

CHAPTER I

INTRODUCTION



The calcium channel blocking agents (calcium antagonists or calcium entry blockers) now become one of the standard first-line treatments for essential hypertension, and together with β -blockers and nitrates, have become the established therapy for angina pectoris. Their effectiveness and low side-effect profile have made these agents among the most widely used cardiovascular drugs (Opie, 1990).

Nifedipine (NFP) is one of the most potent calcium channel blocking agents. It is a coronary and peripheral arterial vasodilator. Its major effects are to increase cardiac output and decrease peripheral resistance. It is a safe and effective drug in a long term treatment and prophylaxis of hypertension and angina pectoris (Opie, 1990 ; Comess and Fenster, 1982). However, NFP is a poorly water-soluble drug whose bioavailability is very low when it is administered in crystalline form. Furthermore, NFP is completely metabolized by the liver to its inactive pyridine metabolites, resulting in a short duration of action. Its biological half-life is in the range of 2-5 hours. These problems are necessary to frequently administer NFP in order to maintain a constant and effective drug plasma concentration. The usual starting dose is 10 mg three or four times daily. The dose is titrated upwards until symptoms are controlled or adverse effects occur. The maximum dose is 120 mg daily (Comess and Fenster, 1982). The frequent oral dose causes patient inconvenience and noncompliance. Therefore, several dosage forms of NFP have recently been developed in an attempt to protect from a first-pass effect, prolong the

duration of action, reduce the incidence and intensity of side effects.

The major advantages of transdermal drug delivery system (TDDs) which may enhance the therapeutic efficacy of NFP are that the drug can be induced into systemic circulation without hepatic first-pass effect with predictable and extended duration of activity, . Furthermore, TDDs is more accessible from its less frequent administrations, and safer from its reduced side effects.

Nifedipine transdermal drug delivery systems were developed by using Pluronic F-127 as hydrophilic gelling agent and Aerosil A-200 as hydrophobic gelling agent. The results indicated that the preparation with highest skin permeation rate could control and exhibit a constant and high skin permeation rate over periods of study. This gel matrix could be used to modified for a-once-daily medication of angina pectoris and/or hypertension (Tattawasart, 1992).

The therapeutic level of NFP in human was 15-100 ng/ml (Opie,1990). To achieve the therapeutic range, the enhancers have to be added as an integral part of NFP transdermal patch. Surface active agents (surfactants) have been classified as one group of the enhancers that may affect the permeation of a solute through biological membranes either by interacting with the solute, with the membrane, or with both (Tan, Liu, and Chien,1993).

This research study aimed to develop the approach of enhancing permeation of NFP transdermal patch by using various surfactants and evaluate in rabbits.

Objectives of this study

1. To develop nifedipine transdermal patch using surfactants as permeation enhancers.
2. To determine whether types and concentrations difference among surfactants studied affect the physical properties of formulations established.
3. To study and compare the release mechanism of nifedipine from patch through pig skin by *in vitro* diffusion cell.
4. To investigate the permeation of nifedipine from patch through rabbit skin, comparing to administration of single nifedipine soft capsules oral dose to rabbits.

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LITERATURE REVIEWS

Transdermal Drug Delivery Systems : General Information

Transdermal drug delivery systems (TDDs) are self-contained , discrete dosage form which, when applied to intact skin, deliver the drug(s) through the skin at a controlled rate to the systemic circulation. Advantages of this system for therapeutic agents are preventive first pass effect and gastrointestinal incompatibility, reproducible and prolonged constant delivery rate, convenience of less frequent administrations, and reduced side effects (Kydonieus, 1987; Chien, 1987; Monkhouse and Huq, 1988).

For a systemical active drug to reach the remote target tissue, some physicochemical properties have to be possessed . These are the sorption of drug by the stratum corneum, the penetration of drug through various skin tissues, and also the uptake of the drug by the capillary network in the dermal papillary layer.

The major parts of a transdermal system can be divided into five components as follows;

- 1. Backing** : It is an impermeable membrane or film, which is the backing support of the system.
- 2. Drug reservoir** : It may be a single or polylayer where the required amount of drug is stored in a stable form.
- 3. Control membrane** : This film controls the rate of drug flux from the dosage form.

4. Contact adhesive layer : This layer is applied to provide an intimate device contact to skin surface. It should not irritate to the skin.

5. Protective peel strip : It is used for protecting the TDDs from the environment before administration.

Several technologies have been developed to provide a rate-control over the release and the skin permeation of drugs. These technologies can be classified into four approaches as outlined in the following.

1. Membrane-Moderated Transdermal Drug Delivery Systems

In this method, the drug reservoir is totally encapsulated in a shallow compartment molded from a drug impermeable metallic plastic laminate and a rate-controlling polymeric membrane (Fig.1). The drug molecules are permitted to release only through the rate-controlling membrane. In the drug reservoir compartment, the drug solids are dispersed in a solid polymer matrix or suspended in an unleachable, viscous liquid medium to form a paste-like suspension. The rate-limiting membrane can be a microporous or a nonporous polymeric membrane with a defined drug permeability property. On the external surface of the polymeric membrane, a thin layer of drug-compatible, hypoallergenic adhesive polymer may be applied to achieve an intimate contact of the transdermal patch with the skin. The rate of drug release from this type of transdermal drug delivery system, can be tailored by altering the polymer composition, permeability coefficient, and thickness of the rate-limiting membrane and adhesive.

MEMBRANE-MODERATED
TRANSDERMAL DRUG DELIVERY SYSTEMS

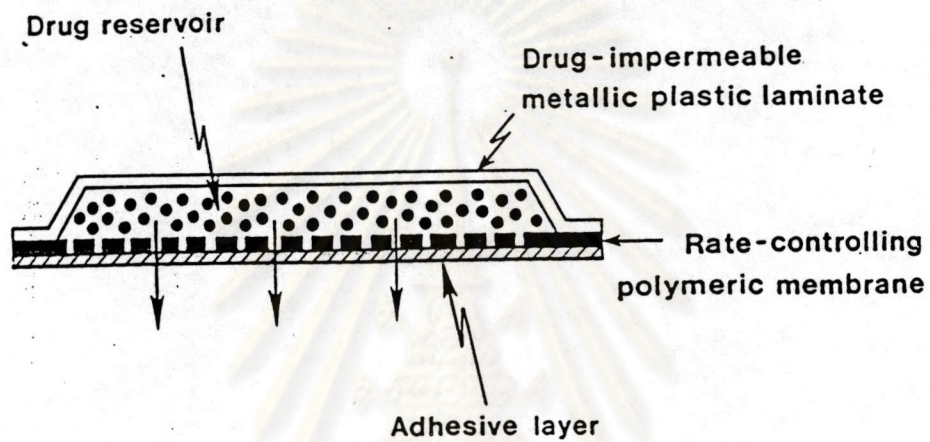


Figure 1 The cross-sectional view of a membrane-moderated transdermal drug delivery system, showing various major structural components

2. Adhesive Diffusion-Controlled Transdermal Drug Delivery Systems

This type of drug delivery system is a simplified version of the membrane-moderated drug delivery system just described in the first approach. Instead of completely encapsulating the drug reservoir in a compartment fabricated from a drug-impermeable metallic plastic backing, the drug reservoir is formulated by directly dispersing the drug in an adhesive polymer and then spreading the medicated adhesive by solvent casting onto a flat sheet of drug-impermeable metallic plastic backing to form a thin drug reservoir layer (Fig.2). On the top of drug reservoir layer, layers of non medicated, rate-controlling adhesive polymer of constant thickness are spread to produce an adhesive diffusion-controlled drug delivery system.

