CHAPTER II

LITERATURE REVIEW

1. Anatomy and Physiology of the Skin

The human skin comprises three tissue layers: the epidermis, the dermis, and the subcutaneous tissue.

The Epidermis

The epidermis is the most superficial layer of the skin, thick range from 0.8 mm on the palm to 0.006 mm on the eyelids. This layer has important roles of aging if the surfaces of epidermis dry or rough. There are keratinocytes (corneocyte) was produced by stem cell, called basal layer. The stem cells divided to daughter cells and migrate upwards to produce the stratum corneum. This process is keratinization. The epidermis layer has a named as its cell shape in each layer. The basal layer is located on base of epidermis and has a cuboidal shape cells. The next layer is referred to a spinous layer because the cells have prominent spiny attachments called desmosomes. Desmosome is complex structure made of adhesion molecules and other proteins, and is important in cell adhesion and cell transport. The next layer is the granular layer because it has visible keratohyaline granules. The last outermost layer is the stratum corneum (SC), its cells no nuclei and granules (figure 1).

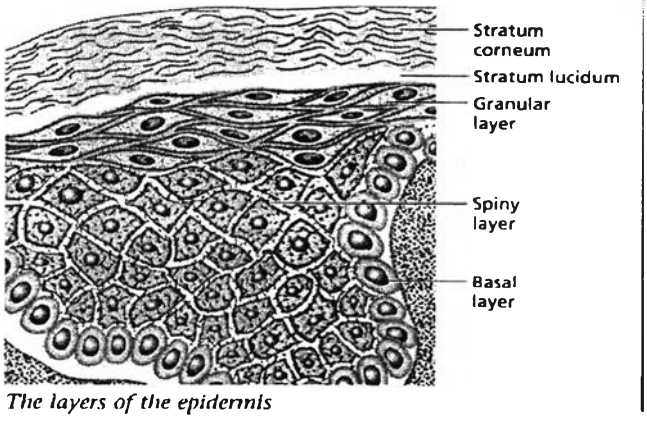


Figure 1 The Structure of the Epidermis shown the various layer (Gray, Gummer, Matts, and Marks, 2000)

The SC is composed of protein-rich corneocyte embedded in bilayer lipid matrix. This layer functions as a protective barrier to prevent transepidermal water loss (TEWL). Amino acids and their metabolites, which was broken down from fillagrin, given a substance known as the natural moisturizing factor (NMF). NMF released by the lamellar granules and can absorb large amount of water. It has an important role to help the enzyme function in aqueous environment. Besides NMF, the surface lipid of stratum corneum is involved in preventing TEWL and the entry of harmful bacteria and water soluble substances. The major lipids found in SC are ceramides, cholesterol and the fatty acids (Baumann, 2002).

The Dermis

The dermis, 3-5 mm thick, consists of mostly a connective tissue (collagen, elastin and reticulin) and laden with nerves, blood vessels and sweat glands. The dermis needs an efficient blood supply to convey nutrients, remove waste product, control temperature and pressure, mobilize defense forces and contribute skin color. Fibroblast has an important role to produce fiber elements, collagen, elastin, other matrix proteins and enzymes such as collagenase and stromelysin(Aulton, 2002). The dermis that has been exposed to ultraviolet light also showed disorganization of collagen fibrils and the accumulation of abnormal elastin-containing material(Fisher, et al,1997; Baumann, 2002).

Collagen

The major fibrous constituent of the dermis, accounting for 75 percent of the dry weight and 18-30 percent of the volume, is collagen. Under the light microscope collagen fibres appear as colourless, branching wavy bands about 15 μm in width. Collagen fibers can be disintegrated by 0.01 percent acetic acid, forming molecules with a molecular weight of 300,000-360,000, about 180 nm long. When these acid solutions of tropocollagen are neutralized, the 64 nm periodicity reappears, which may be explained on the hypothesis that native collagen is composed of molecules of tropocollagen associated side by side with a regular overlap of a quarter of their length. Skin collagen is characterized by a high content of glycerin, which forms a third of all the residues and of proline and hydroxyproline which together make up a further fifth. Tropocollagen molecules consist of three polypeptide chains each

containing about 1000 amino acids. The fibroblasts produce a precursor known as procollagen which has 300-400 additional amino acids in each of its chains; these extensions are removed after secretion.

Elastin and Reticulin

Elastin fibres make up 4 percent of the dry weight and 1 percent of the volume of the dermis. They are delicate, straight, freely branching fibres which can be stretched by 100 percent or more but return to their original length when the stress is removed. Elastin differs from collagen in having only about a quarter or a third the amount of basic and acid amino acids, only one tenth the amount of hydroxyproline, a relatively large amount of valine, and an amino acid known as desmosome which appears to be unique to it and to be concerned with cross-linkage. Not all fibrous constituents can be clearly identified as collagen or elastin on the basis of their tinctorial properties. In addition to true elastin, two other similar fibres have been distinguished and given the names of oxytalan and elaunin. Moreover, about 0.4 percent of the dry weight of the dermis is made up of fine branching fibres which unlike collagen, stain black with silver nitrate, and are known as reticulin. Their axial periodicity is identical with that of collagen.

Ground Substance

The amorphous ground substance in which the fibres and cells lie contains a variety of carbohydrates, proteins and lipids, of which the most important are the acid mucopolysaccharides. These are macromolecules made up of two different saccharide units which alternate regularly. In dermis the major forms are hyaluronic acid, in which D-glucosamine, with an acetylated amino group, alternates with D-glucoronic acid, and dermatan sulphate in which L-iduronic acid alternates with D-galactosamine

Fibroblasts

The term fibroblast should designate a cell at any early stage and fibrocyte one which is fully differentiated, but most authors use fibroblast to describe an actively secreting cell and fibrocyte for an inactive one. Fibroblasts are derived from the mesenchyme. It is not doubted that fibroblasts secrete collagen. It is probable that they are the source of elastin and though Asboe-Hansen has implicated the mast cell, also of mucopolysaccharides. (Wilkinson, and Moore, 1982)

The Subcutaneous Tissue

The subcutaneous fat provides a cushion and a thermal barrier; it synthesizes and stores readily available high energy chemicals (Aulton, 2002).

Rational Approach to Drug Delivery to and via the Skin

There are three main ways to attack the problem of formulating a successful topical dosage form:

1. We can manipulate the barrier function of the skin for example, topical antibiotics and antibacterial help a damaged barrier toward off infection; sunscreen agents and the horny layer protect the viable tissues from ultraviolet radiation; and emollient preparations restore pliability to desiccated horny layer.

2. We can direct drugs to the viable skin tissues without using oral, systemic or other routes of therapy.

3. The third approach uses skin delivery for systemic treatment. For example, Transdermal therapeutic systems provide systemic therapy for conditions such as motion sickness, angina and pain.

