such as nitroglycerine, scopolamine etc, no such dosage form of nifedipine has been developed since then. Thus, this study has been purposed with the main objective as to develop the preparation of nifedipine to be the transdermal drug delivery system.

Objectives of this study :

1. To develop a controlled release dosage form of nifedipine in transdermal delivery system using hydrophilic polymers.
2. To study the effect of different hydrophilic polymers on the release of drug.
3. To study the influence of the concentration of single polymer, or combined polymers on the release of drug.
4. To investigate the release mechanism of nifedipine transdermal delivery system *in-vitro*.
5. To investigate the penetration of nifedipine through skin *in-vivo* , using rabbit as the animal model.

A. *General Information of Transdermal Drug Delivery System* :

Transdermal drug delivery system (TDDS) is a system for delivering of drugs to the systemic circulation via the skin. The basic compositions of TDDS is shown in Figure 1 (11). TDDS consists of five compositions as outlined below ;

1. Backing membrane : It is an impermeable membrane as backing support of the system.

2. Drug reservoir : This may be a single or poly layer where the required amount of drug is stored in a stable form.

3. Rate controlling polymeric membrane : This can establish and maintain the prescribed rate of drug administration through the operational life of the system.

4. Contact adhesive layer : This component is applied to provide an intimate contact with the skin surface. It should not be irritant to the skin.

5. Protective pool strip : It protects the TDDS system from the environment until the system is used.

From the compositions of TDDS, it is considered that the important composition which affect the control release rate is either drug reservoir or rate controlling polymeric membrane. Therefore, controlled release system can be divided into two system for developing of TDDS. One is a membrane permeation-controlled TDDS and the other is monolithic-controlled TDDS.

1. *Membrane Permeation- Controlled TDDS.* (12)

The drug reservoir is prepared by dispersing the drug homogeneously in a solid polymer or suspending the drug in an unleachable, viscous liquid medium to form a clear solution. The

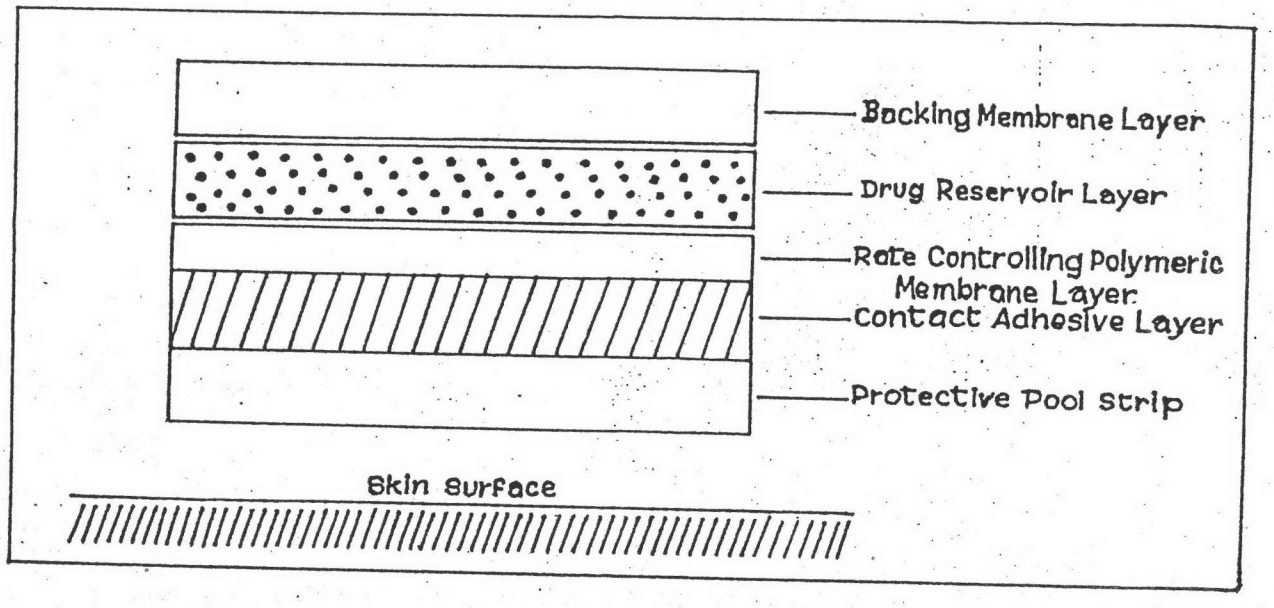

Figure 1 Schematic Illustration of Basic Composition of TDDS

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drug in reservoir is migrated through a rate-controlling membrane to the skin. This membrane can be either a microporous or a non-porous polymeric membrane. The rate of drug release from this system is followed:

$$\frac{dQ}{dt} = \frac{K_{m/r}K_{a/m}D_aD_m}{K_{m/r}D_mh_a + K_{a/m}D_ah_m} C_R \qquad (1)$$

where dQ/dt = rate of drug release

C_R = drug concentration in the reservoir compartment

$K_{m/r}, K_{m/a}$ = partition coefficients for the interfacial partitioning of drug from the reservoir to membrane and from the membrane to the adhesive, respectively

D_m, D_r = diffusion coefficients in the rate-controlling membrane and in the adhesive layers of the membrane, respectively

h_m, h_r = thickness of the rate-controlling membrane and the adhesive layer, respectively

2. Monolithic - Controlled TDDS. (12)

This system controls the release rate of drug by drug reservoir. It can be subdivided to be drug reservoir gradient-, matrix diffusion-, and microreservoir dissolution-controlled TDDS.