3. Matrix Dispersion-Type Transdermal Drug Delivery systems

In this approach, the drug reservoir is formed by homogeneously dispersing the drug solids in a hydrophilic or lipophilic polymer matrix. The medicated polymer is then molded into a medicated disc with a defined surface area and controlled thickness. This drug reservoir-containing polymer disc, is glued onto an occlusive base plate in a compartment fabricated from a drug-impermeable plastic backing (Fig.3). Instead of spreading the adhesive polymer onto the surface of the medicated disc as discussed earlier in the first two systems, the adhesive polymer is applied along the circumference to form a strip of adhesive rim around the medicated disc.

ADHESIVE DIFFUSION-CONTROLLED
TRANSDERMAL DRUG DELIVERY SYSTEMS

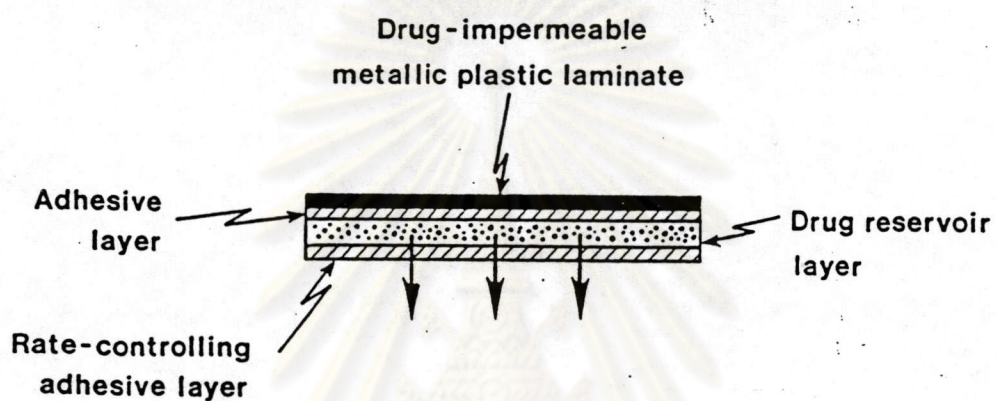


Figure 2 The cross-sectional view of an adhesive diffusion-controlled transdermal drug delivery system, showing various major structural components

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MATRIX DISPERSION-TYPE
TRANSDERMAL DRUG DELIVERY SYSTEMS

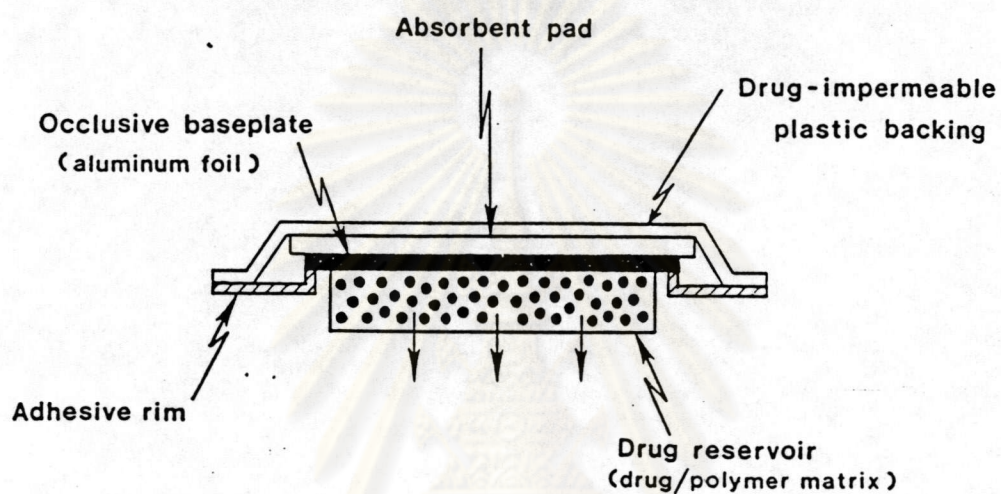


Figure 3 The cross-sectional view of a matrix dispersion-type transdermal drug delivery system, showing various major structural components

The rate of drug release from this matrix dispersion type drug delivery system (dQ/dt) is defined as:

$$dQ / dt = (ACpDp/2t)^{1/2} \quad \text{_____ (1)}$$

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, so C_p is essentially equal to C_R , where C_R is the drug concentration in the reservoir compartment.

At steady state, a Q vs. $t^{1/2}$ drug release profile is obtained as defined by:

$$Q/t^{1/2} = [(2A - C_p)C_pD_p]^{1/2} \quad \text{_____ (2)}$$

4. Microreservoir-Type Transdermal Drug Delivery Systems

This type of drug delivery system can be considered as the hybrid of reservoir and matrix dispersion-type drug delivery systems. In this strategy, the drug reservoir is formed by suspending the drug solids in an aqueous solution of water-soluble liquid polymer. The drug suspension is then dispersed homogeneously in a lipophilic polymer, by high-shear mechanical force, to form thousands of unleachable, microscopic spheres of drug reservoirs. This thermodynamically unstable dispersion is stabilized by immediately cross-linking polymer chain in situ (Fig.4), which produces a medicated polymer disc with a constant surface area and a fixed thickness. A transdermal therapeutic system thus is formed with the medicated disc at the center and surrounded by an adhesive rim (Fig.5).

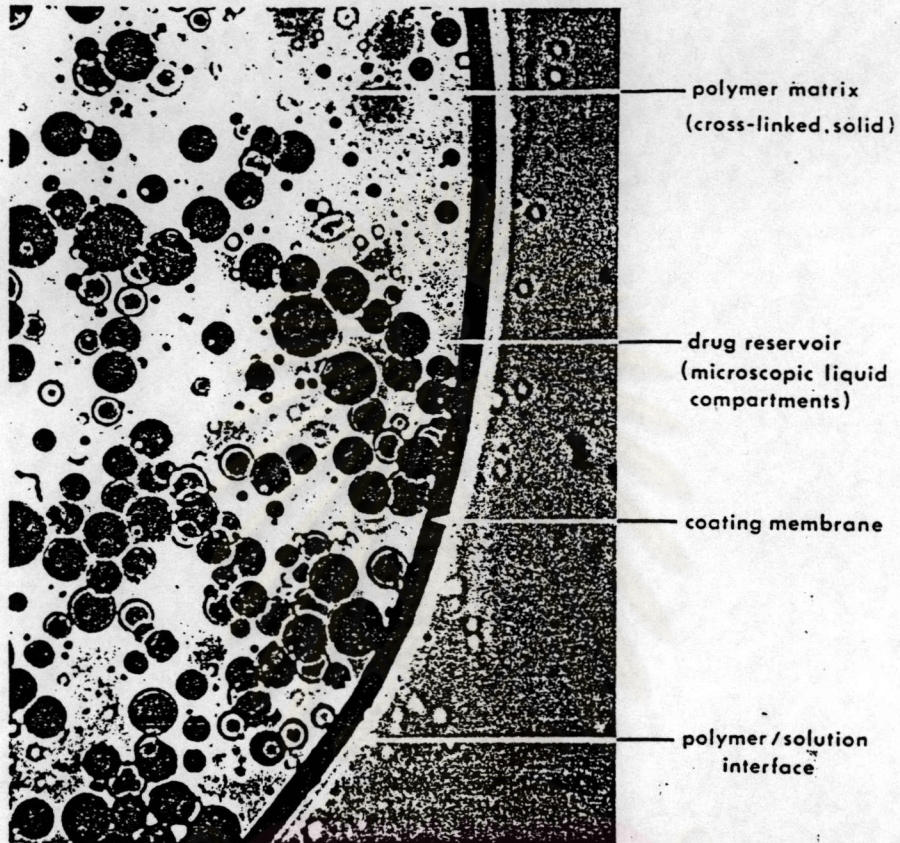


Figure 4 Photomicrograph of a microreservoir-type drug delivery system, showing the microscopic structure of the ions of

