CHAPTER II

LITERATURE REVIEW

Gels, or jellies, are semisolid systems consisting of suspensions made up of small inorganic particles or large organic molecules interpenetrated by a liquid. The jellies are a class of gels in which a structurally coherent matrix contains a high proportion of liquid, usually water (4).

Gels and jellies exhibit various characteristics, including imbibition, swelling, syneresis, and thixotropy. Imbibition is the taking up of a certain amount of liquid without a measurable increase in volume. Swelling is the taking up of liquid by a gel, with an increase in volume. Only liquids that solvate a gel can cause swelling. The presence of electrolytes and pH influence the swelling of protein gels. Syneresis is a form of instability in aqueous and nonaqueous gels. It occurs when the interaction between the particles of the dispersed phase becomes so great that, on standing, the dispersing medium is squeezed out in droplets and the gel shrinks. Thixotropy is a reversible gel–sol formation with no change in volume or temperature (4, 35).

Gels can be classified into two groups by the number of phases of gel. These are the one–phase and two–phase gels. The one-phase gels consist of organic macromolecules uniformly distributed throughout a liquid in such a manner that no apparent boundaries exist between the dispersed macromolecules and the liquid. The particles are primary held by Van Der Waal forces. This produces a crystalline and amorphous region. The gel can not separate the dispersed molecules from the liquid or dispersion medium (35). Another name of this dispersion type is a single-phase system. The one–phase gels may be made from synthetic macromolecules or from natural gums

(mucilage). The continuous phase is usually aqueous, but it can also be alcoholic or oleaginous. The two-phase gel is a gel whose mass consists of a network of small, discrete particles. If the particle size of the dispersed phase is large, the product is referred to as a magma or milk. The stability of this system is poor but is easily redispersed and has thixotropic properties. This system is also called an inorganic gel. If the dispersion medium is water the gel is called a hydrogel, but if the gel is dispersed in an organic solvent it is called an organogel (4, 35).

Gel can be generally classified into two groups; organic and inorganic, depends on its dispersion phases. The organic gels consist of large organic particles dispersed in liquid. These are usually one–phase systems and may include such gelling agents as carbomer and tragacanth and those that contain an organic liquid, such as Plastibase®. The inorganic gels consist of small inorganic particles including gelatinous precipitation and inorganic jellies dispersed in liquid. These are usually two–phase systems such as aluminum hydroxide gel and bentonite magma (4, 35).

Gels can be also divided into two groups; hydrogel and organogel, depend on the dispersion medium. Hydrogels include ingredients that are dispersible as colloids or soluble in water such as natural and synthetic gums. Organogels include hydrocarbons, animal/vegetable fats, soap–base greases and hydrophilic organogels. Jelene® or Plastibase® is a combination of mineral oils and heavy hydrocarbon waxes with a molecular weight of about 1300 (4).

Loyd V. Allen has classified and described gel as shown in Table 1.

Table 1 Classification and description of gels (4)

Class	Description	Examples
Inorganic	Usually two-phase systems	Aluminum hydroxide gel, bentonite magma
Organic	Usually single-phase systems	Carbopol®, tragacanth
Hydrogels	Contain water	Silica, bentonite, pectin, sodium alginate, methylcellulose, alumina
Organogels	Hydrocarbon type	Petrolatum, mineral oil/ polyethylene gel, Plastibase®
	Animal/vegetable fats	Lard, cocoa butter
	Soap-base greases	Aluminum stearate with heavy mineral–oil gel
	Hydrophilic organogels	Carbowax bases (PEG ointment)
Hydrogels	Organic hydrogels	Pectin paste, tragacanth jelly
	Natural and synthetic gums	Methylcellulose, sodium carboxy methylcellulose, Pluronic®F-127
	Inorganic hydrogels	Bentonite gel (10% to 25%), Veegum®