For drug reservoir gradient-controlled TDDS, the drug reservoir is formulated by proportionally increasing the drug loading level to form a gradient of drug reservoir in order to compensate the increasing in diffusional path of drug during the release time. The rate of drug release can be expressed by

$$\frac{dQ}{dt} = \frac{[K_{a/r}D_a]\, A\, [h_a]}{h_a(t)} \qquad (2)$$

where dQ/dt = rate of drug release

$K_{a/r}$ = adhesive reservoir partition coefficients

D_a = diffusion coefficients in the adhesive layer

$A(h_a)$ = drug loading level which is increasing proportionally

$h_a(t)$ = the increased thickness of diffusional path to adhesive layer

t = time

In the case of matrix diffusion-controlled TDDS, drug reservoir is formed by homogeneously dispersing the drug in a hydrophilic or lipophilic polymeric matrix. The medicated polymeric disc is mold with a defined surface area and thickness. The rate of drug release from matrix diffusion-controlled TDDS is defined as

$$dQ/dt = [(AC_pD_p)/\, 2t]^{1/2} \qquad (3)$$

where dQ/dt = rate of drug release

A = initial drug loading dose

C_p = solubility of drug in polymer

Dp = diffusivity of drug in polymer

t = time

The drug reservoir of microreservoir dissolution-controlled TDDS is complicately formulated. Drug is suspened in an aqueous solution of a water-soluble polymer. This suspension is dispersed homogeneously in a lipophilic polymer by high shear mechanical force to form thousands of unleachable microsopic drug

reservoir. These microscopic drug are unstable so they are quickly stabilized by cross-linking the polymer chains in situ. Release of drug from this system can follow either a partition control or matrix diffusion controlled process. So, a Q vs. t or Q vs $t^{1/2}$ release profile is resulted.

In consideration of both requirements to control the release rate of drug and formulation of TDDS preparation, the monolithic, matrix diffusion-controlled TDDS is interesting. The reason is that drug reservoir is easily prepared by dispersing drug into polymer and forming medicated polymer disc with a defined surface area and control thickness. The release profile from this system follows a typical square-root time pattern as shown in equation 3. This equation implies that solubility and diffusivity of drug in polymer depend upon the type of polymer. There are various kinds of polymers available for formulating matrix. Furthermore, in large-scale production the monolithic, matrix diffusion-controlled TDDS is easily produced with the less expense than the other systems which require the complicated instruments (13).

Therefore, the monolithic,matrix diffusion-controlled TDDS is the selected system for this study.

Among the raw materials available to a pharmaceutical or cosmetic formulation, polymers play a very important role almost in every formulation. Both natural and synthetic polymers are widely used in pharmaceutical systems as adjuvants, suspending agent, emulsifying agent, and coating agent (14). In novel drug delivery system, polymers are frequently used to achieve rate controlled release of drug because they act as the barrier for drug movement (15).

Hydrophilic polymers are good candidate for the development of nifedipine transdermal delivery system. They were

reported to control the release of drug by diffusion (16,17) and the zero-order release pattern could be achieved (18). Nifedipine is not soluble in water, as the hydrophobic particle so the probability of the drug released from the hydrophilic polymers was increased more than from the hydrophobic polymers according to the poor affinity between the hydrophobic drug and the hydrophilic polymeric matrix. Moreover, the hydrophilic polymers have more or less polyhydroxyl groups in their molecules which could form hydrogen bonding with water molecule. When the drug has been dispersed or entrapped into these polymers, the solubility of drug is likely to be increased according to the surrounding water molecule attached to these polymers. Therefore, more drug are dissolved and likely to diffuse through the polymers into the medium or the skin.

B. Criteria in Selection Polymers for Matrix Development (14).

1. The physico-chemical properties of the polymer such as ; molecular weight, glass-transtion temperature, and chemical functionality must be appropriate enough in allowing the proper diffusion and release of the specific active agent.