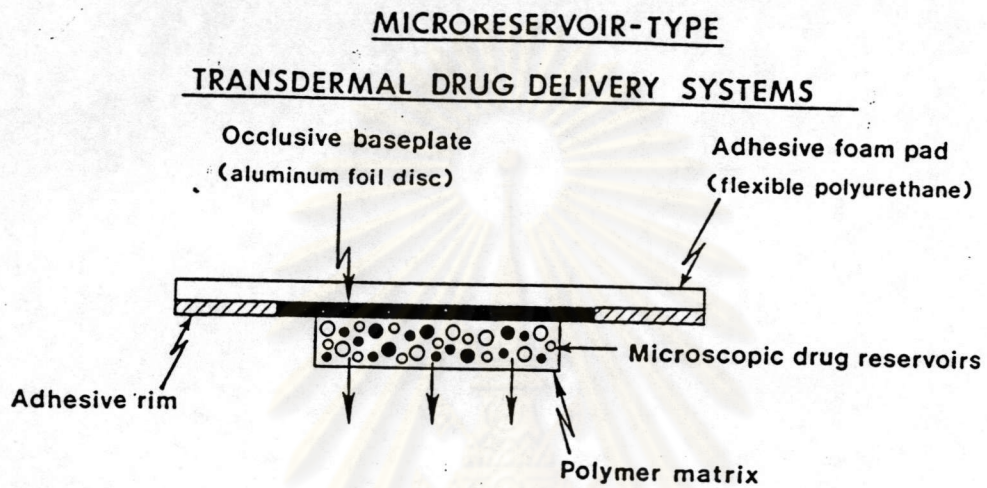


Figure 5 The cross-sectional view of a microreservoir-type transdermal drug delivery system, showing various major structural components

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The selected type of NFP TDDs in this research study is matrix dispersion type.

Gopferich and Lee (1992) studied about the transdermal uptake of clenbuterol from a matrix-type delivery device. The application of a fresh device every 7 days was found to produce a pseudo-steady state drug plasma profile after approximate three changes of device. Of the matrix properties, only drug loading had substantial effect on the drug plasma profile.

The drug release profile of ibuprofen hydroxypropylmethylcellulose (HPMC) matrices with different ratios of polymer-to-drug using HPMC of various molecular weights were studied. Increase in the ratio of polymer-to-drug decreased the release rate in a nonlinear manner. HPMC altered the drug release profile by forming a gel layer, its composition being dependent on polymer content and the molecular weight. Erosion of this gel layer led to enhanced drug release from matrix by increasing the surface area exposed to the dissolution medium. The polymer that formed a gel which was least susceptible to surface erosion and dissolution showed greatest retardation in drug release. In systems where the polymer gel remained intact, the drug diffused through the gel, and the release pattern was linear with the square root of time. Thus, the ratio of the dissolution medium entering the compressed matrix, the rate of formation of the gel layer, the rate of diffusion of drug in the gel, the thickness and the integrity of gel would influence the drug release pattern (Wan, Heng, and Wong, 1990).

Permeation Enhancers

The transdermal route has many advantages for the administration of drugs in local and systemic therapy. However, the outermost layer of skin, the stratum corneum, forms a strong barrier to most external substances including

drugs. One approach to deliver an effective dose of drug through skin is to temporarily reduce the barrier function of skin by the aid of permeation enhancers or accelerants (Okamoto, 1988). Permeation enhancers are substances that temporarily reduce the impermeability of the skin, hence promoting the passage of drug through it (Viegas, Hikal, and Cleary, 1988).

Permeation enhancers can be defined as an agent which reduces the diffusional resistance of the stratum corneum by reversibly damaging it or by altering its physicochemical nature (Shah, 1992). The chemicals used to enhance skin permeability can be classified into three main groups as follow.

1. Solvents such as water, some of the lower alcohol, alkyl methyl sulfoxides, pyrrolidones, laurocapram, and miscellaneous solvents.
2. Surfactant that would be described later.
3. Miscellaneous chemicals e.g. urea, N,N-dimethyl-m-toluamide, calcium thioglycolate, and anticholinergic agents.

An ideal penetration enhancer in addition to being safe, nontoxic, and nonirritating should also be pharmacologically inert reversibly modifying the barrier properties of the skin.

Surfactants

Surfactants are characterized by the presence of both polar and non-polar groups on the same molecule. The common theme of many publications on the biological effects of surfactant is the existence of a concentration-dependent biphasic action such that an enhancement of membrane transport occurs at low concentrations of surfactant, but this decreases at higher concentrations, generally above the critical micelle concentration (CMC) of the surfactant. Increase in membrane transport at low surfactant concentrations

is normally attributed to the ability of the molecule to penetrate and eventually disrupt the cell membrane structure.

Reduction of transport of a permeant in surfactant systems is attributed to the ability of the surfactant to form micelles, and is normally only observed if interaction between micelle and permeant occurs. It can be considered, therefore, that the overall effect of the surfactant is the result of two opposing effects—interaction with the membrane and that of the permeant with the micelle. Thus, there are five possible profiles of surfactant-induced effects on membrane permeability as a function of surfactant concentration (Fig 6). In this figure, profile A is the typical of other systems where permeation is increased at pre-micellar concentrations, but a high level of complexation or solubilization of the permeant occurs at micellar concentrations, resulting in reduced thermodynamic activity of the permeant in the donor medium. Profiles B and C are obtained when the surfactant increases permeability, but has little or no affinity for the permeant. For a surfactant with no membrane permeability-enhancing effect, profile D would be obtained if micellar interaction occurs, or profile E if the permeant has no affinity for the micelles.

The hydrophobic portion of surfactants usually consists of flexible alkyl or aryl chains. The classification of these compounds is based on the charge carried by the hydrophilic head group. Thus, they can be anionic (e.g., sodium dodecyl sulfate), cationic (e.g., cetyltrimethyl ammonium bromide), or nonionic, in which case the polar group is normally a polyoxyethylene chain (e.g., polyoxyethylene sorbitan monopalmitate). Occasionally, a further classification is necessary because a surfactant has the ability to behave either as a cation or anion depending on the pH of the solution. These zwitterionic forms are termed ampholytic and can exist as a nonionic form e.g., N-dodecyl-