The gelling agents in this study were Carbopol® 2020 ETD, Carbopol® 980 NF and Hydroxypropylmethylcellulose (HPMC or Methocel®). Carbopol® is a hydrophilic polyacrylic acid polymer. Its carboxyl groups become highly ionized after neutralization, forming a gel due to electrostatic repulsion among charged polymer chains. The Carbopol® has enabled the formulation of aesthetically appealing topical pharmaceutical products for over forty years, and is widely used globally. It has a long history of safe and effective use in topical gels. It has been shown to have extremely low irritancy properties and is non-sensitizing even with repeated use. It provides an excellent vehicle for drug delivery without penetration into the skin or incompatibility with

the active ingredients. Carbopol®2020 ETD and Carbopol® 980 NF are the new products of Carbopol® from BF.Goodrich Company. The Carbopol®2020 ETD is easier to disperse than the traditional Carbopol® when used as the gelling agent (5).

HPMC belongs to a family of inert hydrophilic non-ionic polymer that is widely used in oral, topical pharmaceutical formulations (6, 7). It was used as a binder and a gelling agent in the manufacture of hydrophilic matrix tablets for controlled released systems (8). It can be divided in several grades, which vary in viscosity, molecular weight, and substitution groups. In topical gels and ointments, HPMC is used as emulsifier, suspending agent, stabilizing agent and gelling agent (7). HPMC E4M has a methoxyl degree of substitution about 1.86-1.90 (29 %w/w) with a viscosity of 2% w/w aqueous solution at 20°C is approximately 4000 cP (9). The release rate of drug from gel layer in controlled released tablet depends on the hydration rate, gel strength, and polymer concentration in the matrix (9).

The skin is an important organ in the body that protects the internal organs from the external environment. It acts as a thermostat in maintaining body temperature, plays a role in the regulation of blood pressure, and protects the transportation of various compounds including medications. For the average adult, the area of the skin is approximately two square meters and is the most readily accessible organ of the human body. It receives about one-third of the blood circulating through the body with a thickness of only a few millimeters (2.97 ± 0.28 mm) (10 ,11).

The skin is a multilayered organ. It is traditionally divided into three major regions: the epidermis, the dermis, and the hypodermis (Figure 1). The epidermis is divided into five anatomical layers with the outer most layer of stratum corneum exposed to the external environment. It provides a barrier against the permeation of most substances. It is composed of compacted, flattened, dehydrated, non-viable, and keratin-filled cells (squames) or keratinized cells that are roughly shaped like pentagonal

plates 0.5 μm thick and 30 to 40 μm across. Filling the intercellular space between these cells are bilayer-structured lipids. The structure of these lipids has proved to be important to the moisture-retaining ability of the stratum corneum. The viable epidermis lies below the stratum corneum. This layer does not contain blood vessels, relying on nourishment by cell fluid from the deeper dermis layer. The deepest layer of skin is the dermis. It consists of dense, irregularly arranged connective tissue, and it nourished directly by blood vessels. Combined, these layers form the skin and are connected to the subcutaneous tissue by bundles of collagen fibers (10, 12).

Embedded in the skin are eccrine sweat glands, apocrine glands, hair follicles and sebaceous glands. Eccrine sweat glands are simple tubular glands distributed over almost all of the human body. Each gland has a secretory part located below the dermis in the subcutaneous tissue and an excretory duct that opens directly on the skin surface. Apocrine glands produce characteristic body odors and are primarily located in the axilla. The human hair can be classified into two major groups: terminal hairs and vellus hairs. Terminal hairs are the courser hairs of the scalp and male trunk hair. The root of terminal hair may extend more than 3 mm below the skin surface into the subcutaneous fatty tissue. Vellus hair is the fine, often unnoticed, body hair that populates regions such as the forehead, and extends less than 1 mm into the dermis. The hair follicle comprises the hair, combined with its surrounding root sheath. The sebaceous gland empties directly into the upper portion of the hair follicles, and the combination of sebaceous glands is the major source of skin surface lipid in adults (10, 12).