2. Polymer functional group should not react chemically with the active agent.

3. The polymer and its degradation product must be non-toxic.

4. The polymer must not decompose during the entire shelf-life.

5. The polymer must be easily manufactured or fabricated into a desired product.

6. The cost of polymer should not be expensive as to make the sustained drug release devices very expensive.

7. The polymer should be readily available.

C. *The Development of Nifedipine TDDS*

Nifedipine TDDSs were formulated using both natural and synthetic hydrophilic polymers. The natural polymers are methylcellulose and hydroxypropyl methylcellulose. The synthetic polymers are poloxamer 407, polyethylene glycol 4000 and 400, polyvinyl alcohol and polyvinyl pyrrolidone. These polymers were selected according to the aforementioned criteria such as the generally available in laboratory's drug store. They are reported to be a good polymeric matrix producing which could also achieve the controlled release rate of drug.

1. Nifedipine

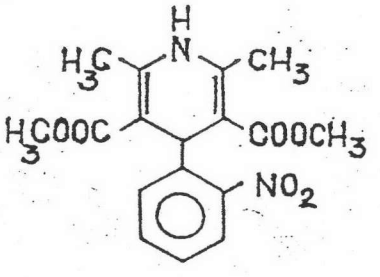

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Figure 2 : Structural Formula of Nifedipine

Nifedipine, $C_{17}H_{16}N_2O_6$, is a yellow crystalline powder, odorless and tasteless. Its melting piont is between 171-175 C. It is soluble in alcohol,acetone and chloroform. Nifedipine must be stored in tight, light-resistant container at a temperature of 15-25 C due to its photo-decomposition property (2). It is used for treatment and prophylaxis of angina pectoris and hypertension.

Presently, the available nifedipine preparations in Thailand are in the form of soft gelatin capsules in the dosage of 5 and 10 mg under the following trade names ; Adalat(R), Avenol(R), Calcegard(R), Nelapine(R) and in the form of retard tablet in the dosage of 20 mg under the name as Adalat(R), Calcegard(R).

The sustained release dosage forms of nifedipine had also been developed. These preparations could be concluded as following.

For the development of nifedipine sustained release granules (5,6), two kinds of granule were prepared by using the same ingredients of nifedipine and ethylcellulose. The different ingredients of pH-independent release granule were hydroxypropyl methycellulose and corn starch. Those of pH-dependent release granules were hydroxypropyl methylcellulose phthalate and microcrystalline cellulose. It was found that the release rate of drug was decreased with an increment of ethylcellulose in both kinds of granules. The release pattern of drug from these matrix granules *in-vitro* study was first order kinetices. Furthermore, both kinds of granules could sustained the release of nifedipine up to 8 hours. The plasma profile of nifedipine in rabbits indicated that the pH-independent release granules were superior to the pH-dependent release granules with respect to prolong action of the effective drug concentration in plasma. Clinical study in healthy subjects with oral administration of the pH-independent release granules, the plasma drug concentration was detected over 2-12 hours peroid. Twice-daily dosing of 20 mg for this kind of granule was sufficient for therapeutic effectiveness.

By the use of solid dispersion system, a sustained release of nifedipine granule form was prepared by spraying the enteric coating agent on an inert core material. Hydroxypropyl methylcellulose phthalate and Eudragit$^{(R)}$L were used as carriers of this system. Oral administration of these granules could provide a good bioavailability of nifedipine according to the gastric fluid resistance. Also, a prolongation of therapeutic plasma drug concentration was obtained in the period of 8 hours (7).

Nifedipine sustained release in double layer suppositories was developed by using a solid dispersion system. Cellulose acetate phthalate (CAP) - polyethylene glycol 4000 (PEG) matrix was prepared for a suppository base with PEG as a water-soluble carrier and CAP as a poorly water-soluble carrier. *In-vivo* study using rabbit as a model, this matrix suppository enhanced the bioavailability of nifedipine and gave a sustained release characteristic without causing an excessively high peak level in plasma (8). It has been reported that the pharmacokinetics of rectal administration of this double layer suppository of nifedipine to healthy volunteers follows a one-compartment model with first order kinetics (9).

Nasal absorption of nifedipine gel preparation was developed from polyethylene glycol 400 (PEG 400) and carbopal 941. *In-vivo* study with rat model the gel preparation with either PEG 400 or carbopal 941 produced a high or low plasma drug concentration, respectively. The gel preparation with a combination of 50% w/v PEG 400 and 0.05% w/v carbopal 941 showed a plasma drug concentration in the range of 0.4-2.2 ug/ml and a prolonged action of 6 hours (10).

2, Hydrophilic Polymers Used in This Study :

2.1 Methylcellulose, MC

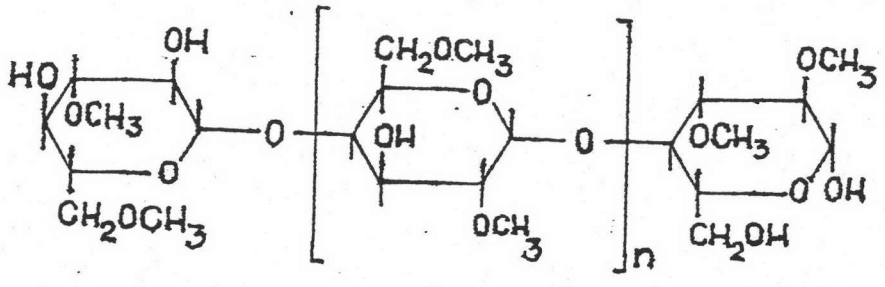

Figure 3 : Structural Formula of Methylcellulose.