Dermatologists aim at five main target regions: skin surface, horny layer, viable epidermis and upper dermis, skin glands and systemic circulation. (Aulton, 2002)

2. Wrinkle

Definition of Wrinkles

The observed fine and coarse indented lines of the skin of the face called "aging". There is much scientific evidence of distinct dermal structural alterations of collagen and elastin that correlate with wrinkled skin. Because of this reason, the damaged infrastructure of the skin can caused skin weaknesses allows various length and depth in foldings of the skin to occur as a result of repetitive and chronic contractions of the exceptionally varied superficial musculature of facial expression.

Causes of Wrinkles

All scientific evidence points to ultraviolet irritation (UVR) as the primary cause of wrinkles and other stigmata of photoaging. UVR exposure induces collagen damages through its generation of reactive oxygen species and damage to membrane lipids, various cellular proteins and DNA. UVB exposure up-regulates the production of several types of collagen-degrading enzymes known as matrix metalloproteinases (MMP) (Fisher, and et al, 1997). MMPs are a group of zinc-requiring enzymes that includes collagenase, elastases, and several other proteinases, their induction, required cofactors, and potential inhibitors are logically of considerable interest in wrinkle causation, prevention and treatment. Repetitive UVR radiation can lead to dermal scars and the wrinkle formation (Griffiths, Russman, Majmudar, and et al, 1993; Nelson, et al, 1994; Fisher, and et al, 1996; Kligman, Zheng, and Lavker, 1997; Craven, Watson, Jones, and et al, 1997; Contet-Audonneau, Jeanmaire, and Pauly, 1999; Baumann, 2002).

Representative Products for Wrinkles

Although wrinkles appear after some years of exposure and are noticeable in the second or third decade of life, other can be signs of photoaging, as freckling. Complete avoidance of UVR is impractical, but avoids the peak solar flux of midday is possible. Protective hats and clothing are practical and desirable. Sunscreens of various types have definite utility in reducing UVR damage. The vitamins and antioxidants can be consideration.

Cosmetics

There are many products to claim to affect wrinkles and some can minimize the appearance of wrinkle. Cosmetics substance may fill in the wrinkle valleys, the other are of color that changed reflected light from wrinkle to hide its appearance. The effect of removing dead, loosely coherent surface keratinocytes, or of stimulating epidermal or dermal processes, may improve the appearance of wrinkles.

Moisturizer

The moisturizer can improve the stratum corneum structure and hydration, and decrease in transepidermal water loss (TEWL) that may results in improvement of

wrinkle appearance. There are two main types of ingredients: occlusive and humectants. A good moisturizer should contain both of them.

The occlusive ingredients include paraffin, squalene, dimethicone, soybean oil, grapeseed oil, propylene glycol, lanolin and beeswax. These ingredients help in decreasing the water loss and effective while present on the skin, when removed, the TEWL returns to the normal level. It is undesirable to decrease TEWL not more than 40 percent because it can cause bacteria growth. Therefore it should combine with humectants.

Humectants are water-soluble materials with water absorption capability. They can attract water from the atmosphere and from underlying epidermis, but they can take water from the deeper epidermis and dermis in the low-humidity environment resulting in skin dryness. For this reason, it should combine with occlusive. In the product, humectants are the additive to prevent product evaporation and thickening, some of them have bacteriostatic activity. They can draw water to the skin causing a slight swelling of the stratum corneum that gives the perception of smoother skin with fewer wrinkles. Examples of humectants include glycerin, sorbitol, sodium hyaluronate, urea, propylene glycol, α-hydroxy acids and sugars.

Retinoids

Retinoic acid, a naturally derivative of vitamin A, is a lipid - soluble molecule known to affect cell growth, differentiation, homeostasis, apoptosis and embryonic development. Retinol, the parent compound, may require metabolism to the purported active transretinoic acid for pharmacological effect and is increasingly incorporated in cosmetic products claiming benefit in wrinkle appearance.

Vitamins

Many vitamins including vitamin A, C, and E are vital in metabolic processes and clinical skin changes resulting from their deficiencies. Some of these changes can cause to impaired collagen synthesis.

- *Vitamin E*

Vitamin E or tocopheral found in vegetables, oils, seeds, nuts, corn, soy, whole wheat flour, magarine and in some meat and dairy product, it is a group of compounds comprised of tocol and tocotrienol derivatives. In 1993, Tanaka reported that reactive oxygen species induce changes in the biosynthesis of collagen and glycoaminoglycans (GAGs) in human dermal fibroblasts. The alteration was prevented by addition of vitamin E to the fibroblast.

A 4-week study of 5 % alpha tocopheral naturally occurring oil-in-water (o/w) cream applied to the crows feet area showed, by optical profilometry, decreased skin roughness, length of facial lines and depth of wrinkles compared with placebo.

- *Vitamin C*

Vitamin C (ascorbic acid) is widely used to antioxidant. Clinically the relevance of these effects on collagen and elastin is unknown. There is only one study in the literature that examines the effects of topically applied vitamin C on wrinkles. In the study, Cellex-C decreased wrinkles when applied topically for three months. The volunteers were evaluated by photography assessments and optical profilometry. However there was a significant difference in the wrinkles on the treated and untreated side of the skin. The mechanism of this effect is not understood. It might be explained by increased collagen synthesis.

Hormones

The wrinkle effacements have been convincing in the subjects that was on the estradiol therapeutic for their menopause especially on epithelium of the skin and vagina. The topical application of 0.01% estradiol or 0.3% estriol-containing preparation shown the wrinkle reduced. Other studies have shown beneficial changes in skin thickness and texture with topical estrogen application.

Minerals

There are many minerals such as sodium, potassium, calcium, magnesium, selenium and zinc are critical in normal mammalian physiology is well established. A potential cosmeceutical role in improvement of skin appearance has been suggested and requires confirmation.

Miscellaneous Agents

Hyaluronic acid is a normal compound of epidermis and especially dermis. Stimulation of hyaluronic-acid production in skin by a device that produces a specific pulsed electromagnetic field produced improvement in appearance of wrinkles in a small study. Natural cartilage polysaccharides as oral formulations derived from cartilage of marine fish have purposed to improve dermal thickness and elasticity (Cunningham, 2001).

