# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Hair

Hair is a keratinized fiber that emerges from the skin surface. Human hair is a complex biological fiber with well-characterized microstructures. Human hair consists of two main parts; hair shaft, which is the fiber of hair emerging from the scalp and hair root which is the part under the scalp.

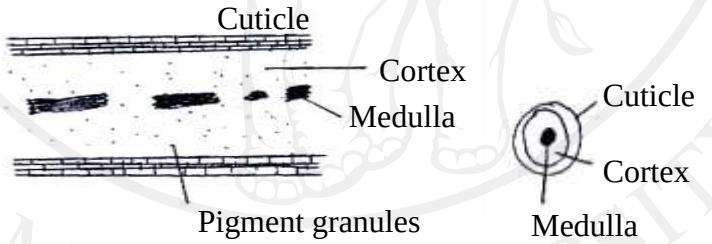


**Figure 1** Hair shaft structure showing the three layers; cuticle, cortex, and medulla

Hair shaft consists of three layers: cuticle, cortex, and medulla as shown in Figure 1. Cuticle is the outermost layer; human hair usually surrounded by 6 – 10 cuticle cells, each approximately 0.2-0.5 μm thick. Cuticular cells are overlapped on the skin surface and oriented liked the fish scales. Cuticle layers have an important role on hair's physical characteristics such as optical properties, combabilty, and feeling.

The middle layer is cortex, which is the bulk component of hair and supplies mechanical strength to hair. Cortex cells are closely-packed macrofibrils, which are oriented along the axis of the hair. Macrofibrils are composed of rodlike microfibril arranged in whorls shape and embedded in an intermicrofibilar matrix in a parallel longitudinal oriented within microfibrils like a rope.

The innermost layer is medulla, which can be found only in terminal hair. Medulla is usually composed of spongy keratin cells bounded with air spaces (Dawber, 1996).

Another main part of human hair is hair root or hair follicle. Hair follicle is the most critical part of hair; it supplies nutrition to hair and support hair fiber growth and elongation. One hair usually comes from one hair follicle. Hair follicle is an appendage of a skin and has a complex and dynamic structure as shown in Figure 2.

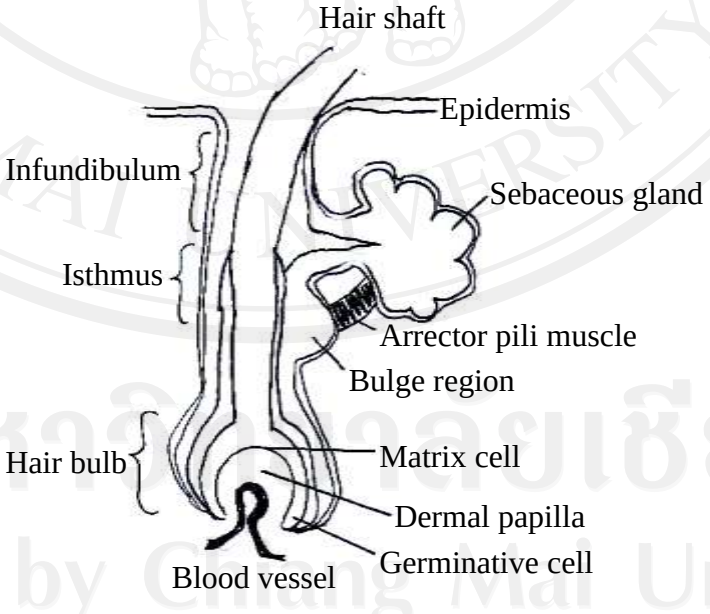


**Figure 2** Schematic diagram of human hair follicle unit

Hair follicle contains the permanent superficial structure and transient cycling component which changes during the hair growth cycle. The permanent portion of the hair follicle can be divided into two sections: i) the infundibulum, which is the part between skin surface and the opening of the duct from sebaceous gland, and ii) the isthmus, which is the part between the opening of the duct from sebaceous gland and the bulge region. The transient portion is the area extends from the bulge region to the base of hair follicle bulb. The epithelial hair bulb is situated around the papilla and contained the matrix cells and the germinative cells (Patzelt et al., 2008).

