CHAPTER II

LITERATURE REVIEW

1. Skin

Skin is the largest organ of the human and it performs many vital roles as both a barrier and a regulating influence between the outside world and the controlled environment within our bodies. As our major interface with the environment, the skin is composed of specialized epithelial and connective tissue cells. It has functions in thermoregulation, protection, metabolic functions, and sensation. The skin is composed of three primary layers, which are the epidermis, the dermis, and subcutaneous tissue (Baumann, 2002).

Structure of the skin

1. Epidermis

This is the outermost layer. In most parts of the body the epidermis is about 0.1 mm thick but on the soles of the feet and the palms of the hands it can be 1mm thick or more. Epidermis contains no blood vessels, and is nourished by diffusion from the dermis (Dan, 2005). It is formed by an ordered arrangement of cells called keratinocytes, whose basic function is to synthesize keratin, a filamentous protein that serves a protective function (Odom, James and Berger, 2000). The epidermis is the most superficial layer of the skin and provides the first barrier of protection from the invasion of foreign substances into the body. It is very important from a cosmetic standpoint because it is the layer that gives the skin its texture and moisture, and contributes to skin color.

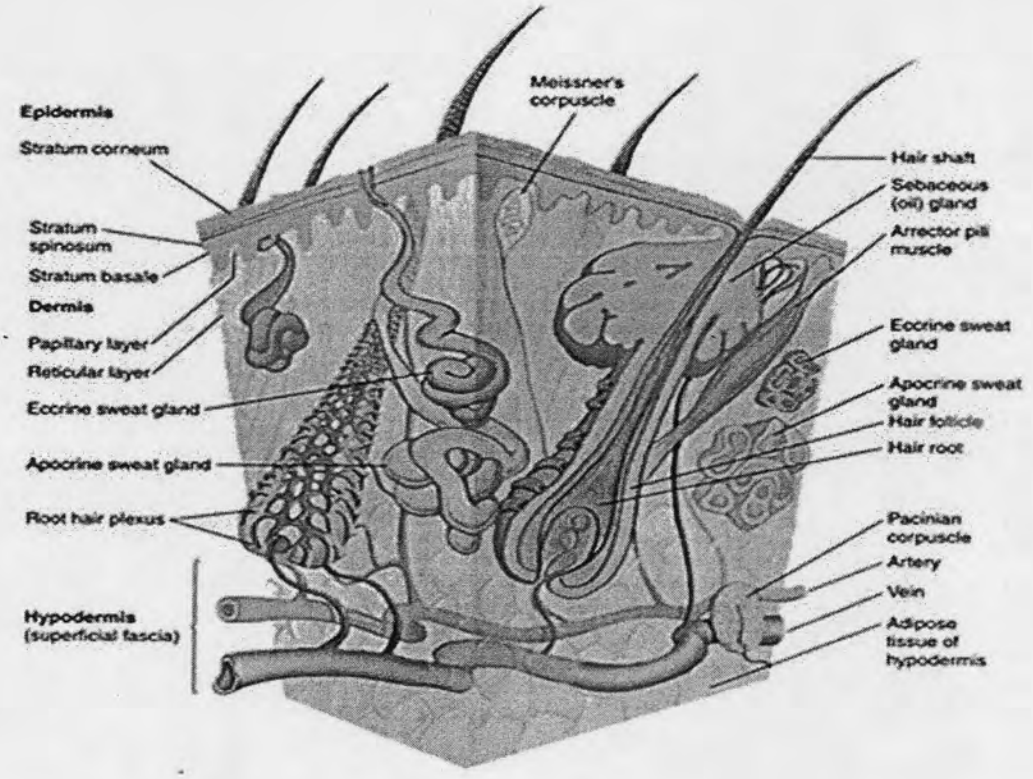


Figure 1 The basic diagram of skin structure (Gartner and Hiatt, 2007)

1.1 Layers of the epidermis

The epidermis is subdivided into five layers or strata, which are the stratum basale (basal layer), the stratum spinosum (spinous layer), the stratum granulosum (granular layer), the stratum lucidum (clear layer), and the stratum corneum (horny layer). The principal cell of the epidermis is called a keratinocyte, which it gradually migrates to the surface and is sloughed off in a process called desquamation.

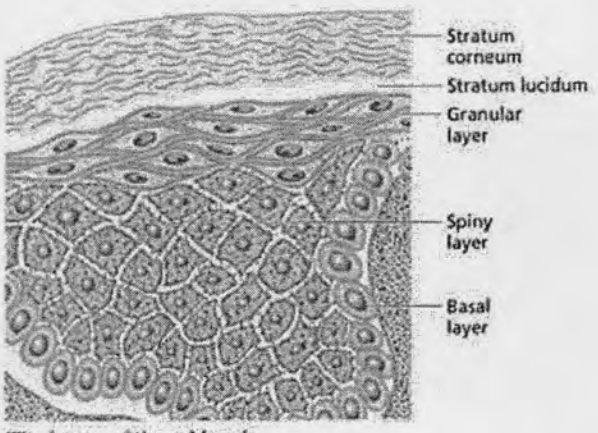


Figure 2 The layers of the epidermis (Gray, 2006)

1.1.1 The stratum basale (basal layer)

The stratum basale (basal layer) is the deepest layer. This layer is comprised of a single layer of mitotically active cuboidal or columnar epithelia. This is a major region of mitotic activity. Roughly half of the basal cell keratinocytes are mitotically active. Basal cells are attached to each other by desmosomes that contain cadherins. Basal cell layers attach to the basement membrane (basal lamina) on which they reside via hemidesmosomes that contain integrins. Desmosomes and hemidesmosomes anchor intermediate filaments, which are comprised of keratins in epithelial cells, to the cell surface. Two types of keratins, type I keratins, which are acidic and type II keratins, which are basic, are required to form an intermediate filament. The basal cells are responsible for maintaining the epidermis by continually renewing the cell population (Gartner and Hiatt, 2007).

1.1.2 The Stratum spinosum (spinous layer)

The cells in this layer arise from the migration from the basal layer and lose their adhesion to the basement membrane and adhere to other keratinocytes. Also, the cells in this layer attain a more flattened shape and have increased amounts of keratin

containing intermediate filaments and desmosomes, which provide the characteristic "prickles" of the stratum spinosum, which is often called the prickle-cell layer.

1.1.3 The stratum granulosum (granular layer)

The granular layer (stratum granulosum) is characterized by the presence of keratin, called keratinization. The cells of the stratum granulosum accumulate dense basophilic keratohayalin granules. These granules contain the protein filaggrin, which serves to bundle the keratin filaments together. The proteins of the cornified cell envelope (involucrin, keratolinin, pancornulins, and loricrin) are cross-linked in this layer by the calcium-requiring enzyme transglutaminase to form the cell envelope. In addition, this is the most superficial layer of the epidermis that still has nuclei.

1.1.4 The stratum lucidum (clear layer)

The stratum lucidum is a thin, clear layer of dead skin cells in the epidermis, and is named for its translucent appearance under a microscope. It contains a clear substance called eleidin, which eventually becomes keratin. This layer is found beneath the stratum corneum of thick skin, and as such is only found on the palms of the hands and the soles of the feet. The keratinocytes of the stratum lucidum do not feature distinct boundaries and are filled with eleidin, an intermediate form of keratin.

The cells of the stratum lucidum are flattened and contain an oily substance that is the result of exocytosis of lamellar bodies accumulated while the keratinocytes are moving through the stratum spinosum and stratum granulosum. It is this substance that gives the stratum lucidum its waterproof properties, and, thus, it is also called the barrier layer of the skin.

1.1.5 The stratum corneum (horny layer)

The most superficial layer of the epidermis is the stratum corneum. The keratinocytes that reside in this layer are the most mature and have completed the keratinization process. These keratinocytes have no organelles, and their arrangement resembles a brick wall. The stratum corneum is composed of protein-rich corneocytes embedded in a bilayer lipid matrix arranged in a "brick and mortar" fashion. The "bricks" are composed of keratinocytes, and the "mortar" is composed of the contents extruded from the lamellar granules, including lipids and proteins. The stratum corneum is described as the "dead layer" of cells because these cells do not demonstrate protein synthesis and are unresponsive to cellular signaling (Baumann, 2002).

1.2 Cell types in epidermis

1.2.1 Keratinocytes

Keratinocytes is the major cell type of the epidermis, making up about 90% of epidermal cells. Keratinocytes originate in the basal layer from the division of keratinocyte stem cells. They are pushed up through the layers of the epidermis, undergoing gradual differentiation. While they move to the surface of the skin the keratinocytes are enucleated, flattened and highly keratinized. In this form they are called squamous cells. Eventually they die off and form the stratum corneum. This layer of dead cells forms an effective barrier to the entry of foreign matter and infectious agents into the body and minimizes moisture loss. The migration process normally takes approximately 28 days (Odom et al., 2000).

1.2.2 Melanocytes (pigment cell)

Melanocytes are neural crest derived cells that produce melanin, the brown pigment that produces skin color. Melanocytes reside in the basal layer of the epidermis and extend processes that contact many keratinocytes in and immediately above the basal layer. They do not form desmosomal connections. Melanocytes produce melanin from tyrosine in specialized organelles called melanosomes, which

contain tryosinase, an enzyme critical for melanin production. Keratinocytes are the reservoir for melanin in the skin. Variations in type and density of melanosomes lead to the wide range of human skin color. Chronic sun exposure can stimulate the melanocyte to produce larger melanosomes, thereby making the distribution of melanosomes within keratinocytes resemble the pattern seen in dark-skinned individuals (Odom et al., 2000; Walters and Roberts, 2002).