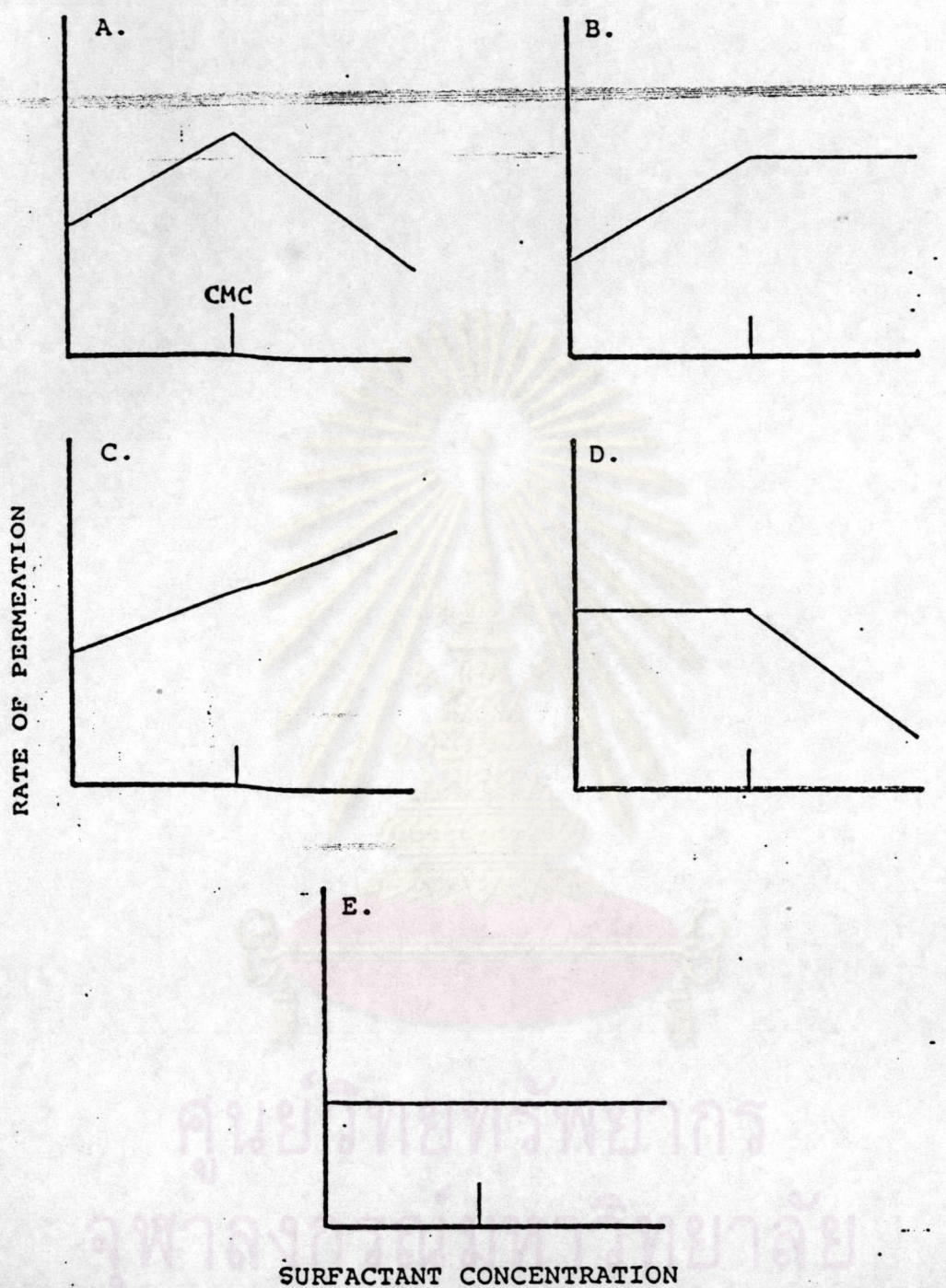


Figure 6 Possible profiles of surfactant induced alterations in biological membrane barrier properties

N,N-dimethyl betaine). In the following sections the skin permeability-enhancing activity of these groups is discussed separately along with a consideration of fatty acids and fatty alcohols.

Surfactants have also been found to enhance permeation especially for hydrophilic drugs. Surfactants are, however, skin irritants, therefore a balance between permeation enhancement and irritation has to be considered if any success can be expected when using these chemicals.

1. Anionic Surfactants

Anionic surfactant can penetrate and interact strongly with skin. (Bettley, 1965; Gibson and Teall, 1983) The penetrating amount of drug is highly influenced by surfactant structure, principally on the alkyl chain length. For example, those surfactants with a decyl or dodecyl chain are more efficient permeants than those based on tetradecyl, hexadecyl, and octadecyl chains. (Howes, 1975) The hydrophilic head group of the surfactant can also influence penetration rate. (Prottey and Ferguson, 1975) Once the surfactants have penetrated the skin they can induce large alterations in the barrier function. The most widely studied surfactants in this group are the alkyl sulfates, which can penetrate and destroy the integrity of the stratum corneum within hours following application. For example, sodium dodecyl sulfate can significantly enhance the permeation of several diverse chemicals, including water, sodium chromate, chloramphenicol, naproxen, and naloxone. As with their intrinsic permeability, the degree of barrier alteration induced by these surfactants is dependent on the alkyl chain length. This is well demonstrated in the work of Scheuplein and Dugard (1973), who showed that the electrical conductance of human skin could be enhanced considerably in the presence of

5 mM sodium dodecyl sulfate and sodium tetradecyl sulfate, but not by the octyl, decyl, or hexadecyl analogs. These effects are somewhat reversible upon removal of the surfactants, suggesting that removal of stratum corneum lipids is not significant to their mechanism of action. Rather, the action of these surfactants on skin is undoubtedly related to their ability to interact with and bind to epidermal proteins. This is particularly true for sodium dodecyl sulfate, which is widely used in biochemical research as a tool for isolating membrane-bound proteins and as an adjuvant in polyacrylamide gel electrophoresis. A detailed model for the interaction of surfactants with keratinous has been given by Breuer (1979), and a unique theory to explain the optimal interaction by dodecyl based surfactants has been presented by Dominguez et al.(1977). The latter group have postulated that by adopting a compact configuration the dodecyl chain can penetrate deeper into skin and interact more easily with epidermal proteins. This theory, however, is speculative and, as pointed out by Cooper and Berner (1985), the mechanism is questionable because maxima in other solution properties (partition coefficients, CMC) do not occur. Whatever the mechanism whereby the dodecyl chain produces optimal interaction with proteins, it remains to be discussed why this interaction should lead to a reduction in the barrier properties of skin.

Most anionic surfactants can induce swelling of the stratum corneum and the viable epidermis (Gibson and Teall, 1983; Rhein et al., 1986; Blake-Haskins et al., 1986). This is not the case for cationic or nonionic surfactants, suggesting that ionic effects play a primary role in the swelling mechanism. It has been postulated that the hydrophobic interaction of the alkyl chains with the substrate leaves the negative end group of the surfactant exposed, creating additional anionic sites in the membrane (Rhein et al., 1986). This results in the development of repulsive forces that separate the protein matrix, uncoil the

filaments, and expose more water-binding sites, possibly increasing the hydration level of the tissue. Separation of the protein matrix would also result in a disruption of the long-range order within the keratinocyte, possibly leading to increased intracellular diffusivity and enhanced percutaneous absorption. Although significant increases in skin penetration can be achieved using anionic surfactants, it is unlikely that they will find a use in transdermal therapeutic systems, primarily because of their irritation potential (Novak and Francom, 1984).

Surfactants can influence the barrier function of the skin and induce an increased transepidermal water loss. Loden (1990) studied the simultaneous penetration of water and sodium lauryl sulfate (SLS) (0.1, 1.0, and 10.0 % w/w) through isolated human skin by using tritiated water and ^{35}S -labeled SLS. The amount of SLS penetrating the skin was 50-100 times higher from 1.0 % SLS than from 0.1 %. Increasing the concentration from 1.0 to 10 % increased the amount penetrating ten times. The rate of penetration also increased with respect to time, indicating increasing damage to the skin over the duration of contact. The results indicating that skin damage from washing solutions is not linearly related to the surfactant concentration in the solution. The monomer activity of SLS was not constant above critical micelle concentration (CMC) but increases when the concentration of micelles increases.

