CHAPTER II

REVIEWS OF RELATED LITERATURE AND RESEARCH

Alpha hydroxy acids (AHAs)

General knowledge

Alpha hydroxy acids (AHAs) are organic carboxylic acids with one hydroxyl group attached to the alpha carbon atom next to the carboxyl group (Figure 1). AHAs range from simple aliphatic compounds to complex molecules. Many of these substances can be derived from natural sources and are often referred to as fruit acid. (Van Scott et al., 1996)

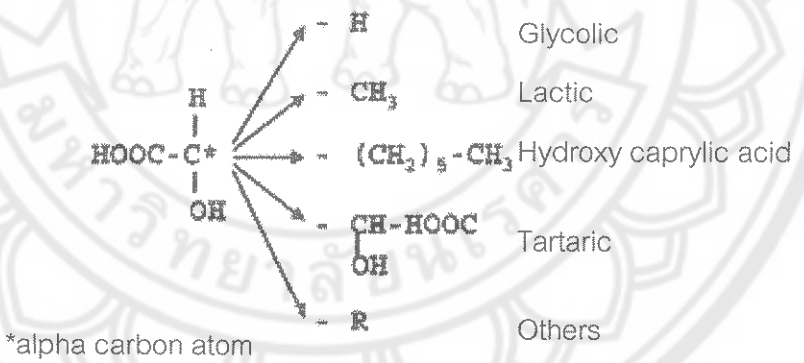

Figure 1 Structure of alpha hydroxy acids.

Representative AHAs are described as follow;

Glycolic acid

Glycolic acid occurs in sugarcane and is the smallest molecule among the other members of the AHAs. It is commercially available as a white crystalline compound with 99% purity and also as a 70% aqueous solution. Glycolic acid is very soluble in water. Aqueous solutions of glycolic acid exist in different pHs depending on acid concentration. For example, glycolic acid aqueous solution has a pH of 1.7 at the concentration of 5% and pH of 0.6 at 70% (Table 1).

Lactic acid

Lactic acid is present in sour milk and tomato juice, etc. It is nontoxic and a normal carbohydrate metabolite in the skin. Owing to the nonsymmetric nature of the molecule, lactic acid has isomeric forms such as D-lactic acid, L-lactic acid, and D,L-lactic acid. It is commercially available as 90% in liquid and very soluble in water.

Table 1 Concentration and pH of AHA and AKA in water

Concentration (%)	pH		
	Glycolic acid	Lactic acid	Pyruvic acid
5	1.7	1.8	1.6
10	1.6	1.8	1.5
20	1.5	1.7	1.4
30	1.4	1.6	1.4
40	1.3	1.6	1.3
50	1.2	1.5	1.2
60	1.0	1.4	1.1
70	0.6	1.2	1.0
80		1.0	0.8

Malic acid

Malic acid is a dicarboxylic acid with one hydroxyl group at the alpha position of the acid, similar to the compound formed from one molecule of glycolic acid and one molecule of acetic acid. It naturally occurs in apple, is nontoxic and present as a carbohydrate metabolite in the skin. Owing to the nonsymmetric nature of the molecule, malic acid has isomeric forms such as D-malic acid, L-malic acid, and D,L-malic acid. It is commercially available as 90% in liquid and is very soluble in water and alcohol.

Tartaric acid

Tartaric acid is a dicarboxylic acid with two hydroxyl group at the alpha position of the acid. It is similar to the compound formed from two molecule of glycolic. Tartaric acid has found in grape and tamarind, nontoxic, and present as a carbohydrate metabolite in the skin. Owing to the nonsymmetric nature of the molecule, tartaric acid has isomeric forms such as D-tartaric acid, L- tartaric acid, and D,L- tartaric acid. It is commercially available as 90% in liquid and is very soluble in water and alcohol.

Citric acid

Citric acid is a tricarboxylic acid with one hydroxyl group at the alpha position to one carboxyl group. Hydroxyl groups are also at the beta position to the two remaining carboxyl groups. Therefore, citric acid can be called an alpha-hydroxyacid or a beta-hydroxyacid, depending on which carboxyl group is referred to. Citric acid occurs in citrus fruits. This AHA is present in 5-8% concentration in lemon juice and also present in oranges and pineapples.

Mandelic acid

Mandelic acid is also called phenylglycolic acid. In combination with methenamine (the product called mandelamine or methenamine mandelate), it has been used as a urinary antiseptic. Mandelic acid is soluble in alcohol and water.

Pyruvic acid

This alpha-keto acid (AKA) is chemically related to lactic acid in that the hydroxyl group can be replaced by a keto group at the alpha position of the acid. Pyruvic acid is converted to lactic acid by lactic dehydrogenase enzyme.

Ascorbic acid

Ascorbic acid is a lactone form of 3-keto-2,4,5,6-tetrahydroxyhexanoic acid, which is both an AHA and a beta-ketoacid. L-ascorbic acid is also called vitamin C which is both regulator and stimulator of collagen synthesis.

Acid strength of AHAs and AKAs

The acid strength of AHA or AKA is determined by proton dissociation in solution and is usually expressed as pKa of the AHA or AKA. An AHA or AKA is a stronger acid when the pKa number is lower. For example, glycolic acid (pKa 3.83) is stronger than lactic acid (pKa 3.86).

Antioxidative effect of AHAs

An antioxidant can be defined as a compound that is capable of preventing or inhibiting oxidation of other substances. Many AHAs are antioxidant such as, malic acid, tartaric acid, citric acid and ascorbic acid, etc. However, several other AHAs are not antioxidant such as glycolic acid and lactic acid.

Mechanism of action of AHAs on skin

AHAs exfoliate dead skin cells and moisturize the skin.Their main action is to facilitate desmosomal degradation leading to increases in corneocyte desquamation, cytokines and epidermal proliferation. There is also an increase in hyaluronic acid (which holds 1000x times its weight in water) and this might be one of the causes of increased skin 'plumpness'. By normalizing corneocyte cohesion, the stratum corneum is thinned

and smoother and more flexible (even at low relative humidity), and the formation of dry flaky scales is reduced. These result in better appearance and feeling of the skin.