Methylcellulose is a long chain substituted cellulose ether of 50-15,000 anhydroglucose units. It is a white to slightly off-white, essentially odorless and tasteless powder or granule. It swells in the cold water and can produce a clear to opalescent, viscous, collodial suspension. MC is slightly hygroscopic so it should be stored in a well-closed container. Irreversible decreasing in viscosity is caused by heating and cooling (19).

Low or medium viscosity grades of MC are preferred to use as a binder. It may be used in solution or in powder form to modify disintegration / dissolution patterns. Usual concentration of 1-20% MC may be used as a gelling, suspending, thickening, emulsifying, and tablet coating agent (20). High viscosity grades of MC may act as a disintegrant by swelling or contacting with disintegration medium. The usual concentration of MC is 1-5% (21).

2.2 Hydroxypropyl methylcellulose, HPMC

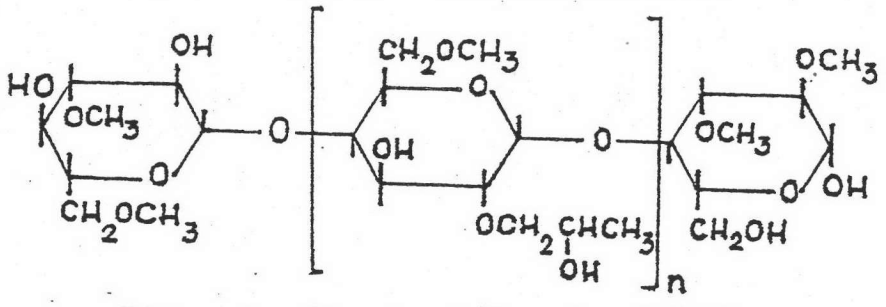

Figure 4 : Structural Formula of HPMC

HPMC is an odorless, tasteless white or creamy-white fibrous or granular powder. It is soluble in cold water forming a viscous colloidal solution. The solution of HPMC is stable at pH 3-11. It is compatible in the extreme pH conditions and with oxidizing materials (14). HPMC can be used as a film-former, thickening agent, protective colloid, emulsifying agent, suspending agent and stabilizer. High viscosity grades are used to retard the release of water soluble drugs (19).

The use of HPMC in various formulations with many kinds of drug have been studied.

A formulation containing theophylline as active drug, glycerol palmitostearate, mannitol and HPMC was studied (22). The results indicated that the release of the drug followed diffusion pattern because of the difference between the fraction of mannitol and HPMC. When both of them were used, the release of drug changed to first-order kinetics according to the changing of HPMC fraction.

Dissolution studies of indomethacin controlled release tablets showed that not only the ratio of the drug and HPMC but also the viscosity of HPMC was important in the system. The particle size of the drug was recognized greater than the solubility of drug. It was suggeted that the poorly soluble drugs were released from the matrix of HPMC by erosion (23).

HPMC was used to produce hydrophilic matrix of propanolal HCl , aminophylline (24) and promethacin HCl (25). It was found that a plot of the percentage of the dissolved drug against the square root of time produced a straight line. The major factor for controlling drug release was the ratio of drug and HPMC.

Notice : In this study, MC and HPMC under the name of Methocel$^{(R)}$, the cellulose ethers manufactured by the Dow Chemical Company were used. Various types of Methocel$^{(R)}$ and their properties are represented in Table 1,2 (19).

2.3 Poloxamers.

Poloxamer is a series of nonionic polyoxyethylene-polyoxypropylene copolymers with the following chemical structure

Table 1 : Various Types of Methocel and Viscosity (19)

Type	Methocel (Premium grades)	Viscosity* (cps)
Methylcellulose, USP	A 15 LV	15
	A 4 C	400
	A 15 C	1,500
	A 4 M	4,000
Hydroxypropylmethylcellulose, USP 2910**	E 5	5
	E 15 LV	15
	E 50	50
	E 4 M	4,000
Hydroxypropylmethylcellulose, USP 2906**	F 50	50
	F 4 M	4,000
Hydroxypropylmethylcellulose, USP 2208**	K 35 LV	35
	K 100 LV	100
	K 4 M	4,000
	K 15 M	15,000
	K 100 M	100,000

* Normal vicosity of 2% aqeous solution at 20°C

** For USP grade hydroxypropylmethylcellulose (HPMC) , the name is followed by a four digit number. The first two digits refer to the approximate content of the methoxy group ($-OCH_3$) in percent. The second two refer to the approximate content of the hydroxypropoxy group($-OCH_2CHOHCH_3$) in percent, calculated on a dried basis.

