CHAPTER II

LITERATURE REVIEW

A. Botanical and Chemical Aspects of *Tamarindus indica* Linn.

Tamarindus indica, known as Ma-kam, is in Family Leguminosae which prefers tropical climate regions such as South East Asia, Africa and South America. Tamarind is a large tropical tree with a short massive trunk, ferny pinnate leaves, small yellow flowers and fat reddish brown pods. The tree can be 90 ft (27.4 m) high but is usually less than 50 ft (15.2 m). It has a short, stocky trunk, drooping branches and a domed umbrella shaped crown about as wide as the tree's height. The leaves are approximately 10 in (25.4 cm) length with 10-18 pairs of 1 in (2.5 cm) oblong leaflets. The flowers are about 1 in (2.5 cm) diameter, pale yellow with purple or red veins. They have five unequal lobes and borne in small drooping clusters. The velvety cinnamon brown pods are 2-6 in (5.1-15.2 cm) long, sausage shaped and constricted between the seeds. The pulp around the 8-10 seeds may be sweet but some extremely sour (Figure 1).

Figure 1 *Tamarindus indica*

Tamarind seed is covered with a black-brown husk (Figure 2). Tamarind seeds consist 30% of husk and 70% of kernel or endosperm. The husk contains 40% water soluble, 80% of which is a mixture of tannin and coloring matter. Kernel or

endosperm is white and component with 65% of non-fiber carbohydrate, 17% of protein, 7% of fat, 5-6% of crude fiber, 2-8% of ash and 4-5% of other components. The carbohydrates of seeds consist mainly of a polysaccharide composed of D-galactose, D-xylose and D-glucose in the ratio 1:2:3 (Lewis and Neelakantan, 1964).

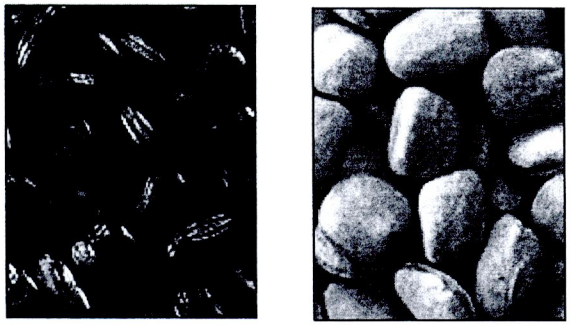


Figure 2 Tamarind seeds and kernel of tamarind seeds

B. Chemistry, physicochemical properties and application of xyloglucan from tamarind seeds

Xyloglucan is major structural polysaccharides found in the primary cell walls of higher plants such as barley, rice, corn, onion, apple and tamarind seed, etc (Picout et al., 2003; Hoffman et al., 2005). Xyloglucans from plants are differentiated by the distribution of side chain residues and molecular weights. It has even been displayed to be different in properties such as viscosity, solubility, gelling, freeze–thaw stability and film formation (Sims et al., 1998).

1. Chemical structure of xyloglucan

Xyloglucan derived from tamarind a seed is composed of a (1-4)-β-D-glucan backbone chain which has (1-6)-α-D-xylose branches. The (1-6)-α-D-xylose branches are partially substituted by (1-2)-β- D-galactoxylose. The tamarind seed xyloglucan consists of three units of xyloglucan oligomers with heptasaccharide, octasaccharide and nanosaccharide, which have different numbers of galactose side-chains (Figure 3) (Kawasaki et al., 1999).