Claims that AHAs reverse photodamage and reduce wrinkles, brown spots and roughness are somewhat controversial and are currently being reviewed by the Cosmetic, Toiletry and Fragrance Association (CTFA), the US.FDA and the Federal Trade Comission (FTC). Several aspects concerning the mechanism of action of AHAs are still unknown. In particular, little is known about the correlation between the histopathological and functional changes in the stratum corneum induced by AHA treatment. Studies have suggested that treatment with AHAs produce significant reversal of epidermal and dermal markers of photoaging.

Effects on pigmentation

Interestingly, an in vitro study showed that glycolic acid and lactic acid in doses of 300 or 500 ug/mL suppressed melanin formation by directly inhibiting tyrosinase activity. Adjusting the pH up to 5.6 did not affect tyrosinase activity and this effect was then deemed independent of these AHAs acidic nature. The authors postulate that glycolic and/or lactic acid might work on pigmented lesions by accelerating epidermal turnover and by directly inhibiting melanin formation in melanocytes. There have been conflicting clinical studies showing no benefit to a positive benefit in patients with hyperpigmented skin conditions. It is this author's belief that the AHAs may actually work clinically by enhancing penetration of other bleaching agents such as hydroquinones or retinoids and/or by directly inducing skin turnover which improves the appearance of hyperpigmentation due to hyperkeratinization (Ditre C.M., 1996).

Therapeutic use

AHAs have been used as a cosmeceutical, a dermatologic application and as a chemical peel. Formulation is more important than concentration alone. Bioavailability of the AHA is a major determinant. For example a high concentration of AHA near neutral pH is ineffective because the bioavailability is miniscule. At the other extreme, at low pH even small concentrations can be effective because a major amount of the AHA is available. The higher free acid, the higher biological activity.

Thai FDA requirement

AHAs are safe in cosmetic products at concentrations of 10% or less, at a pH of 3.5 or greater, and formulated to avoid increasing the skin's sensitivity to the sun or accompanied by directions to use sun protection daily.

Stronger formulations of AHAs (concentrations up to 30% and a pH as low as 3.0) are safe if applied by trained professionals. Such use should be brief, discontinuous, and followed by thorough rinsing and accompanied by directions to use sun protection daily. Stronger concentrations are sometimes needed for the thickened stratum corneum seen in some dermatologic diseases.

Adverse effects

AHAs are acids and can cause mild to moderate irritation unless they are neutralized in the final product. Low concentrations of AHAs appear to be less irritating than tretinoin, and no other adverse effects have been reported. However long-term studies have not been done. As is the case with tretinoin, AHAs can sometimes cause stinging/burning in nasolabial and sub-orbital areas and local contact irritation. If an acid peel has been accomplished using glycolic acid, then photosensitivity is a concern for about two weeks after the peel. In any case, sunscreen and sunblock agents should always be used to protect against solar damage.

Tamarind (*Tamarindus indica* L.)

Family : Leguminosae (Fabaceae)

Common Names: Tamarind, Tamarindo, Tamarin, Sampalok.

Distant affinity: Carob (*Ceratonia siliqua*).

Figure 2 Fruit pulp of tamarind (*Tamarindus indica* L.).

Origin: Tamarind is native to tropical Africa and grows wild throughout Sudan. It was introduced into India so long ago. It has often been reported as indigenous there also. It is extensively cultivated in tropical areas of the world. Sometime during the sixteenth century, it was introduced into America and today is widely grown in Mexico.

Adaptation: Tamarind is well adapted to semiarid tropical condition, although it does well in many humid tropical areas of the world with seasonally high rainfall. Young trees are very susceptible to frost, but mature trees will withstand brief periods of -2°C without serious injury. A tamarind tree in the Quail Botanical Gardens in San Diego County flowers, but rarely sets fruit, possibly because of the cool coastal climate. Dry weather is important during the period of fruit development. The tree is too large to be grown in a container for any length of time.

Growth Habit: Tamarinds are slow-growing, long-lived, evergreen trees that under optimum conditions can grow 80 feet high with a spread of 20 to 35 ft., in its native eastern Africa and Asia. However, in Southern California it seldom reaches more than 15 to 25 ft. in height.

Foliage: The bright green, pinnate foliage is dense and feathery in appearance, making an attractive shade tree with an open branch structure. The leaves are normally evergreen but may be shed briefly in very dry areas during the hot season. There are usually as many as 10 to 20 nearly sessile 1/2 - 1 inch, pale green leaflets per leaf. The leaflets close up at night.

Flowers: The inconspicuous, inch-wide, five-petalled flowers are borne in small racemes and are yellow with orange or red streaks. The flower buds are pink due to the outer color of the 4 sepals which are shed when the flower opens.

Fruit: The 3 - 8 inch long, brown, irregularly curved pods as shown in Figure 2 are borne in abundance along the new branches. As the pods mature, they fill out somewhat and the juicy, acidulous pulp turns brown or reddish-brown. When fully ripe, the shells are brittle and easily broken. The pulp dehydrates to a sticky paste enclosed by a few coarse stands of fiber. The pods may contain from 1 to 12 large, flat, glossy brown, obovate seeds embedded in the brown, edible pulp. The pulp has a pleasing sweet/sour flavor and is high in both acid and sugar. It is also rich in vitamin B and high in calcium as shown in table 2. There are wide differences in fruit size and flavor in seedling trees. Indian types have longer pods with 6 - 12 seeds, while the West Indian types have shorter pods containing only 3 - 6 seeds. Most tamarinds in the Americas are of the shorter type. (Dassanayake M. D.,1991)