**2.2 Hair growth cycle**

Normal hair growth cycle can be classified into 3 stages; the growth phase (anagen), regression phase (catagen), and resting phase (telogen). Duration of each phase depends on many factors such as types and location of hair follicle, and its physiological status. In a normal condition, around 85% of scalp hair follicles are in anagen phase, while other 15% are in telogen phase. The duration of anagen phase is ranged from 2 – 6 years, which is the main factor determining individual's hair length. When anagen phase end, it is followed by short resting phase, catagen phase, which is characterized by a cessation of protein and pigment production, involution of the hair follicle and a transient restructuring of extracellular matrix, turning into club hair. In a telogen phase, hair follicles regress to a less than half of their anagen size; the remaining here are remnants of epithelial cell overlying the cluster of the dermal papilla cell. During the telogen phase, hair papilla tends to restart a new anagen phase,

this is why seasonal hair moulting cannot be seen in human (McElwee and Sinclair, 2008).

The recent research suggests that hair fiber shedding process is an active and highly controlled process. The new "Exogen stage" has been introduced to define the hair fiber shedding event, which involving a proteolytic event in the cells of telogen shaft base leading to hair loss during combing or others mechanical stress applying to them. The duration after hair shedding and the onset of a new anagen is termed "Kenogen". Kenogen can be observed in healthy skin, but in individuals with AGA kenogen frequency and duration are much greater.

In healthy individuals, each hair follicle will continue their cycle throughout their entire life. Hair cycles are regulated by many complex biological factors such as hormones, cytokines, neurotransmitters and their receptors as well as transcription factors and enzymes, which act via endocrine, paracrine, or autocrine routes. The impairment in these factors is the cause of the various forms of alopecias.

**2.3 Alopecia**

There are various forms of alopecias, the most common is androgenic alopecia (androgenetic alopecia, AGA), which affects millions of people not only in men, but also in women, however the severity of AGA in women is usually lower than in men. Hair loss in both men and women can be seen as early as a teenager, but it can start even in a later decade of life. AGA can be psychologically devastating to accept, giving less overall body-image satisfaction, and making them difficult to cope and retain integrity of personality functioning.

The hair loss pattern in AGA is usually distinctive and recognizable in most cases. The progression of the hair loss occurs in an orderly manner and well studied. Initially, genetically predisposed men develop bitemporal recession. Next they develop diffuse frontal loss and thereafter a bald patch over the vertex of the scalp. Finally, all the hair over the crown is lost.

The biochemical changes in AGA are concluded by the following. Scalp hair follicle units have different amount of local steroid metabolizing enzyme, which are aromatase, and 5α-reductase. Androgen metabolic pathway in the hair follicle is shown in Figure 3.

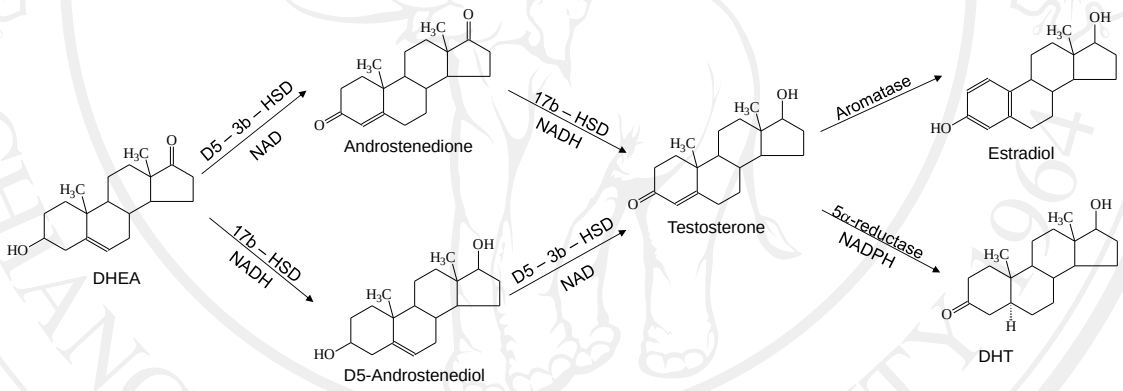


**Figure 3** Androgen metabolic pathways in hair follicle

From Figure 3, weak androgen precursor dehydroepiandrosterone (DHEA) is metabolized into testosterone (T), T is further metabolized by two pathways; i) aromatase enzyme is the enzyme that is responsible for the metabolism of T into estradiol, which is estrogen. ii) 5α-reductase enzyme is the enzyme that is responsible for the metabolism of T into dihydrotestosterone (DHT); this compound is a more potent androgen. Excessive DHT production can cause many androgen-related disorders such as hirsutism, benign prostatic hypertrophy, AGA, and acne. Activity

of 5α-reductase enzyme is concentrated in dermal papilla of a hair follicle and in sebaceous gland. Therefore, the skin has potential to mediate androgen action without relying on elevated systemic levels of T and/or DHT (Sawaya, 1998; Sawaya and Shapiro 2000).