1.2.3 Langerhans cells

Langerhans cells are monocyte derived dendritic cells present in the living layers of the epidermis, especially are found scattered among keratinocytes of the stratum spinosum, or prickle cell layer of the epidermis. They intimately involved in immunological response in the skin by providing for the recognition, uptake, processing, and presentation of antigens to sensitized T lymphocytes (Odom et al., 2000; Gartner and Hiatt, 2007).

1.2.4 Merkel cells

Merkel cells are present in the basal layer of thick skin of hands, soles, the oral and genital mucosa, the nail bed, and the follicular infundibula. The Merkel cells are located directly above the basement membrane. They are modified keratinocytes that possess keratins and form desmosomal attachments to keratinocytes. They are associated with sensory nerve terminals and function in touch sensation (Odom et al., 2000).

2. Dermis

The dermis has the thick range from 3 – 5 mm consisting of mostly connective tissue (collagen and elastin) and laden with nerves, blood vessels and sweat glands. The dermis lies between the epidermis and the subcutaneous fat. The dermis needs an efficient blood supply to convey nutrients, remove waste product, control temperature and pressure, mobilize defense forces, and contribute skin color. The upper portion of this layer, which lies beneath the epidermis, is known as the papillary dermis and the lower portion is known as the reticular dermis.

2.1 Layers of the dermis

The dermis gives the skin its mechanical strength. There are two main layers of the dermis.

2.1.1 Papillary layer

The papillary dermis contains vascular networks that have two important functions. The first one is that the papillary dermis contains capillary loops being to support the vascular epidermis with vital nutrients and, secondly, to provide a network for thermoregulation. In the papillary dermis, collagen fibers are loosely bundled (Baumann, 2002).

2.1.2 Reticular dermis

The reticular layer of the dermis is tightly packed with collagen and elastin for strength and elasticity. This layer also contains glycosaminoglycans to bind water (Baumann, 2002).

2.2 Components of the dermis

2.2.1 Cellular components

Fibroblasts are the primary cell type in the dermis. They have an important role produce fiber elements, collagen, elastin, ground substance, other matrix proteins, and enzymes such as collagenase and stromelysin (Aulton, 2002). Immune cells such as mast cells, polymorphonuclear leukocytes (PMNs), lymphocytes, and macrophages are also present in the dermis (Odom et al., 2000).

2.2.2 Extracellular matrix

The extracellular matrix is the material that forms the bulk of the dermis, excluding water and cells (Bernstein and Uitto, 1996). This extracellular matrix (ECM), produced by keratinocytes and fibroblasts, works not only as a physical

support, but also as an exchange and communication area that allows nutrients, metabolites and growth factors to diffuse between cells. Formation of ECM is essential for processes like growth, wound healing, and fibrosis. The main molecules forming the ECM are collagen, elastin, proteoglycans, fibronectins, and other glycosylated proteins (Thibodeau, 2000). Most of the dermal ECM is synthesized by fibroblasts, which respond to variety of stimuli, such as growth factors elaborated by keratinocytes, inflammatory cells, and dermal fibroblast themselves.

2.2.3 Collagen

Collagen is one of the long fibrous structural proteins whose functions are quite different from those of globular proteins such as enzymes. Tough bundles of collagen called collagen fibers are a major component of the ECM that supports most tissues and gives cells structure from the outside, but collagen is also found inside certain cells. Collagen has great tensile strength, and is the main component of fascia, cartilage, ligaments, tendons, bone and teeth. Along with soft keratin, it is responsible for skin strength and elasticity, and its degradation leads to wrinkles that accompany aging. It strengthens blood vessels and plays a role in tissue development. Collagen fibers are always seen in the final, mature, state of assembly as opposed to elastin, whose immature fibers are seen in the superficial dermis and whose more mature fibers are found in the deeper layer of the dermis. Each type of collagen is composed of three chains. Collagen is synthesized in the fibroblasts in a precursor from called procollagen. The skin drives its strength from the fibrillar collagens, chiefly collagen type I and III, which from the bulk of the dry weight of the dermis.

2.2.4 Elastic fiber

Elastic fibers form a fine network throughout the dermis. Although, they make up only 3-4 percent of the dry weight of the skin and 1 percent of the volume of the dermis, they are crucial to provide resilience and elasticity. Elastic fibers are composed of at least two distinct proteins, elastin and fibrillin. Elastic fibers are found at the periphery of collagen bundle and endow the skin with recoil properties. These fibers are assembled on bundles of microfibrils composed of fibril. Fibrillin constitutes the fibrillar component of elastic fibers and is analogous to the cloth

surrounding a bungee cord, while the stretchy inner component corresponds to elastin. Elastic fibers form a fine network that extends vertically in the dermal papillae and surrounds dermal bloods vessels, while in the reticular dermis the fibers are much thicker and run parallel to the epidermis surrounding the larger collagen fibers (Bernstein and Uitto, 1996; Baumann, 2002).

2.2.5 Glycosaminoglycans (GAGs)

Glycosaminoglycans are a family of endogenous mucopolysaccharides that constitute the fundamental substance of the connective tissue. These are electronegative polymers constituted of repetitive units of disaccharides including an amino-sugar and uronic acid. In international chemical nomenclature, the term "mucopolysaccharides" has been substituted with "glycosaminoglycans" (GAG) as the first was less precise and included products elaborated from epithelial cells and mucous secretion. Moving on to GAG, It can be referred to a narrower and more defined series of products, the sulphurated and asulphurated acid mucopolysaccharides and more precisely the following: Chondroitin sulphate, Dermatan sulphate, Keratan sulphate, Heparin, Heparan sulphate, Hyaluronic acid. GAGs associated with a protein core are aptly termed proteoglycans. Proteoglycans and GAGs maintain dermal hydration, as well as possibly participating in collagen and elastic fiber formation (Curri, 1966; Bernstein and Uitto, 1996; Baumann, 2002).

2.2.6 Matrix metalloproteinases (MMPs)

Matrix metalloproteinases (MMPs) constitute a protein family that participates in the degradation ECM macromolecules. Collectively they are capable of degrading all kinds of ECM proteins, but also can process a number of bioactive molecules. There are more than 200 known metalloproteinases, almost all of them dependent on zinc at the active centre for their catalytic function.

3. Subcutaneous layer (hypodermis)

The hypodermis is the deepest section of the skin. The hypodermis refers to the fat tissue below the dermis that insulates the body from cold temperatures and provides shock absorption. Fat cells of the hypodermis also store nutrients and energy. The hypodermis is the thickest in the buttocks, palms of the hands, and soles of the feet. As we age, the hypodermis begins to atrophy, contributing to the thinning of aging skin. The blood vessels and nerves that it contains are larger than those in the dermis. It may also house the hair follicles when they are in the growing phase (Gray, 2000). One of the functions of this fatty layer may be to act as an insulation to conserve body heat. This layer also contains collagen type I, III, and V. As human age, some of the subcutaneous fat is lost or redistributed into undesired areas. This phenomenon contributes to the aged appearance.

4. Skin appendages

Skin appendages are appendages that are associated with the skin and serve a particular function. In humans some of the more common skin appendages are hairs, sebaceous glands, sweat glands, and nails.

4.1 Hair follicle

Epidermal buds grow into the dermis. The developing follicle forms at an angle to the skin surface and continues its downward growth. The hair is formed from cells just above the bulb, which also give rise to concentric zones of differentiated epithelial cells destined to form the inner and outer root sheaths. A tiny bundle of muscle fiber that attached to the follicle called the *arrector pili* that is responsible for causing the follicle to become more perpendicular to the surface of the skin, and causing the follicle to protrude slightly above the surrounding skin. This process results in goose bumps or goose flesh. Stem cells are located at the junction of the arrector and the follicle, and are principally responsible for the ongoing hair production during a process known as the Anagen stage. The average growth rate of healthy hair follicles on the scalp is .04 cm per day (Walters and Roberts, 2002).

4.2 Sebaceous glands

The sebaceous glands are found in greatest abundance on the face and scalp, though they are distributed throughout all skin sites except the palms and soles. Sebaceous glands secrete an oily substance called sebum that is made of fat (lipids) and the debris of dead fat-producing cells. In the glands, sebum is produced within specialized cells and is released as these cells burst; sebaceous glands are thus classified as holocrine glands. Sebum acts to protect and waterproof hair and skin, and keep them from becoming dry, brittle, and cracked. It can also inhibit the growth of microorganisms on skin (Aulton, 2002; Walters and Roberts, 2002).

4.3 Sweat glands

Sweat glands are normally found in almost every part of the skin, forming tiny coiled tubes embedded in the dermis or subcutaneous fat. The skin contains two different groups of sweat glands, which are apocrine sweat glands and eccrine sweat glands. Apocrine sweat glands are limited to specific body regions and are coiled tubular glands that produce a viscous, cloudy, and potentially odorous secretion. The eccrine sweat glands have three primary functions. First, the glands sweat to cool the surface of the skin and reduce body temperature. This cooling is the primary function of sensible perspiration, and the degree of secretory activity is regulated by neural and hormonal mechanisms. Second, eccrine sweat gland secretion can also provide a significant excretory route for water and electrolytes, as well as for a number of prescription and nonprescription drugs. Last, eccrine sweat gland secretion provides protection from environmental hazards by diluting harmful chemicals and discouraging growth of microorganisms (Walters and Roberts, 2002).