Green tea

Green tea contains the polyphenolic compound and known as epicatechins. The polyphenolic component thought to be responsible for the biochemical or pharmacological effects is (-)-epigallocatechin -3-gallate (EGCG); this is the most studied agent. The benefits of green tea treatment in human skin have been studied and it has been shown photoprotective effects. (Mukhatar, and Admad, 2000; Baumann, 2002)

Beside these constituent, in Thailand there are many substance from the plants that can give collagen synthesis one of them is *Centella asiatica(L.) Urb.*

Centella asiatica

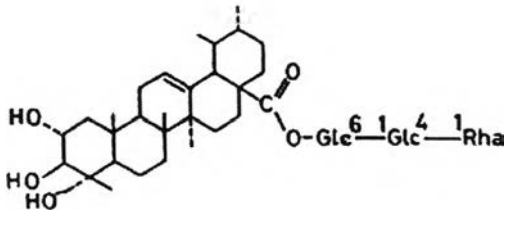
Centella asiatic (L.) Urb. also known as gotu kola and Indian pennywort, is a perennial herbaceous creeper , which grows to a length of 50 cm with fan shaped leaves. It is a plant native to parts of India, China, Indonesia, Sri Lanka, Thailand, the western South Sea Islands, Australia, Madagascar and southern and middle Africa. It is commonly used firstly as the medicinal plant in India. It is presently being used in numerous herbal energy stimulants for strengthening the body, significantly improving the learning abilities in mentally retarded children. Other clinically tested uses include improving circulation by thinning the blood, exerting limited sedation on the cholinergic mechanism in the central nervous system. Considering what gotu kola has done from a medicinal perspective for skin problems in other countries, one is tempted to speculate whether these same therapeutic successes could be translated into similar cosmetic miracles. *Centella asiatica* contains vallejin, a bitter constituent and a mixture of triterpenoid glycosides, the most abundant of which asiaticoside ($C_{48}H_{78}O_{19}$) and madecassoside ($C_{48}H_{78}O_{20}$) which on hydrolysis produces asiatic

acid, glucose and rhamnose. It also contains traces of an alkaloid, volatile oil and pectin. The active principle in Gotu kola — asiaticoside and madecassoside act as detergents and dissolve the waxy covering of the bacillus that causes leprosy and skin tuberculosis and penetrates the greasy film surrounding the herpes virus.

Asiaticoside

Asiaticoside ($C_{48}H_{78}O_{19}$) one of the principle terpenoids in *Centella asiatica* belonging to the *Umbelliferae family*, is known to stimulate collagen synthesis in fibroblast (Baek, Rho, and Kim, 1990). It enhanced tensile strength in wound tissue (Shukla, and Rasik, 1999) and facilitated the wound healing process. It can help healing ugly skin lesions common to lupus erythematosus and herpes simplex by promoting a rapid thickening of the skin and an increased blood supply to the connective tissue (Boiteau, and Ratsimamamga, 1956). This component is also resulted in accelerated growth of hair and nails in the same result.

Structure of Asiaticoside (Inamdar, and Yeole, 1996)



Chemical Name	Asiaticoside
Molecular Formula	$C_{48}H_{78}O_{19}$
Molecular Weight	959.13
Structure	Stereo compound
Compound Type	Heterocyclic
Melting point	235-237 °C

In traditional Chinese medicine, it was believed that *C. asiatica* provided longevity, and thus called it the "fountain of youth" herb. Recent studies show that *C. asiatica* has positive effect on the circulatory system; it seems to improve the flow of blood throughout the body by strengthening the veins and capillaries. It has been used successfully to treat phlebitis, as well as leg cramps, swelling of the legs, and heaviness or tingling in the legs. This is particularly useful for bedridden people. *C. asiatica* has even been used as 'food for the brain' after a nervous breakdown to rebuild energy reserves, or to prevent a nervous breakdown. It has an energizing effect on the cells of the brain, relieves high blood pressure, mental fatigue, senility, and helps the body defend itself against various toxins. It works as a blood purifier and in strengthening the heart, as well as with bowel problems, rheumatism skin problems, and also promotes blood circulation in the lower limbs and reduces the pain and swelling due to phlebitis. Researchers have founded that it contains several glycosides that exhibit wound healing and anti-inflammatory activities, and in large doses it can act as a sedative. Other researchers have shown that fresh leaves of the *C. asiatica* plant are effect in healing chronic skin ulcers and other wounds. *C. asiatica* contains a group of triterpenes called asiaticoside that possess strong antioxidants properties. In modern health care *C. asiatica* is used primarily for venous insufficiency, localized inflammation and infection, and post-surgery recovery. *C. asiatica* is also used for the following:

- Skin: Open wounds, sores, ulcers, other infections and radiation ulcers.
- Confinement: Bedsores, phlebitis, and tingling, night cramps.
- Vein problems: Phlebitis, varicose veins, cellulite and edema.
- Gynecology: Lesions during pregnancy, delivery and obstetric manipulations, and episiotomies tears.

C. asiatica affects various stages of tissue development, including keratinization that is the process of replacing skin after sores or ulcers. Because it has asiaticoside that stimulates the formation of lipids and proteins which are necessary for healthy skin. *C. asiatica* has been found to have significant results in healing of skin, other connective tissues, lymph tissue, blood vessels (decreasing capillary fragility), and mucous membranes. *C. asiatica* is believed to be a rejuvenate and

restoring 'anti-aging' herb, and is naturally consumed as a preferred food by South Indian elephants which are animals with exceptional memories and longevity. In general, it is traditionally used as a cooling, soothing, relaxing, antispasmodic diuretic.

Its historical applications include treatment for a sore or inflamed throat, and for skin, liver and urinary tract diseases. It is excellent for both internal and topical application.

French scientist recently did some breakthrough research to show it stimulates synthesis of collagen, for powerful anti-aging effect for the skin. In addition to its intellect promoting and anxiolitic effects, the plant is also used in chronic cough, eczema, psoriasis, and boils. It is in preparation given for anemia, dyspnea, emaciation, splenic enlargement, and rheumatic joint pain. The leaves of this swamp plant have been used around the world of centuries to threat leprosy, cancer, skin disorders, arthritis, hemorrhoids, and tuberculosis.

In normal as well as delayed-type wound healing, asiaticoside isolated from *C. asiatica* has been proven for its activity. *In vitro* and *in vivo* wound healing activity of guinea pig punch wounds topical applications of 57% solution of asiaticoside produced 56% increase in hydroxyproline, 57% increase in tensile strength, increased collagen content and better epithelisation. In streptozotocin diabetic rats, where healing is delayed, topical application of 0.4% solution of asiaticoside over punch wounds increased hydroxyproline content, tensile strength, collagen content and epithelisation thereby facilitating the healing. Asiaticoside was also improved the guinea pig punch wound model (Shukla, and Rasik, 1999) by oral administration of 1 mg./kg dose. It promoted angiogenesis in the chick chorioallantoic membrane model. These result indicate that asiaticoside exhibits significant wound healing activity in normal as well as delayed healing models and is the main active constituents of *C. asiatica* .

3. Polysaccharide Gel (PG)

Durian (Durio zibethinus Murr.) , a tropical fruit native to Southeast Asia, is one of popular fruit and has an distinct flavour and delicious. The fruit is ovoid or ovoid-oblong to nearly round shaped with an average size weighing between 2 and 4.5 kg depending on their varities. The waste of the rind could lead to a big problem to manage (Pongsamart and Panmaung, 1998).

Polysaccharide gel was isolated from durian fruit-hulls by using the method that investigated by Pongsamart and Panmaung in 1998, is a water soluble polysaccharide. PG is comprise of long chain polygalacturonan with branch chain neutral sugars such as galactose, glucose, rhamnose, fructose and arabinose (Hopputsa, et al.,2004 and Gerddit, et al.,2001). PG can use as cellulose derivative in preparation of food and pharmaceutical products such as jelly, tablet, suspension and emulsion(Pongsamart and Panmaung, 1998; Umprayn et al,1990). Lertchaiporn, Vayamhasuwan and pongsamart(2002) have successfully formulated vitamin E gel and lotion using PG as a emulsifier. Toxicity test of PG have been reported, a high oral dose(2g/kg) did not induce severe toxicity in male mice and rats(Pongsamart, Sukrong and Tawatsin,2001). No toxic effects have been observed in subacute treatment in male mice (Pongsamart, Jesadanont and Markman, 1989) and subchronic toxicity test of durian polysaccharide gel in male and female mice has not found to induce toxic effect (Pongsamart, Sukrong and Tawatsin,2002).