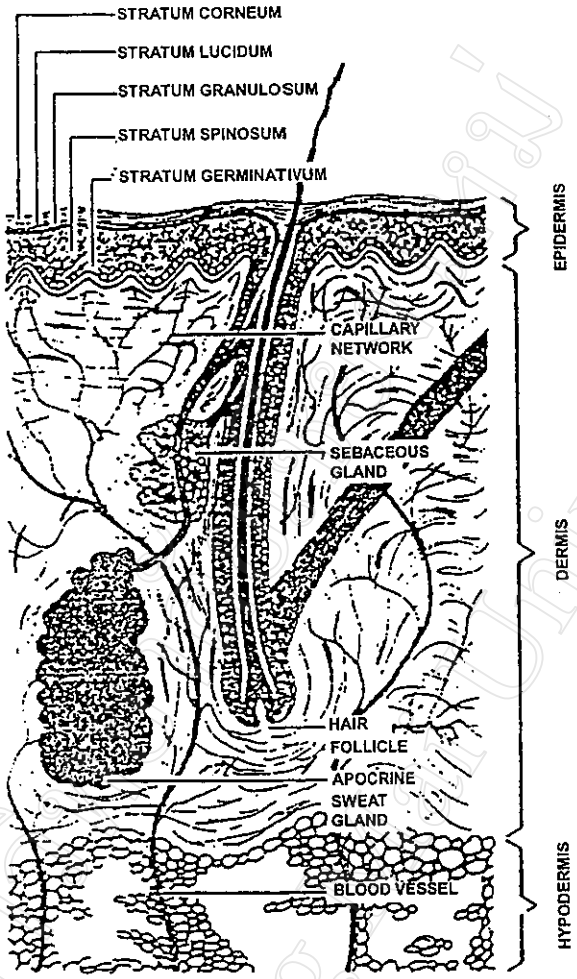

Figure 1 Cross-sectional view of human skin, showing various skin tissue layers and appendages(10)

The stratum corneum has a water content of only 20% as compared to the normal physiological level of 70%, such as in the physiologically active stratum germinativum. Every square centimeter of human skin contains, on the average, 10-70 hair follicles and 200-250 sweat ducts. These skin appendages actually occupy, grossly, only 0.1% of the human skin surface (10).

Human skin comprises a supporting dermis consisting of connective tissue, an overlying, stratified, avascular, cellular epidermis and a fatty, subcutaneous tissue beneath the dermis. The dermis layer supports apocrine and eccrine sweat glands, which reach the skin surface through pores, sebaceous glands and hair follicles, also. However, the most important layer for drug permeation is the stratum corneum, or horny layer, which usually provides the rate-limiting step for penetration into normal skin (13).

The possible macro routes for drug permeation are shown in Figure 2. These are the transepidermal pathway; across the horny layer either intracellularly or intercellularly, and the hair follicles and sweat glands. In the permeation study of a drug, every pathway should be concomitantly considered. There are many factors affected drug permeation pathways, these included the physicochemical properties of the drug, density of follicles and sweat glands, integrity and thickness of the horny layer, skin metabolism, hydration and vehicle effects (13).

Figure 3 shows the micro routes of drug penetration pathway for medications; the intercellular and transcellular or intracellular pathways. The stratum corneum represents as a wall-like structure and consists of protein-filled bricks and a lipid mortar. The hydrated protein within the cells and the lipid between the corneocytes plays an important role in skin permeability (13).

Two possible routes for drug permeation are also shown in Figure 3. These are through protein-filled cells and across lipid-rich regions in tandem (transcellular route) or between cells (intercellular route). Water-soluble agents may be able to penetrate the skin via transappendageal route faster than through the intact area of the stratum corneum. This route of percutaneous absorption has provided a very limited contribution to the overall kinetic profile of transdermal permeation. Therefore, most neutral molecules can be permeated through the intact stratum corneum in the interfollicular region by passive diffusion (13).