4.4 The nails

Anatomically fingernails and toenails are made of a tough protein called keratin and have many different parts. Certainly, nail plate composition, layers of flattened keratinized cells fused into a dense, but somewhat elastic mass, will afford some protection to the highly sensitive terminal phalanx in the keratinization process the cell undergo shape and other changes, similar to those experienced by epidermal

cells forming the stratum corneum. The nail plate comprises two major layers (the dorsal and intermediate layer) with, possibly, a third layer adjacent to the nail bed. There are differences in the chemical composition of the two layers, which further suggests that applied drugs may possess differing partitioning tendencies between the layers (Walters and Roberts, 2002).

2. 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. The first way is to 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. The second way is to direct drugs to the viable skin tissues without using oral, systemic or other routes of therapy. The last approach is to use 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, which are skin surface, horny layer, viable epidermis and upper dermis, skin glands, and systemic circulation (Aulton, 2002).

3. *Phyllanthus emblica*

Family : Euphorbiaceae

Synonym : *Emblica officinalis* Gaertn.

English names : Emblic myrobalan, Malacca tree, Indian gooseberry

Common name: Amalaka, Amla, Avla

In Malaya the emblic is called *melaka*, *Asam melaka*, or *amlaka*. In Thailand, it is known as *ma-kham-pom*; in Laos, *mak-kham-pom*; in Cambodia, *kam lam* or *kam lam ko*; in South Vietnam, *bong ngot*; in North Vietnam, *chu me*. In the Philippines, it is called *nelli* (Morton, 1987).

Morphological and characteristics

P. emblica is a small to moderate size deciduous tree, reaching 8 to 18 m in height, with crooked trunk and spreading branches. Its fairly smooth bark is a pale grayish-brown and peels of in conchoidal flakes (Morton, 1987; Scartezzini and Speroni, 2000). The branchlets are glabrous or finely pubescent, 10-20 cm long, usually deciduous; the leaves are linear-oblong blunt and closely set along branchlets, light green, resembling pinnate leaves, 8-12 mm or more long and 2-5 mm broad, stipulate, entire, obtuse, or round at the base, subacute or apiculate apex, hairless, pale green or often pubescent beneath, almost stalk-less. Leaves fall in November-December and grow in February-March (Scartezzini and Speroni, 2000). The flowers are greenish-yellow and are borne in compact clusters in the axils the lower leaves, blossom March-May, unisexual, 0.5-1.5 cm long. Usually, male flowers occur at the lower end of a growing branchlet, with the female flower above them, but occasional trees are dioecious. The nearly stemless fruit is round or oblate, nearly spherical or globular, indented at the base, light greenish yellow, quite smooth and hard on appearance, with 6 vertical stripes or furrows. Normally, fruit is 12-25 mm wide and 15-20 mm long, ripen from November-February. When ripened the mesocarp is yellow and the endocarp is yellowish brown. The mesocarp is acidulous in flesh fruit and acidulous astringent in dried fruit .The berries are harvested by hand after climbing to upper branches bearing the fruits. The taste of Indian gooseberry is sour,

bitter and astringent, and is quite fibrous (Morton, 1987; Scartezzini and Speroni, 2000).

Distribution

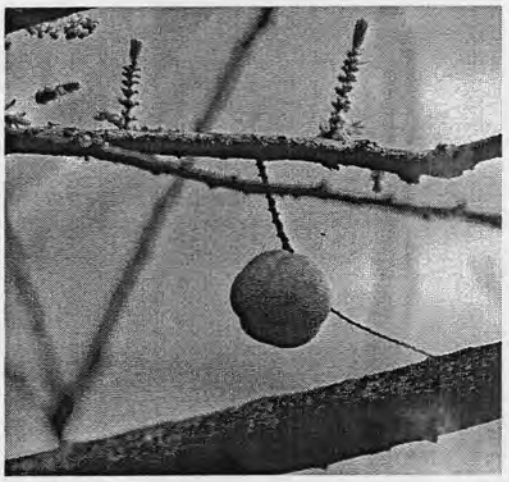
P. emblica is a shrub or tree distributed in subtropical and tropical areas of Southeast Asia, particularly in China, India, Indonesia, The Malay Peninsula, Bangladesh, Nepal, Pakistan, Uzbekistan, Sri Lanka and Thailand (Scartezzini and Speroni, 2000, Zhang et al., 2001., Zhang et al., 2004). It is commonly cultivated in home gardens throughout India and grown commercially in Uttar Pradesh. In India, and to a lesser extent in Malaya, the *P. emblica* is important and esteemed, raw as well as preserved, and it is prominent in folk medicine. Fruits from both wild and dooryard trees and from orchards are gathered for home use and for market. In southern Thailand, fruits from wild tree are gathered for marketing (Morton, 1987).

Phytochemistry

A wide range of plant species belonging to the genus *Phyllanthus* have been phytochemically investigated. Among the studied species, *P. niruri, P. urinaria, P. emblica, P. flexuosus, P. amarus*, and *P. sellowianus* have received the most phytochemical and biological attention. According to the literature, research has either been focused on isolating all the substances in these plants, or on determining a specific class of natural products (Calixto et al. 1998). The *P. emblica* tree contains different classes of constituents listed in Table 1. The complexity of the mixture of compounds and the presence of several compounds in small concentrations can make the isolation and identification of the substances present in this genus very laborious. Different environment condition of the spectral data of the complex structures has been reported, resulting in considerable confusion (Summanen, 1999).

Chemical constituents present in different parts of the plant:

P. emblica



Fruit

Flowers

New leaves

Figure 3 *Phyllanthus emblica*

Tree trunk

Habit

Figure 4 *Phyllanthus emblica* (barks and trees)

Table 1 The classes of chemical constituents reported in *Phyllanthus emblica* (Summanen, 1999; Zhang et al., 2004).

Class	Compound	Occurrence
Alkaloid	Phyllantine Phyllantidine Zeatin Zeatin nucleotide	Leaves, fruit, and tissue cultures Leaves Fruit
Benzenoid	Chebulic acid Chebulinic acid Chebulagic acid Gallic acid	Leaves
	Ellagic acid	Leaves
	Amlaic acid	Fruit
	Corilagin 3-6-di-O-galloyl-glucose ethyl gallate	Fruit
	ß-glucogallin	Leaves, fruit
	1,6-di-O-galloyl-ß-D-glucose 1-di-O-galloyl-ß-Dglucose putranjivain A digallic acid	Fruit
	Phyllemblic acid Emblicol Music (=galactaric) acid	Fruit
Furanolactone	Ascorbic acid	Fruit, leaves
Diterpene	gibberellin A-1 gibberellin A-3 gibberellin A-4 gibberellin A-7 gibberellin A-9	
Sesquiterpene	Phyllanemblic acid and its	Roots

	glycosides phyllanemblicuns A-C	
Triterpene	Lupeol	Fruit, leaves
Flavonoid	Leucodelphinidin Kaempherol Kaempherol-3-glucoside Rutin Quercetin	Leaves
	Kaempherol-3-O-ß-D-glucoside Quercetin-3-O-ß-D-glucoside	Fruit
Hydrolysable tannins	Emblicanin A, B Punigluconin Pedunculagin	Fruit
Sterol	ß-sitosterol	Leaves
Carbohydrate	Acidic and neutral polysaccharides Glucose	Fruit Leaves

1. Fruits

Fruits are composed of moisture 81.2%, protein 0.5%, fat 0.1%, mineral matter 0.7%, fiber 3.4%, carbohydrates 14.1%, Ca (0.05%), K (0.02%), Fe (1.2 mg/100g), nicotinic acid (0.2 mg/100g), phyllemblin, phyllemblic acid, gallic acid, emblicol, ellagic acid, pectin, putranjivatin A, two new hydrolysable tannins, vitamin C-like, called emblicanin A and B (not ascorbic acid as it was believed by mistake until 1996), punigluconin and pedunculagin (Ghosal, 1996; Scartezzini and Speroni, 2000). The fruit pulp, which constitutes 90.97% of the whole fruit, contains 70.5% moisture. The total soluble solids constitute 23.8% of the juice. The acidity of *P. emblica* is 3.28% on pulp basis. The pulp contains 5.09% total sugars and 5.08% reducing sugars (Parmar and Kaushal, 1982). New organic acid gallates and polyphenols including L-malic acid 2-O-gallate, mucic acid 2-O-gallate together with

hydrolysable tannins, 1-O-galloyl-ß-D-glucose, corilagin, and chebulagic acid were found to be the major phenolic constituents of fruit juice (Zhang et al., 2004).

2. Seeds

Seeds contain a fixed oil, phosphatides, and some essential oil. The fixed oil (yield 16%) has the following physical and chemical characteristics: acid value 185; iodine value 139.5; acetyl value 2.03; unsaponifiable matter 3.815; sterol 2.70%; saturated fatty acid 7%, linolenic 8.78%, linoleic 44.0%, oleic 28.40%, steric 2.15%, palmitic 2.99%, and miristic acid 0.95%. Proteolytic and lipolytic enzymes are also present in seeds (Scartezzini and Speroni, 2000).

3. Leaves

Leaves contain phyllantine, zeatin, chebulic acid, chebulinic acid, chebulagic acid, gallic acid, ellagic acid, ascorbic acid, ß-glucogallin, lupeol, flavonoid, and sterol (Summanen, 1999; Zhang et al., 2004).

4. Bark

The bark contains leukodelphinidin, tannin, and proanthocyanidin (Scartezzini and Speroni, 2000).