The further investigation shown the antibacterial activity against both gram positive and gram negative bacteria such as *Staphylococcus aureus*, *Escherchia coli, Staphylococcus epidermidis, Bacillus subtilis, Micrococus luteus, Lactobacillus pentosus and Proteus vulgaris* (Lipipan, Nantawanit and Pongsamart,2002; Nuntawanit,2001). In 2001 Gerddit investigated the film-forming property of PG to give satisfactory dressing film product and evaluated the efficacy in pig skin *in vivo* (Nakchat, 2002). Siripokasupkul, 2004 had developed the dressing film and evaluated its effect on wound healing skin in dog skin.

The interesting study of Tachatawepisarn, 2003 and Tinmanee, 2004 show the film forming property of PG to prepare the mucoadhesive films that can widely use constituent on cellulose derivative in present day. Tinmanee developed the mucoadhesive film to heal aphthous Stomatitis that gave a sustained release film and evaluated the efficacy in *in vitro* using Franz diffusion cell and *in vivo* in the volunteers.

4. Dermal Therapeutic Systems (DTS)

Dermal therapeutic system is self-adhesive-patches to treat the topical skin pathologies. The simplest design of DTS consists of a flexible backing layer, an adhesive matrix layer containing the drug and a removable protecting layer. The backing layer serves as a platform or carrier for the matrix and it is essential for the application and the removal of the system from the skin. Generally, the DTS should not be occlusive, because occlusion may result in a maceration of the skin due to water accumulation and may favor growth of pathogenic microorganisms (Hurkmans, *et al*,1985; Minghetti, *et al*,1997).

Hjartstam et al. reported that alterations in cellulose film structure influenced both drug transport and the mechanical properties. In addition, moisture permeability and moisture uptake of transdermal film may influence the drug release rate as well as the adhesion of film to the epidermis. It has been shown that the type and level of plasticizer, temperature and relative humidity, all effect drug release, moisture uptake and mechanical properties of films formed from aqueous dispersions (Wheatley, and Steuernagel, 1997; Amighi, and Moes, 1992; Repka, and *et al*, 2000).

The dermal delivery is to localize a drug within skin to enhance the local effect, and the Transdermal delivery is to increase the penetration of a drug through the skin for the systemic effect (Carafa, and *et al*,2002). According, the topical dosage forms should be designed depending on what the target sites are. In case of dermato-pharmacotherapy for the treatments of skin inflammation, skin fungal infection, hair growth disorder and acne, the dermal delivery of active ingredients is desirable. The liposomal encapsulation of corticosteroids, antifungal, minoxidil and retinoids are

reported to enhance penetration of the active ingredients into the skin, localizes the drug at the sites of action, and reduces percutaneous absorption (Mezei,1992).

The other type of dermal system is bioadhesive patch. Bioadhesion is generally defined as the ability of a biological or synthetic material to 'stick' to the skin or a mucous membrane. This results in the adhesion of the material to the tissue for a prolonged period of the time (Bulletin, 1994). As one would expect, this concept has received considerable attention in the pharmaceutical field due to the potential for applications in drug delivery and wound care. A widely used approach to explain the adhesive properties of dermal or Transdermal systems is based on the belief that inter-atomic or inter-molecular forces are established at the interface of the adhesive and the substrate or skin in these applications (Shultz ,and Nardin,1994).

5. System Manufacture and Testing

The manufacturing processes for reservoir, matrix and drug-in-adhesive Transdermal systems are similar. All involve the following stages: preparing the drug; mixing the drug (with other excipients and penetration enhancer

5.1 Device Design

Manufacturers design patches in a variety of ways, but for simplicity they may be categorized into one of two main types, the monolith (or matrix) or the rate-limited membrane configuration. In considering these two designs, it is convenient initially to accept the original assumption that the skin under the patch operates as a perfect sink, even though no Transdermal Therapeutic System (TTS) produced to date works perfectly on this basis.

5.1.1 Monolith or Matrix System

In these patches, the Higuchi square root of time law is usually obeyed. Equations (1.- 6.) below illustrate the relationships when the drug is dissolved in the matrix or exists as a suspension;

Eqn 1. Represents the relationship between *m*, the quantity of drug released to the sink per unit area of application, and C_0, the initial concentration of solute in

$$m = 2C_0\sqrt{\frac{D_v t}{\pi}} \qquad \text{——— Eqn 1.}$$

the vehicle, D_v, the diffusion coefficient of the drug in the vehicle, and *t*, the time after application.

Differentiating this equation provides the release rate *dm/dt*:

$$\frac{dm}{dt} = C_0\sqrt{\frac{D_v}{\pi t}} \qquad \text{——— Eqn 2.}$$

We can obtain equations that relate *m* to t in the form:

$$m = \sqrt{D_v t}(2A - C_s)C_s \qquad \text{——— Eqn 3.}$$

A : Total amount of drug (soluble and suspended) per unit volume

C_s: The solubility of the drug

This equation holds essentially for all times less than that corresponding to complete depletion of the suspended phase. If we differentiate Eqn 3. With respect to time, we obtain the instantaneous rate of release, dm/dt, given by:

$$\frac{dm}{dt} = \frac{1}{2}\sqrt{\frac{D_v(2A - C_s)C_s}{t}} \qquad \text{——— Eqn 4.}$$

For a common condition in which the solubility of the drug in the vehicle is very small and A is appreciable (i.e. A >> Cs) Eqn.4. simplifies to:

$$m = \sqrt{2AD_v C_s t} \qquad \text{——— Eqn 5.}$$

Then Eqn 5. Becomes:

$$\frac{dm}{dt} = \sqrt{\frac{AD_v C_s}{2t}} \qquad \text{——— Eqn 6.}$$

These equations indicate that the formulator can manipulate drug bioavailability from ointment suspensions by altering the diffusion coefficient, the total concentration or the solubility, However, Eqn 6. predicts that dm/dt α A1/2; doubling A only increases dm/dt by about 40%

For obvious reasons Eqns 1-6 are often refered to as 'square root of time' relationships; they may also be called Higuchi equations, after the pharmaceutical scientist who developed them. The amount of drug released is proportional to the square route of time: the flux is an inverse function of $time^{1/2}$. Figure 2 illustrates release profiles, plotted both linearly and as square route functions of time. Figure 3 illustrates the fundamental construction for a suspension-type TTS.