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Table 2 : *Degree of Substitution and Typical Weight Percent Substitution for Methocel Premium Grades (19)

Product	Methoxyl D.S.*	Methoxyl %	Hydroxypropoxyl Molarsubstitution	Hydroxypropoxyl %
Methocel A	1.79-1.83	29.9	-	-
Methocel E	1.86-1.90	29	0.22-0.25	8.5
Methocel F	1.71-1.81	28.4	0.12-0.15	5.0
Methocel K	1.36-1.42	22.1	0.18-0.23	8.1

* D.S. (Degree of Substitution) Weight percent or average number of substituent groups attached to each anhydroglucose unit along the chain.

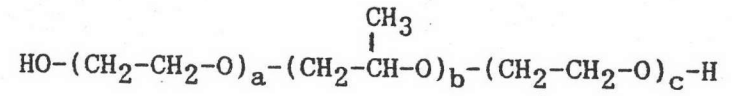

Figure 5 Structural Formula of Poloxamer

;where a and c are statistically equivalent (26). The other name is Pluronic(R). Available grades of the poloxamers are varied from liquids through pastes to solid waxy flaskes. Their properties range from the hydrophobic liquid which is almost insoluble in water to solid which is very soluble in water and give high HLB values. The solution of poloxamer can be sterilized by autoclaving. It is incompatible with phenol, resorcinol, and betanaphthol in certain concentration. It has low acute oral toxicity and low potential to cause irritation or sensitization. Pluronic(R) can be used as a dispersing agent, emulsifier, solubilizer, thickening agent, gel former,and dissolution controlling agent (27). A distinguishing property of poloxamer is that it can be liquified by merely lowering their temperature without cocommitant loss of product integrity. Also, it can be reversed to their original consistency. Therefore it is called as " reversible gel ". One of the advantages of a reversible gel product is that it can eliminate any air bubbles which may have been accidentally incorporated during the processing.

Pluronic(R)F 127 (Poloxamer 407) is one of the Pluronic series that have average molecular weight of 12500. The composition of this copolymer is 70%w/w polyoxyethylene and 30% w/w polyoxypropylene. It is the most efficient gellant in the Pluronic(R) series (27).

Pluronic(R)F 127 was formulated for the novel dosage form in dermatological preparations. The release of lidocaine from Pluronic(R)F 127 gel in the *in-vitro* release model which did not utilize a membrane had been studied. The drug was released by

diffusion. It had been found that the drug release rate was inversly proportional to the concentration of Pluronic$^{(R)}$F 127 and the electrolyte (28).

Hadgraft and Howard (29) investigated a potential use of Pluronic$^{(R)}$F 127 gel as sustained release depot preparation for barbiturates.It was found that the linear relationships between the amount of barbiturate released and the square root of time existed. The increasing concentration of Pluronic$^{(R)}$F 127 made an apparent diffusion coefficient of butabarbital decreased.

Pluronics$^{(R)}$ were evaluated as a vehicle for rectal administration of indomethacin in the *in-vitro* release model (30). It had been reported that the apparent release rate of the drug was decreased by increasing the concentration of Pluronic$^{(R)}$F 127 but it was increased by increasing the temperature from 20 to 44 C and / or the drug concentration.

2.4 Polyvinyl Alcohol (PVA)

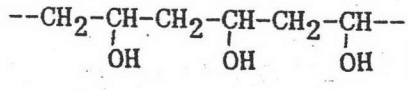

Figure 6 : Structural Formula of Polyvinyl Alcohol

Polyvinyl alcohol,$(C_2H_4O)n$, is odorless, white to cream-colored granular powder or granules. Its average molecular weight from low to high viscosity is 30,000-200,000. It is essentially soluble in hot or cold water. It is stable to light,resistant to most organic solvent, but can be decomposed in strong acid. It is incompatible to most organic salts, especially sulfates and phosphates (31). PVA can be used as a suspending and/or

emulsifying agent. It is commonly ultilized as a lubricant and protectant in various opthalmic preparations,e.g., decongestant, artificial tears, and contact lens products (32).

2.5 *Polyvinyl Pyrrolidone (PVP)*

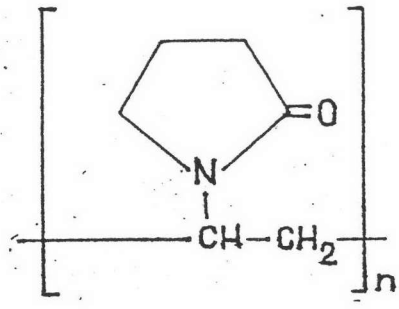

Figure 7 : Structural Formula of Polyvinyl Pyrrolidone

Polyvinyl pyrrolidone (PVP), or povidone, $(C_6H_9NO)_n$, is an odorless, hygroscopic, white to creamy white powder. It is readily soluble in water,freely soluble in many organic solvents. Several grades of PVP are determined by K value that implies to the relative viscosity of its solution. The K values are related to the average molecular weight of PVP as shown in Table 3 (32).