Chowhan and Pritchard (1978) studied about the effect of surfactants on percutaneous absorption of naproxen through excised human skin. Four skin cells, taken from the same piece of skin, were used for rate control and for each surfactant under study. For comparison, the mean flux of control in such experiment was taken as one, and the relative flux was then calculated. The

concentration of all surfactants was above the critical micelle concentration (CMC). The effect of nonionic, cationic, and amphoteric surfactants on the relative flux of naproxen was small. The lower relative flux with certain surfactants may be due to the lowering of the thermodynamic activity of naproxen by complexation or other interactions with the skin. Above of CMC, proportionately less surfactant is available for interaction. The anionic surfactants, sodium laurate and sodium lauryl sulfate, increased the *in vitro* flux of naproxen appreciably.

2. Cationic Surfactants

Cationic surfactants are reputedly more irritating than the anionics and they have not, therefore, been widely studied as skin penetration enhancers. Despite this, they have been shown to enhance the permeation of several chemicals, including sodium and potassium ions, sodium chromate, and naloxone. Cetyltrimethylammonium bromide (CTAB), for example, has been shown to bind more readily to epidermal protein than sodium dodecyl sulfate (Wood and Bettley, 1971), but does not appear to cause swelling, suggesting a different mode of action for the two ionic surfactants. In contrast to the alkyl ammonium bromides some cationic surfactants, such as long-chain alkyl amines, appear to be much less irritating, and are widely used in cosmetic formulations.

The fact that many of these long-chain alkyl amines are protonated at the slightly acidic pH of the skin surface (pH = 5-6) yet become deprotonated at neutral pH has stimulated investigations into the possibility of facilitating the transport of ionized drugs across skin (Barker and Hadgraft, 1981; Hadgraft, Walters, and Wotton, 1986). At the outer skin surface the amine has

the capacity to form an ion pair with the drug. The ion pair, which has an intrinsically higher solubility in the skin than does the parent anion, diffuses down its own concentration gradient to the inner layers of the stratum corneum. In this region the pH is 7.4, and the amine deprotonates, liberating the anion. Once it has deprotonated, the amine is free to travel back to the skin surface. Only small amounts of amine are required for this carrier mechanism, which has been shown to function in artificial membranes. There are also indications that it can function in skin. In the latter case the influence of Ethomeen S12 (N,N-bis [2-hydroxyethyl] oleylamine) on the skin permeation of sodium salicylate was studied using conventional Franz-type diffusion cells. Pretreatment of the skin with the amine markedly increased the permeability coefficient of the salicylate. However, it was also reported that the skin permeation of caffeine, a weakly basic drug, could also be significantly enhanced following pretreatment with the amine, although permeation of the xanthine across an artificial lipid membrane was unaffected. It is likely, therefore, that Ethomeen S12 exerts an effect upon the stratum corneum which is unrelated to its carrier properties. The skin penetration rate of salicylate can also be enhanced by several related amines, including oleylamine (a primary amine with a monounsaturated C18 alkyl chain), polyoxyethylene-5-oleylamine (Ethomeen S15), and bis-(2-hydroxyethyl) cocoamine (Ethomeen C12), the latter being the most effective. From these limited data it is difficult to confer any definite structure activity relationship for the alkyl amines, but it is apparent that increasing the degree of ethoxylation reduces the efficiency of these compounds to act as skin-penetration enhancers. (Hadgraft, Walters, and Wotton, 1986) With the primary amines, as with most surfactant species, the dodecyl analog appears to be a more effective enhancer than those with a longer alkyl chain (Aungst, Logers, and Shefter, 1986).

In an attempt to enhance the skin permeation rate of ionized indomethacin by ion-pairing, a group of cationic surfactants were evaluated to determine their potential as counter-ions. Permeation rates through hairless rat skin were measured *in vitro*. The ion-pairing agents used were all quaternary ammonium compounds. Most enhanced the skin permeation of indomethacin to varying magnitudes and through different mechanisms. Some involved increased drug solubilization in the aqueous vehicle while others demonstrated permeability increase as a function of partitioning. One surfactant did not exert an apparent effect on any of the permeation parameters. Of Arquad[®] 12, Arquad[®] 16, Cetylpyridinium chloride[®], and Benzalkonium chloride[®], only the last surfactant clearly indicated its ability to pair with indomethacin (Tan, Liu, and Chien, 1993).

3. Nonionic Surfactants

On the three major classes of surface-active agents, nonionics have long been recognized as those with the least potential for irritancy. They can, however, have a profound effect on the permeability characteristics of several biological membranes, including gastro-intestinal mucosa (Walters, Dugard, and Florence, 1981; Wilson and Thomas, 1984; Whitmore, Brookes, and Wheeler, 1979; Touitou, Donbrow, and Rubinstein, 1980), erythrocytes, and goldfish gill epithelium (Florence and Gillan, 1975; Walters, Florence, and Dugard, 1982). The mode of action of these surfactants appears to be linked to their ability to increase membrane fluidity and their capacity to solubilize and extract membrane components. While this can sometimes result in severe toxicological responses, their action on skin is somewhat less dramatic. Polyoxyethylene nonionic surfactants possess a greater intrinsic skin permeability than anionic counterparts (Black and Howes, 1979), and they are excreted mainly via urine.

Although there are many different types of nonionic surfactants, the majority of studies concerning their effects on biological systems are limited to four principal series. These include the polysorbates, which are mixtures of partial esters of sorbital and its mono- and dianhydrides condensed with approximately 20 mol of ethylene oxide; polyethoxylated alkyl ethers and esters, in which the alkyl chain can be either saturated, unsaturated, branched or linear; polyethoxylated alkyl phenols, in which the hydrophobic group is normally octyl or nonylphenol; and poloxamers, polyoxyethylene-polyoxypropylene block copolymers, in which the polyoxypropylene chain acts as the hydrophobic moiety.

As with many surfactant systems, the polysorbates have been shown to enhance, retard, or have no effect on skin permeability rates. In his studies on the biochemical consequences of surfactant exposure, Mezei observed that polysorbate 85 could increase the rate of transepidermal water loss across both rabbit and human skin (Mezei and Ryan, 1972; Ryan and Mezei, 1975). This was attributed to surfactant-membrane interaction, and it was found that the effect was only partially reversible on cessation of surfactant exposure. Similarly, polysorbate 20, 40, 60, and 80 increase the flux of hydrocortisone and lidocaine across hairless mouse skin (Sarpotdar and Zatz, 1986). When applied in conjunction with propylene glycol, the surfactants were more effective enhancers, presumably because of the higher concentration of monomer in these systems. When present at concentrations of 0.5% and 1.0%, polysorbate 80 can increase the skin penetration rate of chloramphenicol, but at lower concentrations causes a slight reduction (Aguilar and Weiner, 1969). Polysorbate 20 has no effect on the permeation of naloxone, nor was polysorbate 60 effective on the percutaneous absorption of naproxen. The conflicting results for the polysorbates can be partially explained by a

consideration of their surface properties. There is little doubt that monomers of this group can interact with and alter the barrier properties of skin the micelles, however, have a marked solubilizing capacity which for interacting molecules can lead to a significant decrease in permeant thermodynamic activity in the vehicle.

To assess the penetration-enhancing effect of the nonionic surfactants Cetiol HE and cetareth 20 (Eumulgin B3) on the stratum corneum, interaction of these surfactants and their 1:1 mixture with human stratum corneum was assessed using differential scanning calorimetry. It was found that Cetiol HE influenced the lipid bilayers. In contrast, neither cetareth 20 nor the surfactant mixture disturbed the stratum corneum thermal behavior. It was concluded that Cetiol HE has possible penetration-enhancing capacities (DeVos and Kinget, 1993).