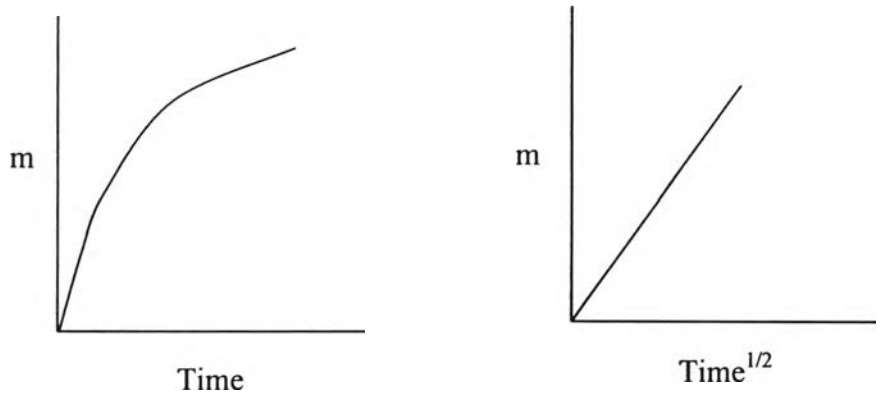


Figure 2 Release rate profile, plotted both linearly and as square route function of time, for matrix or monolith patches operating under Higuchi conditions. (Aulton, 2002)

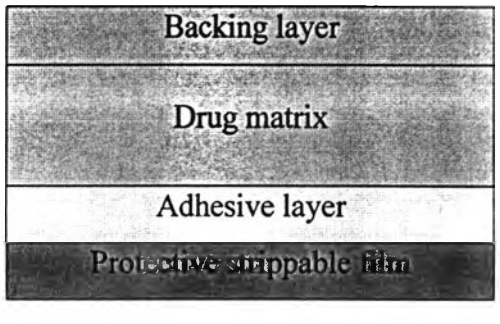


Figure 3 Fundamental construction for suspension type Transdermal Therapeutic System based on a matrix or monolith design(not to scale) (Aulton, 2002)

An occlusive backing layer protects the drug matrix, which comprises a suspension of drug in equilibrium with its saturated solution (maximum thermodynamic activity). An adhesive layer contains dissolved drug in equilibrium with that in the matrix, and attach the patch to the skin.

5.1.2 Rate-limiting Membrane System

As these patches include a membrane, we might expect that the release profile would follow the simple Fickian conditions. Thus, at steady state we would expect the amount of drug released to the skin to be directly related to the time. However, such a profile only follows when the membrane is initially free of drug. The lag time then represents the period during which the membrane equilibrates with drug after application to the sink (in this case the skin). In practice this does not happen, because the drug equilibrates in all patch components on storage, before the patient receives the patch.

Figure 3 illustrates the situation; a typical patch in this category consists of a backing layer, a reservoir containing the drug, the membrane, a skin adhesive and the protective film. On storage, the drug equilibrates into the membrane and adhesive. This portion of the drug more readily releases into the skin, as it does not have to permeate through all of the membrane. The result is to produce a so called burst effect that leads to the type of plot illustrated in figure 5. such profiles may be confused with Higuchi plots, e.g. with matrix release plots, as illustrated in the first plot of figure 2. An advantage is that the burst component can provide a quick-acting, priming dose of drug.

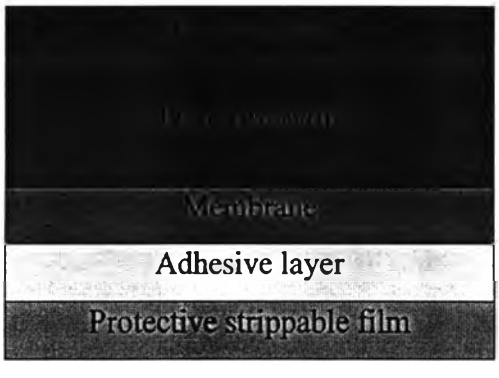


Figure 4 Transdermal Therapeutic System based on a rate limiting membrane design (not to scale) (Aulton, 2002)

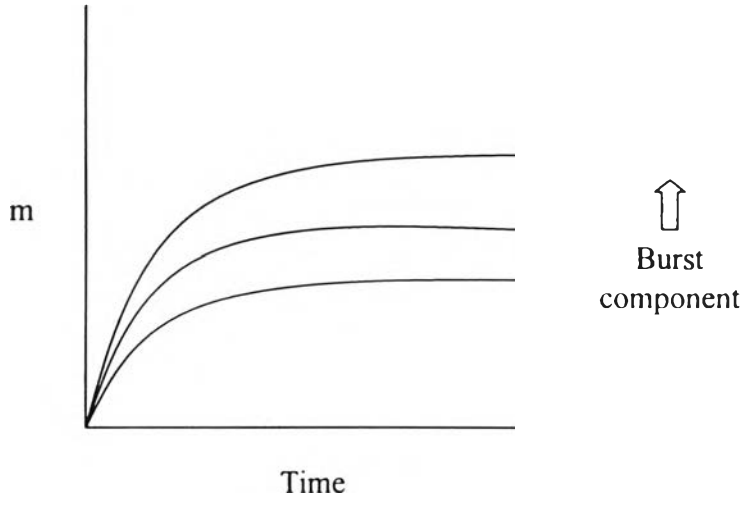


Figure 5 Release profile from rate-limiting membrane patches; effect of increasing amount of drug partitioned into the membrane and adhesive on storage (which provide the 'burst' component) (Aulton,2002)

5.2 Mechanical Properties

The tensile testing process is for apply increasing tensile load at a constant rate to a film strip which know dimensions in the dimension perpendicular to the cross-section of the film strip until the failure take place. The load at the film failure can be measured in term of force / unit cross-section area of the film. The tensile test gives an indication of the strength, toughness and elasticity of the film reflected by the parameters such as tensile strength, Young's modulus and % elongation. The method of the preparing tested film is cast film method. Cast film method gives a more perfect specimen, uniform thickness and free from bubbles and defects. Casting films are reproducible because environmental factors affect to the film preparation less than with sprayed films. Casting is therefore a better means of obtaining accurate data on the fundamental properties of the polymer and polymer formulation (Aulton, 2002). Polymers are divided into 5 categories according to a qualitative description of their mechanical behavior and corresponding stress-strain characteristics as showed in Table 1 and figure 6.

Table 1 Qualitative description of polymer and it's stress-strain characteristics (modified from ASTM D882-95a; Aulton and Abdul-Razzak, 1981)

Polymer Description	**Characteristics of stress-strain curve**			
	Young's Modulus (MPa)	Yield Stress	Tensile Strength (Mpa)	Elongation to Break (%)
Soft, weak	Low	Low	Low	Low to modulate
Soft, tough	Low	Low	Moderate	Very high (20-100)
Hard, brittle	High	None (break around yield point)	Moderate to High	Very low (<2%)
Hard, strong	High	High	High	Moderate (5%)
Hard, tough	High	High	High	High

Hard or stiff polymers are characterized by high modulus as opposed to soft ones. Strong (as opposed to weak) polymers have high tensile strengths. Tough (as opposed to brittle) polymers have large area under their stress-strain curves and require large amounts of energy to break under stress, combining high or at least amounts of energy to break under stress, combining high or at least modulate tensile strength with high elongation. The desirable hard, tough film must have a high yield stress large extension before breaking and high elastic modulus.