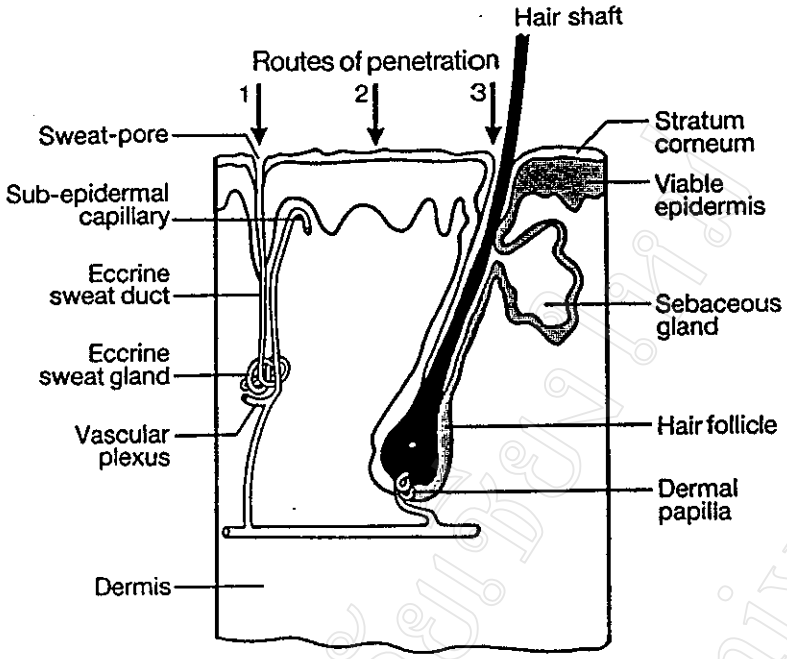

Figure 2 The macro routes for drug permeation through skin; via 1. the sweat gland, 2. across the intact stratum corneum, and 3. the hair follicles (13)

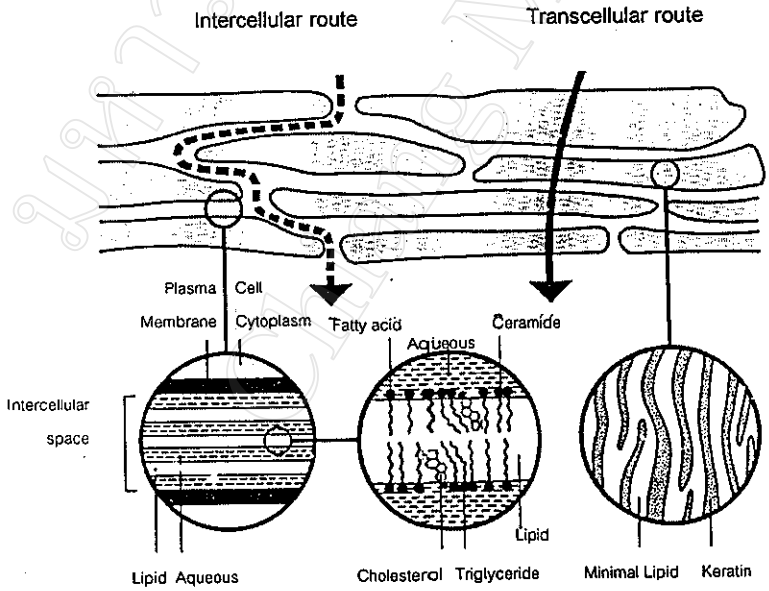

Figure 3 The micro routes for drug permeation across the intact stratum corneum (13)

The stratum corneum usually provides the rate-limiting step for the penetration process. Skin penetration enhancers are important and widely used to increase the permeation of various drugs. For example, ethanol (ETOH), propylene glycol (PG), some surfactants such as Tween®20, Tween® 80 (TW80) and Span®20, and organic acid such as L-lactic acid (L-LA) (14-18).