5. Roots

Roots contain ellagic acid and lupeol. Sesquiterpene (phyllanemblic acid and its glycosides phyllanemblicuns A-C) are the major sesquiterpenoids from the roots (Scartezzini and Speroni, 2000; Zhang et al., 2004).

Ethnopharmacology

P. emblica has long been used in folk medicine to treat a broad spectrum of disorders, and there are numerous references to controlled assays. Active principles or extracts of *P. emblica* L. have been shown to possess several pharmacological actions, e.g. analgesic, anti-inflammatory, antioxidant, chemoprotective, hypolipidaemic, anti-HIV-1 (Human immunodeficiency virus-1) activities, etc as shown in Table 2 .

P. emblica fruit extract has been shown to have multi-function benefits. Low molecular weight tannins (<1000), namely Emblicanin A and Emblicanin B, along with Pedunculagin and Punigluconin are the key ingredients of *P. emblica* fruit extracts (Chaudhuri, et al., 2004). The tannin-based *P. emblica* extract has been found to have broad-spectrum antioxidant activity (Chaudhuri, 2002). This fruit also exhibits inhibitory effect on the activity of collagenase and stromelysin-1 (collagen-degrading enzymes), which is increased by UV light. Reduction in matrix-metallopreoteinase (MMPs) activity helps reduce wrinkling and loss of elasticity of skin. *P. emblica* thus helps younger looking skin and act as anti-ageing agent. Though its primary applications within the skincare industry are as a photo-protective agent and skin lightener, it offers a host of other benefits for the skin (Chaudhuri, et al., 2004, Chaudhuri, EMD chemicals, Inc., 2004).

Table 2 Reported biological effects of *P. emblica* (Adapted from Summanen, 1999)

Biological effect	References
Antagonistic activity against genotoxic chemicals, anticlastogenicity	Giri and Banergee, 1986; Dhir et al. 1990 and 1991; Roy et al., 1992; Nandi et al., 1997.
Antimicrobial activity	Admad, Mehmood, and Mohamed, 1998
Antioxidant activity	Jose and Kuttan, 1995; Ghosal et al., 1996; Kumar and Muller, 1999; Bhattacharya et al., 2002; Wattanapitayakul et al. 2005.
Anti-inflammatory activity	Asmawi et al., 1992; Jantan et al., 1996; Ihantola-Vormisto et al., 1997; Perianayagam et al., 2004.
Anti-atherogenic	Daun, Yu and Zhang, 2005.
Anti-diabetic	Sabu and Kuttan, 2002; Suryanarayana et al., 2004.
Anti-ulcerogenic	Sairam et al., 2002; Bandyopadhyay and Pakrashi, 2000; Bafna and Balaraman, 2005.
Anti-tussive	Nosal et al., 2003
Anti-tumor and anti-proliferative	Jeena and Kuttan, 2001; Khan et al., 2002; Lambertni et al., 2004; Zhang et al., 2004; Kaur el at., 2005;
Hepatoprotective activity, prevention of hepatocarcinogenesis	Roy et al., 1991; Gulati et al., 1995, Jose et al., 1997 and 1999; Jeena et al., 1999; Jeena and Kuttan, 2000.
Hypolipidaemic	Thakur, 1985; Mand et al., 1991; Jacob et al., 1988; Mathur et al., 1996; Anila and Vijyalakshmi, 2002.
Immunomodulating	Suresh and Vasudevan, 1994; Ganju et al., 2003.
Inhibition of human immunodeficiency	El- Mekkawy et al., 1995; Kusumoto et

virus-1 (HIV-1) reverse transcriptase, *in vitro*	al., 1995
Prevention of experimental acute pancreatitis, *in vivo*	Thorat et al., 1995.
Protection against radiation-induced chromosome damage, *in vitro*	Yadav, 1987.

4. Tannin

Tannins are astringent, bitter plant polyphenols that either bind and precipitate or shrink proteins. Tradition use of tannins as plants for converting animal hides to leather (tanning), their ability to interact with and precipitate protein, including the proteins found in animal skin. The term tannin comes from the ancient Celtic word for oak, a typical source for tannins for leather making, however, the term is widely applied to any large polyphenolic compound containing sufficient hydroxyls and other suitable groups such as carboxyls to form strong complexes with proteins and other macromolecules.

Bate-Smith defined tannins as "water-soluble phenolic compounds having molecular weights between 300-500, the usual phenolic reactions, special properties such as the ability to precipitate alkaloids, gelatin and other proteins". There are two groups in tannins, condensed tannins are polymeric flavonoids. The flavonoids are a diverse group of metabolites based on a heterocyclic ring system derived from phenylalanine and polyketide biosynthesis. Hydrolysable tannins are derivatives of gallic acid (3, 4, 5-trihydroxyl benzoic acid). Gallic acid is esterified to a core polyol, and the galloyl groups may be further esterified or oxidatively crosslinked to yield more complex hydrolysable tannins (Hagerman 1998, 2002).

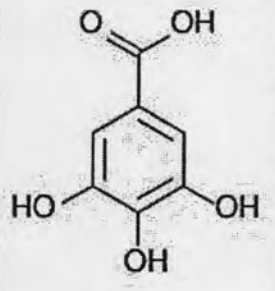
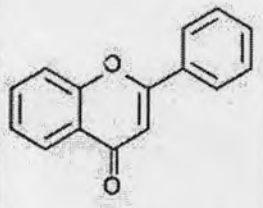
Base Unit:	 Gallic Acid	 Flavone
Class/Polymer:	Hydrolyzable Tannins	Condensed Tannins

Figure 5 Structure of hydrolyzable tannins and condensed tannins

5. Gallic acid

Gallic acid is an organic acid, also known as 3,4,5-trihydroxybenzoic acid, found in gallnuts, sumac, witch hazel, tea leaves, oak bark, and other plants. The chemical formula is $C_6H_2(OH)_3COOH$. Gallic acid is found both free and as part of tannins. Salts and esters of gallic acid are termed gallates. Despite its name, it does not contain gallium. Recent reports indicate the presence of gallic acid in the fruits of *P. emblica*. The gallic acid in this fruit is present either in free form of bound tannin form (gallotannins and ellagotannins). The amount of free gallic acid present in triphala formlations which vary by the extraction process. During the extraction process tannins present in the fruit samples may get hydrolyzed to give free gallic acid or they may remain in bound form. Thus the exact quantification of free gallic acid in triphala is necessary (Polewski, et al., 2002). Gallic acid is widespread in plant foods and beverages such as tea and wine was proven to be one of the anticarcinogenic polyphenols presented in green tea. The consumption of France of a diet high in saturated fat coupled with an apparently low incidence of coronary heart disease has been associated with the consumption of red wine. Antioxidants presented in red wine have been shown to have a protective role against oxidation of LDL in vitro. It is a strong chelating agent and forms complex of high stability with iron. It has shown

phytotoxicity and antifungal activity against Fusarium semitectum, F. fusiformis and Alternarea altternata. It is used for arteriosclerosis prevention. Otherwise, gallic acid has been used in pharmaceuticals industry, such as in the synthesis of Trimethoprim, in food and feed industries, in ink dyes, in laboratories for absorbing oxygen, in the production of azo dyes, and in photography (Polewski, et al., 2002).

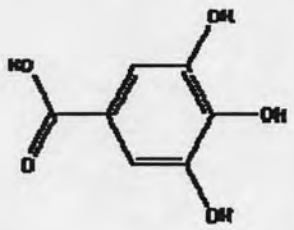


Figure 6 Structure of gallic acid

6. Nanoparticles

Nanoparticles are solid colloidal particles with the size ranging from 1 to 1000 nm (Lockman *et al.*, 2002). They could be classified into polymeric nanoparticles, nanocapsules, solid lipid nanoparticles, and nanogels (Date and Patravale, 2004). Nanoparticles made from lipid material or lipospheres represent an alternative coilloidal drug carrier system since 1990s period. This carrier combines the advantages but avoids the disadvantages of other traditional colloidal carriers such as emulsions, liposomes and polymeric microparticles. The proposed advantages include protection of incorporated labile drugs from degradation, controlled release capacity, low cytotoxicity, excellent tolerability and high entrapment efficiencies, especially for water-insoluble drugs. At the same time, this lipid-base nanoparticles also avoid the problem of organic solvent and biotoxicity of carrier (Mehnert and Mader, 2001; Wissing, Kayser and Muller, 2004).

Nanoparticles can be used to deliver hydrophilic drugs, hydrophobic drugs, proteins, vaccines, biological macromolecules, etc. They can be formulated for targeted delivery to the lymphatic system, brain, arterial wall, lungs, liver, spleen, and skin or made for long-term systemic circulation. Therefore, numerous protocols exist

for synthesizing nanoparticles based on the type of drug used and the desired delivery route. Once a protocol is chosen, the parameters must be tailored to create the best possible characteristics of nanoparticles. Four of the most important characteristics of nanoparticles are their size, encapsulation, zeta potential (surface charge), and release characteristics. Additionally, numerous methods exist for incorporating drugs into the particles. Nanoparticles made from lipid material or lipospheres is called nanoliposomes.

7. Liposomes

Liposomes were first described by Bangham in 1965 while studying cell membranes. He found that liposomes are vesicular structures consisting of hydrated bilayers which form spontaneously when phospholipids are dispersed in water. Since this, further studies into liposomes and their application in various fields such as medicine and research have been explored (Crommelin, 1978).