Tensile strength, ultimate strength or breaking stress is the maximum stress applied to a point at which the film specimen breaks. The determination of tensile strength alone is not very useful in predicting mechanical performance of the films, however higher values of tensile strength of the films are desirable for abrasion resistance.

$$\text{Tensile strength} = \frac{\text{load at failure}}{\text{Film thickness} \times \text{film width}}$$

Strain or elongation is a measure of the ductility of the film. It is calculated by dividing the increase in length by original length. It can also be expressed as a percentage.

Young's modulus or elastic modulus is the most basic and structurally important of all mechanical properties and is a measure of stiffness and rigidity of the film. It is calculated as applied stress divided by the corresponding strain in the region of linear elastic deformation (slope). The greater slope of the curve the higher the elastic modulus. The high value of the elastic modulus indicates the stiffness and the strength of film.

$$\text{Elastic modulus} = \frac{\text{slope}}{\text{fim thickness} \times \text{film width} \times \text{cross-head speed}}$$

Work of failure is a function of work done in breaking the film specimen and is representative of the film toughness. It can be calculated from the area under the stress-strain curve.

$$\text{Work of failure} = \frac{\text{area under curve} \times \text{cross-head speed}}{\text{Film thickness} \times \text{film width}}$$

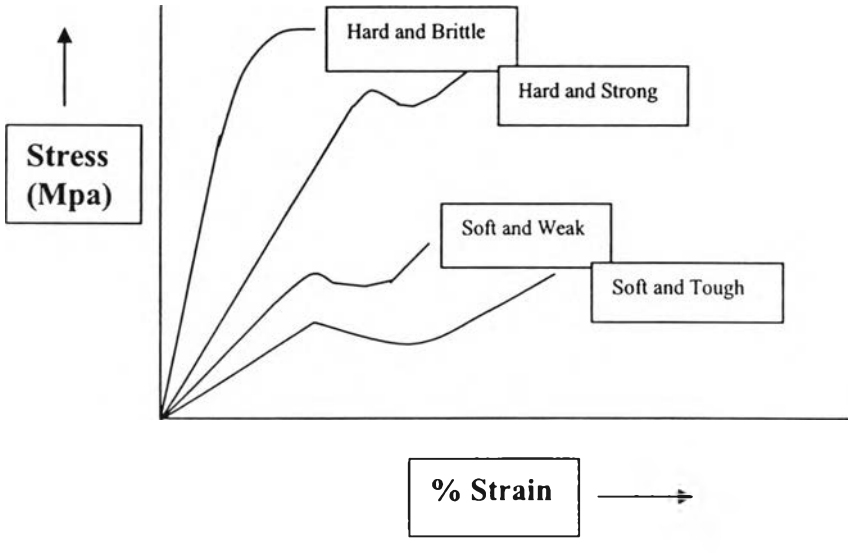


Figure 6 Mechanical behavior and corresponding stress-strain characteristics of polymers (Aulton and Abdul-Razzak, 1981; Tachatawepisarn, 2003)

6. Methodology in Evaluation of Dermal Delivery Systems

6.1 *In vitro* Drug Release Study from the Patch

The simplest in vitro experiment that can be used to evaluate a transdermal system is to measure its intrinsic ability to release the active agent. The simple dissolution tests recommended by FDA (Shan, *et al.*, 1986) can be used and are useful to compared batch to bath variation. Hence this type unable to provide *in vivo* predictions.

There are potential problems in designing experiments to assess intrinsic release rates. If the delivery systems contain hydrophilic components, they may lead to rapid patch degradation on contact with the dissolution medium. Very rapid drug release will result, and the rates obtained will not be representative of what happens in the absence of erosion. It may not be possible to assess batch to batch conformity if this occurs. This may be circumvented by inserting an inert, but not rate-controlling, membrane between the delivery system and the receptor medium. Rapid-release kinetics may also be found if the delivery system contains a high loading of the active. Addition to, inert membranes can be selected to give appropriate analytical quality control of the patches (Aulton , 2002).Other techniques that widely used to study the release profile are diffusion cell.

Diffusion Cell

In diffusion cells designed to examine steady-state flux and deduce fundamental parameters, a well stirred donor solution at constant concentration releases penetrant through a membrane into an agitated 'sink' receptor liquid. The donor compartment may be closed or open to ambient conditions or to controlled temperature and humidity; the skin or membrane should be washed, or materials added during the experiment. The test formulation should be a solid deposited from volatile solvent, a liquid, a semisolid, a film or a drug device.

A technique such as HPLC analytical method may also be used to measure passage across the membrane or the skin.

The diffusion cell that widely used in the experiment is Franz diffusion cell or modification of this technique.

Franz Diffusion Cell (Chien. Y.W.,1987)

The vertical type cell developed by Franz and commercial by Crown Glass used for study the percutaneous absorption. The cell composes of the donor and the receptor compartment that have a skin or membrane between both of them. The receptor volume is approximately 10-12 mL and has an intact surface area 1.57 to 4.71 cm^2. There is a magnetic stirrer rotates at a speed of 600 rpm in a low viscosity receptor fluid such as normal saline solution, the temperature of the solution can be maintained at a constant level by circulation thermostated water through the water jacket surrounding the receptor compartment.

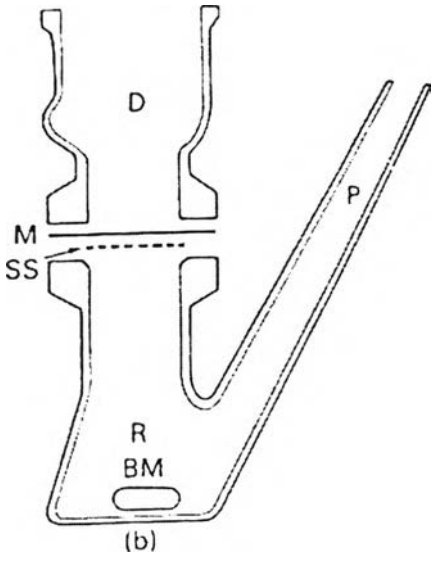


Figure 7 Modified Franz Diffusion cell (Aulton, 2002)

Modified Franz Diffusion Cell

Another vertical type diffusion cell had been developed from Franz cell by Keshary and Chien shown in figure 7. They improve the efficiency of fluid mixing. The modified cell (K-C cell) has an effective receptor solution volume of 12 mL and an intact surface area of 3.14 cm^2. The receptor solution is stirred by a star-head magnet rotating at the constant speed of 600 rpm by the same driving unit originally designed for Franz diffusion cell (Chien.Y.W.,1987)

There are generally considerations that apply for the enhancement of permeation across any membrane. In permeation, one is flux and the other is concerned with the problem of increasing the flux across membranes. For any region within a membrane the flux, the equation of this accumulative amount as follows:

$$J = -D\frac{dC}{dX} \qquad \text{——————} \text{ Equn 7}$$

For flow in one dimension;