ETOH is widely used as a disinfectant, antiseptic, preservative, cosolvent and enhancer for various medications (14, 18). In 1989, Bret Berner , *et al.*, found that the optimum concentration range of aqueous ETOH produced a 5-10 fold increase in nitroglycerin flux across the skin. The absorption of ETOH and water into stratum corneum, and delipidization of stratum corneum were investigated in their experiment by using triolein as a simple model lipid (15).

In 1990, Kurihara-Bergstrom T. ,*et al.*, studied the percutaneous absorption enhancement of an ionic molecule using an ETOH-water system. They concluded that ETOH-water systems enhanced permeation of the salicylate ion through the human stratum corneum. The optimum enhancement of salicylate ion has been observed when the ETOH volume fractions was about nearly 0.63. They suggested that enhancement effects might be involving the alteration of the polar pathway. This alteration may occur in either or both the lipid polar head and proteinaceous regions of stratum corneum and ion-pairs formation. It may also increase permeation. However, the permeation of the ion decreased at higher volume fractions of ETOH. This phenomenon may contribute to decrease the uptake of permeant into the stratum corneum (16).

In 1996, Eiichiro Manabe , *et al.*, observed that the combination of ETOH and water increased the skin penetration of drug and these effects could be explained by hydrodynamic pore theory. They also suggested that the clearance of lipophilic drug, isosorbide dinitrate, was dependent on the flux when the ETOH was more than 40%w/w (18).

In 1989, Bret Berner, *et al.*, concluded the skin permeation of other lipophilic drug, such as nitroglycerin, was decreased when the higher concentration of ETOH was used, these because of the delipidization of the skin (15, 17). In 1997, Yoichi Kobayashi, *et al.*, suggested that the ETOH increased drug permeability through the entire region of the skin (19).

Recently, in 1999, Angela K Levang , *et al.*, studied the influence of ETOH and PG on the *in vitro* percutaneous absorption of aspirin in biophysical changes and on the macroscopic barrier properties of the porcine epidermis. They found the flux of aspirin increased with increasing concentrations of ETOH and the maximum flux of aspirin was achieved by using 80% ETOH in combination with 20 % PG beyond which there was no increase in the flux. Also the results from the biophysical study showed that 80% ETOH and 20 % PG can perturbed the macroscopic barrier integrity of the stratum corneum and the stratum corneum lipids was lost (14).

PG has widely used as a solvent, cosolvent and skin enhancer. Its structural formula is $CH_3CHOHCH_2OH$ and its molecular weight is 76.09. It is a clear, colorless, viscous, practically odorless liquid with a sweet slightly acid taste resembling glycerin. It is miscible with ETOH (95%) and water, immiscible with light mineral oil or fixed oils but can dissolve in some essential oils. PG is chemically stable when mix with 95% ETOH, glycerin or water. It is generally regarded as a nontoxic material, low irritant. It is broadly used as a cosolvent for lipophilic drugs and potential enhancers. Barry reported that the enhancement action of PG is supposed to be by increasing the solution capacity in the stratum corneum (13, 14).

Barry B.W. examining PG-skin interaction using Differential Scanning Colorimetry (DSC), permeation studies, and the vasoconstrictor bioassay. From the results, he suggested PG can act as a penetration enhancer under suitable conditions. It is effective when the stratum corneum is not fully hydrated. PG probably operates by

solvating the keratin and occupying hydrogen-bonding sites, thus reducing drug/tissue binding, alter lipid structure. When PG was applied alone to fully hydrated tissue, it could not increase drug penetration. In this situation excessive amounts of water operated as a penetration enhancer. He also found that PG might enhance drug partitioning into the skin, thus providing higher flux (13).