Liposomes are simply vesicles in which an aqueous volume is entirely enclosed by a membrane composed of lipid molecules (usually phospholipids). They form spontaneously when these lipids are dispersed in aqueous media, giving rise to a population of vesicles which may range in size from tens of nanometers to tens of microns in diameter. They can be constructed so that they entrap quantities of materials both within their aqueous compartment and within the membrane. The value of liposomes as model membrane systems derives from the fact that liposomes can be constructed of natural constituents such that the liposome membrane forms a bilayer structure which is in principal identical to the lipid portion of natural cell membranes. The similarity between liposomes and natural membranes can be increased by extensive chemical modification of the liposomal membrane, and may be exploited in areas such as drug targeting or immune modulation, both in vivo and in vitro.

Liposomes constitute an important potential as drug delivery systems, not only for intravenous delivery but also particularly for topical applications. Liposomes are spherical self-closed structures, composed of curved lipid bilayers, which enclose part of the surrounding solvent into their interior. The range in size of liposomes is from

10 nm up to several micrometers in diameter and they may be composed of one or several concentric membrane, each with a thickness of about 4 nm. Liposomes are made up of amphiphiles, which are characterized by hydrophilic, or water-soluble, head and hydrophobic tail.

Liposomes form spontaneously when phospholipids are hydrated in which an aqueous volume is entirely enclosed by a membrane composed of phospholipids. When mixed in water under low shear condition the phospholipids arrange themselves in sheets, these sheets then join tails-to-tails to form a bilayer membrane, which enclosed some of water in a phospholipid sphere. Particles containing only one bilayer have been termed "unilamellar vesicles" (ULVs), while these containing many bilayers have been termed "multilamellar vesicles" (MLVs). In the case of unilamellar vesicles, one can differentiate between small unilamellar vesicles (SUVs) and large unilamellar vesicles (LUVs) (Bergstrand, 2003).

Type of liposomes (New, 1987)

Liposomes can be characterized by their size and shape. They are range in size from the smallest vesicle (diameter approximate 25 nm) and which are visible under light microscope with a diameter of 1000 nm or greater. Liposomes of different size often require completely different method of manufacturing.

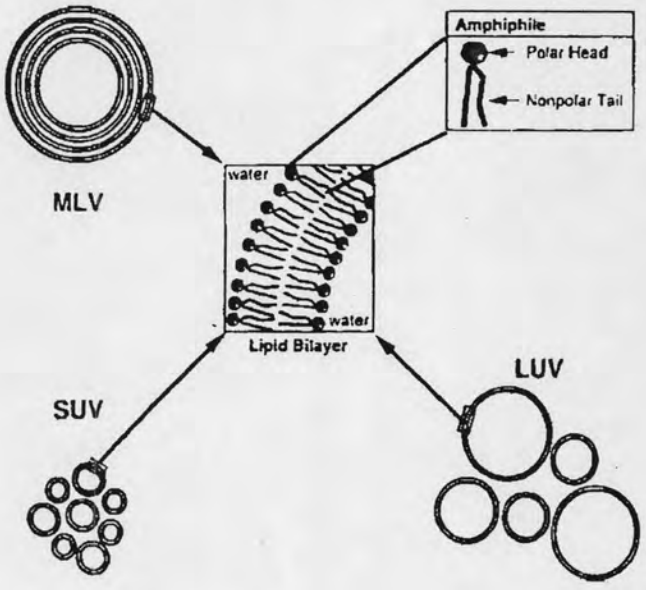


Figure 7 Structures of SUVs, MLVs and LUVs (Lasic, 1997).

Small unilamllar vesicles (SUVs)

They are the lowest limit of size possible for phospholipids vesicles. The ranges of sizes are 15-25 nm. They require high-energy input for production, the best method that does not kinetics or thermodynamics. SUVs are well characterized vesicles population, homogeneous in size and lamellarity. They are thermodynamic unstable, susceptible to aggregation and fusion particularly below the phase transition temperature. The entrapped volume is small; % entrapment of aqueous solute is low. Therefore, SUVs are not recommended for efficacy of entrapment, retention of materials. SUVs are appropriate choice when a high surface area need.

Multilamellar vesicles (MLVs)

The simplest type of liposomes is prepared. They consist of population of vesicles covering a wide range of sizes (100-1000 nm). Each vesicle generally consists of five or more concentric lamellae. Mechanically stable upon storage for long period of time. The internal space occupied by lipid membrane, suitable to acting as a carrier for lipophilic compound.

MLVs can be prepared by hand shaking of lipid in aqueous media and can be included in lipid mixture at time of dry down. The ultimate size can be control by extrusion. Percentage capture of aqueous compartment is not very high. The higher value can be achieved (>50%) by very large MLVs.

Large unilamellar vesicles (LUVs)

LUVs show large size of vesicles (1000 nm), they have a high aqueous: lipid compartment ratio, larger volume. So, they are suitable for entrapment of aqueous materials. LUVs can be prepared by REV technique.

Phospholipids

Phospholipids are the major structural components of biological membranes. The most common phospholipids are phosphatidylcholine (PC) molecules which have amphipathic molecules in which a glycerol bridge links a pair of hydrophobic acyl hydrocarbon chains, with a hydrophilic polar head group, phosphocholine. Molecules of PC are not soluble in water. Phosphatidylcholines contrast markedly with other amphipathic molecules (detergents, lysolecithin) in that bilayer sheets are formed in preference to micellar structures. This is thought to be because the double fatty acid chain gives the molecule an overall tubular shape, more suitable for aggregation in planar sheets compared with detergents with a polar head and single chain, whose conical shape fits nicely into aspherical micellar structure.

Phosphatidylcholines is a phospholipid which is the major component of a phosphatide fraction which may be isolated from either egg yolk or soy beans from which it is mechanically or chemically extracted using hexane (Rose and Jenkins 2006). Phosphatidylcholine that is extract from soy beans forms as they do the major phospholipid component of many cell membranes, they are often used as the principal phospholipid in nanoliposomes for a wide range of applications, both because of their low cost relative to other phospholipids, and their neutral charge and chemical inertness, Lecithin from natural sources is in fact a mixture of phosphatidylcholines, each with chains of different length and varying degrees of unsaturation. Lecithin

from plant sources has a high level of polyunsaturation in the fatty acyl chains, while that from mammalian sources contains a higher proportion of fully saturated chains.

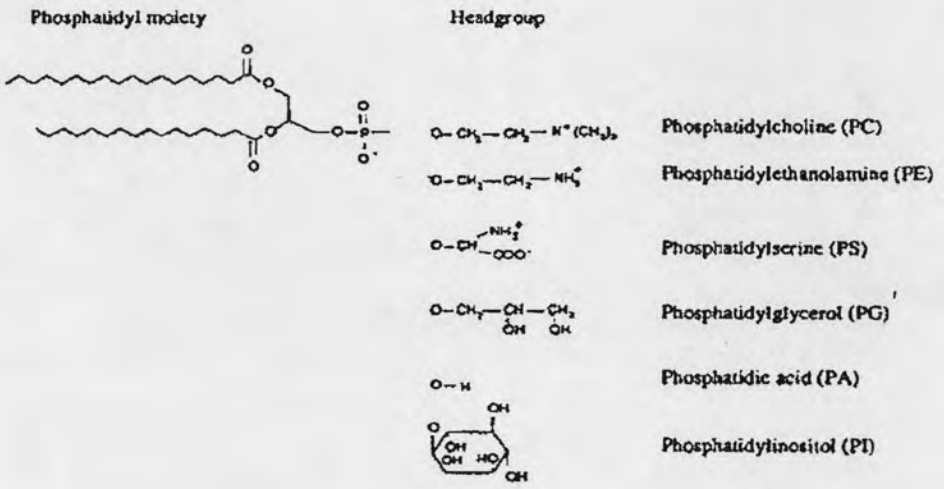


Figure 8 Structure of a number of phospholipids regularly used in liposomes (Grit and Crommelin, 1993).

Phosphatidylcholines membranes

Phosphatidylcholine, also known as lecithin can be derived from both natural and synthetic sources. They are readily extracted from egg yolk and soy bean but less readily from bovine heart and spinal cord. At different temperature, lecithin membranes can exist in different phase, and transition from one phase to another as the temperature is increased. That one occurring at the highest temperature is the membrane passes from a tightly ordered 'gel' or 'solid' phase to liquid-crystal phase, where the freedom movement is higher. The method for determining the phase transition temperature (T_c) is microcolorimetry. The hydrocarbon chain length and unsaturation as well as head group influence on the value of T_c. Generally, increasing the chain length or increasing the saturation of the chain length increase the transition temperature.

The liposome membranes are semi-permeable membrane. For molecule with a high solubility in both organic and aqueous media, phospholipid membranes are a

weak barrier. On the other hand, for polar solute and high molecular weight compound slowly pass through the membrane. A smaller molecule with neutral charge (water, urea) can diffuse across quite rapidly. Proton and hydroxyl ion cross the membrane fairly quickly.

Other neutral phospholipids

Neutral lipid bilayers may be composed of sphingomyelin or alkyl ether lecithin analogues. Replacement of ester grouping by ether linkages increases the resistance of the lipid to hydrolysis, while not affecting the physical properties. When the content of saturated fatty acid increased, the phase transition temperature was increased. Mixtures of different chain lengths give a combined phase transition temperature below that of individual components. Sphingomyelin membranes are more stable and tightly packed than lecithin, decreased value for permeability to solute, greater resistance to lyses and lower membrane fluidity.

Preparation of nanoliposomes

The main point of preparation of nanoliposomes is to getting the membranes to form vesicles of the right size and structure. Moreover, to entrap materials with high efficiency and in such a way that these materials do not leak out of the nanoliposomes once formed.

