CHAPTER II

LITERATURE REVIEW

1. Literature review of minoxidil

Minoxidil, a piperidino-pyrimidine derivative, is a strong vasodilator used for treatment of resistant hypertension. Minoxidil has gained the attention of dermatology because it causes a reversible hypertrichosis in most patients who received systemic therapeutic doses for one month or longer (Dargie, Dollery, and Daniel, 1977). In one of many case reports noticed that body hair became more profuse, longer, coarser and darker, particulary on the forearms (Burton and Marshall, 1979).

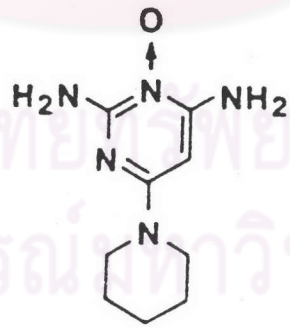


Figure 1. Structure of minoxidil ($C_9H_{15}N_5O$, MW = 209.3)

Weiss et al. (1984) reported that in 48 patients with alopecia areta treated with 1% minoxidil topical solution, twenty-five patients had terminal hair regrowth. In 11 of these 25 patients, it was cosmetically acceptable. Hair regrowth began approximately 2 months after the initiation of treatment and was not uniformly well maintained after the treatment was terminated. No systemic side effect was seen. Also, several studies have indicated that patients with male pattern baldness using topical minoxidil solution show a significant increase in the number of terminal hairs (Olsen et al., 1985; De Villez, 1985; Katz et al., 1987; Rumsfield, West, and Fiedler-Weiss, 1987; Kreindler, 1987)

Shupack et al. (1987) studied in 58 men with Hamilton scale type III vertex or type IV male pattern baldness to determine the dose response activity of low concentrations of topical minoxidil in promoting hair regrowth and found that patients using 0.1%, 1%, and 2% topical minoxidil solutions showed a significantly greater difference in the mean increase of nonvellus hair growth in comparison with those using 0.01% minoxidil or placebo. Moreover, there was a clear dose-response correlation for the increase of nonvellus hairs in the 0.1%, 1% and 2% minoxidil treatment groups. However, only patients treated with 1% and 2% solution were clinically perceptible in hair growth. Katz et al. (1987) also reported that 2% minoxidil solution was equal to or more efficacious than 3% minoxidil solution .

Potential mechanisms proposed of minoxidil-induced hair growth in such divergent diseases as alopecia areata and alopecia androgenetica are that it either acts on the hair follicles per se or has more widespread mechanisms such as a direct effect on cellular function. There was no any evidence for a systemic androgen effect (Weiss et al., 1984). In vivo, topical minoxidil therapy was associated with changes in the follicular epithelium, suppressed effect of lymphocyte-mediated immunologic phenomena and reopening of previously closed lumina of perifollicular vessels (Fiedler-Weiss, 1987)

In 1984, there was a 1% minoxidil solution used at Torbay hospital which was prepared from Loniten® tablets in mixtures of propylene glycol, indrustrial methylated spirit 95%, IMS and purified water. This preparation has been routinely assayed using a reverse phase HPLC method which comprised of Spherisorb 5ODS column, a mixture of 75 : 25 acetonitrile : 0.01 M KH_2PO_4 as solvent, adjusted to pH 3 with phosphoric acid, dectected with UV spectrophotometer at 280 nm and flow rate was 2 ml/min.

Stability data after 6 months indicated that minoxidil did not undergo any chemical degradation, however, the clear alcoholic solution developed a slight yellow coloration after 3 months, which gradually darkening (Haines-Nutt, Adams, and Bendell, 1984).

Another stability study was done by Pimolpan Pithayanukul (1988). Hydroalcoholic solution and hydroalcoholic gel of topical minoxidil were formulated and investigated the color change at room temperature (30°C) with naked eyes. From this study, it was found that minoxidil preparations containing propylene glycol turned into "pink" color which resulted from the oxidation reaction between minoxidil and propylene glycol. This oxidation reaction was catalysed by heavy metal impurities in propylene glycol and minoxidil themselves. However, the reaction could be inhibited by adding a chelating agent, 0.01% EDTA sodium and/or an antioxidant, sodium bisulfite more than 0.5%

Beside stability problems, topical minoxidil solutions also have low bioavailability since topically applied minoxidil is poorly absorbed through the skin. In man 97% of orally administered minoxidil was excreted in urine, but urinary excretion of 1% and 5% solutions of minoxidil labeled with ^{14}Carbon following two single applications was low, with mean values ranging from 1.6-3.9% of applied dose (Franz, 1985)

Other studies were done in patients with alopecia areata or alopecia androgenetica. These patients were treated with topical minoxidil 1%, 3% or 5% once or twice daily. Serum minoxidil concentrations very rarely exceeded 5 mcg/l and were frequently below detectable levels (Fiedler-Weiss et al., 1986; Novak et al., 1985; Weiss et al., 1984).