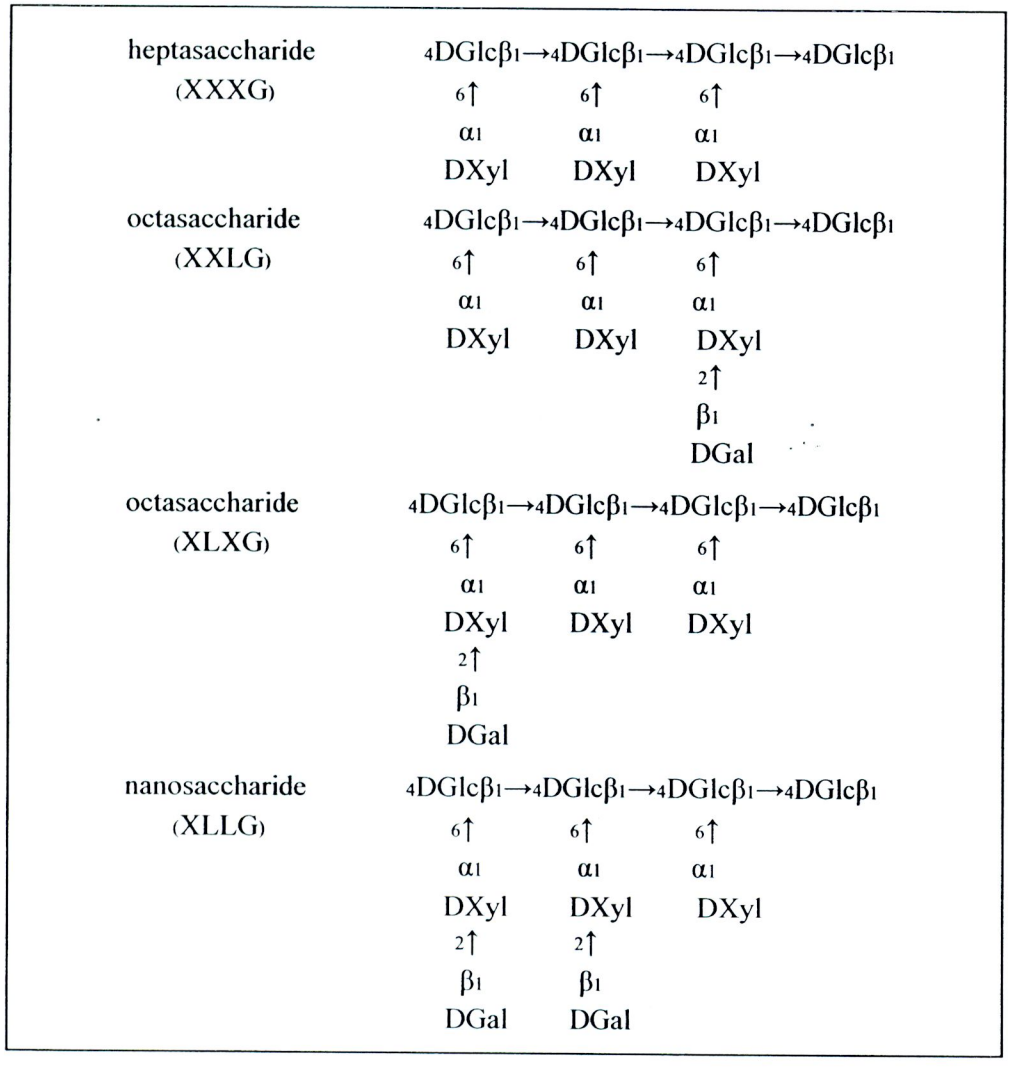


Figure 3 The unit structures of oligosaccharides from tamarind xyloglucan

2. Physicochemical properties of xyloglucan

Physicochemical properties of tamarind seed xyloglucan have been widely studied and exhibited to be a new excipient for pharmaceutical and food industries. There are several beneficial properties of tamarind seed xyloglucan which is a high molecular weight polysaccharide, such as water-solubility, high viscosity, broad pH tolerance, stability against shear, sol to gel transition, adhesivity, non-carcinogenicity, mucoadhesivity, biocompatibility, high drug holding capacity and high thermal stability (Sumathi and Ray, 2002).

In 1998, Sims et al studied rheological properties, viscosity and effects of temperature and pH on viscosity of three xyloglucans from the extracellular medium

of suspension cultured *Nicotiana plumbaginifolia* cells, apple pomace and tamarind seeds with different structural features and molecular weights. They stated that viscous solution of tamarind seed xyloglucan displayed non-Newtonian behaviour. It has the highest molecular weight (880 kDa) and the highest viscosity among three xyloglucans. The viscosity of *Nicotiana* xyloglucan at 5% w/v and apple pomace xyloglucan at 5% w/v were almost equivalent to tamarind seed xyloglucan at 0.5% and 2% w/v, respectively. In addition, the viscosity of xyloglucan solution decreased when the temperature increased. There was no effect of pH on viscosity of three xyloglucans and stability over the acid pH range.

The toxicity and carcinogenicity of tamarind seed polysaccharide were studied in the diet to B6C3F_1 mice of both sexes for 78 weeks. This study showed that tamarind seed polysaccharide is neither toxic nor carcinogenic in mice with long-term dietary exposure (Sano et al., 1996).

Shirakawa et al. (1998) studied the changes in physiological function and properties of tamarind seed xyloglucan when it was modified by fungal β-galactosidase. The result demonstrated that the changes in solution viscosity accompanied with the removal of terminal galactose from tamarind seed xyloglucan. Xyloglucan by removing galactose residues had sol-gel transition points. It formed a gel on heating and reverted to a solution on cooling below the low temperature transition point. Subsequently, over the high temperature transition point, the gel reverted to solution again. For example, xyloglucan which a galactose removal ratio of 35%, solution changed to gel on heating at 40 °C and reverted to solution again at 80 °C. Xyloglucan which a galactose removal ratio of 58%, solution reverted to gel on heating at 5 °C and transformed to solution again at 110 °C.

In addition, tamarind seed xyloglucan forms gel at low temperature in the presence of ethanol. As a result, tamarind seed xyloglucan in 15% ethanol aqueous solution had gel-sol transition at 47 °C (Yamanaka et al., 2000).

3. Applications of Xyloglucan

Tamarind seed xyloglucan is used in food and pharmaceutical industries since its solution is very stable against heat, pH and shear. In the food industry, tamarind seed xyloglucan is widely used as a thickener, stabilizer, fat replaces, or starch modifier to improve rheological and thermal properties of many products, for example, ice cream, salad dressing, mayonnaise, noodles, stew, etc (Shirakawa and Yamatoya, 2003). Due to low stability against heat or shear, the physical properties of native starch pastes often limit commercial applications in products. Addition of hydrocolloids such as xyloglucan tamarind seed may improve textural properties and stability of native starch pastes in food products.