J = amount of the drug

D = diffusion coefficient

C = permeant concentration

X = spatial coordinate

Although the solution J with various boundary conditions and membrane heterogeneities can be very complex, the basic concepts regarding flux enhancement can be found in equation 7. The concentration of gradient is thermodynamic in origin, and the diffusion coefficient is related to the size and shape of the permeant and to the energy required to make a pore for diffusion. Thus enhancement of flux across membranes reduces to considerations of:

1. Thermodynamics (lattice energies, distribution coefficients)
2. Molecular size and shape
3. Reducing the energy required to make a molecular pore in the membrane

This technique is widely used in the drug release profile study. In 2003, Bian and coworker study the release of gentisic acid from the patch using the Keshary-Chien diffusion cells, which have a diffusion area 2.14 cm^2 at 37 °C. A patch was mounted between the donor and receptor cell with the adhesive side facing the receptor cells. Each receptor cell was filled with 12 mL 40% w/v propylene glycol in phosphate buffer solution (PBS). At predetermined time intervals, 0.5 ml of the receptor solution was withdrawn and refilled with the same volume of fresh receptor solution. Samples were kept in the freezer until analyzed by HPLC.

In 2004, Tinmanee.R., had studied in vitro release of triamcinolone acetonide from the buccal mucoadhesive films using modified Franz diffusion cell and analyzed the amount of the drug by HPLC analytical method.

Artificial membrane

Because human skin is variable and difficult to obtain, researcher often used other materials, for example cellulose acetate, silicone rubber or isopropyl myristate; or lamellar systems designed to mimic the intracellular lipid of the stratum corneum.

The Selection and Use of Synthetic Membranes for in vitro Diffusion Experiments

Advances in the sciences of the drug design, drug delivery technology, penetration enhancement and topical vehicle formulation have allowed the stratum corneum, classically considered to be a total barrier to the ingress of chemical substances, to be used as a portal for the delivery of a select group of drugs. The increased interest in topical drug delivery systems has important the development of new experimental procedures for the assessment of these products.

In fact the testing of topical delivery system should be used *in vivo* model, but there are many variation involved. The *in vitro* experimental procedure is important to this field because of the multitude of problems associated with *in vivo* protocols. Laboratory test systems require a membrane to mimic the barrier function of the stratum corneum. To this end, many different types of membrane, both biological and synthetic, have been investigated for potential usefulness in *in vitro* test systems. These membranes range in barrier function from negligible resistivity to permeabilities approximating that of human skin. Generally, membranes from animal sources tend to have higher resistivities to drug diffusion compared with synthetic membranes, probably because of the more complex biochemical composition of the former. The main objective of *in vitro* experimentation is to stimulate diffusion conditions in man, thus obviating the requirement for *in vivo* research using humans or animals. Clearly, if an *in vivo/in vitro* correlation can be established then routine testing can be conducted in the laboratory. It should be emphasized, however, that the establishment of an *in vivo/in vitro* correlation is unique for a specific membrane, drug and delivery vehicle.

Synthetic Membrane

It may be possible to adequately stimulate the *in vivo* permeation of a drug using a specific diffusion system and synthetic membrane. The commercial availability, stability, interbatch uniformity and case of usage make the use of synthetic media highly desirable (Sato and Wan Kim, 1984). The barrier potential of porous membranes is dictated by the probability of a diffusant molecule entering and diffusing through the pores, and the factors governing selectivity to diffusion would be the relative molecular size, molecular shape and its electrostatic interactions with the membrane. Conversely, aporous media appear to offer some rate-limiting factor to permeation and may, therefore, more closely simulate diffusion through biological tissue. The barrier properties are generally relate to the solubility of the diffusant in the polymer matrix (partition coefficient between donor vehicle and membrane) and the case of diffusant passage through the polymer. There are many types of synthetic membranes such as cellulose media, filter membranes, synthetic polymers.

Cellulose Media

Cellulose is a relatively rigid structure consisting of glucopyranose rings joined by β-1,4-linkages. This conformation allows only two types of movement in the chains: inversion of the pyranose ring or rotation around the glycosidic linkage. In addition, the cellulose chains exist in a partially crystallized form due to interchain hydrogen bonding (Sato and WanKm,1984). Commercial cellulose membranes have a cut off of 8000-15000 daltons for molecular dialysis and on purchase normally contain a number of softener, preservative and plasticizer additives which may affect drug permeation depending on the membrane pretreatment prior to experimentation. These plasticizer and preservative additives are usually ultraviolet radiation absorbing substances that may leach into the receptor the receptor chamber solution and interfere with the subsequent analysis procedure. The removal of these substances is therefore imperative and since they are mainly water-soluble compounds they may be removed by soaking the membrane in water, or as recommended by some manufacturers, boiling the membrane.

It can reasonably be assumed that the degree of additive extraction may influence the flux of diffusants passing through the membrane. If additives are only

partially removed, they may interact with the diffusant by absorption or may occupy interchain positions thereby hindering the passage of other moieties. Generally, cellulose membranes are reported to be more permeable than biological membranes or porous synthetic media(Touitou and Abed, 1985b) and are nondiscriminatory to the characteristics of the diffusant molecule. These membranes have been used for quality control release studies (Shah *et al.*,1989; Haigh and Smith ,1994).

Filter Membranes

Porous filter membranes have been relatively little usage in diffusion systems in comparison to the synthetic polymers (De Meere and Tomlinson, 1984; Viegas *et al.*, 1986). Turakka, Prepponen, and Kahela, 1984 used a polycarbonate filter membrane to separate media in a simple drug release apparatus. Interestingly, they state that this membrane was chosen for investigation in preference to cellulose or silicone media because hydrocortisone acetate was not found to diffuse through the latter two membranes into propylene glycol receptor phase using their particular diffusion cell.

This membrane preparation has to soaking the membrane in purified water at the temperature and for the time recommended by the manufacturer. After that, the membrane should be rinsed with fresh water and blotted dry before use. The soaking procedure is conducted simply to maintain uniformity in membrane preparation as the filter material is reported to be non-hygroscopic and has very low adsorptive potential. The degree of hydration, which the medium undergoes during immersion is, therefore, thought to be minimal.

In fact, porous filter media appear to be most useful as a support medium where the release rate of drug from the delivery system is under investigation, and not the actual transdermal kinetics of the permeant. In these cases the filter medium does not simulate the skin and provides no significant barrier to diffusant passage.

Synthetic Polymers

Diffusion a molecule through synthetic polymer is analogous in many ways to diffusion through unstirred liquids. Mass transfer through the matrix is dependent on the frequency of void formation of sufficient size to accommodate the

diffusant. Voids are formed by the random oscillation of polymer chains and the larger the diffusant species the greater the number of neighboring polymer units which would have to move in a specific manner in order to chains generate a void of sufficient volume to accommodate the diffusant. The degree of bonding interaction between the polymer chains will determine the rigidity of the matrix and, thus, the propensity for hole formation and resultant permeability (Lee, Ulman, and Larson, 1986; Sun, Tojo, and Chien, 1986).