Animal studies have confirmed the low level of percutaneous absorption (between 5 and 36%) and showed that for rats and monkeys 30 to 78% of an administered dose was retained in the skin (Novak et al., 1985).

From the facts that minoxidil is insoluble in water (2.2 mg/ml), but slightly soluble in ethanol (29 mg/ml) and propylene glycol (75 mg/ml), the most frequently used solvent in topical solutions is ethanol. Since topical minoxidil solutions contained ethanol up to or more than 60%, propylene glycol 20% and water, volatile ethanol and water could rapidly evaporate from the application site. It caused relatively rapid compositional changes and hence the thermodynamic activity of minoxidil in the vehicle was forced through changes and crystallization might occur.

Chiang et al. (1989b) have confirmed this phenomena by observing crystal formation of minoxdil solutions under a microscope and following the weight of minoxidil solutions applied to a glass surface. It was found that 65% of the weight of the solution vehicle evaporated after the first 30 minutes and about 75% evaporated after 2 hours. For the 5% minoxidil solution, crystals were evident within 6-7 minutes, but no crystal was seen in 3% minoxidil solution after 2 hours.

These were accompanied to and confirmed the results from the study that the flux of minoxidil increased systematically from 0.5 to 3% concentration and then fell back abruptly at 5% concentration. In addition., there was no significant difference in the flux of minoxidil when its concentration in the non-volatile component, propylene glycol, was kept constant. This suggested that the evaporation

of the volatile ethanol and water in the vehicle played an important role in the delivery. However, the vapor pressure of propylene glycol (0.32 mmHg at 25°C) was sufficiently high to evaporate at an appropriate rate relative to it mass when it was spread as a thin film (Tsai, Cappel et al., 1992).

The original composition of 2% minoxidil in propylene glycol : ethanol : water (20 : 60 : 20, v/v) barely contained enough propylene glycol to dissolve all minoxidil after ethanol and water had evaporated. Thus, evaporation of propylene glycol further concentrated the solution to supersaturation, precipitated out the drug, and then stabilized the thermodynamic activity of the drug in the vehicle (Tsai, Cappel et al., 1992). The precipitation limited the amount of minoxidil that could be absorbed and led to poor percutaneous absorption.

Bioavailability assessment was done in vitro with 2 dosage forms of minoxidil preparations, ointment and solution. Chiang et al. (1989a) demonstrated that the flux of minoxidil through human cadaver skin from minoxidil suspended in ointment attained a steady state after an initial lag period of between 6 and 8 hours but steady delivery was not apparent for the minoxidil solution.

Indeed, the steady state for the ointment was unexpected since minoxidil was present in amounts greatly exceeding its solubility in the vehicle and the vehicle was compositionally stable. On the other hand, for minoxidil solution, the concentration of drug and composition of solvents varied systematically upon application to the skin. Thus, the thermodynamic activity of drug would be expected to radically change over the course of experiment.

Tsai, Flynn et al. (1993) found that minoxidil 0.02% solution was more efficiently delivered into hairless mouse skin than 2% solution. This was postulated that evaporation of the vehicle after application of the 2% minoxidil solution led to composition changes so drastic that saturation was attained quickly reaching

saturation within 30 minutes. Hence, it was quite resonable to assume that minoxidil permeation was from a saturated suspension in the case of initially 2% solution.

On the contrary, the percent saturation of minoxidil on skin surface from 0.02% solution gradually and continuously increased but never attained 50% of the saturation level.

Considering from what was mentioned, the increased efficiency of delivery of minoxidil from the lower concentration formulation suggested that the advantage of 2% formulation which had higher thermodynamic activity than the 0.02% solution disappeared substantially and abruptly upon precipitation of the drug. The driving force following minoxidil's precipitation no longer reflected all the minoxidil presented, and thus the delivery rate from the 2% formulation dropped.

2. Literature review of cyclodextrins

Cyclodextrins (CDs) are cyclic oligosaccharides, containing a mininum of 6 D-(+)-glycopyranose units attached by α-1,4-linkages produced by the action of the cyclodextrin-trans-glycosidase enzyme on a medium containing starch. Due to their particular conformation, CDs have characteristic of being able to include various kinds of molecule inside their hydrophobic cavity. These inclusion compounds have completely new pharmaceutical properties, but the most important one is increasing in water solubility and bioavailability.