Pongsawatmanit et al (2006) investigated the influence of xyloglucan obtained from tamarind seed on rheological properties and thermal stability of tapioca starch. Tapioca starch and xyloglucan were prepared at different mixing ratios (10/0, 9/1, 8/2, 7/3, and 6/4) with 5% total polysaccharide concentration. The viscosity and thermal stability of these solutions were determined. Consequently, the viscosity of the tapioca starch paste was lower than those of the tapioca starch and xyloglucan mixture. The tapioca starch and xyloglucan mixture gave higher apparent viscosity with increasing xyloglucan concentration. The change in the apparent viscosity with temperature of the tapioca starch alone was greater than those of the tapioca starch and xyloglucan mixture. The addition of a small amount of xyloglucan to topioca starch might be useful to control the texture of food and to prevent the retrogradation of starch. Moreover, there were some studies on the effect of xyloglucan on rheological and thermal of corn starch and tapioca (Yoshimura et al., 1999; Temsiripong et al., 2005; Pongsawatmanit, Temsiripong and Suwonsichon, 2007) and the effect of xyloglucan on the concentration of gellan and formation of a network of gellan (Ikeda et al., 2004).

For the pharmaceutical application, xyloglucan was studied to be used as an alternative pharmaceutical excipient for sustained release. For example, xyloglucan was used to prepare mucoadhesive buccal patch, matrix tablet of both water-soluble and water insoluble drugs and in situ gelling formulations for rectal, ocular,

transdermal, intraperitoneal and oral drug delivery, it showed sustained release (Miyazaki et al., 2001; Suisha et al., 1998).

Different mucoadhesive polymers such as tamarind seed xyloglucan, polycarbophil, polyacrylic acid and xanthan gum were studied to investigate an innovative mucoadhesive controlled-release device for topical buccal drug delivery. Benzydamine and lidocaine were used as a model drugs in this study. Mucoadhesive properties of four non-medicated matrices prepared by different polymers were determined. The tamarind seed xyloglucan containing matrics was the best mucoadhesive properties. The devices containing the salts of benzydamine and the complex of lidocaine showed zero-order release kinetics in vitro. The patches adhered for over 8 hours to the upper gums of the volunteers, and were perfectly tolerated (Burgalassi et al., 1996).

Tamarind seed xyloglucan and crosslinking tamarind seed xyloglucan with epichlorohydrin were used to prepare the microspheres of both water-soluble drugs; such as acetaminophen, caffeine, theophylline and salicylic acid, and water insoluble drugs such as indomethacin. Releasing behaviors of these drugs were determined. About 50% of total loading of drug releases in 5, 5.5, 7 and 10 hours for caffeine, acetaminophen, theophylline and salicylic acid, respectively. The total release of indomethacin in the first 5 hours is about 10% of total load of the tablet. As a result, tamarind seed xyloglucan can be used for controlled releasing both water-soluble and water insoluble types of drugs. Crosslinking tamarind seed xyloglucan could be sustained for longer period than with tamarind seed xyloglucan (Sumathi and Ray, 2002). In addition, there was the study of controlled caffeine released from tamarind seed xyloglucan tablets and sustained release of verapamil hydrochloride (Sumathi and Ray, 2003; Kulkarni et al., 1997).

A sustained release of gel formation in situ following the oral administration of diluted aqueous solutions of tamarind seed xyloglucan was investigated by the in vitro and in vivo studies. Xyloglucan was modified by partially degraded by β-galactosidase in order to eliminate 44% of galactose residues. The enzyme-degraded xyloglucan could form gels at concentrations 1.0% and 1.5% w/w at 37 °C. In vitro,

the releasing indomethacin and diltiazem from the enzyme-degraded xyloglucan gels followed square root-time kinetics over a period of 5 hours at 37°C and pH 6.8. In vivo, plasma concentrations of indomethacin, after oral administration to rats of chilled 1% w/w aqueous solutions of the enzyme-degraded xyloglucan containing dissolved drug were determined. The release of indomethacin was sustained over a time period of at least 7 hours. Bioavailability of indomethacin from xyloglucan gel formed in situ was increased approximately threefold compared with the suspension. As a result, the enzyme-degraded xyloglucan gel had potential as vehicles for oral delivery (Kawasaki et al., 1999).

4. Xyloglucan extraction

Many methods of xyloglucan extraction were report. For example, Suttananta (1986) extracted xylolglucan from seed kernel by hot water at 92-98 °C for 60 minutes. The slurry was filtered through muslin or sieves no. 80 and was then centrifuged at 3,200 rpm for 30 minutes. The solution was precipitated by 1-1.5 volumes of ethanol. The precipitant was dried by oven at 50-60 °C for 8-12 hours and milled. A yield of approximately 30% was obtained.