Table 3 : Several Grades of Polyvinyl pyrrolidone and Molecular Weight

K value	Average Molecular Weight
15	10,000
30	40,000
60	160,000
90	360,000

The molecular weight and the concentration of PVP affect the viscosity of the product. PVP can be stored under the ordinary condition without undergoing decomposition or degradation. It is an inert and non-toxic agent. It has no irritating effect on the skin and it causes no sensitization. PVP does not irritate the mucous membrane of rabbit eyes. It is used as a carrier for drug, dispersing agent, binder and coating agent for tablet.

Viegas et al (33) indicated that there was large difference in the release rate of levodopa between the PVP polymers and the silicone polymers. The PVP polymers showed the drug release rate faster than did the silicone polymers. It was indicated that there was no significant in the difference grades of PVP between the K 26-28 grade and the K 29-32 grade on the release pattern.

Nitro-dur is one of the dosage form of nitroglycerine in TDDS., which is formulated from the PVP and PVA polymers. Its action follows zero-order kinetics with the prolong release drug action within 24 hours (34).

Jin-Shing Lai et al (35) had investigated the release kinetics of three different concentrations of 1, 2 and 4% of indomethacin preparations from the polymer matrices. The matrices were composed of PVP and PVA polymers with glycerine as plasticizer. It was described that the drug release pattern could follow the Higuchi's equation and could prolong the drug release for 24 hours. These polymeric matrices were suggested to be a potentially developed transdermal delivery system.

Bhalla and Toddywala (36) investigated that the polymer of PVP and PVA with glycerine as plasticizer could form a polymeric matrix type for the TDDS of ephedrine. These polymers were found to be free from skin irritation and stable at room temperature.

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2.6 Polyethylene Glycols (PEGs)

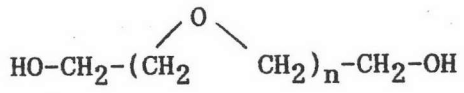

Figure 8 : Structural Formula of Polyethylene glycols

Several grades of polyethylene glycol depend on the average number of oxyethylene groups (n). Low molecular weight grades (200-600) of PEGs are clear, colorless or slightly yellowish and viscous liquids. The odor is slight but characteristic,and the taste is bitter and slightly burning. High molecular weight grades (1,000-20,000) or the solid grades are white or off-white in color. The appearant range is consistent between pastes and waxy flaskes. Their odor is faint and sweet (21).

As the molecular weight of PEGs are high, their water solubility, vapor pressure, hygroscopicity, and the solubility in organic solvents are decreased (37). On the contrary, the freezing point, the melting range, the specific gravity,the flash point and the viscosity are increased. PEGs are dissolved in water forming a clear solution. They are soluble in various kinds of organic solvents. An aqueous solution of the high molecular weight grades of PEGs may form gels which are stable in air and in the solution. PEGs cause mild irritation to skin and also a local stinging effect can occur when they are used as the suppository base due to their hypertonicity.

PEGs have the wide range of solubility and compatibility. These advantages make them available in many pharmaceutical and cosmetic preparations. The liquid PEG 400 and the solid PEG 3350 which are widely used in the official PEG ointment, can provide a water-soluble ointment base. This base is used in the formulation of many dermatologic preparations (37).

The study of Umeda et al (38,39) on the release of nifedipine from the formulation containing 15% cellulose acetate phthalate-polyethylene glycol 4000 copolymers as matrix in double layer of nifedipine suppositories have shown that the copolymer could enhance the bioavailability and also give a sustained-release plasma level of nifedipine.

D. Diffusion Cell Used for In-Vitro Release Study.

1. Principle of The Diffusion Cell.

According to the assumption that the release of drug obtained from the *in-vitro* study is similar to drug release obtained from *in-vivo* study, the system of diffusion study is designed to provide a mimic *in-vivo* sitution. Usually, the system of diffusion study consists of the following compositions ;

- skin or 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 to be 37°C
- stirring system for ensuring homogeneity of drug concentration in the medium solution.

Diffusion cell is the only composition which was modified to achieve the convenient and suitable pattern for each diffusion system proposed. Michaels et al (40) fabricated a diffusion cell which was equipped with a pair of Teflon impellers to stir the solution in both donor and receptor compartments. In this model, the speed of the Teflon impellers was controlled by the external synchronous moters, and also the sampling ports were open. Durrhein et al (41) developed a miniature diffusion cell in which each donor and receptor compartment had a volume of only 1.5 ml. The cell was equipped with

two open ports : one was used to accomodate the externally driven impeller and another for solution filling and sampling. All aforementioned skin diffusion cells have the following drawbacks : (a) both the donor and the receptor solutions are maintained at the same temperature by either immersing the whole cell in a temperature regulated water bath or retaining it in thermally controlled oven. This setup could not simulate exactly the clinical setting in which the donor compartment is exposed to an ambient environment and (b) the solution in the donor and receptor compartment is constantly exposed to the atmosphere through the opening for stirring and sampling. The loss of solvent through the opening could be significant and may affect the drug concentration in the solution.