2.1 Structure and physical properties

Three natural CDs are α-CD, β-CD and γ-CD, consisting of 6,7 and 8 glucose units respectively. These glucose molecules are linked by α-1,4-bonds, as a consequence of the C1-conformation of the α-D-glycopyranosyl residues and the lack of free rotation around glycosidic bonds, causing the formation of torus molecules (cone-shaped). The secondary hydroxyl groups (on the C2 and C3 atoms of the glucose unit) are situated on one edge and all primary hydroxyls on the other, as shown in Figure 2.

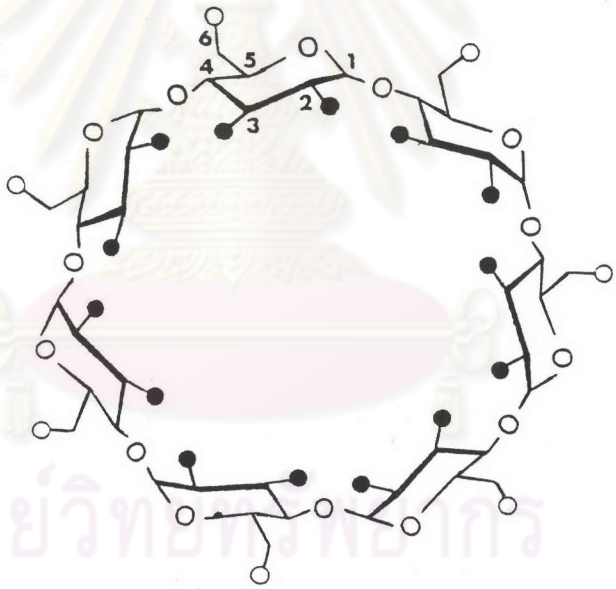


Figure 2. The structure and numbering of the atoms of β-CD;
(—O primary and —● secondary hydroxyl groups)

The cavity of the torus consist only of a ring of C3-H group, C5-H group and a ring of glucosidic oxygens. For this reason, the cavity of the torus is non-polar. This makes CDs exterior decidedly hydrophilic where as the interior of the cavity is rather hydrophobic. Free rotation of primary hydroxyls reduces the effective diameter of the cavity on the side they occur, while the secondary hydroxyl groups on the relatively rigid chains cannot rotate (Szejtli, 1982).

The dimensions of the CDs alter with the number of glucose units. Because of their different internal cavity diameter, each CD shows a different capability of complex formation with different sized guest molecules. Table 1 lists the some physical properties of CDs and some of their derivatives.

Table 1 shows that β-CD is the least soluble which results from intramolecular hydrogen bonds between the C2-OH group of one glucopyranoside unit and C3-OH group of the adjacent glucopyranoside unit. These intramolecular H-bonds stabilize the macrocycle of the CD molecule and turn it into a rigid structure.

2.2 Requirements for complex formation

The minimum requirement for inclusion complex formation is that the guest molecule must fit, entirely, or at least partially, into the CD's cavity. Stable complexes will not be found if guest molecules are too small because they would slip out of the cavity. Complex formation is also possible with bulky molecules if certain groups or side chains of those molecules can penetrate into CD's cavities (Szejtli, 1982).

In general, hydrophobic or non-ionized molecules have higher affinity to CD's cavities than hydrophilic ones or ionic species in aqueous solution (Bekers et al., 1991)

Table 1. Physical properties of the CDs and some derivatives.

	α	β	γ	DM-β[1)]	HP-β[2)]
Number of glucose residues :	6	7	8	7	7
Cavity dimensions (A°)					
cavity diameter :	5	6	8	6	6
height of torus :	7.9	7.9	7.9	10.0	
diameter of periphery	14.6	15.4	17.5		
Molecular weight :	973	1135	1297	1331	±1300
Aqueous solubility[3)] :	14.5	1.85	23.2	57	>50
Melting point (°C) :	275	280	275	295-300	
pKa[4)] :	12.3	12.2	12.1		
Half-life of ring opening[5)] (h) :	6.2	5.4	3.0	8.5	
Enzymetic hydrolysis[6)] :	negli-gible	slow	rapid		

1) heptakis-2,6-di-O-methyl-β-CD
2) 2-hydroxypropyl-β-CD
3) in grams per 100 ml water at ambient temperature
4) pKa : by potentionmetry at 25°C
5) Half-life of ring opening : in 1 N HCl at 60°C
6) by Aspergillus oryzae α-amylase