In 1990, Molinarolo, Thompson and Stratton reported the extraction of xyloglucan from tamarind seed after the defatting. Then treated tamarind kernal powder (TKP) was dispersed in a small amount of distilled water prior to extraction for one-half hour in boiling distilled water. The suspension was filtered through coarse grade filter paper in a Buchner funnel and then through a layer of diatomaceous earth in a medium grade fritted glass filter funnel. The solution was precipitated by 96% ethanol and drained on cheesecloth and squeezed out to remove as much alcoholic liquid as possible. The precipitate was stirred with 50% ethanol and the liquid was squeezed out. The sample was then concentrated in vacuum to remove ethanol and then freeze dried. Approximately 33% yield was obtained.

Sumathi and Ray (2002) extracted xyloglucan from tamarind seed powder. Tamarin kernel powder was dispersed in 200 ml of cold water. The slurry was added into 800 ml of boiling water. The solution was boiled for 20 minutes under stirring

condition in a water bath. It was kept overnight so that most of the proteins and fibers settled out and was then centrifuged at 5000 rpm for 20 minutes. The supernatant was separated and was precipitated by twice volume of absolute ethanol. The precipitant was washed with absolute ethanol, diethyl ether and petroleum ether and then dried at 50-60 °C under vacuum. The dried material was ground and sieved.

Tamarind seed kernels were dissolved in water by stirring at room temperature. Subseuently, the slurry was centrifuged for 15 minutes at 8,000 rpm to remove insoluble ingredients. Isopropanol was added drop by drop to the supernatant up to a isopropanol concentration of 70 %. The precipitant was separated by centrifugation at 8,000 rpm for 10 minutes, washed by 70 % isopropanol aqueous solution, and dried in a vacuum at 500 °C for 24 hrs (Hiroshi et al., 2002).

In addition, xyloglucans were extracted by mixing tamarind seed powder with water at 25 °C. The slurry was centrifuged at 10,000 rpm for 20 minutes and the supernatant passed sequentially through Millipore filter membranes with pore sizes of 3 and 0.8 mm. Then, the solution was precipitated with two volumes of 96% ethanol and washed with acetone (Freitasa et al., 2005).

C. Spray drying technique

Spray drying is the most common industrial process for removal of solvent involving particle formation and drying such as beverages, flavors, milk, plant extracts, pharmaceuticals, plastics and polymers etc. The spray dryer system consists of a feed pump, an atomizer, an air heater, an air dispenser, a drying chamber and a system for exhausting air cleaning and powder recovery. There are three main phenomena in spray dry process as follows: (Shabde and Hoo, 2008).

1. Atomization of the liquid feed
2. Drying of the droplets once they are formed
3. Motion of the droplet to model the spray drying process

For this process, a sample such as solution, emulsion, slurry and suspension, etc, is atomized in a spray drying chamber. Heat is supplied to the liquid mixture by

passing a hot air to the liquid under controlled temperature and airflow conditions. The contact between the droplets and the hot air results in the solvent in the liquid mixture evaporates and the droplets converted into solid particles. The solid particles are continuously discharged from the drying chamber and recovered from the exhaust air using a cyclone or a bag filter.

The parameters influencing yield and powder properties such as particle size and size distribution, particle shape, moisture content and bulk density are concentration of the sample, inlet and outlet air temperatures, main air flow rate and liquid feed flow rate.

The spray drying parameters were determined in relation to particle size and the yield of the resultant powder. Chawla et al. (1994) investigated on pump speed, aspirator level, inlet temperature and the concentration of the aqueous salbutamol sulphate solution dried. The yield percentages of the spray dried material relied on the several factors such as the aspirator level, the feed concentration and the interaction between the pump and the feed concentration. An increase in any of these factors, or interacting factors, led to an increase in product yield. It was found that spray drying produced spherically shaped particles of salbutamol sulphate with a mass median diameter of 4.5 μm (laser diffraction), mean Feret's diameter (image analysis) of 1.58 μm and a mass median aerodynamic diameter of 9.7 μm (cascade impaction), i.e., particles sufficiently small in diameter for use in inhalation formulation.

Billona et al. (2000) investigated the effects of five parameters on production yields and moisture contents of spray-dried products. These factors concerned concentration of solution feed (drug concentration, colloidal silica concentration and polymer:drug ratio), inlet temperature and feed rate. Yields could be considerably increased by reducing feed rate. An increase in inlet temperature improved yields when the feed rate was high. An increase of feed rate led to higher moisture contents, especially at low inlet temperature; however, a higher inlet temperature promoted a decrease of residual moisture. The total feed concentration (drug concentration and colloidal silica concentration) also greatly affected yields and

moisture content. When the concentration of the solution feed increased, the drying process is greater. Polymer:drug ratio had significant effect of the formulations.

The effect of process variables on the degradation and physical properties of spray dried insulin intended for inhalation was investigated by Stahl et al. (2002). Factorial experimental design was applied to evaluate the effects of feed flow rate, nozzle gas flow rate, inlet air temperature and aspirator capacity on the degradation and physical properties of spray dried insulin intended for inhalation. It was found that the degradation of insulin was not dependent on the nozzle gas flow rate. However, a decrease in the nozzle flow resulted in an increase in the yield. They also stated that the atomization energy could be decreased by decreasing nozzle flow and thus producing enlarged droplets. It was also found that larger particles dried by these droplets enable these particles to be easily captured through the centrifugal force in the cyclone. Not only increasing aspirator capacity but also increasing inlet air temperature led to a decrease in moisture content as a result of higher efficient drying caused by an increase in the supplies of heat energy. This relationship between moisture content and outlet air temperature was in agreement with the spray-dried β-galactosidase study of Broadhead et al. (1994). Due to the reduction of the outlet air temperature caused by increasing feed flow, drying capacity was lower but moisture content in the powder product was higher. The investigators also reported that an increase in inlet air temperature led to an expansion of the particle size.