Table 2 Composition of tamarind per 100 g

Composition	Pulp (ripe) *	Leaves (young)	Flowers
Moisture	28.2-52 g	70.5 g	80 g
Fiber	5.6 g	1.9 g	1.5 g
Invert Sugars	30-41 g		
Calcium	35-170 mg	101 mg	35.5 mg
Magnesium		71 mg	
Phosphorus	54-110 mg	140 mg	45.6 mg
Iron	1.3-10.9 mg	5.2 mg	1.5 mg
Copper		2.09 mg	
Sulfur		63 mg	
Sodium	24 mg		
Potassium	375 mg		
Vitamin A	15 I.U.	250 mcg	0.31 mg
Thiamine	0.16 mg	0.24 mg	0.072 mg
Riboflavin	0.07 mg	0.17 mg	0.148 mg
Niacin	0.6-0.7 mg	4.1 mg	1.14 mg
Ascorbic Acid	0.7-3.0 mg	3.0 mg	13.8 mg
Oxalic Acid		196 mg	
Tartaric Acid	8-23.8 mg		
Oxalic Acid	trace only		

Skin structure

Skin structure consists of three layers of epidermis, dermis and hypodermis. Each layer has particular tasks, but works in harmony with the next layer. The structure of the skin is shown in figure 3.

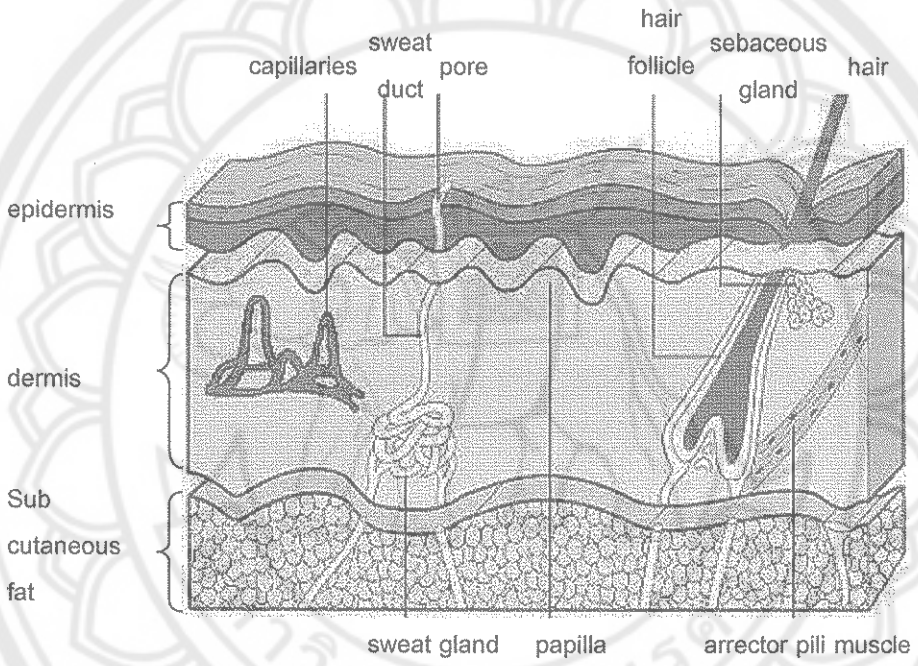

Figure 3 Skin structure (www.talkacne.com).

The Epidermis

The epidermis is a stratified squamous epithelium that mainly serves as a protective barrier. The epidermis is about 0.1 mm thick, but on the palms and soles, the thickness can be greater (0.8-1.4 mm). The keratinocyte is the principal cell of the epidermis and it serves to produce the protein keratin. The represented in the five layers of the epidermis is shown in figure 4.

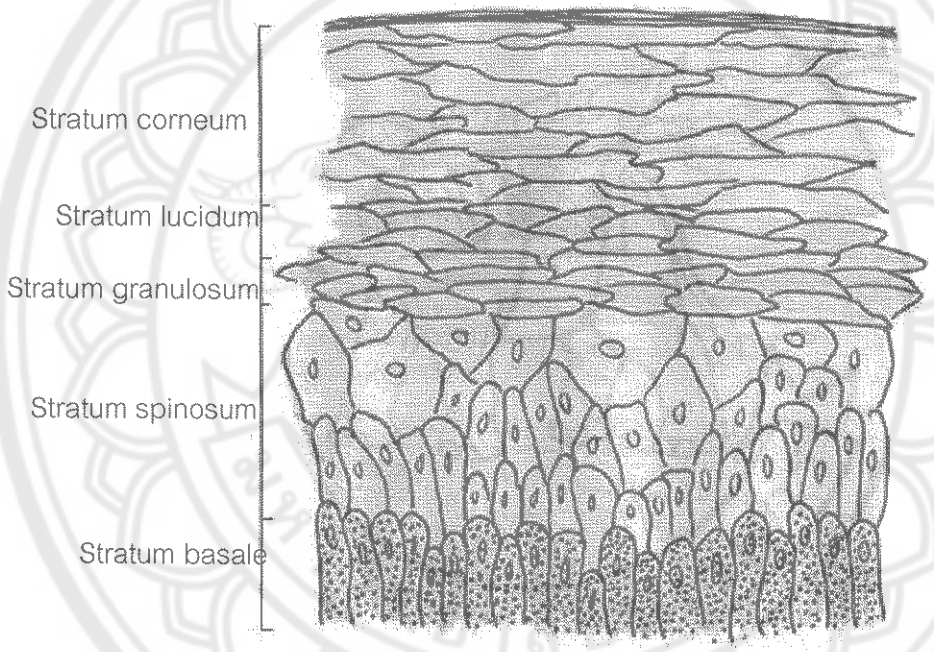

Figure 4 Epidermis structure (www.science.kennesaw.edu).

1) Stratum basale (Basal Cell Layer)

The basal cell layer is comprised mostly of keratinocytes which are either dividing or non-dividing. The cells contain keratin tonofibrils and are secured by hemidesmosomes to the basement membrane.