2.3 Binding forces of the complexes

The inclusion complex formation proceeds by an energetically favoured interaction of a relatively non-polar guest molecule with an imperfectly solvated hydrophobic cavity. Thus, the complexes should be stabilized by various intermolecular forces such as (Szejtli, 1982; Szejtli, 1988) :

a) Van der Waals interaction (including both permanent induced-dipole-dipole interactions and London dispersion forces) between the guest and host

b) Hydrogen bonding between the guest and the host

c) Replacement of high energy water molecules in CD's cavity by guest molecules, resulting in a favourable enthalpy change

d) Release of strain energy in the macromolecular ring of CD (changing from the high energy conformation of the CD-water complex to the lower energy conformation of the CD-guest complex)

2.4 Metabolism and toxicity of cyclodextrins

After oral administration, CDs did not result in acute or long term toxicity. Cyclodextrins were not hydrolysed during transit through the small intestine, hydrolysis occuring only in the colon. It seemed that α-CD was not (or only partially) degraded in the gastro-intestinal tract. Furthermore, α- and β-CD were poorly absorbed in the small intestine (Irie et al., 1988; Nakanishi et al., 1989).

Following parenteral administration of high dose CDs, signs of toxicity were observed (Szejtli, 1987). This toxicity was characterized by nephrosis

and hemolysis. Nephrosis in the kidney was a result of tubular reabsorption of the intact β-CD which precipitated due to its low aqueous solubilty. The hemolytic activity was observed to be in order of β- > α- > γ-CD.

2.5 Cyclodextrin derivatives

Natural CDs can be modified for many different purposes such as to improve water solubility or to decrease toxicity in parenteral applications.

Methylated CDs : introducing methyl groups onto the hydroxyls of C2, C3 or C6 prevents the formation of intra- and intermolecular hydrogen bonds between the hydroxyl groups and considerably changes the physicochemical properties of the initial CDs, especially increasing its solubility. 2,6-di-O-methyl-β-CD (DIMED) was found to be the least parenteral toxic among methylated derivatives (Bekers et al., 1991).

Hydroxyalkylated CDs (HA-CDs) : these derivatives were found to be powerful solubilizers of several drugs and no crystalline complexes precipitated at high concentrations of solubilizers (Yoshida et al., 1988). The nephrotoxicity and hemolytic activities of HA-β-CDs are less than that of natural CDs or DIMED (Yoshida et al., 1988; Uekama and Irie, 1990). Examples of this group are 2-hydroxypropyl-β-CD (HP- β-CD) and hydroxyethyl-β-CD (HE-β-CD).

Branched CDs : example of this groups are glucosyl, maltosyl, glucopyranosyl α- and β-CDs etc. All are more water soluble than γ-CDs. They possess less hemolytic properties than the parent CD.

Cyclodextrin polymers : these polymers contain at least 2 CD units. β-CD polymers with molecular weight of above 10,000 could only swell in water and form insoluble gels. Polymers with high molecular weight can be used as tablet excipients.

3. Literature review of cyclodextrin inclusion compounds

CDs have been in the interest of pharmaceutical sciences from their ability to form inclusion compounds with a wide variety of drug molecules to yield desirable properties on the drugs. Some pharmaceutical applications are given as a short outline here (Bekers et al., 1991; Szejtli, 1988) :

a) Transformation of liquid into powders.

b) Masking an unpleasant taste or odor of a compound.

c) Avoidance of an incompatibility of the uncomplexed compound with other drugs or excipients in a formulation.

d) Enhancement of stability of a compound which could otherwise be sensitive to temperature, hydrolysis, oxidation, etc.

e) Enhancement of water solubility.

f) Improvement in bioavailability because of an increase in water solubility.

3.1 Preparation of inclusion complexes (Darrouzef, 1993)

There are different methods to prepare inclusion complexes depending on the properties of the guest molecules.

a) In case of a water soluble active ingredient, the guest is added to a saturated aqueous solution of CD, and the mixture is agitated for several hours or even days, until spontaneous precipitation of the inclusion takes place. Sometimes

precipitation does not occur spontaneously, it is necessary to cool the medium or to induce evaporation by freeze-drying or spray-drying.

b) When the active ingredient is insoluble, it is necessary to use an organic solution of the active ingredient, which is poured, under agitation, into an aqueous solution of CD. Precipitation is obtained either spontaneously or by evaporation.

c) If the active ingredient is thermosensitive or hydrosensitive, kneading method is suitable. The active ingredient is adding to a slurry of CD and kneaded throughly to obtain a paste, then dried and washed with organic solvent to remove the free active ingredient.

d) Heating in a sealed container is the new method for preparing inclusion compounds. An example of this method is the preparation of CD-benzoic acid inclusion complexes by heating the ground mixture of drugs and CDs in closed system at 125°C for 1 hour.