Advantages of spray drying (Master, 1979):

1. A single-step operation from liquid feed to dry product.
2. An ability to operate in applications that range from aseptic pharmaceutical processing to ceramic powder production.
3. A potential design to virtually any capacity required.
4. A constant powder quality constant during the entire run of the dryer.
5. A continuous and adaptable operation for full automatic control.
6. A great variety of spray dryer designs meeting various product specifications.
7. A possible application for both heat-resistant and heat sensitive products.

8. An endurable process even though the abrasive corrosive, flammable, explosive or toxic feedstock if the pump working
9. An potential application for the different of solution feedstock such as slurry, paste, gel, suspension or melt form.
10. A characteristic particle from the product a high bulk density and, in turn, rapid dissolution (large surface area)
11. A non-contraction metal surfaces until dried, reducing corrosion problems.
12. A dry product specifications meeting through dryer design and operational flexibility.

D. Structure of skin

The skin is the largest organ of the human body, accounting for approximately 10% of total the body mass of an average person, and it covers an average area of 1.7 m^2. Its function is to prevent loss of water and other components of the body to the environment and protect the body from a variety of environmental insults. The skin also has important immune and sensory functions, helps to regulate body temperature, and synthesizes vitamin. The skin composes of 70% water, 25% protein and 2% lipids. The remainder includes trace minerals, nucleic acids, glycosoaminoglycans, proteoglycans and numerous other chemicals. The skin consists of three main layers: epidermis, dermis and subcutaneous tissue as depicted in Figure 4.

The viable epidermis is the topmost layer of the skin. It is a complex multiply-layered membrane, approximately 100-150 μm thick. About 95% of the viable epidermis layer is composed of keratinocytes. Other cell types in its are melanocytes, Langerhans cells and Merkel cells (mechanoreceptors). The viable epidermis contains four layers.

1. Stratum germinativum or basal layer
2. Stratum spinosum or spinous layer or prickly layer
3. Stratum granulosum or granular layer
4. Stratum lucidum

The stratum corneum or the horny layer is the top layer of the skin, where its thickness depends on the region of the body and can be from about 10 microns to several hundred microns. Furthermore, it is consisted of layers of dead, flattened

keratinocytes by surrounding a lipid matrix. The lipid matrix is jointly active as a brick-and-mortar system which has a difficulty of penetrates. It is interesting to note that the stratum corneum is a majority of barrier to transdermic delivery.

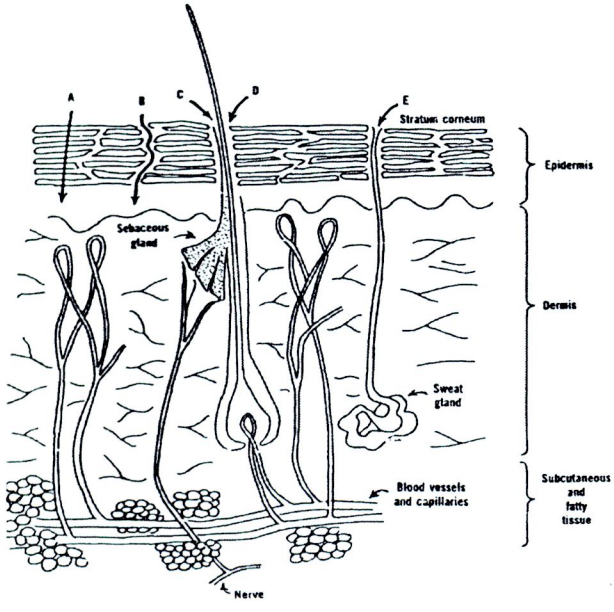


Figure 4 Structure of human skin in crosssection

The central layer of the skin, the thickest of the skin layers, 3-5 mm thick, being location between the epidermis and subcutaneous tissue is called as the dermis or courium. It has a composition of a matrix of connective tissue woven from several fiberous proteins; such as collagen, elastin and reticulin, that tightly surround by an amorphous ground substance of mucopolysaccharide. Moreover, there also are several compositions of the dermis such as sebaceous glands, sweat glands, hair follicles and a small number of nerve and muscle cells. The supply of blood maintains the dermal concentration of a permeated drug as a very low level, the essential driving force for transdemal permeation is contributed by a consequence of different concentration across the epidermis.

Eventually, the deepest layer of the skin being located under the dermis and containing mostly of fat cells is called as subcutaneous tissue. There are several advantages of subcutaneous tissue such as maintenance of regulated temperature,

contribution of nutritional support and mechanic protection , carrying the principal blood vessels and nerving the skin and may contain sensory pressure organs (Williams, 2003; Wille, 2006).