4. Fatty Acids and Alcohols

Long-chain fatty acids have been shown to be effective penetration enhancers for a variety of drugs (Aungst, Rogers and Shefter, 1986 ; Cooper, Merritt, and Smith, 1985; Bennett and Barry, 1985), provided they are applied in a suitable cosolvent such as propylene glycol. This is also true for the corresponding alcohols. In their comprehensive study on naloxone permeation across human skin, Aungst et al. (1986) examined the effects of a series of saturated fatty acids ranging from heptanoic (C7) through stearic (C18). Maximum enhancement of flux was obtained for the C12 analog, lauric acid. This was also true for the long-chain alcohols, but the degree of enhancement was somewhat less. The presence of cis double bonds in the alkyl chain appears to increase the degree of enhancement over the corresponding

saturated acid or alcohol. Thus, for the C18 acids, maximal effects are obtained with linoleic (cis,cis-9,12-octadecenoic), whereas with the alcohols linolenyl (all cis-9,12,15-octadecenoyl) appears optimal.

Cooper (1984) has examined the effect of a series of saturated and unsaturated fatty acids on the skin permeation of salicylic acid. It was found that the degree of penetration enhancement is dependent on several factors, including chain length, position, type (cis/trans) and number of double bonds.

Fatty acids and alcohols have also been shown to be effective skin-penetration enhancers for acyclovir, mannitol, hydrocortisone, and nicardipine. The mode of action is undoubtedly upon the lipids of the stratum corneum, and their relative effectiveness, as with nonionic surfactants, appears related to the ease with which they partition into these phases.

Seki et al. (1990) has examined the enhancement of zidovudine TDDs in rat and human skin. It was found that the permeation of AZT is enhanced in both rat and human by the addition of oleic acid.

A number of compounds including aliphatic esters, alkanols and amides were investigated for their ability to enhance the *in vitro* permeation rate of verapamil across hairless mouse skin. Shah, Tojo, and Chien (1992) found that while most of the compounds increased the solubility of verapamil in skin and hence its partition coefficient from the delivery system, the lauric derivatives also increased its diffusivity in skin. Permeation of verapamil from delivery systems containing these lauric derivatives was further investigated through delipidized and stripped skin. It was found that most of these derivatives enhanced the permeation of verapamil across both whole and delipidized skin, indicating that in addition to their action on the skin lipids, they must also have some effect on the proteins in the stratum corneum.

Soni, Jain, S.K., and Jain, N.K. (1992) studied about effect of penetration enhancers on transdermal delivery of timolol maleate. The results indicated that the drug penetrates poorly through human cadaver skin. However, skin penetration enhancers such as dimethyl sulfoxide (DMSO), Oleic acid (OA), and lauryl chloride (LC) enhanced the permeability of timolol maleate (TM) through human cadaver skin. The permeation enhancement of drug was maximum by lauryl chloride amongst the three enhancers. Moreover, lauryl chloride increase in the time of application and concentration on skin. The change in lag time was also observed.

Urban et al. (1991) studied about the influence of enhancers on the physicochemical properties and on the release of suppositories of clomipramine hydrochloride. These experiments showed in one hand that it was impossible to choose one or several enhancers among the assayed ones, using only the measure of the physicochemical properties of the suppositories containing an active materials as soluble as the clomipramine HCL, in the other hand the *in vivo* experiment could be useful to study the optimization of the formula by the use of several enhancers.

***In Vitro* Skin Permeation Study of Transdermal Drug Delivery System**

The aim of *in vitro* experimentation in TDDs is to understand and/or predict the delivery and penetration of a molecule from the skin surface into the body via the skin of a living animal (Gummer, 1989).

Drug absorption by the skin can be measured directly in living human or animals by analyzing the drug concentration profile in the blood or in the urine following topical administration. However, a quantitative assessment of the mechanisms and rates of transdermal permeation of drug can be achieved

by analyzing the drug permeation profile through an excised skin mounted on a diffusion cell, without any unwanted complications from the specific pharmacokinetic rate processes, such as distribution, metabolism and excretion of a drug in the body (Keshary and Chien, 1984).

In addition, *in vitro* skin permeation study 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.

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

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

Diffusion Cell

A variety of diffusion cells used for the *in vitro* study of percutaneous absorption are commercially available. Early model, have been designed by Scheulein (Chien and Valia, 1984). Later, several diffusion cell have been developed.

Franz (1975) had designed an upright-type diffusion cell, one of the most frequently used for skin permeation studies.

Michaels, Chandrasekaran, and Shaw (1975) fabricated a diffusion cell which was equipped with a pair of teflon impellers to stir the solution in both donor and receptor compartments.

Dunheim et al. (1980) developed a miniature diffusion cell in which each donor and receptor compartment had a volume of only 1.5 ml.

Keshary and Chien (1984) developed a finite-dosing cell for *in vitro* skin permeation studies. For reasons, to overcome the deficiencies observed in the commercially available Franz diffusion cell and to accomplish the solution hydrodynamics and temperature control required in studying the rate profiles of skin permeation.

Results of comparative studies indicated that the improved diffusion cell, named Keshary-Chien diffusion cell, can achieve and maintain the target body temperature on the skin surface and in the receptor solution, which cannot be accomplished by the Franz diffusion cell. Solution mixing efficiency was substantially improved, so the drug distribution and concentration homogeneity could be achieved in the Keshary-Chien diffusion cell within a duration four times shorter than in the Franz diffusion cell; and a 3-fold reduction in the thickness of the hydrodynamic boundary layer was achieved, so the effect of mass transfer in the hydrodynamic boundary layer on the skin permeation rate profiles was minimized.

The controlled release and skin permeation of nitroglycerin from four marketed transdermal therapeutic systems were investigated. As the result of the improvements in solution hydrodynamics and temperature control, the rates of release and skin permeation of nitroglycerin from the controlled-release transdermal therapeutic systems were enhanced substantially; so, the skin permeation rate profiles could be realized with minimal effect from the mass transfer process.

Skin model

The preparation of skin model for use in a diffusion cell is an important step for *in vitro* percutaneous absorption study.

In general, excised human skin is the most valuable, preferred membrane for *in vitro* skin permeation study. However, human skin is short supply and has a variety condition that could make a large variation in permeability between individual. 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).

In vitro permeation studies are possible to work with animal skin, in particular hairless mouse, guinea pig, fuzzy rat, rabbit, monkey and miniature pig. Throughout the history of TDDs investigators have sought to find a predictive correlation between the penetration of molecules through animal and human skin. Although there exists a number of similarities, there is as yet no animal skin that completely mimics the penetration characteristics of human skin (Gummer, 1989).

Skin is usually removed from the animal by blunt dissection, although dermatome sectioning may be used. *In vitro* experiments differ widely in the thickness of skin used though virtually all hinge on the now accepted dogma that the principal barrier to penetration is the stratum corneum.

Often, full-thickness skin is used in an absorption study, most chemicals do not diffuse completely through the skin when applied *in vivo*. Most of an absorbed chemical is taken by the blood in the capillaries of the skin at a depth of 200 μm . This is a much shorter distance for diffusion than

the 2-3 mm of full-thickness human, pig, and rat skin. The stratum corneum is thought to be the primary barrier absorption of water and other polar compounds. It is composed of nonliving cornified tissue that is enriched in its solid composition. The unique barrier properties of the stratum corneum are due chiefly to this lipodal material (Bronaugh and Stewart, 1986).