There are noticeable changes in hair cycle dynamics in people with AGA. For example, the duration of anagen phase is decreased while the duration of telogen phase is remained constant or increased. The number of hair follicle in anagen phase is decreased while a number of hair follicle which is in telogen phase is increased. Moreover, the kenogen phase is prolonged. Additionally, a greater than ten-fold reduction of overall hair follicle cell numbers have been reported, leading to a decrease in the hair follicle size, this process was called "Hair follicle miniaturization".

There are some agents to treat AGA. For examples, 5α-reductase inhibitors such as finasteride (Proscar$^{TM}$, Propecia$^{TM}$) and dutasteride (Avodart$^{TM}$) are commonly used by physicians to treat androgen-related disorders. But 5α-reductase inhibitors have some serious and unaffordable side effects such as impotence (erectile dysfunction), abnormal ejaculation, decreased ejaculatory volume, abnormal sexual function, gynecomastia, testicular pain and myalgia.

Vascular/angiogenic-related compounds such as minoxidil, is also used for the treatment of AGA as a topical formulation, but the mechanism of minoxidil in the treatment of AGA is still unclear, it seems to open potassium channels and increases proliferation and differentiation of epithelial cells in the hair shaft. Moreover, the hair tends to fall again after discontinuation of these medicines.

**Models in the study of hair growth**

In the presents, there are both animal models and cell culture models that have been used in the study of hair growth (Rogers and Hynd, 2001). Within various animal models, C57BL/6 mice are the most popular mice models that have been used by many researcher groups (Datta et al., 2009, Hirata et al., 2007, Matsuda et al., 2002, Shimizu et al., 2000a), since this type of mice contains melanocyte only in their hair and their melanogenesis occurred only in the anagen phase of hair growth (Slominski et al., 1994). When they are in 7 weeks of age, all of their hair follicles are in the telogen stage, after removing the hair only white skin is appeared, and they will become blackening only when anagen phase is reinitiated.

**2.4 5α-reductase enzyme**

Steroid 5α-reductase (5αR, EC 1.3.99.5; $\Delta^4$-3-oxo-steroid 5α-oxidoreductase) is an enzyme that catalyzes the NADPH-dependent reduction of testosterone into a more potent androgen, dihydrotestosterone (DHT), which can bind firmly to androgen receptor with higher affinity and slower dissociation rate than testosterone (Bruchovsky and Wilson, 1968; Liu et al., 2006; McGuire et al., 1960).

There are two different 5αR isozymes, which are 5α-reductase type 1 (5αR1) and 5α-reductase type 2 (5αR2). These two subtypes differ in there distribution; 5αR1 can be found in brain, liver, non-genital skin and in dermal papilla of hair follicle, while 5αR2 can be found only in androgen-dependent tissues, such as

prostate, epididymis, and seminal vesicle (Eicheler et al., 1998; Iehlé et al., 1999; Liu and Yamauchi, 2008).

There are many phytochemical classes with reported anti-5αR activity. For example, fatty acids and phenolic compounds are reported about their anti-5αR activity. Firstly, in fatty acids class, the presence of double bond and free carboxyl group are required for anti-5αR activity and only in *cis* configuration is active. For example, oleic acid ($C_{18:1,\ cis\text{-}9}$) and linoleic acid ($C_{18:2,\ cis\text{-}9,12}$) are active inhibitors while their *trans*-isomer, elaidic acid ($C_{18:1,\ trans\text{-}9}$) and linoelaidic acid ($C_{18:2,\ trans\text{-}9,12}$), are totally inactive. Moreover, from previous report, it was indicated that γ-linoleic acid is a potent inhibitor of 5αR enzyme (Liang and Liao, 1992).