E. Transdermal drug delivery system

Alternatively, the delivery of systemically acting drugs can apply the skin to deliver transdermal drugs. There are several advantages for using skin as an alternative route over oral drug administration (Wang et al., 2002).

1. A circumvention of a variety of influences on gastro-intestinal absorption such as pH, food intake and gastro-intestinal motility.
2. A circumvention of the hepatic metabolism, which be suitable for drugs with a low bioavailability.
3. A delivery of transdermic drug resulting in proving a constant, controlling drug input, decreasing the variation in drug plasma levels, reducing the side effects particularly of drugs with a narrow therapeutic window.
4. An increase in patient compliance.
5. A quick termination of therapy by simple removal of the system from the skin.

There are two traditional designs of transdermic drug delivery devices (Tyle, 1988).

1. Reservoir-type devices

The drug in a reservoir-type system is kept in a reservoir from its diffusion via a rate-limiting membrane to the site of absorption. This form of system can get a benefit, which is the near-constant release rate of drug from device, when the stratum corneum is not the principal rate-limiting barrier for the diffusion of drugs and rate control from the desirable device. There are four main compositions of the drug in a reservoir-type system; backing membrane, reservoir of drug, rate limiting membrane and adhesive layer. The rate-limiting membrane is microporous or nonporous membrane. There are several methods of a control of a releasing rate such as varying the polymer ratios, drug reservoir formulation, permeability coefficient of the rate

controlling membrane and thickness of the adhesive. Many designs in this system are appearances such as

a). Hercon device

b). Multireservoir rate-limiting devices

c). Devices with rate-limiting adhesive layer

d). Drug reservoir gradient transdermal device

e). Microencapsulated drug reservoir-type devices

f). Transdermal devices with Hollow reservoir etc.

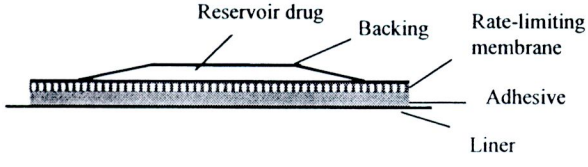


Figure 5 A reservoir-type transdermal delivery device

2. Matrix-type transdermal devices

In a matrix-type of devices, the drug is a uniform dispersion all through a hydrophilic or lipophilic polymer matrix. Subsequently, the hydrophilic or the lipophilic polymer matrix is cured into a polymeric disk of predetermined thickness and surface area. The matrix is subsequently glued to aluminum foil sealed to drug impermeable backing via an absorbent pad. Occasionally, the polymer matrix is not only a reservoir of drug but also adhesive. There are several factors influenced on the release rate of polymer-matrix system such as the initial drug loading dose, solubility and diffusion coefficient of drug in polymer matrix.

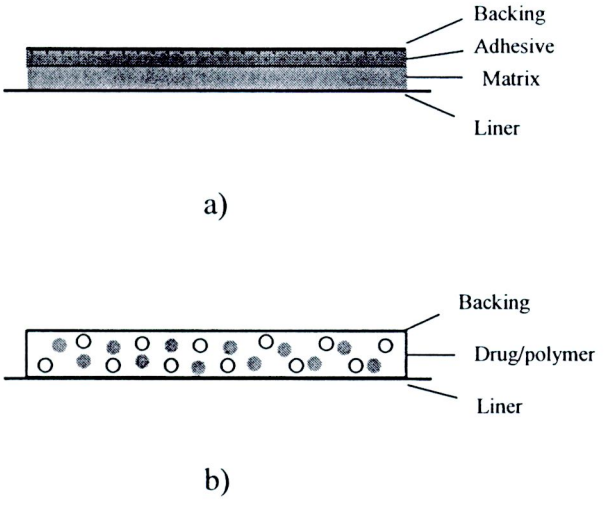


Figure 6 a) A typical matrix-type transdermal delivery device
b) An adhesive matrix device

Furthermore, 'microreservoir or microsealed transdermal delivery device' is the hybrid of the reservoir and matrix types of system. The preparation of an aqueous suspension of drug for this system is in a water-soluble polymer. The dispersion of the suspension into lipid-soluble polymer with high-speed-shear force is the formation of microscopic spherical reservoir with the drug entrapped. Instantly, the system is the crosslink of the addition of polymeric crosslinking agents and the formation of matrix, which is attachment to an aluminum foil plate at the back. There is a peripheral adhesive ring in the system (Mathiowitz et al., 1999; Hadgraft and Guy, 2002).

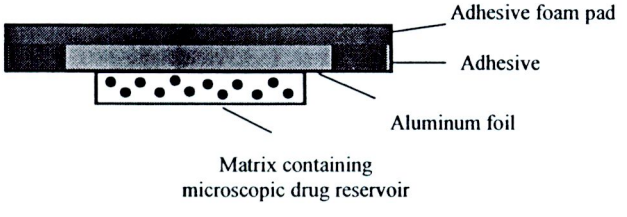


Figure 7 A microreservoir transdermal delivery device.