Owing to the constraints of *in vitro* apparatus on penetration, it is often desirable to reduce skin thickness without disturbing the barrier properties.

In Vitro Studies

Many studies developed TDDs and evaluated *in vitro* experiments, such as;

Mueller, Roberts, and Acott (1990) described about a diffusion cell, term the patch cell, suitable for conducting *in vitro* percutaneous absorption testing of nitroglycerin TDDs using skin from man, hairless mice and Yucatan pigs. In addition, the Patch Cell can be utilized for batch-to-batch quality control testing of nitroglycerin TDDs. The diffusion cell is incorporated into a laboratory robotics workstation that performs unattended, automated sampling of up to 24 diffusion cells over a 24-hour period. The method, referred to as the Patch Cell Method, has been used to measure the *in vitro* percutaneous absorption of nitroglycerin TDDs from two commercially available nitroglycerin delivery patches through hairless mouse skin, Yucatan pig skin and human stratum corneum. The results indicate that the Patch Cell Method, in addition to being useful for *in vitro* percutaneous absorption assays, is also useful for direct release testing into water.

Valia and Chien (1984) studied about long-term skin permeation kinetics of estradiol:(II) kinetics of skin uptake, binding, and metabolism. The results concluded that the stratum corneum plays a rate-limiting role in the uptake and binding of estradiol and its metabolism to estrone by the skin. The mechanisms of the uptake and binding of estradiol to whole skin tissue (with stratum corneum) showed a sex dependence, with the rate higher in the female than in the male mouse. On the other hand, no sex-dependent difference was observed in the mechanism and rate of metabolism to estrone. After stripping off the rate-limiting stratum corneum, the mechanism of the uptake, binding and metabolism of estradiol all became sex dependent with identical rates observed between male and female mice in the initial 14 hrs of the experiment. Between 14 to 21 hrs, the rates increased substantially with the female skin, but not with the male skin. The increase in the rate of uptake of estradiol by the female skin was related primarily to the increased rate of metabolism of estradiol to estrone.

A transdermal drug delivery system of diclofenac was developed for prolonged and controlled release of diclofenac. The designed system essentially based on polymeric pseudolatex dispersion. The formulation variables that could effect the formulation stability vis a vis drug release were studied. To achieve the desired release rate, different combination of hydrophilic and hydrophobic polymer were used for the preparation of pseudolatex system. The designed system exhibited linear relationship between drug release (Q) vs square root of time ($t^{1/2}$). The product having skin permeability rate 0.188 mg/h/cm^2 was selected for the *in vitro* anti-inflammatory activity and *in vivo* evaluation. The system could maintained a constant and effective plasma level for 24 hours. The effective drug plasma concentration was monitored periodically. The study revealed that designed

pseudolatex transdermal drug delivery system of diclofenac could be used successfully with improved performance and hold promise for further studies (Vyas, Gogoi, and Jain, 1991).

Materials Information

1. Nifedipine

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

Chemical name :- 3,5-Pyridinedicarboxylic acid, 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-, dimethyl ester
 :- Dimethyl 1,4-dihydro-2,6-dimethyl-4-(*o*-nitrophenyl)-3,5-pyridinedicarboxylate

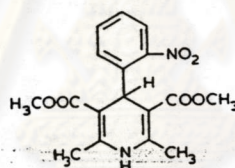


Figure 7 Structure formula of nifedipine

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

Molecular weight :- 346.34

1.2 Physicochemical Properties

NFP is a calcium-channel blocking agent. The drug occurs as a yellow, crystalline odorless and tasteless powder. It has melting range between $171\text{ }^{\circ}\text{C}$ and $175\text{ }^{\circ}\text{C}$ (United States Pharmacopoeia Convention, 1990; American Society of Hospital Pharmacists, 1992; Reynolds, 1989).

Solubility : NFP is practically insoluble in water, soluble in acetone and chloroform, and slightly soluble in ethanol and methanol (Reynolds, 1989;

McEvoy,1987).

Stability : NFP is photo-sensitive compound. NFP solution is more sensitive to light than its crystalline form. NFP undergoes ready photochemical conversion to nitropyridine derivative when exposed to ultraviolet light and to nitrosopyridine derivative when exposed to day light (Majeed et al.,1987; United State Pharmacopoeia Convention,1990).

Storage : NFP should be stored in tight, light-resistant containers at a temperature of 15-20 °C (Reynold,1989).

1.3 Pharmacology

Nifedipine is a dihydropyridine derivative with potent coronary and peripheral arterial vasodilator properties and little or no direct depressant effect on S-A or A-V nodal function. Because of its relatively selective action on vascular smooth muscle, reflex sympathetic activity tends to counteract its pronounce direct negative inotropic effect. Negative inotropic effects may be evident, however, in patients with severe heart failure or aortic stenosis and in those who are receiving other myocardial depressant drugs. Nifedipine reduces blood pressure and may reflexly increase heart rate. It increases myocardial oxygen supply by increasing coronary blood flow and decreases myocardial oxygen demand by reducing afterload (Comess and Fenster, 1982; Reynolds, 1989).

1.4 Pharmacokinetics

Absorption : Approximately 90% of an oral dose of nifedipine is rapidly absorbed from the GI tract following oral administration of the drug as conventional capsules. Only about 45-75% of an oral dose as conventional capsules reaches systemic circulation as unchanged drug since nifedipine is metabolized on first pass through the liver. Times for maximum drug

concentrations usually are within 0.5-2 hours after oral administration of drug as conventional capsules. Food appears to decrease the rate but not the extent of nifedipine absorption administered as conventional capsules.

Distribution : Binding of nifedipine to plasma proteins is concentration dependent and ranges from 92-98%. Protein binding may be reduced in patients with renal or hepatic (e.g., liver cirrhosis) impairment.

Elimination : In patients with normal renal and hepatic function, the plasma half-life of nifedipine is 2-5 hours. The drug is rapidly and completely metabolized at the liver to be the inactive metabolites. Approximately 70-80 and 15% of an oral dose of nifedipine are excreted as metabolites in urine and feces, respectively. Nifedipine appears to be negligibly removed by hemodialysis or hemoperfusion. In patients with hepatic impairment, elimination of the drug may be altered. The elimination half-life of nifedipine has been reported to increase to 7 hours in patients with liver cirrhosis (American Society of Hospital Pharmacists, 1992).

By administration NFP in the form of long-acting slow-release capsules within 24 hours, the steady blood level can be achieved in the range of 20-30 ng/ml within the therapeutic range (15-100 ng/ml) of the drug (Opie, 1990).

1.5 Uses

NFP is use in the treatment and prophylaxis of angina pectoris and hypertention. It is an effective agent for the treatment of angina caused by coronary artery spasm (Bayley, Dobbs, and Robinson, 1982; Comess and Fenster, 1982; Reynolds, 1989).

1.6 Adverse effects

The mild side effects such as headache and flushing occur in about 5 percent of long-term users. Other side effects are uncommon and include hypotension, nausea, vomiting, sedation, and fatigue. Side effects are intolerable to about 5 percent of patients. Serious adverse reactions requiring discontinuance of nifedipine therapy or dosage adjustment are relatively rare. An increase in the frequency, intensity, and duration of angina, possibly resulting from hypotension, has occurred rarely during initiation of nifedipine therapy (Comess and Fenster, 1982; Temkin, 1982; United Pharmacopoeia Convention, 1990; Reynold, 1989).