Barbara Bendas , *et al.*, studied the influence of PG on the mechanism of drug transport from hydrogels. Some drugs and various concentrations of PG in water were used in this study. They found that, for the lipophilic drug (betamethasone 17-valerate), PG acts only as a cosolvent. On the other hand, the hydrophilic drug (hydrocortisone), PG acts as both a cosolvent and an enhancer. They explained that this is because the lipophilic drug has higher solubility in the skin lipid than in PG/water mixture. In contrast, the hydrophilic drug has higher solubility in PG/water mixtures than in skin lipid. When PG concentration increased, the amount of drug penetration into the skin was also increased. PG may serve as an enhancer due to the solvent drag effect (20).

S. Santoyo , *et al.*, studied the effect of penetration enhancer on the *in vitro* percutaneous absorption of piroxicam gel through rat skin. They concluded that 40% PG acted as a cosolvent for piroxicam in Carbopol gels. In addition, oleic acid was added in the piroxicam gel, which contained 40% PG and it was found that this solvent mixture enhanced the flux of piroxicam. They also explained that this mixture might fluidize the stratum corneum lipids and reduce the resistance of stratum corneum to permeation compounds (21).

Surfactant is used as a stabilizer in many topical pharmaceutical formulations (22). It is well known that surfactants have effects on the permeability characteristic of several biological membranes, including skin. So, they enhanced the skin penetration of other compounds. Non-ionic surfactants have been recognized as those with the least toxicity and irritant potential. TW80 or polysorbate 80 is one of hydrophilic nonionic

surfactant widely used as emulsifying agents in the preparation of stable oil in water pharmaceutical emulsions. TW80 is yellow oily liquid. It has high tolerance to electrolytes and weak acids and bases. Polysorbates are seldom reported of their hypersensitivity when applied topically (23).

In 1998, Arellano A. , *et al.*, concluded that TW80 in Carbopol gels containing 40% PG decreased diclofenac penetration rate. This situation due to a decrease in thermodynamic activity as a result of micellar complexation. In contrast, the more hydrophobic sorbitans the more increasing in skin penetration. This might be due to the changes in the barrier properties of the skin and in the vehicle-stratum corneum partition coefficient.. They found the Span®20, a surfactant with C_{12} saturated hydrophobic group, induced the permeation enhancing effect. However, diffusional lag times for all tested surfactant were longer than the gel formulation that without any surfactant (24).

L-LA is the organic acid. Its structural formula is $CH_3CHOHCOOH$, with the molecular weight of 90.08. L-LA is used in beverages, foods, cosmetics, and pharmaceuticals as an acidifying agent. In topical formulations, particularly in cosmetics, it is used for softening and conditioning the skin. L-LA is practically odorless, viscous, hygroscopic, non-volatile liquid, miscible with ETOH 95% and water. A 1%w/w L-LA is harmless when applied to skin and there is no evidence of carcinogenic, teratogenic or mutagenic agent (23).

Hiroyoki Nakamura , *et al.*, studied the influence of L-LA on the increasing ketotifen solubility in the multicomponent vehicles. They found that L-LA in alcohol/isopropyl myristate system did not have a marked effect on alcohol flux. However, it could increase the ketotifen flux and shorten the lag time of ketotifen permeation (25).

In 1997, Yoichi Kobayashi, *et al.*, estimated the action site of L-LA-ETOH-isopropyl myristate mixed system. They concluded that its site of action was on the aqueous domain of the stratum corneum and the lower layer of the skin (19).

KP is a potent nonsteroidal anti-inflammatory drug that widely used for the treatment of acute and chronic arthritic condition (26,1). Recently, various papers reported about the enantiomers of KP. KP has two enantiomers; R-KP and S-KP. S-KP was an enatiomer with COX-inhibitory activity but R-KP was an inactive enantiomer. In addition, M.H. Ossipov, *et al.*, also studied about R-KP and they suggested that R-KP does not have COX inhibitory activity. A spinal COX inhibitory action was the action of S-KP (27).

Like other NSAIDs, the oral administration of KP often causes gastric irritation and unwanted systemic side effects (28). To avoid these disadvantages, the efficacy of KP by topical application had been studied. When KP was applied directly onto the inflamed site it could produce the high local drug concentrations (1).