Melanocytes make up to 5-10% of the layer and produce melanin which is transferred to neighboring keratinocytes via dendritic processes. Melanocytes are of neural crest origin and most numerous on the face and other exposed areas of skin. Merkel cells can also be found in this layer and are closely associated with terminal filaments of cutaneous nerves. Merkel cells have a role in sensation. Neuropeptide granules, neurofilaments, and keratin can be seen in their cytoplasm.

2) Stratum spinosum (Prickle Cell Layer)

Daughter basal cells migrate upwards and differentiate into polyhedral cells in this layer. Desmosomes interconnect these polyhedral cells and give rise to the "prickles/spines" seen at light microscope level. Keratin tonofibrils form the cytoplasmic supportive network. Langerhans cells are mostly found in this layer. They are dendritic, immunologically active cells that play a role in antigen presentation.

3) Stratum granulosum (Granular Cell Layer)

In this layer, cells become flattened and lose their nuclei. In the cytoplasm, there are keratohyalin granules as well as membrane-coating granules which expel their lipid contents into the intercellular spaces.

4) Stratum Lucidum

The stratum lucidum is normally only well seen in thick epidermis and represents a transition from the stratum granulosum to the stratum corneum.

5) Stratum corneum (Horny Layer)

The main barrier of skin stratum corneum, is composed of sheets of overlapping polyhedral cornified cells with no nuclei called corneocytes. This layer is thickest on the palms and soles. The flattened corneocyte develops a thickened cell envelope.

Its cytoplasm is replaced by keratin tonofibrils in a matrix formed from keratohyalin granules. Whereas, the membrane-coating granules produce a lipid glue that keeps the cells stuck together. This forms the hydrophobic barrier membrane that protects the skin, and prevents water loss (science.kennesaw.edu).

The Dermis

The dermis is connected to the epidermis. It is a well-vascularized connective tissue also permeated by nerve fibers and lymphatic vessels. This layer helps maintain the unvasculated epidermis. The structure characteristic dermis is fibrous network of proteins. These proteins are made up of collagen fibers while elastin and reticulin fibers appear only in limited amounts. Since, fibrous proteins are imbedded in a colloidal base, thus, its high water retention properties. It is responsible for the turgidity of the skin.

The Hypodermis

This layer is a layer of fatty tissue. It provides nourishment to the dermis and upper layers of the skin. Blood vessels, nerves, nerves, sweat glands, and deeper hair follicles are found this layer.

Skin pigmentation

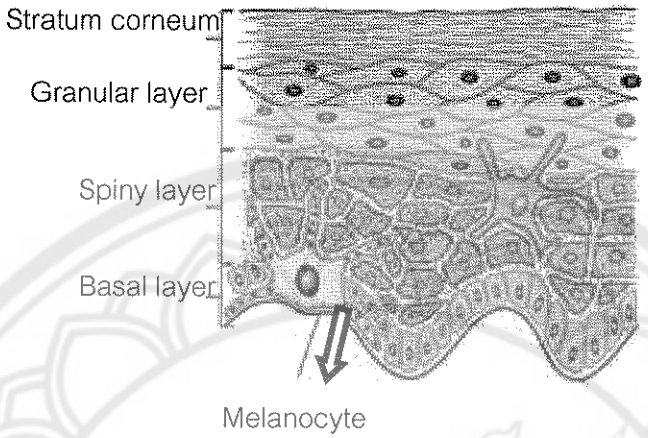

Figure 5. Melanocyte structure (www.katinkauneuskulma.fi).

As shown in figure 5, the keratinocytes are not the only cells in the epidermis. The melanocytes of the epidermis are crucial in determining skin pigmentation.

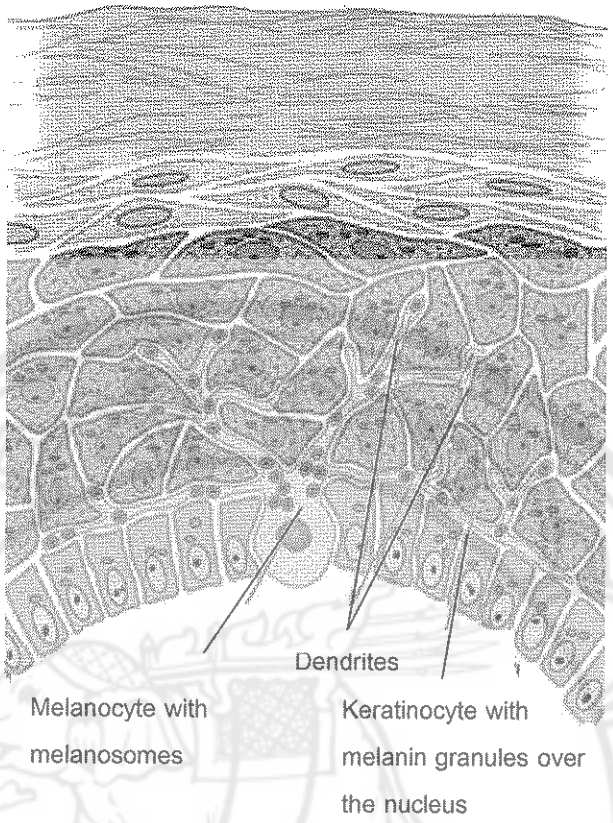

Figure 6 Distribution of melanin in epidermis (www.katinkauneuskulma.fi).

Melanocyte function

Melanocytes are located in the basal layer. In this location, they produce the pigment melanin in elongated, membrane-bound organelles known as melanosomes. Melanin is packed into granules which are moved down dendritic processes and transferred by phagocytosis to adjacent keratinocytes (Figure 6).

In the inner layers of the epidermis, melanin granules form a protective cap over the outer part of keratinocyte nuclei. In the stratum corneum, melanin granules are uniformly distributed to form a UV-absorbing blanket which reduces the amount of radiation penetrating the skin.

Contrary to popular belief, variations in racial pigmentation are not due to differences in melanocyte numbers, but to the number and size of melanosomes produced.