An important parameter to consider when addressing the formation process of nanoliposomes is the rigidity of the bilayer. Hydrated-single component phospholipid bilayers can be in a liquid-crystalline state or in a gel state. By increasing the temperature, the gel state bilayer melts and is converted into the liquid state. This occurs at a temperature known as the transition temperature (T_c). The T_c of a bilayer depends on acyl chain length, degree of saturation, and polar head group. The Tc can vary between 15°C for egg yolk phosphatidylcholine (high degree of unsaturation) to over 50°C for fully saturated distearolylphosphatidylcholine (DSPC) (Crommelin, 1978). The raw material for nanoliposome formation depends on the intended use of the nanoliposomes. Several companies supply reasonable grade and priced lipids

which usually contain at least 98% phospholipid and less than 1% lysophospholipid, low endotoxin and microbial load and trace metals. It is up to the individual investigator to purify the lipid to acceptable standards.

There are many different strategies for the preparation of nanoliposomes, which can be classified into 3 main groups:

1. Mechanical methods – for example film method, and ultrasonication method
2. Methods based on replacement of organic solvent – for example reverse-phase evaporation, and ether vaporisation method.
3. Methods based on size transformation or fusion of preformed vesicles – for example freeze-thaw extrusion method and the dehydration-rehydration method.

The standard manufacturing procedure of liposomes is the film-forming method. Alternatively, solvent injection and reverse phase dialysis are appropriate procedure for the formulation of SUVs and LUVs. Properties of lipid formulations can vary depending on the composition (cationic, anionic, neutral lipid species), however, the same preparation method can be used for all lipid vesicles regardless of composition. The general elements of the procedure involve preparation of the lipid for hydration, hydration with agitation, and sizing to a homogeneous distribution of vesicles.

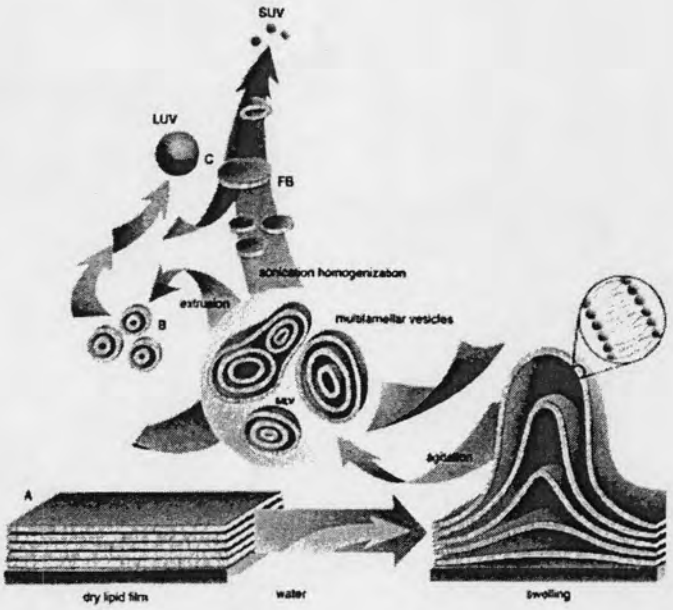


Figure 9 The general procedure involves preparation of liposomes, hydration with agitation, and sizing by sonication and extrusion to a homogeneous distribution of vesicles (Lasic, 1997).

The simplest concept of preparation in mechanical dispersion is drying the lipid composition down onto a round bottom flask and then dispersed by addition of the aqueous medium, followed by shaking (Figure 10). Even before exposure to water, the lipids in the dried-down form are thought to be oriented in such a way as to separate hydrophilic and hydrophobic regions from each other, in a manner not unlike their conformation in the finished membrane preparation. Upon hydration, the lipids are said to swell and peel off the support in sheets, generally to form multilamellar vesicles. In this method, the aqueous volume enclosed within the lipid membrane is usually only a small proportion of the total volume used for compounds to be entrapped, although the absolute yield of material may be satisfactory for practical purposes. Lipid soluble compounds on the other hand, can be encapsulated to 100% efficiency, providing they are not present in quantities which overwhelm the structural components of the membrane.

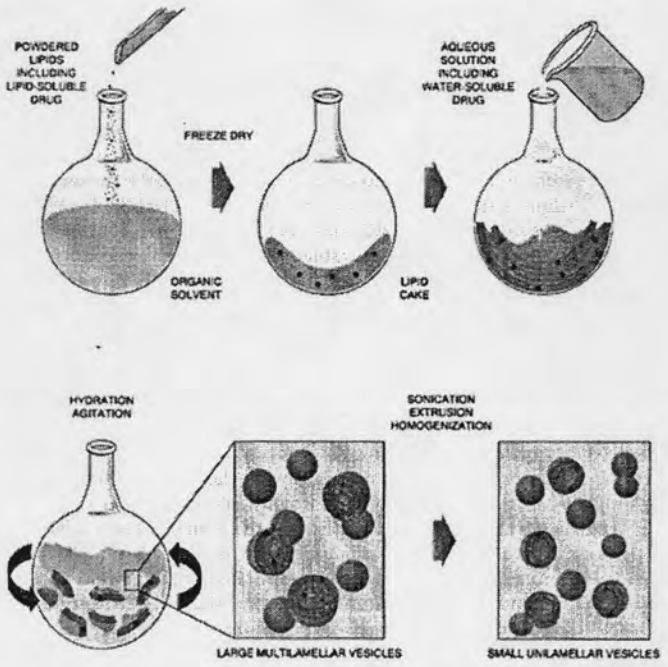


Figure 10 The method for preparation liposomes (Lasic, 1997).

In the group of solvent dispersion, the lipids comprising the liposome membrane are first dissolved in an organic solution, which is then brought into contact with the aqueous phase containing materials to be entrapped within the liposome. At the interface between the organic and aqueous media, the phospholipids align themselves into a monolayer which forms the basis for half of the bilayer of the liposome.

Ethanol injection was originally reported by Batzri and Korn, 1973. An ethanol solution of lipids is injected rapidly into an excess of saline or other aqueous medium, through a fine needle (Figure 11). The force of the injection is usually sufficient to achieve complete mixing, so that the ethanol is diluted almost instantaneously in water, and phospholipid molecules are dispersed evenly throughout the medium. This procedure can yield a high proportion of small unilamellar vesicles with diameter approximately 25 nm, although lipid aggregates and larger vesicles may form if the mixing is not through enough. The method has the advantages of extreme simplicity and very low risk of bringing about degradative changes in sensitive lipids.

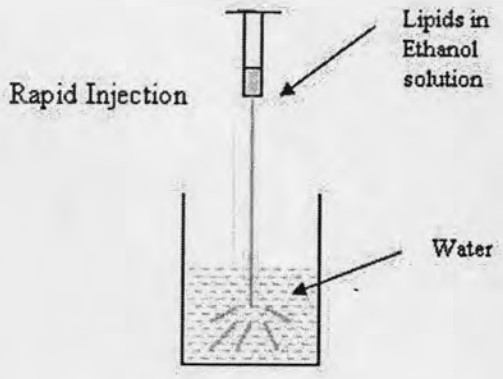


Figure 11 Preparation of liposomes by modified ethanol injection (New, 1987)

Disruption of LMVs suspensions using sonic energy (sonication) typically produces small, unilamellar vesicles (SUVs) with diameters in the range of 15-50nm. The most common instrumentation for preparation of sonicated particles is bath or probe tip sonicators. Bath sonicators are the most widely used instrumentation for preparation of SUVs. Sonication of a LMVs dispersion is accomplished by placing a test tube containing the suspension in a bath sonicator (or placing the tip of the sonicator in the test tube) and sonicating for 5-10 minutes above the T_c of the lipid. The lipid suspension should begin to clarify to yield a slightly hazy transparent solution. The haze is due to light scattering induced by residual large particles remaining in the suspension. These particles can be removed by centrifugation to yield a clear suspension of SUVs. Mean size and distribution is influenced by composition and concentration, temperature, sonication time and power, volume, and sonicator tuning. Since it is nearly impossible to reproduce the conditions of sonication, size variation between batches produced at different times is not uncommon. Also, due to the high degree of curvature of these membranes, SUVs are inherently unstable and will spontaneously fuse to form larger vesicles when stored below their phase transition temperature.

Lipid extrusion is a technique in which a lipid suspension is forced through a polycarbonate filter with a defined pore size to yield particles having a diameter near the pore size of the filter used. Prior to extrusion through the final pore size, LMVs

suspensions are disrupted either by prefiltering the suspension through a larger pore size (typically 0.2μm-1.0μm). This method helps prevent the membranes from fouling and improves the homogeneity of the size distribution of the final suspension. As with all procedures for downsizing LMVs dispersions, the extrusion should be done at a temperature above the T_c of the lipid. Attempts to extrude below the T_c will be unsuccessful as the membrane has a tendency to foul with rigid membranes which cannot pass through the pores. Extrusion through filters with 100 nm pores typically yields large, unilamellar vesicles (LUVs) with a mean diameter of 120-140 nm. Mean particle size also depends on lipid composition and is quite reproducible from batch to batch.

Liposomes for drug delivery

Liposomes possess unique properties owing to the amphiphilic character of the lipids, which make them suitable for drug delivery. These phospholipid vesicles are capable of encapsulating both hydrophilic and hydrophobic drugs. The entrapment of materials into liposomes depends on whether the molecule is hydrophilic or hydrophobic. Liposomes can encapsulate water-soluble actives within the aqueous compartment. Additionally a lipid-soluble or amphiphilic ingredient can be embedded within the bilayer membrane. In Figure 12, it shows the position of drugs that are located in the liposomes. Compounds can probably be accommodated in the membrane to concentration of about 1-10% by weight with out serious disruption of the bilayer structure, although the fluidity or permeability may be altered. The large liposomes entrap a far greater aqueous volume than do small liposomes.