Franz (42) had designed an upright-type diffusion cell. The feature of this diffusion cell was that it could provide the finite-dosing. This led this diffusion cell as one of the most frequently used for the *in-vitro* for the study of the release of the drug or the mechanism of TDDS. Nevertheless, several deficiencies from Franz's model had been noticed. The model could not achieve the solution hydrodynamics, the mixing efficiency, and the controlled temperture which were required in the quantitative evaluation of skin permeation kinetics (43). The other improved diffusion cell shown in Figure 9, called as Keshary-Chien diffusion cell (43) was indicated as the fulfil diffusion cell commonly used until now. It could achieve and maintain the temperature on the membrane and in the receptor solution. Solution mixing efficiency was substantially improved, so the drug distribution and concentration could be homogenized within a duration four times shorter than that in the Franz diffusion cell. A 3-fold reduction in the thickness of the hydrodynamic boundary layer reduced the mass transfer rate profile on the skin permeation.

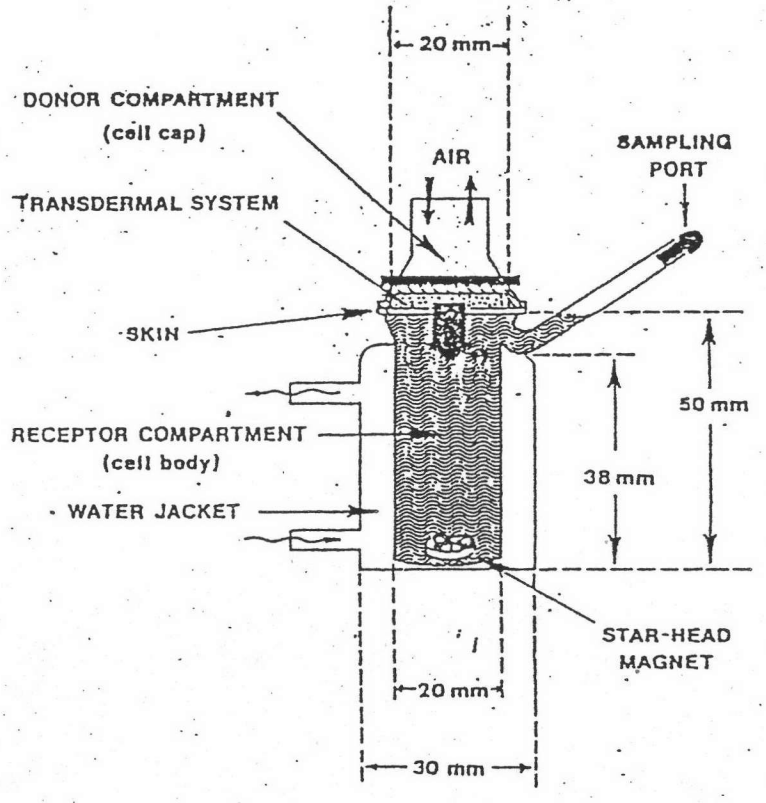

Figure 9 Schematic Illustration of Keshary-Chien Diffusion Cell

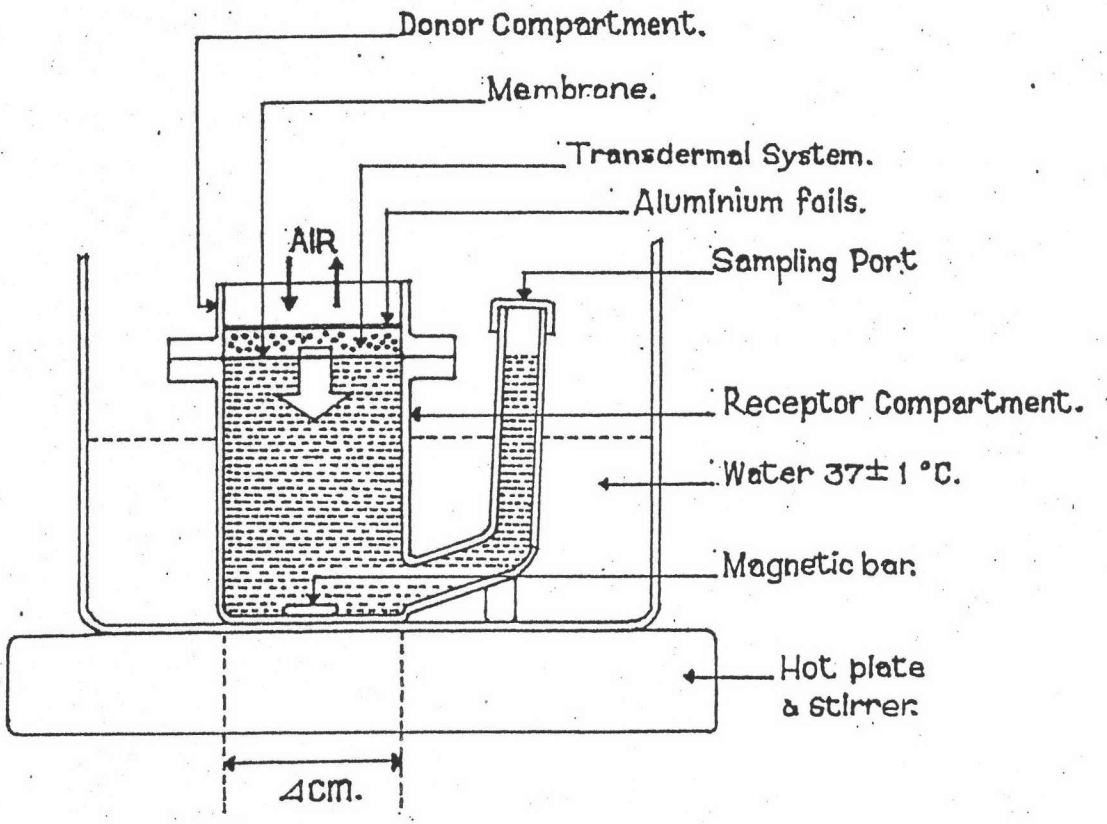

Figure 10 Schematic Illustration of the Modified Diffusion Cell Used in This Study

In this research study, a modified Keshary-Chien diffusion cell was selected to be used. Modification was to solve the problems. First, the volume of sample need for further drug analysis was much greater than the volume drawn from the diffusion cell. The Keshary-Chien diffusion cell could provide only less than 1.0 ml per sampling while the volume needed should be 5 ml. Thus, the inner diameter of the receptor compartment as well as the height were both doubled enlarged from the former Keshary- Chien diffusion cell of 20 mm and 50 mm to 40 mm and 100 mm, respectively. Secondly, due to the bending of the sampling port, the way to pipette the sampling was inconvinience and the air bubbles often entered into the receptor compartment while the pipetting the sample. The sampling port was modified to be straight at the end of the receptor compartment. The other external features of the Keshary-Chien diffusion cell was still retained. This modified diffusion cell in this study was shown in Figure 10.

2. *The Skin Used in Diffusion Cell.*

The skin used in diffusion cell may be an human or animal skin. However, both kinds of the skin used met the same problem of readily unavailable and of limited viability (44).