3.2 Detection of inclusion complex formation

3.2.1 Detection of inclusion complexes in solid state

a) Infrared (IR) spectroscopy

This method is less clarifying than other methods. CD bands often changed slightly upon complex formation, and if the fraction of guest molecules encapsulated was less than 25%, bands which could be assigned to the included part of the guest were easily masked by the bands of CDs (Bekers et al., 1991).

b) X-ray diffraction

Comparison of the diffractograms is only possible if CDs and guest are treated both under identical conditions as the assumed complex. Preparation processes of inclusion compounds may change the crystallinity of the pure substance and may lead to different diffraction patterns (Szejtli, 1988).

c) Thermo-analytical methods

Thermo-analytical methods determine whether the guest undergoes some changes before the thermic degradation of CD. The changes may be melting, evaporation, decomposition, oxidation or polymorphic transition. The method consists of many techniques, such as thermal analytical system (TAS), thermo evolution analysis (TEA), differential scanning calorimetry (DSC), thermogravimetry (TG) and differential thermal analysis (DTA).

d) Thin layer and paper chromatography (TLC and PC)

e) Scanning electron microscopy

f) Wettability and dissolution tests

3.2.2 Detection of inclusion complexes in solution

a) Spectroscopic method

This method consists of Ultraviolet/Visible (UV/VIS) spectroscopy, Fluorescence spectroscopy, Circular dichroism (CD), Nuclear magnetic resonance (NMR) spectroscopy and Electron spin resonance (ESR).

b) pH-potentiometric titration

c) Electrochemistry (polarography and conductivity)

d) Microcalorimetry

e) Solubility method

If the solubility of a guest potentially increases with increasing CD concentrations, complex formation in solution is indicated (Bekers et al., 1991)

f) Surface tension technique

3.3 The complex stability constant

Effects which can be achieved by means of CD inclusion complex formation depend on the stability and solubility of the complex. A complexation may be defined as the reversible association of 'm' molecules of a substrate 'S' with 'n' molecules of a ligand species 'L' to form a new species 'S_mL_n' as shown in Eq. (1)

$$mS + nL \quad \overset{K_{m:n}}{\longleftrightarrow} \quad S_mL_n \qquad (1)$$

The equilibrium constant (complex stability constant, formation or association constant, $K_{m:n}$) for the interaction may be defined as

$$K_{m:n} = \frac{[S_mL_n]}{[S]^m[L]^n} \qquad (2)$$

As defined in Eq. (2), $K_{m:n}$ is a concentration equilibrium constant.

4. Literature review of phase solubility diagram

Many methodologies which have been mentioned in subheading 3.2 can be utilized to study the changes in the properties of the system. Since this study was directed toward use of complexation in altering solubility characteristics, subsequent discussion will be confined to the use of solubility method (Repta, 1981).

4.1 Experiment approach (Higuchi and Connors, 1965)

The common experimental set-up is a series of n containers selected and into each is placed an identical weight or volume of the solvent to be used, and a quantity of substrate well in excess of its solubility in the quantity of solvent used. To each except the first of the container in the series, varying quantities of the ligand is added, which is incrementally increased in each succeeding container. The containers are then tightly closed and equilibrated, usually with agitation at a constant temperature. After equilibrium has been attained, the solution phase in each container is analyzed for total substrate in solution.

Tha data are normally presented as phase diagram where the total substrate S_t in the solution phase is plotted as a function of the total added ligand L_t. The Y- intercept value is $[S]_o$, the inherent solubility of the substrate.

4.2 System type and phase diagram interpretation

Several different phase diagrams may be obtained from system which form complexes. Higuchi and Connors (1965) have divided the system into two main classes : type A and type B diagrams.

The type A phase diagram, shown in Figure 3, is obtained when the complex formed is soluble and does not form a precipitate regardless of the amount of

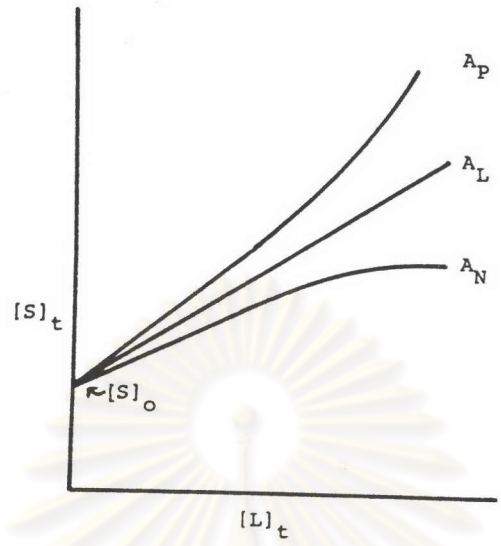


Figure 3. Schematic representation of the A-type phase diagram

($[S]_t$ = the concentration of the total substrate in the solution

$[L]_t$ = the concentration of the total added ligand)