The good examples of plants which contain fatty acids with an anti-5αR activity are saw palmetto or American dwarf palm (*Serenoa repens*, *Sabal serrutala*) and *Boehmeria nipononivea*. Saw palmetto's hexane extract is mainly composes of oleic, lauric, myristic, and linoleic acid with the trace amounts of phytosterol, aliphatic alcohols, and polyprenic compounds (less than 2% by weight). Contrasting to Liang and Liao (1992), lauric acid ($C_{12:0}$) and myristic acid ($C_{14:0}$) in this plant are accounting for 5αR inhibition activity (Raynuad et al., 2002). Saw palmetto is a most popular herbal supplement in many countries for the treatment of lower tract urinary symptoms (LUTS) associated with BPH which is a result from its anti-5αR activity (Lowe and Fagleman, 1999). This plant is well-studied, the results show safety, clinical efficacy, and tolerability profile (Gerber and Fitzpatrick, 2004). The phytochemical compositions in its extract may also have potential in clinically treatment of androgen-related disorder.

Secondly, in polyphenols class, tannins are plant polyphenolic compound that are subdivided into two groups, based on their chemical structure: hydrolysable tannins and condensed tannins. Condensed tannin can be found in the tree bark, and the extraction yield of the bark usually higher than that of the wood. Condensed tannin extracted from the barks of woody plants by acetone-water (1:1 by volume) are reported to be able to inhibit 5αR enzyme with an $IC_{50}$ (50% inhibitory concentration) ranged from 39 to 75 µg/ml, compared to γ-linoleic acid gives $IC_{50}$ at 32 µg/ml (Liu et al., 2008).

In flavonoids class, for example, myricetin, quercitin, baicalein, and fisetin are the flavonoids with activity against 5αR1 with an $IC_{50}$ of 23, 23, 29, and 57 µM, respectively. While in chrysin, morin, and taxifolin are inactive. Moreover, in rutin, which is 3-rutinose glycoside of quercitin, is totally inactive. This is contrast to green tea catechins, epicatechin gallate and epigallogallocatechin gallate, are active against both 5αR1 and 5αR2, while epicatechin and epigallocatechin are totally inactive. However, flavonoids biochanin A, kaempferol, genistein, and daidzein are rather effective against 5αR2 than 5αR1 (Hiipakka et al., 2001).

Chalcone, which is classified as phenolic compound, also reported as 5αR inhibitor. For example, geranylated chalchone (3'-geranyl-2',3,4,4'-tetrahydroxychalcone) isolated from the leaf of *Atrocarpus incisus* (Thai breadfruit) shows more potent inhibitory activity ($IC_{50}$ = 104 µM) than α-linoleic acid ($IC_{50}$ = 116 µM). In addition, geranylated stilbene, chlorophorin, isolated from the heartwood show potent 5αR inhibitory activity ($IC_{50}$ = 37 µM), but non-geranylated one, oxyresveratol, does not (Shimizu et al., 2000b).

Currently, there are many reports indicated that some plant extracts are able to promote hair growth. For example, methanol extract from whole plant of *Eclipta alba* shows hair growth promoting activity in C57BL/6 mice in a dose-depending manner (Datta et al., 2009). The extract of *Asiasari radix* rhizome also shows potent hair growth stimulation both in C56BL/6 and C3H mice (Rho et al., 2005). Additionally, essential oil of *Zizyphus jujube* is also able to promote hair growth in BALB/c mice (Yoon et al., 2010).

There are many scientific evidences indicated that plant extracts or phytochemicals with an anti-5αR activity are also able to promote hair growth as well as inhibit the enzyme. For example, the extract of *Thujae occidentalis* (white cedar) semen is able to inhibit 5αR as well as promote hair growth in B6CBAF1/j mice (Park et al., 2003). The extract from the spore of *Lygodium japonicum* (Japanese climbing fern) is also exerts good activity both in an enzyme inhibition and the hair growth promoting activity in C57Black/6CrSlc strain mice (Matsuda et al., 2002). Moreover, the extract of *Myrica rubra* (red bayberry) (Matsuda et al., 2001), lignan isolated from *Piper nigrum* (Black pepper) leaf (Hirata et al., 2007), are able to inhibit the enzyme as well as promote hair growth. For example of phytochemical, EGCG which is previously known for its enzyme inhibitory activity (Hiipaka et al., 2002) shows potency in hair growth promotion both in human hair follicle cell culture and in human volunteers (Kwon et al., 2007).