1.7 Administration

Nifedipine is administered orally. The drug also has been administered sublingually or intrabuccally (e.g., for rapid reduction of blood pressure). When nifedipine is administered sublingually or intrabuccally, the conventional liquid-filled capsule must be punctured, chewed, and/or squeezed to express the liquid into the mouth. NFP extended-release tablets should be swallowed intact and should not be chewed, crushed, or broken (American Society of Hospital Pharmacists, 1992; United State Pharmacopoeia Convention, 1990).

1.8 Dosage

The usual initial dose is 10 mg, three to four times daily. The dose is titrated upwards until symptoms are controlled or adverse effects appear. The usual maximal dose is 120 mg daily. the dose for extended-release capsules is about the same as the total daily dose of standard capsules (Comess and Fenster, 1982; Opie, 1990).

1.9 Development of NFP Preparations

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

Two kinds of granules were prepared: one with pH-dependent release and the other with pH-independent release. The effects of the mixing ratios of the polymers and the drug contents in the granules were examined *in vitro*. In both granules, the release was decreased with the increment of ethylcellulose. These sustained-release granules and the commercial fine granules were orally administered to rabbits. In the plasma levels of nifedipine detected from rabbits orally administered sustained-release granules, a reduced but sustained level was observed. This indicates the sustained release of the drug from the granules *in vivo*. Furthermore, the plasma profiles of nifedipine indicated that the granules with pH independent release were superior to that with pH-dependent release with respect to prolonging the effective plasma levels and to minimizing the intersubject variations (Kohri et al., 1986).

The release characteristics of nifedipine sustained-release granules, composed of ethylcellulose, hydroxypropylmethyl cellulose and corn starch, were examined *in vitro* as well as in healthy subjects. The release of nifedipine from the granules *in vitro* was not first-order, but a linear relationship up to about 40 % release was obtained based on the Higuchi equation. The release rate was not strongly influenced by pH, stirring speed, surfactant or ionic strength. These granules were administered to healthy subjects and the plasma levels of nifedipine were compared with those after administration of nifedipine soft gelatin capsule. Plasma levels following the administration of the granules were prolonged as compared with those in the case of the soft gelatin capsule, and plasma levels of about 13 ng/ml at 12 hours post-dosing

were detected. The known problem of marked inter-subject variability of the plasma levels was not encountered after administration of the sustained-release granules, presumably due to the multiple-units dosage form and the established low sensitivity of the drug release rate to the *in vitro* environment, i.e., pH, stirring speed, surfactant and ionic strength (Kohri et al., 1987).

Pre-formulation studies were carried out to develop a sustained-release dosage form of nifedipine with good bioavailability. Granules were prepared by spray-coating nifedipine-enteric coating agent solid dispersion on an inert core material. These granules results in prolonged absorption of the drug with good bioavailability in beagle dogs and human volunteers. This absorption characteristic arises from the pH dependency of the dissolution behavior of these solid dispersions. That is, dissolution of nifedipine was suppressed in acidic medium and showed supersaturation in comparatively neutral medium. It was confirmed that the granules were transported through the gastrointestinal tract and showed prolonged drug release behavior with good bioavailability (Hasegawa, Nakagawa, and Sugimoto, 1985)

Attempt was made to develop the solid dosage forms of nifedipine which showed good absorption rate and total bioavailability. The dissolution rate of PVP-nifedipine coprecipitates exhibited rapid dissolution rate. Nifedipine in the coprecipitate was chemically stable to heat and humidity, but the dissolution rate of nifedipine from the coprecipitate stored at 21° and 75 % R.H. markedly decreased. The gastrointestinal absorption of nifedipine in beagle dogs after oral administration of the coprecipitate was increased than that after oral administration of the physical mixture (Sugimoto et al., 1980).

By the use of solid dispersion systems, suppositories having both a fast release and a sustained release of nifedipine were developed. Namely, cellulose acetate phthalate (CAP) - polyethylene glycol (PEG) matrix was prepared as a suppository base by using PEG-4000 as a water-soluble carrier and CAP as a poorly soluble carrier. Conventional suppositories, three kinds of CAP-PEG matrix suppositories, and double layer suppositories (D-15) including nifedipine only in the outside layer were prepared, and the sustained-release effect and bioavailability of each suppository were examined in rabbits. The D-15 enhanced the bioavailability of nifedipine and also gave a sustained-release plasma level of nifedipine (Umeda et al., 1985).

Double layer suppositories (D-15) of nifedipine, prepared by using a solid dispersion system of polyethylene glycol 4000 as a water-soluble carrier and cellulose acetate phthalate as a poorly water-soluble carrier, were administered to healthy volunteers, and their sustained-release characteristics, bioavailability and clinical utility were investigated. It was found that D-15 was able to maintain a therapeutically effective level of NFP from 30 min to 10 hr without causing an excessively high peak level and offered good bioavailability. From the results of pharmacokinetic analysis by using the compartment model method, it appeared that the plasma concentration-time course after rectal administration of D-15 is satisfactorily accounted for by a one-compartment model with first-order release and absorption steps. The value of release rate constant of D-15 obtained was smaller than that of absorption rate constant, and the sustained-release effect was apparently attributed to the slow release of NFP from the suppository. The plasma level of NFP rapidly decreased as removing D-15, and the plasma level showed hardly any irregularities arising from the removal and the renewal of suppositories. Accordingly, it was concluded that D-15 is an effective

sustained-release dosage form and represents a convenient mode of therapy with reduce frequency of drug administration and reduced risk of side-effects (Ohnishi et al., 1987).

Kleinbloesem et al.(1984) studied NFP kinetics and dynamics during rectal infusion to steady state with an osmotic system. NFP was given rectally through an osmotic system at a zero-order rate for 24 hr. Steady-state plasma concentrations of approximateley 20 ng/ml were achieved within 6 to 8 hr. Our data indicate that nifedipine lowers blood pressure in subjects with normotension and that it is possible by infusing the drug at a relatively low rate to dissociate its effect on blood pressure from that on heart rate.

2. Poloxamer

2.1 Formula

Chemical name :-

Polyoxyethylene-polyoxypropylene-polyoxyethylene block copolymers

Empirical formula :-

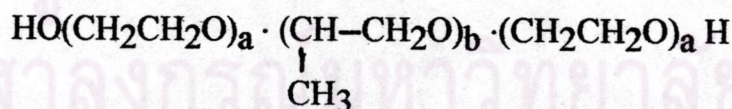


Figure 8 Structure formula of poloxamer

For poloxamer F-127 :- a=35, b=30

Average molecular weight :- 11,500

Trade name :- Pluronic, Lutrol

2.2 Characteristics

Poloxamer is white, waxy, free-flowing prilled granules or cast solid; practically tasteless and odorless. Poloxamers are more soluble in cold water than hot water. In general, they are soluble on aromatic solvent such as benzene, toluene and xylene, chlorinated solvents, acetone, alcohol, propylene or hexylene glycol, cyclohexanone. They are insoluble in ethylene glycol, kerosene, mineral oil. Most of them are physically stable, stable to acids, alkalies and metabolic ions. They have low acute oral toxicity and low potential to cause irritation or sensitization (BASF, 1987).

Reverse thermal gelation is one of the characteristics of aqueous solutions of this compound, e.g. 20-30% Pluronic F-127 gels are fluid at refrigerator temperature (4-5°C), but are highly viscous gels at room temperature and body temperature. The gelation at elevated temperatures is reversible upon cooling. This reversible sol-gel property allows cool solutions to flow onto the skin or into wounds, where they can seek intimate surface contact before warming to form a non-occlusive gel (Chen-Chow and Frank, 1981).