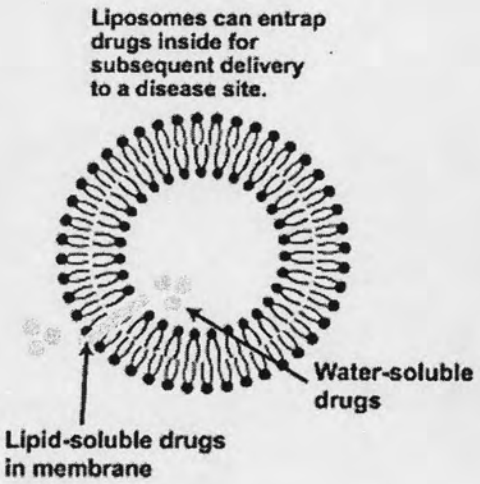


Figure 12 The positions of drugs which are located in the liposomes (Zang, 2005).

The effective of liposomes for enhancement of therapeutic efficacy should be considered to retain their content as long as possible, suitable for interaction with some biological system, have ability to take up material of all shape, size and for immunological effect. Inclusion of other membrane component may be considered useful in view of the specific interaction may occur which could modify the behavior of liposomes with regard to either its contents or it mode of action (Ranade and Hollinger, 2003).

Encapsulation of drug into liposomes can lead to the enhancement of therapeutic efficacy of drugs by reduction of toxicity and prolongation of their therapeutic effect. Tretinoin which was entrapped in liposomal preparation was more stable to photodegradation than when dissolved in caster oil (Brisaert et al., 2001).

8. Transdermal Therapeutic Systems

Probably the most innovative practical step in the science of transdermal delivery in recent years has been introduction into medicine of skin patches.

The original transdermal therapeutic system (TTS) or transdermal drug delivery system (TDDS) was introduced as a device which would release drug to the skin at a controlled rate, well below the maximum that the tissue can accept. Thus, the device, not the stratum corneum, would control the rate at which a drug diffuses through the skin, as the intended flux would be much lower than the maximum skin flux.

A TTS tried to provide systemic therapy in a more convenient and effective way than parenteral or oral therapy. The claimed advantages for the percutaneous over the oral route include the following:

1. Drug administration through skin eliminates variables which influence gut absorption, such as changes in pH along the gastrointestinal tract, food and fluid intake, stomach emptying time and intestinal motility and transit time, and the presence of human and bacterial enzymes.

2. Drug enters the systemic circulation directly, eliminating the 'first-pass' effect of enzymes in the gut and the liver, the body's main metabolizing organ.

3. Transdermal input may provide controlled, constant drug administration, displaying a pharmacological effect. The continuity of input may permit the use of drugs with short half-lives and improve patient compliance.

4. Percutaneous administration could eliminate pulse entry into the circulation. Peaks in plasma concentrations often produce undesirable effects and troughs may be subtherapeutic.

5. The transdermal route can use drugs with a low therapeutic index.

6. Patches may be readily removed, although there is a reservoir effect and blood levels do not fall immediately to zero after TTS removal

Transdermal systems usually contain potent drugs that should not irritate or sensitize the skin. They must be stable and have the correct physicochemical properties to partition into the stratum corneum and permeate o the vasculature (Aulton, 2007).

Dermal Therapeutic System (DTS)

Dermal therapeutic system (DTS) is self-adhesive-patches to treat the topical skin pathologies. The simplest design of DTS consists of a flexible backing layer, an adhesive 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).

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 et al., 2002). According, the topical dosage forms should be designed depending on what the target sites are. In case of dermatopharmacotherapy 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 time (Bulletin, 1994).

Device design

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

Monolith or matrix system

In these patches, the Higuchi Square Root of Time Law is usually followed. Equations 1-6 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, with C_0, the initial concentration of solute in the vehicle, D_v, the diffusion coefficient of the drug in the vehicle, and t, the time after application:

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

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

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

We can obtain an equation that releases m to t in the form:

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

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

C_s: The solubility of the drug in the vehicle

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, it will obtain the instantaneous rate of release, d*m*/d*t*, given by:

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

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

$$m \approx \sqrt{2ADvCst} \qquad \text{Eqn.5}$$

Then Eqn.5 becomes:

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

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 $dm/dt \propto A^{1/2}$; doubling A only increases d*m*/d*t* by about 40%. For obvious reasons Eqn. 1-6 are often referred to as "square root of time" relationship; 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 13 illustrates release profiles, plotted both linearly and as square route functions of time. Figure 14 illustrates the fundamental construction for a suspension-type TTS. 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 attaches the patch to the skin. A protective strippable film is removed immediately prior to application (Aulton, 2007).

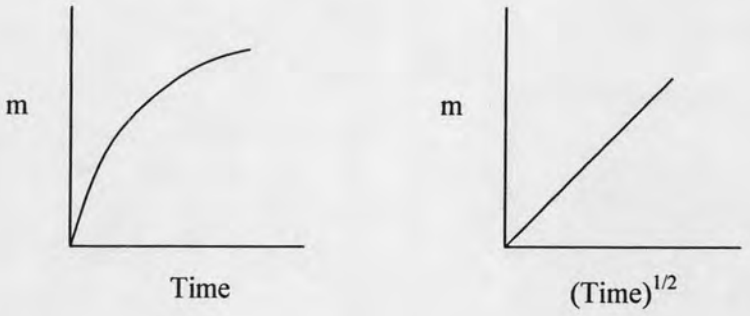


Figure 13 Release profiles, plotted both linearly and as square route functions of time, for matrix or monolith patches operating under Higuchi condition (Aulton, 2007).

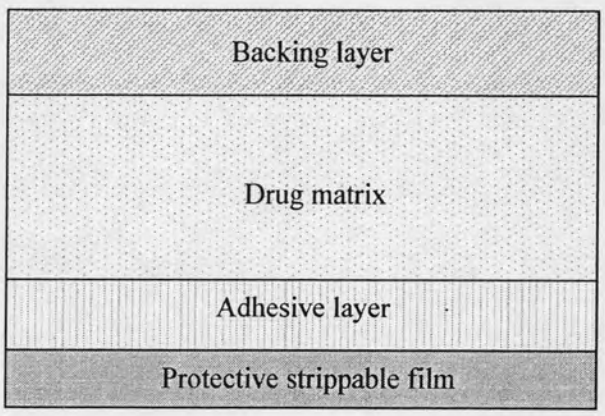


Figure 14 Fundamental construction for suspension type transdermal therapeutic system based on a matrix or monolith design (not to scale) (Aulton, 2007).

Rate-limiting membrane system

As these patches include a membrane, it might expect that the release profile would follow the simple Fickian conditions. Thus, at steady state it would expect the amount of drug released to the skin to be directly related to 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 (the skin in this case). In practice, this does not happen because the drug equilibrates in all patch components on storage before the patient receives the patch.

Figure 15 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 release 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 16. Such profiles may be confused with Higuchi plots, i.e. with matrix release plots, as illustrated in the first plot of Figure 13. An advantage is that the burst component can provide a quick-acting, priming dose of drug.

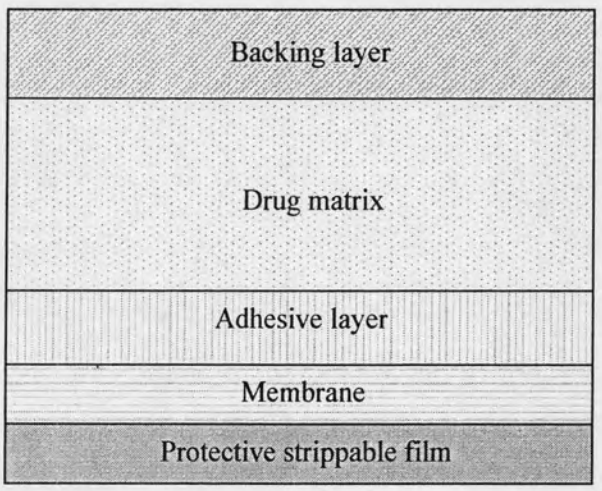


Figure 15 Transdermal therapeutic system based on a rate-limiting membrane design (not to scale) (Aulton, 2007)

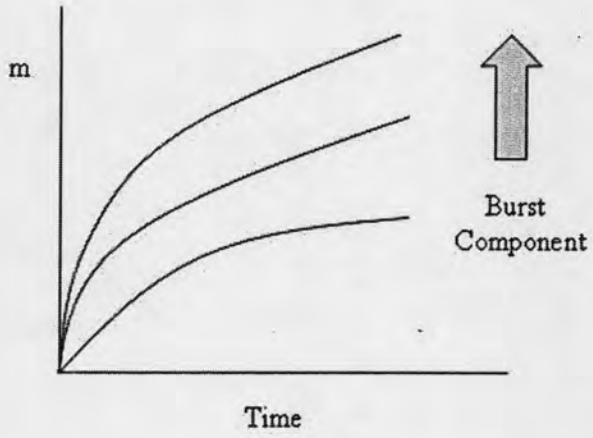


Figure 16 Release profile from rate-limiting membrane patches; effect of increasing amount of drug partitioned into the membrane and adhesive on storage which provides the 'burst' component (Aulton, 2007).