UV radiation - mainly the wavelengths of 290 to 320 nm (UVB) - darkens the skin firstly by immediate photo-oxidation of preformed melanin, and secondly over a period of days by stimulating melanocytes to produce more melanin. UV radiation also thickens the epidermis by inducing keratinocyte proliferation.

Melanin

Melanin is the pigment of skin color which is synthesized in the melanosomes of melanocyte. Melanin synthesis is regulated by melanogenic enzymes such as tyrosinase, tyrosinase-related protein 1 (TRP-1), and tyrosinase-related protein 2 (TRP-2). The starting material for the production of melanin, both the brown-black eumelanin and the yellow-red pheomelanin, is the amino acid tyrosine. The level and type of melanin production relate to the activity of the various enzymes as well as MSH (α-melanocyte stimulating hormone), agouti signaling protein, basic fibroblast growth factor (bFGF), endothelin-1 and ultraviolet light (Bolognia J.L., 1999).

Melanogenesis Inhibitory Activity Tests

Nowadays, several methods have been used to screen depigmenting effect of the compounds. There are shown in Table 2. However, *in vitro* assay of malanogenesis inhibition is the most commonly used as the following:

1. Mushroom Tyrosinase Assay

Mushroom tyrosinase assay is the determination of dopachrome content occurred due to the reaction of DOPA substrate and tyrosinase enzyme. When some chemical is added to this reaction mixture and the dopachrome color disappears, it means that the added substance could successfully block the activity of tyrosinase enzyme (Elsner P., 2002).

2. Melanin Content Assay

Melanin content assay is the determination of the melanin amount in melanocyte cells which are treated with the whitening agents. Cultured B16 melanoma cells have been used in the melanogenesis studies and are useful in demonstrating several new mechanisms of melanogenesis inhibition. The cell concentration, cell morphology and the extracts of melanin pigment in cultured cell indicate the potent of tested whitening agents. Whole melanin was determined by using two condition assays. For extracellular melanin content, the secretion of melanin in the medium was estimated whereas the intracellular melanin was quantified in pelleted cells.

Permeability pathway

There are two main pathways by which drugs can cross the skin and reach the systemic circulation (Figure 7). The more direct route is known as the transcellular pathway. By this route, drugs cross the skin by directly passing through both the phospholipid membranes and the cytoplasm of the dead keratinocytes that constitute the stratum corneum. Although this is the path of shortest distance, the drugs encounter significant resistance to permeation. This is because the drugs must cross the lipophilic membrane of each cell, then the hydrophilic cellular contents containing keratin, and then the phospholipid bilayer of the cell one more time. This series of steps is repeated numerous times to traverse the full thickness of the stratum corneum. Few drugs have the properties to cross via this method.

The more common pathway through the skin is via the intercellular route. Drugs crossing the skin by this route must pass through the small spaces between the cells of the skin, making the route more tortuous. Although the thickness of the stratum corneum is only about 20 μm, the actual diffusional path of most molecules crossing the skin is on the order of 400 μm.[5] The 20-fold increase in the actual path of permeating molecules greatly reduces the rate of drug penetration.

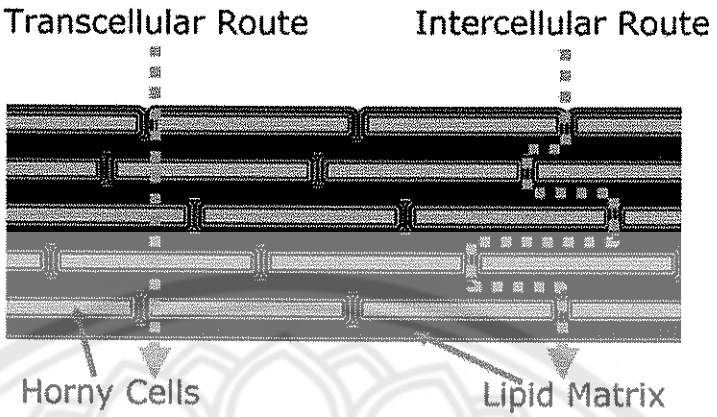

Figure 7 Transcellular and intercellular route.

A less important pathway of drug penetration is the follicular route (Figure 8). Hair follicles penetrate through the stratum corneum, allowing more direct access to the dermal microcirculation. However, hair follicles occupy only 1/1,000 of the entire skin surface area. Consequently, very little drug actually crosses the skin via the follicular route.

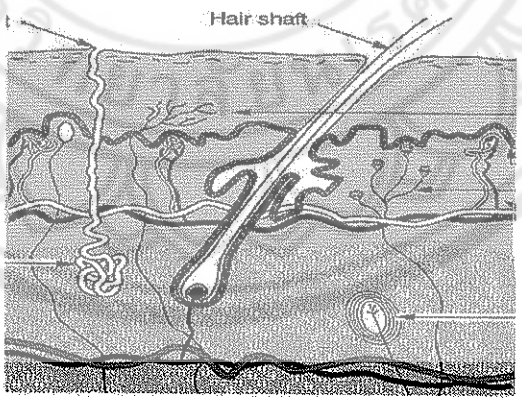

Figure 8 Follicular route.

Liposomes

Bangham discovered liposomes in 1963 and the use of lipid vesicles as systemic and topical drug delivery systems has attracted increasing attention. The application in skin treatment is based on the similarity of the bilayer structure of lipid vesicle to that of natural membranes.

A liposome is defined as a structure consisting of one or more concentric spheres of lipid bilayers separated by water or aqueous buffer compartments (Figure 9). These spherical structures can be prepared with diameters ranging from 80 nm to 100 um. When phospholipids are dispersed in and aqueous phase, hydration of the polar head groups of the lipid result in a heterogeneous mixture of structures, generally referred to as vesicles, most of which contain multiple lipid bilayers forming concentric spherical shells. These were the liposomes first described by Bangham and are now referred as multilamellar vesicle (MLVs). Sonication of these lipid dispersions results in size reduction of these liposomes to vesicles containing only a single bilayer with diameters ranging from 25-50 nm. These structures are referred to as small unilamellar vesicles (SUVs). Since MLVs and SUVs have curtain limitations as model membrane systems as delivery systems, a number of laboratories have developed single bilayer liposomes which exhibit a size range of 100-500 nm in diameter. These vesicles are referred to as large unilamellar vesicles (LUVs) (Weiner, 1989).