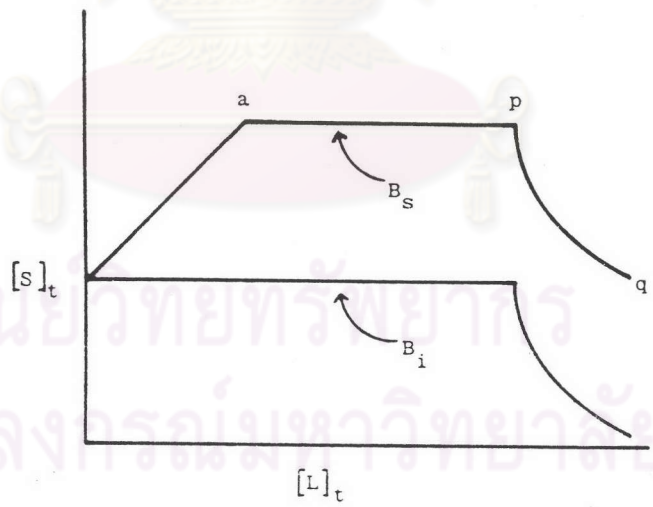


Figure 4. Schematic representation of the B-type phase diagram

($[S]_t$ = the concentration of the total substrate in the solution

$[L]_t$ = the concentration of the total added ligand)

ligand added. This can be subdivided according to the nature of the obtained phase diagram. The A_L type, exhibiting a linear relationship between S_t and L_t, is obtained when the complexes are a first order dependence on $[L]_t$. The A_P type, showing a positive deviation from linearlity, is obtained when the complexes formed containing more than one molecule of ligand. As the ligand concentration increases, the contribution of the higher order complexes increases. The remaining diagram, A_N type, exhibits a negative deviation which represents a decreasing dependence on ligand added at higher ligand concentrations. This may be explained on the basis of self association of the ligand at high concentrations (Higuchi and Connors, 1965).

In all systems giving rise to the A type diagram, the total solubility $[S]_t$ is determined by 3 factors.

1) the inherent solubility of the substrate $[S]_o$
2) the stability constant $K_{m:n}$
3) the concentration of ligand [L]

The maximun solubility [S] can be achieved for an A_L system involving formation of a 1 : 1 complex as shown in Eq. (3)

$$[S]_{t\,(max)} = [S]_o + K_{1:1}[S]_o[L]_o \qquad (3)$$

where $[L]_o$ is the initial solubility of the ligand.

When the concentration of free ligand in solution equals its inherent solubility, further addition of ligand has no effect on $[S]_t$ since it does not dissolve, and the solution composition become invariant.

The B type diagram, shown in Figure 4, results when the system develops the third phase consisting of the complex. If the complex exhibits some solubility, the diagram shows an initial rise in $[S]_t$ and the diagram is said to be a B_S diagram. If the complex is significantly soluble relative to the inherent solubility of the substrate, the system gives rise to the B_I diagram.

In the B-system diagram, the plateau region represents solution compositions which are invariant due to formation and precipitation of the complex. The concentration of S in solution remains constant due to replenishment from the excess substrate present. The decrease in [S] following the plateau is due to the disappearance of any undissolved substrate, and an attendant decreased in [S] is due to continued formation and precipitation of the complex.

As in the A-type diagram, there are also identifiable factors controlling the maximum solubility of substrate $[S]_{t(max)}$, which can be realized in B-type diagrams. These are presented in Eq. (4) for the case of a 1 : 1 complex.

$$[S]_{t\,(max)} = [S]_o + [SL]_o \qquad (4)$$

The only controlling factors are the inherent solubility of the substrate and of the complexes formed.

5. Literature review of drug stability and chemical kinetics

If a product is sufficiently stable to be market, it will require relatively long storage at room temperature or actual temperature at which it will be stored prior to ultimate use to permit observation of the rate at which the product degrades under normal storage conditions.

Prediction of stability based on data obtained at elevated temperature is generally satisfactory for solution dosage forms. While it is usually desirable to determine drug stability by analysing samples for the amount of intact drug remaining, a stable solution must retain its original clarity, color and odor throughout its shelf life.

Kinetic principles are a great importance in stability programs. The goal of chemical kinetics is to elucidate reaction mechanisms. It is important to lay the proper kinetic foundation before discussing the phenomena encountered in actual dosage forms.

In a stability indicating assay methodology, it is usual to deliberately decompose the drug in a solution and also desired to establish the kinetic order of the decomposition.