Synthetic membrane model have been proposed to be used in studying drug diffusion kinetics. Early studies using dimethyl polysiloxane membrane were pioneered by Garrett and Chemburkar (45). Barry et al (46) used a cellulose acetate membrane to study the influence of temperature and nonionic surfactants on the permeation rate of various steroids. There are the other synthetic membranes which are made of various polymers such as; silicone rubber and polyethylene vinyl acetate (47,48).

Durapore is the synthetic membrane consisted of by polyvinylidene difluoride (49). Usually its utility is membrane filter for HPLC sample, antibiotic, virus, sterilizing filtration for insulin, DNA solution, etc. In this research study, Durapore is selected to be used as a barrier in stead of the excised skin. The reasons are, it is designed to provide the maximum purity and strength. It is compatible with most of the organic reagent except ketone, amines and esters. It contains the uniform pore size of 0.45 μm. It is not soluble in dissolution medium used in this study. The other benefit of Durapore is that it can be fitted to the diffusion cell with 4 cm.

3. *The Medium in Receptor Compartment of Diffusion Cell*

It is important to ensure that the release profile of drug from the preparation is not limited by the solubility of drug in the receptor medium. Therefore, the receptor medium in diffusion cell is essentially act as a perfect sink condition. In diffusion study of nifedipine, a poor water-soluble drug, a volume of diffusion cell used in this study is limited of 63 ml, so it is neccessary to find out a solvent dissolved nifedipine completely.

Nifedipine is soluble in ethanol, methanol, chloroform and acetone (50). These solvents are carcinogenic agents. The evaporation of them affect the drug concentration. It is considered that co-solvent can be used to increase the solubility of the drug. The intravenous preparation of nifedipine was reported that it could be prepared by using either 30%v/v polyethylene glycol 400 (38) or the co-solvent of polyethylene glycol 400, ethanol and water in the ratio of 15:15:17 as a vehicle (10). However, neither of both could not dissolve 50 mg of nifedipine completely, but the trial and error of the cosolvent of polyethylene glycol 400 and ethanol in the ratio of 1:1 could. (as shown in Appendix 1) This co-solvent dissolved 50 mg of nifedipine in the volume of 4.5 ml. Therefore, this co-solvent was used as a medium in diffusion cell.