F. Wound healing

Wound healing is necessarily applied to remove invaded pathogen from the damaged tissue of the body and completely or partially remodel injured tissue. The phases of normal wound healing consist of hemostasis, inflammation, proliferation, and remodeling.

Initially, tissue injury clearly responds to the wound of devitalized tissue and foreign material and then the stage for subsequent tissue healing and regeneration are formed. The initial vascular responds to both a brief and transient period are vasoconstriction and hemostasis. A 5-10 minute period of intense vasoconstriction is subsequent to active vasodilation in company with increased capillary permeability. An aggregation of platelets within a fibrin clot generate a variety of growth factors and cytokines, by setting the stage for an orderly series of events resulting in tissue repair.

The second phase of wound healing is the inflammatory phase which represents as erythema, swelling, and warmth. It is frequently liked to the pain. An increase in vascular permeability, which leads to migration of neutrophils and monocytes into the surrounding tissue, is affected from inflammation. The first line prevention of infection is contributed by the neutrophils engulfing debris and microorganisms. In case of non–contamination after the first few days post-injury, neutrophil migration ends. Furthermore, in case of persistiveness of acute inflammatory phase as result of wound hypoxia, the release of hypoxia, infection, nutritional deficiencies, medication use, or other factors is to the patient's immune response, possibly interfering with the late inflammatory phase. The convention of monocytes in the tissue to macrophages, which is used for a digestion and a kill of bacterial pathogens, scavenged tissue debris and an elimination of remain neutrophils, is in the late inflammatory phase. In order to transit wound inflammation of macrophages to wound repair, a variety of chemotactic and growth factors including the stimulation of cell migration, proliferation, and formation of the tissue matrix, are repaired.

The subsequent proliferative phase is dependent on the formation of granulation tissue and epithelialization. The size of the wound also has effect on its period. The stimulation of the migration and activation of wound fibroblasts for the production of a variety of substances essential to wound repair, involving glycosaminoglycans (mainly hyaluronic acid, chondroitin- 4-sulfate, dermatan sulfate, and heparan sulfate) and collagen, is released by chemotactic and growth factors. The formation of an amorphous, gel-like connective tissue matrix is necessity for cell migration. It is essential that new capillary growth associated with the advancing fibroblasts into the wound for the contribution of metabolic needs. Both vascular integrity and strength of new capillary beds has been a responsibility to improper cross-linkage of collagen fibers. Near the beginning of the proliferation phase fibroblast activity is limitable to cellular replication and migration. Approximately the third day after wounding the growing mass of fibroblast cells start for synthesizing and secreting determined quantities of collagen. A continue rise in collagen levels last approximately three weeks. The tensile strength of the wound is evaluated by the quantities of secreted collagen over this approximately three weeks.

Wound remodeling, involved by a reorganization of new collagen fibers, a formation of more organized lattice structure which continue progress for an increase in wound tensile strength, is the final phase of wound healing. The remodeling process lasts no more than 2 years. It is interesting to note that 40-70 percent of the strength of undamaged tissue can be achieved in four weeks (MacKay and Miller, 2003).

G. Botanical, Chemical and Pharmacological Aspects of *Centella asiatica* (Linn.)

1. Botanical aspects of *Centella asiatica* (Linn.)

Centella asiatica (Linn.) of the family Apiaceae (Umbelliferae) is commonly found in parts of India, Asia and the Middle East. It is known as Buabok, Pegaga, Luei Gong Gen, Tung Chain, Vallarai and Daun Kaki Kuda.

Centella asiatica (Linn.) is a perennial, herbaceous creeper growing up to 30 cm in height with fan-shaped leaves. The stems are slender, creeping stolons, green to reddish green in color. It has long-stalked, green, reniform leaves with rounded apices which have smooth texture with palmately netted veins. The leaves are borne on pericladial petioles, around 20 cm. The rootstock consists of rhizomes, growing vertically down. They are creamish in color and covered with root hairs. The flowers are pinkish to red in color, born in small, rounded bunches (umbels) near the surface of the soil. Each flower is partly enclosed in two green bracts. The hermaphrodite flowers are minute in size (less than 3 mm), with 5-6 corolla lobes per flower. Each flower bears five stamens and two styles (Figure 8).

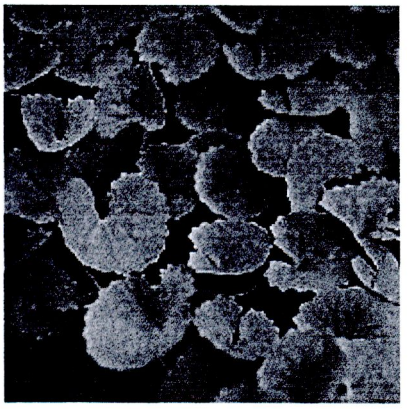
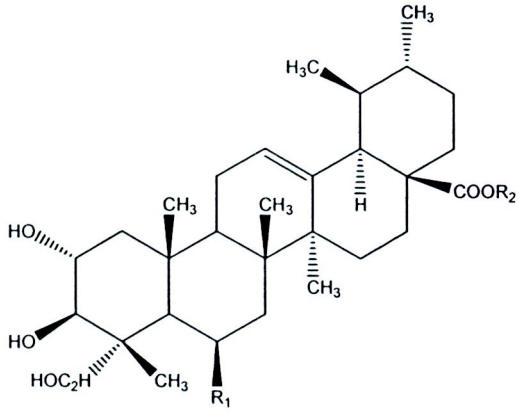