Silicone polymers such as polydimethylsiloxane is interesting because they are lipophilic in nature and highly permeable to many non-ionic drugs which dissolve in the barrier matrix and diffuse across it (Di Colo.et. al.,1980;Liu. et. al., 1985; Touitou and Abed,1985 ;Julian and Zentner,1986;Kneczke.et.al.,1986) (Haigh. and Smith,1994).

6.2 Toxicity and Irritation Studies

There are many methods to predict the potential activity of substance to induce irritation in man. Most of them are done in animal, but in some test are made on man. Predictive tests have been very effective for many years in detecting substances or products likely to be harmful to human. It should be understood that, despite all care in examination of products, such is the diversity of human susceptibility that a few persons will show adverse reactions if sufficient numbers are exposed to the products. Nevertheless, predictive tests for safety in use have ensured that products are harmless to the very great majority.

In Vivo Irritation Test

The most popular test that used to the primary irritants is Draize's test or slightly modifications of it. Albino rabbits are clipped and the test substance is applied to intact skin and to abraded or lightly scarified skin, and covered with a closed patch for 24 hours. The sites of application are then examined at intervals, and the changes seen are assessed in severity according to a scale of numerical values for various features.

The skin of the rabbit is more susceptible to irritation than that of human, so that it is possible to identify any substance likely to have an effect on man.

However the method of assessing results may lead to false positives and rejection of materials harmless to man. It is preferable to compare the effect of the test substance with that a similar substance known to be harmless to users, rather than to use the score system incorporated in the test procedure of the USA or France. In Europe proposals are being debated that the period of application of the test substance should be four hours or less, which is just as effective for including irritation and is milder treatment for the animals.

Having obtained the results of tests on animals and those of other tests to determine the safety of the substance or product, the product may be tested for its irritation potential in man by several methods such as repeated application to the skin, patch tests, arm immersion tests and simulated in-use tests. Such tests on human confirm the results of the animal tests. The result of patch test determine the amount of the suitable concentration of the reagents should they be required by dermatologists to examine any adverse effect, allergy or irritation occurring in an individual user of the product (Wilkinson and Moore, 1982).

6.3 *In Vivo* Tests on Human Volunteers: Clinical Studies

The most direct proof of a claim is to show the product effect directly on the human volunteers using the product. Many test protocols may be used depending on the objective. Most protocols have been established in scientific literature and are well-established tests. The applications are used in volunteers at home or in the laboratory (Jackson and Robillard, 1982; Paye *et al*, 1999). It is obvious that the more realistic the application condition, the more powerful the demonstration of the effect. Besides the application procedure, these protocols can also differ by the assessment technique of the claimed effect: scoring of the effect by an expert evaluator, objective measurement of the property by a biometric technique, or self-assessment of the subjective effect by the user.

Test for Skin Hydration

Experimental models used for measuring skin hydration are basically clinical models incorporating or not invasive bioengineering measurements. To ensure effective results, the protocol of the intended studied should be of modern design

incorporating blinding, randomization and a suitable statistical control (particularly if different products are to be compared). This last point means including a predetermined adequate number of subjects in the study. The general ethical and legal frames of such clinical studies required for claim support are well defined in corresponding monographs or publications covering extensively the general procedures to be followed and the pre requisite information needed about the products to be tested (COLIPA,1997; Seidenschnur,1995; Davis *et al.*,1998).

According to method used, a further important point concerns standardization of the experimental conditions to obtain acceptable and reproducible results, measurement should be performed with relaxed volunteers already acclimatized for at least 20 minutes to controlled ambient temperature and relative humidity conditions. Both factors mainly affect sweat gland activity, but other parameters should equally be considered with attention to, e.g., anatomical skin site, test products remaining or not on the skin, and correct handling of the measuring equipment if any. All these possible influences on measurement outcome have been discussed in detail in recent guidelines (Serup, 1995; Berardesca, 1997; Wilhelm, 1998).

Incorporating Bioenginerring Methods

A large number of bioengineering methods are now available to evaluate hydration (or dryness) of the skin directly or indirectly. Inclusion of these methods in the study protocol opens many possibilities for getting meaningful results such as design variations, optimization of the claim support, and importantly improvement of cost effectiveness by shortening the duration of experiment, using a lower number of subjects, and strengthening the statistical evaluation.

The technique based on the electrical properties of the stratum corneum or on measurement of transepidermal water loss (TEWL) is widely used for the skin hydration measurement. There are many devices in commercially available such as the Corneometer, the Skicon, the Dermal Phase Meter, the DermaLab® Moisture probe. These devices have a small difference that can give different performance under varying experimental or physiological conditions.

Another important difference not yet mentioned is the measuring depth of the various instruments. This depth is between 30 and 45 μm for Corneometer, less than 15 μm for the Skicon and between 45 μm and 60 μm for DermaLab® Moisture probe. The ultimate choice of the instrument depends on the conditions one would like to measure.

Statistical of Skin Hydration Studies

Within-treatment analyses are conducted to assess changes from the baseline hydration levels. Student's T-test for paired data is typically used to determine if these changes are statistically significant. Non-parametric alternatives as well as binomial statistics (for instance, when testing improvement in hydration versus no improvement) are also employed depending upon the design, number of subjects enrolled and the objectives of the study.

Between-treatment analyzes are conducted to evaluate differences in hydration levels between the test articles, whether they are ingredients or final formulations. Typical analyses employed with hydration data use the changes or percent changes from baseline hydration levels (analysis of variance) or they use the baseline hydration level as a covariate in an analysis of covariance.

The appropriate statistical analysis should flow from the study design and the objectives of the study (Barlow and Wiechers, 1999).

Objective Methods for Assessment of Human Facial Wrinkles

Wrinkles can be easily visualized and many clinical studies have involved the use of ranking scales that rely on subjective assessments by expert grader. To improve the validity and reproducibility of this approach, more complex ordinal scales with semiquantive word descriptors and reference photographs have been devised by several investigators (Daniell, 1971; Griffiths *et al*, 1992; Larnier *et al* et, 1994). For example, Daniell devised a set of reference photographs that illustrates his six-point grading scheme for evaluating crow's feet wrinkles in the lateral periorbital area. Such reference photographs can be used to train inexperienced graders and periodically review the competency of all evaluator. In contrast, the major drawback

to this type of approach is that it provides no permanent records that fully describe the skin surface features or allow retrospective analysis. Instead, we must rely on the volunteer judgments of trained graders and their ability to recall from memory the full range of changes in skin- surface features that might occur in each situation.

This problem can be overcome by taking standardized photographs before treatment and at various intervals during the treatment period. This provides a series of photographs that not only documents the study but can also be used to quantify the therapeutic response. This can be done by a panel blinded, independent readers who are remote from the study environment as was done for photodamaged skin treated with isotretinoin(Armstrong *et al*, 1992) or alpha-hydroxy acids(Stiller *et al* , 1996).