The order of a chemical reaction determines the shape of the concentration-time profile of a drug or drug products, whereas the rate constant determines its slope. An example of a chemical reaction can be expressed as :

$$uA + vB + wC \xrightarrow{k} \text{products} \quad (5)$$

and if its reaction rate is

$$\text{rate} = k[A]^U[B]^V[C]^W \quad (6)$$

where A, B and C are the reactants and k is a rate constant; u, v and w will be the order of reaction with respect to A, B and C, respectively. If u, v and w have values of 2, 1 and 0, respectively, it is said to be second-order with respect to A, first-order to B and zero-order to C. The overall order of the reaction is the sum of power of the concentration terms affecting the experimentally determined rate. In the above example, the reaction would be third-order.

Graphic method is useful to determine the order of reaction. If a straight line results when concentration is plotted against time, it is zero-order; if log or ln (concentration) versus time yield a straight line, it is first-order; if 1/(concentration) versus time gives a straight line, it is second-order. When a plot of $1/(\text{concentration})^2$ against time produces a straight line, with all reactants at the same initial concentration, the reaction is third-order.

6. Literature review of in vitro skin permeation

In vitro permeation absorption can be studied by placing a skin section between donor and receptor phase of a kind of diffusion cell. The permeation of a chemical from the donor phase to the receptor phase is followed carefully and the permeation rate (flux) is determined either from the depletion of the concentration of the chemical in donor phase or from the accumulating concentration of the chemical in receptor phase.

There have been a wide variety of diffusion cell designs for in vitro measurement of skin permeation. The Franz diffusion cell (Figure 5) is one of the most widely used systems for in vitro studies. A skin sample is sandwiched between the donor and the receptor compartments. The drug permeation through skin is followed by sampling the receptor solution via an open sampling port at a scheduled time interval.

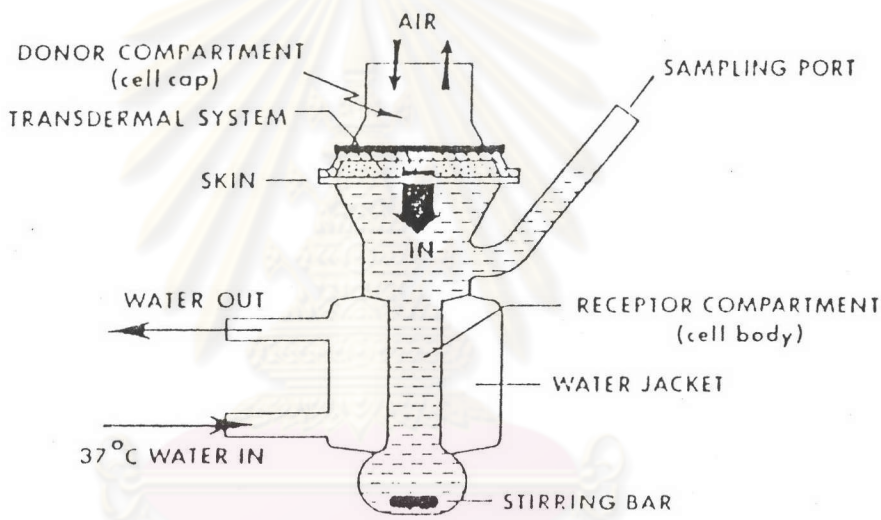


Figure 5. Schematic representation of Franz diffusion cell. Top is open to ambient environment.

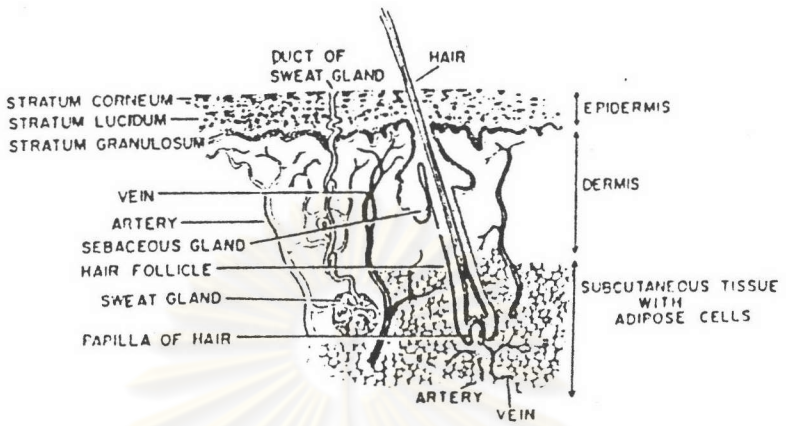
6.1 Membranes used in in vitro permeation studies

The stratum corneum consisting of dead, anucleate and keratinized cells embedded in a lipid matrix is essential for controlling the percutaneous absorption of most drugs and other chemicals. Diffusion through the stratum corneum tends to be the rate-limiting step in percutaneous absorption.