Mechanical properties

Tensile strength measures the amount of stress applied to a material at its breaking point or the point at which it fails. The tensile strength of a material is the point at which a material under the stress of an applied force, snaps, breaks or can no longer maintain its structural integrity. It is, in other words, the amount of force the material can withstand without breaking.

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 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, 2007).

Polymers are divided into five categories according to a qualitative description of their mechanical behavior and corresponding stress-strain characteristic as showed in Table 3 and Figure 17.

Table 3 Qualitative description of polymer and its stress-strain characteristics (modified from Aulton and Adul-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.

$$\text{Tensile strength} = \frac{\text{Load at failure}}{\text{Film thickness x film width}}$$

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.

$$\%\ \text{Elongation} = \frac{\text{Extension at the moment of rapture of the sample x 100}}{\text{Initial gauge length of the specimen}}$$

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 the film.

$$\text{Elastic modulus} = \frac{\text{Slope}}{\text{Film thickness x film width x cross-head speed}}$$

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.

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

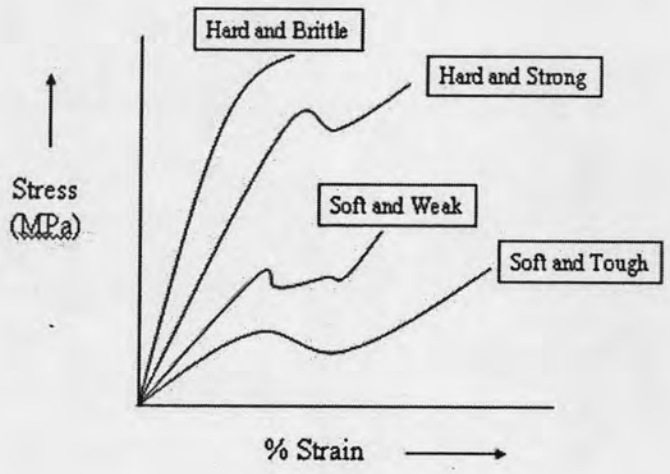


Figure 17 Mechanical behavior and corresponding stress-strain characteristics of polymers (Aulton and Abdul-Razzak, 1981)

9. *In vtiro* drug release study

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 compare batch to batch variation. Hence, this type unable to provide *in vivo* predictions.

There have been several methods reported in literature for percutaneous penetration enhancement and its qualification. These include diffusion experiments (Biruss and Valenta, 2005; Contreras et al., 2005; Foco et al., 2005), visualization by electron microscopy (Bhatia and Singh, 1999; Sinico el al., 2005; Van Den Bergh et al., 1999).

In vitro diffusion cells, method for determining topical absorption have been used to understand and/or predict the delivery of drug from the skin surface into the body of living animals or humans. The advantages of *in vitro* experiments are easier to perform, less expensive, and easier to control the environment factors such as temperature. Accurate absorption rates can be determined, since sampling can be done

frequently directly beneath the skin (Venter et al., 2001). Although a potential disadvantage is that little information on the metabolism, distribution, and effects of blood flow on permeation can be obtained (Brain et al., 1998).

Diffusion cell design

The diffusion cell consists of an upper donor and a lower receptor chamber, separated by a membrane. The cells are made preferably from an inert non-adsorbing material, and glass is most common, although Teflon and stainless steel are also used. In all case excised membrane is mounted as a barrier between a donor chamber and a receptor chamber. Temperature control of the receptor fluid is crucial throughout the experiment. Efficient mixing of the receptor fluid is essential and sample removal should be simple. The amount of compound permeating from the donor to the receptor side is determined as a function of time. Continuous agitating of the receptor medium, sampling from the bulk liquid rather than the side arm, and accurate replenishment after sampling, are important practical considerations. It is essential that air bubbles not be introduced below the membrane during sampling.

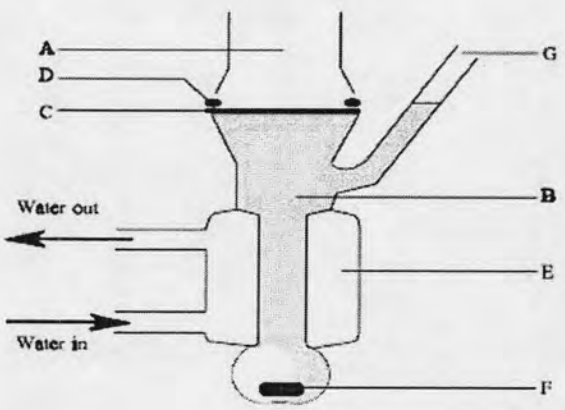


Figure 18 The static Franz diffusion cell. A = donor compartment; B = receptor compartment; C = membrane; D = O-ring; E = water jacket; F = stirring bar; G = sampling port (Frantz, 1990)

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

Receptor chamber and medium

A large receptor volume may ensure sink conditions but will reduce analytical sensitivity unless large samples can be taken and subsequently concentrated. The ideal receptor phase provides an accurate simulation of the conditions pertaining to *in vivo* permeation of the test compound. Excessive receptor phase concentration can lead to a decrease in the rate of absorption, which may result in an underestimate of bioavailability. The most commonly used receptor fluid is pH 7.4 phosphate-buffered saline (PBS), although this is not always the most appropriate material. It has been postulated that if a compound has a water solubility of < 10 μg/mL, then a wholly aqueous receptor phase is unsuitable and the addition of solubilizers becomes necessary. One particularly useful receptor fluid is 25% (v/v) aqueous ethanol, which provides a reasonable 'sink' for many permeants, whilst removing the need for other antimicrobial constituents. Other examples of modified receptor phase include 3% bovine serum albumin (Sinico et al., 2005), 1.5% to 6% Triton X-100, and 6% Poloxamer 188. A nonionic polyethylene glycol-20-oleyl ether surfactant did not disrupt the barrier function of rat skin to hydrophilic compounds.

The composition of the receptor fluid is chosen so that it does not limit the extent of diffusion of the test substance, such as the solubility and the stability in the receptor fluid of the chemical under investigation have to be guaranteed. Saline or buffered saline solutions are commonly used for hydrophilic compounds. For lipophilic molecules, serum albumin or appropriate solubilizers/emulsifiers are added in amounts which do not interfere with membrane integrity (Brain et al., 1998).

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.

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 selective 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 membrane

Cellulose is a relatively rigid structure consisting of glucopyranose rings joined by ß-1,4-linkages. This conformation allows only two types of movement in the chains, which are 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 Wan Kim, 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 effect 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 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, it may interact with the diffusant by absorption or occupy interchain positions thereby hindering the passage of other moieties. Normally, cellulose membranes are reported to be more permeable than biological membranes or porous synthetic media (Touitou and Abed, 1985) and are nondiscriminatory to the characteristics of the diffusant molecule. These membranes have been used for quality control release studies (Shan et al., 1989).

Filter membrane

Porous filter membranes have been relatively little usage in diffusion systems in comparison to the synthetic polymers (Demere and Tomlinson, 1984). This membrane preparation has to soak 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 dependant 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 whole formation and resultant permeability (Lee, Ulman and Larson, 1986). Silicone polymers such as polymethylsiloxane 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; Haigh and Smith, 1994).

Experiment duration

The normal exposure time is 24 h. The longer duration may result in membrane deterioration and requires membrane integrity to be carefully checked. Sample intervals should be of an appropriate frequency to be allowed realistic assessment of such parameters a lag time and steady stage. For a compound with unknown permeation characteristics, samples should ideally be taken at 2 h intervals for the duration of the experiment. *In vitro* release experiments are normally achieved by maintaining the receptor solutions at 37°C, either by immersing cells in a water bath or by using cell jackets perfuse at the correct temperature (SCCNFP, 2003).

10. *In vivo* efficacy tests on human volunteers

The most direct proof of a claim is to show the product effect directly on the human volunteers using the products. 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 (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, for example, 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.

Skin hydration test

Experimental models used for measurement 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. 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 by the prerequisite information needed about the products to be tested (Colipa, 1997; Davis et al., 1998). According to method used, a further important point concerns standardization of the experiment conditions to obtain acceptable and reproducible results, measurement should be performed with relaxed volunteers already acclimatized for at least 20 minutes to control ambient temperature and relatively humidity conditions.

A large number of bioengineering methods are now available to evaluate hydration or dryness of the skin directly and 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 moisture content is widely used for the skin hydration. There are many devices in commercially available such as the Corneometer, the Skicon, the Derma Phase Meter, the DermaLab® Moisture probe, or Skin Diagnostic SD 27. These devices have a small difference that can give different performance under varying experimental or physiological conditions. This ultimate choice of the instrument depends on the conditions one would like to measure.

Within-treatment analyses are conducted to assess changes from the baseline hydration levels. ANOVA is typically used to determine if these changes are statistically significant. Non-parametric alternatives as well as binomial statisticals are also employed depending upon the design, number of subjects enrolled and the objectives of the study. The appropriate statistical analysis should flow from the study design and the objectives of the study (Barlow and Wiechers, 1999).

Skin elasticity test

The measurement of the elastic property of the skin was conducted by the DermaLab® Elasticity probe. To measure skin elasticity, a measuring head is placed on the area of skin of interest. A partial vacuum is created with the help of a vacuum pump. The suction and release times are specified and set before starting the measurement. The depth of penetration or deformation of the skin into the 2 mm wide measuring probe is measured optically by means of a mirror and a light detector. The time taken for the skin to return to its original state (recovery) after release of the vacuum is quicker or slower according to the skin condition.