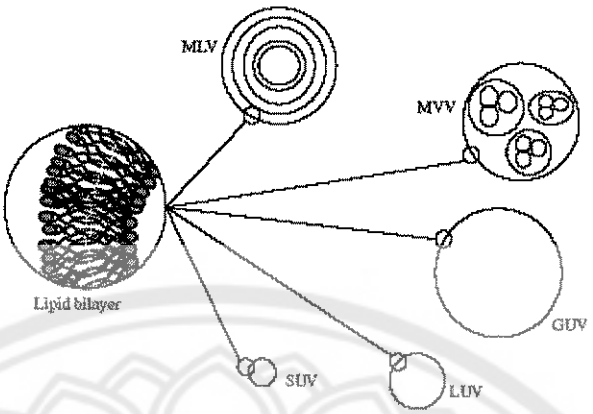

Figure 9 Lipid bilayer structure and types of liposomes ; MLV (Multilamellar vesicle), MVV (Multivesicular vesicle), GUV (Giant unilamellar vesicle), LUV (Large unilamellar vesicle), SUV (Small unilamellar vesicle) (modified from Gomez-Hens A., 2005).

Phospholipids

The main material used in liposome preparation is phospholipids. These lipids present in biological membranes. As shown in figure 10, the general chemical structure of these types of lipids is exemplified by phosphatidic acid. The backbone of the molecule resides in the glycerol moiety. At position number 3 of the glycerol molecule the hydroxyl is esterified to phosphoric acid, hence the name glycerophospholipids. The hydroxyls at position 1 and 2 are usually esterified with long chain fatty acids giving rise to the lipidic nature of these molecules. One of the remaining oxygens of phosphoric acids may be further esterified to a wide range of organic alcohols including glycerol, choline, ethanolamine, serine and inositol. The most abundant glycerol phosphatides in plants and animals are phosphatidylcholine (PC), also called lecithin, and phosphatidyl-ethanolamine (PE), sometimes referred to as cephalin. Table 3 shows the fatty acid composition of two common phosphatidylcoline, one extracted from egg yolk and the other from soya bean oil. Notice the difference in the degree of unsaturation between egg and soy PC. Soya bean PC contains a greater proportion of unsaturated bond.

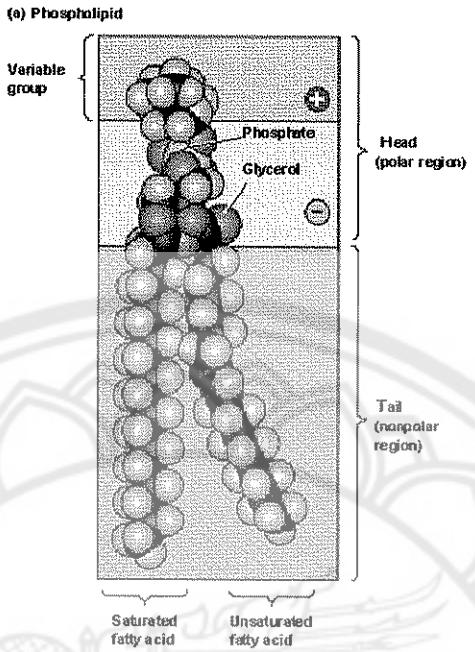

Figure 10 Structure of phospholipids (www.nicerweb.com)

Table 3 Composition of two common phosphatidylcoline

Fatty acid composition		Egg PC	Soya bean PC
16:0	Palmitic	32	12
16:1	Palmitoleic	1.5	<0.2
18:0	Stearic	16	2.3
18:1	Oleic	26	10
18:2	Linoleic	13	68
18:3	Linolenic	<0.3	5
20:4	Arachidonic	4.8	<0.1
22:6	Dodosapentaenoic	4.0	<0.1

Why liposomes are formed

As shown in figure 11, lipid capable of forming liposomes (or other colloidal structures) exhibits a dual chemical nature. Their head groups are hydrophilic and their fatty acyl chains are hydrophobic. It has been estimated that each zwitterionic head group of phosphatidylcholine has on the order of 15 molecules of water weakly bound to it, which explains its overwhelming preference for the water phase. The hydrocarbon fatty acid chains, on the other hand, vastly prefer each other's company to that of water. This phenomenon can be understood quantitative terms by considering the critical micelle concentration (CMC) of PC in water. The lipid forms micelles or bilayer structures rather than remaining in solution as monomers. It should be point out that under proper conditions, relative large amounts of lipids which normally tend to form hexagonal or micellar structures can be successfully incorporated into liposomes (Weiner, 1989).

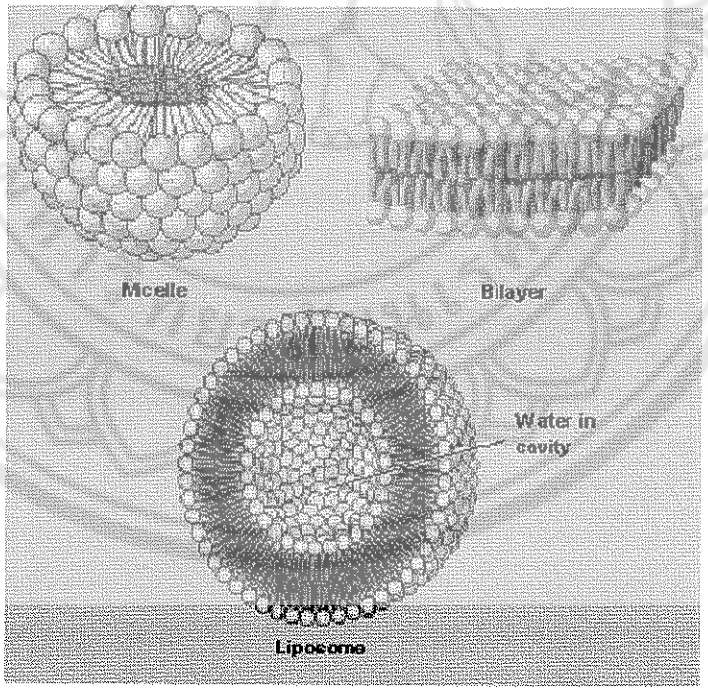

Figure 11 Spontaneous arrangement of liposome (www.avantilipids.com).