Figure 8 *Centella asiatica* (Linn.) Urban

2. Chemical components of *Centella asiatica* (Linn.)

Centella asiatica (Linn.) contains a wide range of other substances such as triterpenoid glycosides (saponins) , phytosterols and a volatile oil consisting of vallerin, camphor, cineole and an unidentified terpene acetate. The substances of therapeutic interest are the saponin-containing triterpene acids and their sugar esters, the most important being asiatic acid, madecassic acid, asiaticoside and madecassoside (Figure 9) (Shim et al., 1996; Lawrence, 1967).



Compound	R_1	R_2
Asiaticoside	H	glu-glu-rham
Madecassoside	OH	glu-glu-rham
Asiatic acid	H	H
Madecassic acid	OH	H

Figure 9 Structures of the triterpenoids from *Centella asiatica* (Linn.)

3. Pharmacological activities of *Centella asiatica* (Linn.)

Centella asiatica has been used for hundreds of years as the traditional medicine of many asiatic countries to treat dermatological conditions. It is used to support faster healing of small wounds, chaps and scratches, superficial burns and, as an oral preparation, for atonic wounds and hypertrophic healing. Centella also has been used traditionally as an anti-inflammatory, particularly for eczema, and also for minor itching and insect bites. The active ingredients of *Centella asiatica* which claimed to possess wound healing properties are madecassic acid, asiaticoside and asiatic acid (The European Agency for the Evaluation of Medicinal Products Veterinary Medicines Evaluation unit, 1998). They promoted fibroblast proliferation and type-I collagen synthesis. The overall effects contributed to the restoration of elastic connective tissue tissue, a reduction in fibrosis and a short in the time necessary for wound healing (Lu et al., 2004).

Ether undefined alcohol or aqueous extracts or one of the following extracts; TECA, TTFCA, or TTF are mostly applied for clinical studies of *Centella asiatica*. The extracts TECA (titrated extract of *Centella asiatica*) and TTFCA (total triterpenoid fraction of *Centella asiatica*) combine a comprisal of asiatic acid (30%), madecassic acid (30%), and asiaticoside (40%). The centella extract TTF (total triterpenic fraction) is a comprisal of asiatic acid and madecassic acid (60%) in a doubt definition of ratio, in association with asiaticoside (40%) (Brinkhaus et al., 2000). Centella has potentially developed connective tissue integrity by elevating antioxidant levels in wound healing and enhancing capillary permeability (Shukla et al., 1999; Belcaro, Grimaldi and Guidi, 1990).

Shukla et al. (1999) studied the effect of asiaticoside on the wound healing in guinea pigs punch and streptozotocin diabetic rats. The 0.2% asiaticoside solution in guinea pig punch wounds produced 56% increase in hydroxyproline, 57% increase in tensile strength, increased collagen content and better epithelisation. The 0.4% asiaticoside solution in streptozotocin diabetic rats increased hydroxyproline content, tensile strength, collagen content and epithelisation thereby facilitating the healing. These results indicated that asiaticoside exhibits significant wound healing activity in normal as well as delayed healing models and was the main active constituent of *Centella asiatica*.

The investigation of *Centella* extract affecting on the protectiom of ethanol induced gastric lesions in rats. The 50% ethanol in the gastric ex-vivo chamber model was used the reduction of gastric transmucosal potential difference and an acceleration of its recovery resulted from *Centella* extract. Before a significant inhibition of gastric lesions formation (58% to 82% reduction) by ethanol administration and a reduce in mucosal myeloperoxidase (MPO) activity in a dose dependent manner, Oral administration of *Centella* extract (50 mg/kg, 0.25 g/kg and 0.50 g/kg) was applied. Consequently, it was suggested by Cheng and Koo (2000) that the prevention of ethanol by *Centella* extract induced gastric mucosal lesions by strengthening the mucosal barrier and a decease in the damaging effects of free radicals.

The potentiality of the wound-healing ethanolic extract of *Centella asiatica* in both normal and dexamethasone suppressing wound healing was investigated by Shetty et al. (2006). Wistar albino rats were incision, excision, and dead space wounds models. A significant increase in the wound breaking strength in incision wound model compared with controls ($P < 0.001$) resulted from the extract of *Centella asiatica*. It was found that the extract-treated wounds epithelize faster, and a significant increase in the rate of wound contraction was apparent when compared with control wounds ($P < 0.001$). The content of wet and dry granulation tissue weights, granulation tissue breaking strength, and hydroxyproline in a dead space wound model resulted in an increase in statistically significant levels.

The extract of *Centella asiatica* affected on attenuating the known effects of dexamethasone healing in all wound models ($P < 0.001$, $P < 0.05$). As a result, wound healing is significantly promoted by the extract of *Centella asiatica*. This extract has ability for defeating the wound-healing suppressing action of dexamethasone in a rat model.