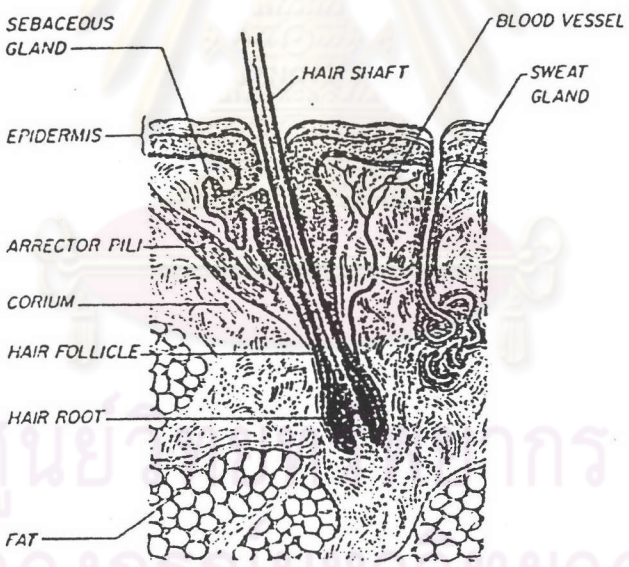
A molecule may use two diffusion routes to penetrate normal intact skin : the skin appendages (sweat glands and hair follicles) and the intact skin. The role of hair follicles and sweat glands must be considered; however, their effect is minimized by the relatively small fractional areas occupied by these appendages. In the very early stages of absorption, transit through the appendages may be comparatively large, particularly for lipid-soluble molecules and those whose permeation through the stratum corneum is relatively low. Drug permeation through the intact skin involves either an intercellular or transcellular path in the stratum corneum. Lipophilic compounds transfer preferentially into the lipoidal intercellular phase of the stratum corneum, while relatively more hydrophilic compounds transfer into the intracellular domain of the stratum corneum.

Several investigators (Mc Greesh, 1965; Tregear, 1966; Campbell, Watanabe, and Chandrasekeran, 1976; Wester and Maibach, 1987) have determined the permeability through excised skins of different species. The studies showed that the skins of pig and monkey approximated the permeability of human skin more closely than the skins of other animals.

Membranes selected to use in these studies were newborn pig skin. Frandson et al. (1986) and Warren and Walker (1974) found that the skin compositions of pig and man were remarkably similar. The pig skin is divided into three major regions (Figure 6) : the stratum corneum, the viable epidermis and the dermis. It also has skin appendages like human skin. However, the human and the pig skins may have some differences in tissue thickness, hair density, gland density, lipid composition, metabolism etc.



(a) human skin



(b) pig skin

Figure 6. Microscopic vertical section of skin : (a), human skin; (b), pig skin.

6.2 Factor influencing the in vitro permeation studies

Some important factors must be considered in an in vitro permeation study (Broanugh and Collier, 1990) as follows

a) Maintenance of physiological temperature since the percutaneous absorption of a molecule is temperature dependent.

b) Provision for adequate mixing of receptor fluid to prevent local concentration of drug and to minimize static diffusion boundary layers.

c) Choice of receptor fluid : an ideal receptor fluid is isotonic saline buffered to pH 7.4

d) Preparation of skin : a suitable technique for preparation of skins is the seperation of skin by a dermatome. Dermatome slices of 200 to 300 μm thickness from surface of the skin have been routinely used in the studies. The skin should be measured for the thickness prior to the experiments.

Franz (1975) concluded that freezing the skin up to 3 months did not damage the barrier properties of the excised skin.

6.3 Theory of diffusion

The stratum corneum can be regarded as a passive diffusion membrane but not an inert system. The absorption isotherm is frequently linear in dilute concentration ranges. The correlation between external and surface concentratons is given in terms of the solvent membrane distribution coefficient K_m. The integrated form of Fick's Law is given as

$$Jss = \frac{K_m D C_s}{h} \quad (7)$$

and

$$K_p = \frac{K_m D}{h} \quad (8)$$

where - K_p is the permeability coefficient, Jss is the steady state flux of solute, C_s is the concentration difference of solute across membranes, h is the membrane thickness,

- K_m is the $\frac{\text{solute sorbed per ml of tissue}}{\text{solute in solution per ml of solvent}} = \frac{C_m}{C_S}$,

and D is the average membrane diffusion coefficient for solute.