Liposome preparation method

There are many method used to prepare liposome such as Hydration, Sonication, French pressure cell, Solvent injection and Reverse phase evaporation. The obtained different size and structure by different method, but the main preparation step are similar as show in figure 12.

Reverse Phase Evaporation Technique (REV)

LUVs can also be prepared by forming water in oil emulsion of phospholipids and buffer in excess organic phase followed by removal of the organic phase under reduced pressure (the so called "Reverse Phase Evaporation or REV method). The two phases are usually emulsified by sonication but other mechanical means have also been used. Removal of the organic solvent under vacuum causes the phospholipids-coated droplets of water to coalesce and eventually form a viscous gel. Removal of the final traces of solvent results in the collapse of the gel into a smooth suspension of LUVs. With some lipid compositions, the transirion from emulsion to LUV suspension is so rapid that the intermediate gel phase appears not to form. This method was developed by Szoka and Papahadjopoulos in 1978 and has been used extensively for applications which require high encapsulation of a water soluble drug. Entrapment efficiencies up to 65% can be obtained with this method

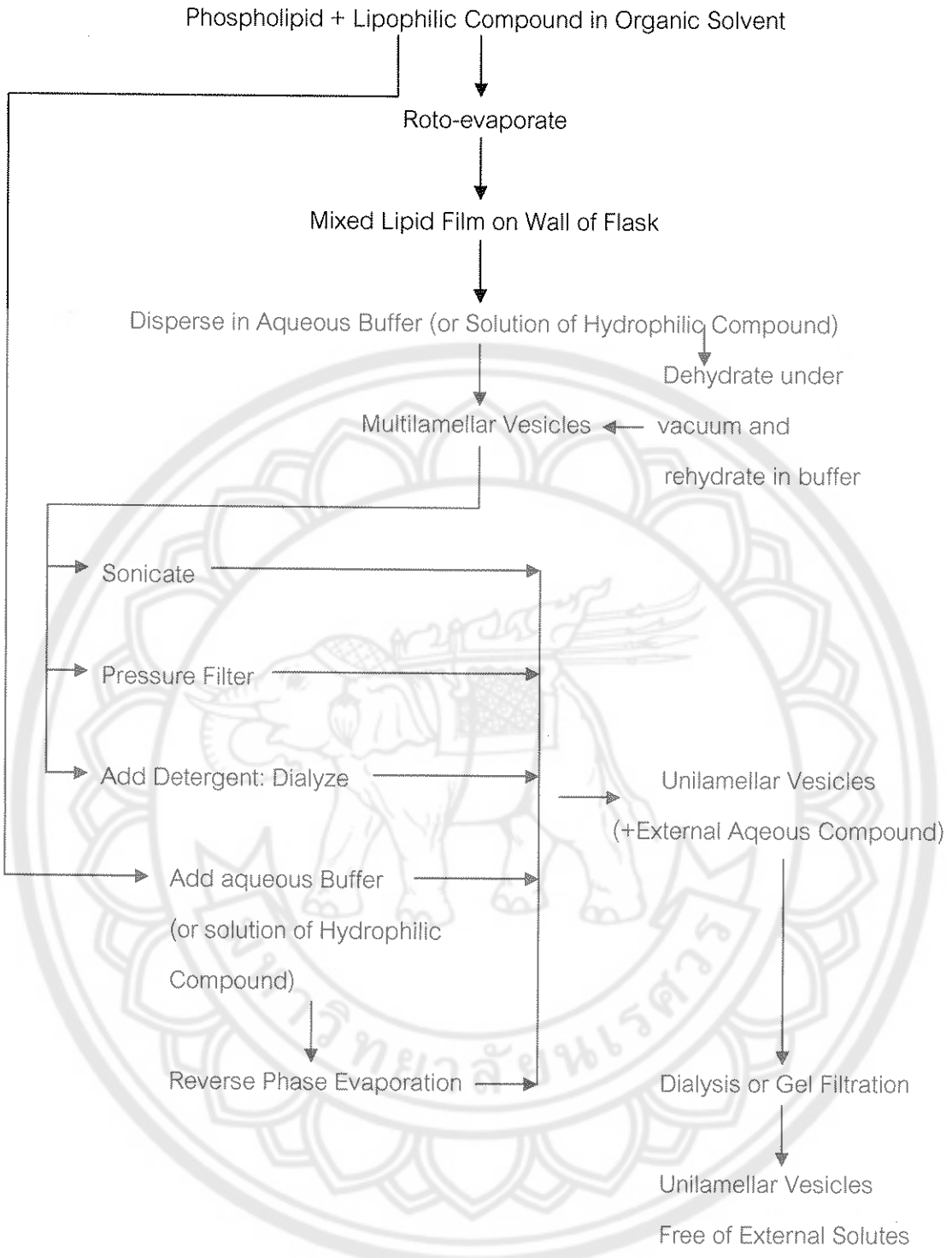

Figure 12 Flowsheet for preparing drug or cosmetic carrying liposomes.