In 1990, S.C. Chi and H.W. Jun evaluated the anti-inflammatory activity of 1% KP gel in 20 % pluronic acid F-127 having pH5 using the carageenan-induced rat paw edema method. It was found that the 1% KP gel in pluronic base inhibited 53% of the carrageenan-induced edema formation as compared with 38% inhibition obtained with a 3% KP gel in a Carbopol-based formulation (26). In 1991, they studied the stability of KP in PF-127 gel base and also evaluated some formulation variables such as polymer content, drug and ETOH concentrations, pH, and temperature of the gel on drug release. They suggested that the release of KP decreased exponentially as the polymer concentration increased (29). In the same year, Masakazu Kawata. *et al.*, studied about *in vitro* release of KP from Eudragit L and S organogels by using the rotation disk method. They observed that the dissolution pattern of KP from the Eudragit L organogels followed apparent zero order kinetics. On the other hand, the dissolved

percent KP released from Eudragit S organogels was a linear function of the square root of time, which agree with Higuchi's model for the release of a drug from semisolid (29).

G.E. Hildebrand and C.C. Moller-Geymann studied the characteristic of ketoprofen sodium (KNa) in solutions, hydroxypropyl cellulose (HPC) gels and liquid crystals (LC) as potential topical formulations. Due to the high solubility of KNa in water, a distinct concentration gradient between a topical formulation and the skin can be created enhancing drug absorption. They found that the release rate of KP from the solutions was faster than LC, and the release rate of drug from HPC gel was the slowest. Therefore, to enhance the bioavailability of KNa in novel delivery systems, HPC gels or LC was chosen (30).

Recently, in 1999, Gye Ju Rhee, *et al.*, studied about *in vitro* and *in vivo* permeation of KP topical oleo-hydrogel preparations. The oleo-hydrogels were prepared by dissolving KP in N-methyl pyrrolidone, suitable surfactant, and oil mixture to prepare the emulsion preconcentrate. Separately, prepared the hydrogel from gelling agent such as Carbopol. After that, the emulsion preconcentrate was added into the hydrogel under stirring to prepare the oleo-hydrogel. It was found the KP oleo-hydrogel was more beneficial than conventional products in enhancing transdermal permeation. Furthermore, there was a good correlation between *in vitro* permeation data and *in vivo* data. For example, the *in vivo* penetration rate and *in vitro* steady state flux have a very high correlation and the bioavailability AUC_{24hr} exhibited a good correlation with the *in vitro* cumulative amount at 8 hr. Thus, *in vivo* permeability parameter data may be predicted from the *in vitro* permeation parameter data (2).

In 2000, Estelle Beetge, *et al.*, studied the influence of the physicochemical characteristics and pharmacokinetic properties of selected NSAIDs included KP on their transdermal absorption. They concluded that the most reliable parameter for transdermal absorption was the lipophilic character of a drug. The molecular mass,

solubility constraint and percentage un-ionized moiety can only be used in combination with other properties in the prediction of possible transdermal drug delivery (31). In the same year, E.G. de Jalon, *et al.*, determined the amount of KP on *in vitro* rat skin permeation by using high performance liquid chromatography (HPLC). They found that the detector response was linear in the concentration range of 0.02-40 μg/ml. The HPLC stationary phase was 5 μm Kremasil 100 C_{18} column and the mobile phase was the mixture of acetonitrile 0.01 KH_2PO_4 adjusted to pH1.5 with ortho-phosphoric acid 85% (60:40 v/v) (32).

In 2001, Yasuko Obata, *et al.*, suggested that the lipophilicity among some physicochemical properties contribute the most in promoting enhancing activity of cyclohexanol derivatives on percutaneous absorption of KP using artificial neural networks. They concluded that the lipophilicity of an enhancer might effect on percutaneous absorption of KP (33).