Stability of Liposomes

The stability of any cosmetic product is usually defined as the capacity of the formulation to remain within defined limits for a predetermined period of time (shelf-life of the product). The first step in designing any type of stability testing program is to specify these limits by establishing parameters defined in terms of chemical, physical and microbial stabilities. General observations about liposomal stability include:

1. There are very few published reports on long-term stability studies of liposomes.
2. There are no published reports on the establishment of detailed protocols for stability testing.
3. There are no published reports on the establishment of protocols for accelerated stability testing.
4. MLVs and REVs appear to be more stable than SUVs (with respect to leakage on storage).
5. Use of saturated phospholipids and incorporation of cholesterol into the bilayer generally improves stability.
6. Liposomes stored at 4^{0}C, at times, appear to be more stable than liposomes stored at room temperature.

Vesicle-mediated skin transport

Encapsulation of active agent in phospholipids vesicle suspensions (liposomes) has attracted considerable attention as a novel strategy for topical delivery.

Generally, three principal mechanisms can be envisaged for the vesicle-mediated skin uptake of an associated penetrant :

1. The intact vesicle transports across the skin carrying its payload with it.
2. The vesicle dissociates at or near the skin surface and then the penetrant is absorbed in association with fragments (or constituents) of the carrier.
3. Post-application to the skin, the penetrant partitions rapidly from the vehicle into the stratum corneum and crosses the barrier alone.

From many studies reported clearly that, the second and third mechanisms also allow for the possibility that vesicle components may themselves permeate the membrane and even disrupt its barrier function (i.e. they may act as penetration enhancers). Furthermore, it is conceivable that vesicles may break down and reform in situ incorporating thereby, for example, lipids originating from the skin surface (e.g. sebaceous lipids) or lipids from the intercellular domains of the stratum corneum (Alvarez-Roman R. et al., 2004)

Chitosan

Chitosan and chitin are polysaccharide polymers containing more than 5,000 glusosamine and acetylglucosamine units, respectively, and their molecular weights are over one million Daltons depend on the processing conditions. Chitin is found in fungi, arthropods and marine invertebrates. Commercially, chitin is derived from the exoskeletons of crustaceans such as shrimp, crab and other shellfish. Chitosan is obtained from chitin by deacetylation process.

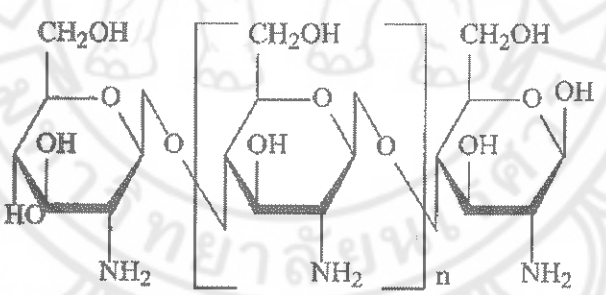

Figure 13 Structure of chitosan.

Chitin, the polysaccharide polymer from which chitosan is deriverd, is a cellulose-like polymer consisting mainly of unbranched chains of N-acetyl-D-glucosamine. Deacetylated chitin, or chitosan, is comprised of chains of D-glucosamine.

Standard grades of chitosan require the addition of acid to solubilize in water. Acetic acid is commonly usedd as a reference, but other organic acids such as citric acid, formic acid, lactic acid, tartaric acid etc., as well as mineral acids, can be used successfully.

The advantage of using chitosan in such products is firstly based on its ability to form tough, clear and very flexible film, more stable at high humidity and nontoxic. Nowaday, chitosan has been used in the cosmetic and the pharmaceutical industries for its potential use in controlled delivery systems

In addition to the advantage of chitosan described above, No interactions are known. However, chitosan might bind to certain drugs, especially lipophilic drugs.

In vitro evaluation of liposome

Dialysis

In biochemistry, dialysis is the process of separating crystalloids and colloids in solution by the difference in their rates of diffusion through a semipermeable membrane. Dialysis is a common laboratory technique, and operates on the same principle as medical dialysis. Typically a solution of several types of molecules is placed into a semipermeable dialysis bag, such as a cellulose membrane with pores, and the bag is sealed. The sealed dialysis bag is placed in a container of a different solution, or pure water. Molecules small enough to pass through the tubing (often water, salts and other small molecules) tend to move into or out of the dialysis bag, in the direction of decreasing concentration. Larger molecules (often proteins, DNA, or polysaccharides) that have dimensions significantly greater than the pore diameter are retained inside the dialysis bag. One common reason for using this technique would be to remove the salt from a protein solution. The technique will not distinguish between proteins effectively.

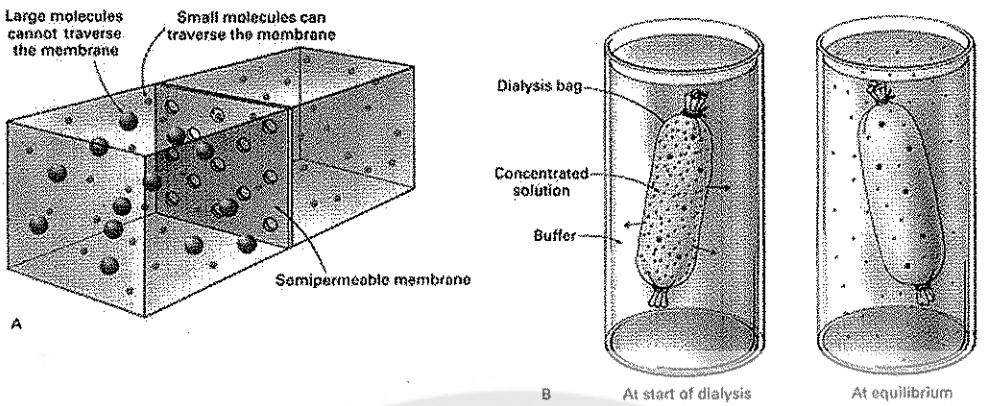

Figure 14 Dialysis process (departments.oxy.edu).

This dialysis technique can used to evaluate the release pattern of active agent from liposome (Perugini et al., 2000)