In this study, 26 Thai plants were selected based on the criteria of the reported usage involving in hair caring and/or their dominant phytochemicals which may be able to inhibit the 5αR enzyme. The 17 plants with the reported uses involving in hair

caring are listed in Table 1. The 9 plants with dominant phytochemical classess that could inhibit the 5αR enzyme are listed in Table 2.

**Table 1** The plants with reported uses involving in hair caring

| Botanical name | Family | Usage |
|---|---|---|
| *Acacia concinna* Wall. | Leguminoceae | Anti-dandruff and Hair nourishment |
| *Alpinia galanga* Willd. | Zingiberaceae | Anti-fungi and Hair growth promotion |
| *Andrographis paniculata* Nees | Acanthaceae | Hair growth promotin and Hair loss prevention |
| *Averrhoa carambola* L. | Oxalidaceae | Anti-dandruff and Hair nourishment |
| *Carthamus tinctorius* L. | Asteraceae | Hair color enhancement |
| *Cassia siamea* Lam. | Ceasalpiniaceae | Scalp oil control and Hair nourishment |
| *Citrus hystrix* DC. | Rutaceae | Hair conditioner and Hair growth promotion |
| *Clitoria ternatea* L. | Fabaceae | Hair growth promotion |
| *Cymbopogon citratus* Stapf | Poaceae | Scalp oil control |
| *Ipomoea aquatica* Forssk. | Convovulaceae | Hair loss treatment |
| *Lawsonia inermis* L. | Lythraceae | Hair colorant |
| *Phyllanthus emblica* L. | Euphorbiaceae | Hair growth promotion |
| *Rhinacanthus nasutus* Kuntze | Acanthaceae | Hair growth promotion |
| *Sapindus rarak* DC. | Sapindaceae | Hair cleansing |
| *Tinospora rumphii* Boerl. | Menispermaceae | Hair growth promotion |
| *Trichosanthes cucumerina* L. | Curcubitaceae | Hair growth promotion |
| *Zingiber officinale* Roscoe | Zingiberaceae | Hair growth promotion |



In traditional uses of these listed plants, there are many simple traditional methods for preparing the plants, for examples, used in fresh form, decoction, or fried in oil. In this study, semipolar solvent, ethyl alcohol or ethanol, will be used in the

extraction of active phytochemicals from these plants to obtain various phytochemical classes. Moreover, ethanol is considerably safer than any of other organic solvents and more compatible to other composition in the cosmetic formula. In addition, the reactions that used for determining the 5αR inhibitory activity in this study contains water more than 97% by volume, using the organic solvent that immiscible to water may result in phase separation of the tested sample and the enzyme suspension, substrate and the other composition of the tested reaction.

**Table 2** Plants with suspected phytochemical classes that could inhibit 5α-reductase

| **Botanical name** | **Family** | **Dominant phytochemical classes** |
|---|---|---|
| *Centella asiatica* (L.) Urb. | Apiaceae | Flavonoids |
| *Terminalia chebula* Retz. | Combretaceae | Polyphenols, tannins |
| *Terminalia bellirica* (Geartn.) Roxb. | Combretaceae | Polyphenols, tannins |
| *Oryza sativa* L. | Poaceae | Red strain containing anthocyanins |
| *Garcinia mangostana* L. | Guttiferae | Tannins and xanthones |
| *Ocimum basilicum* L. | Lamiaceae | Terpenoids |
| *Piper nigrum* Wall. | Piperaceae | Alkaloids |
| *Citrus reticulata* Blanco | Rutaceae | Flavonoids |
| *Curcuma longa* L. | Zingiberaceae | Flavonoids |

Although there are many researches indicating that substance in fatty acids and sterol classes are potent 5αRI, these substance may be immiscible with the study model since these compounds show only a little water solubility. Thus these compounds were excluded from this study.

**2.5 Hair follicle permeation (Follicular penetration)**

Follicular penetration is a complex process. The effectiveness in drug delivery via hair follicle depends on many synergistic different factors, such as follicular density and size, activity status of hair follicles, and physicochemical properties of the penetrating substances (Patzelt et al., 2008).

1. Follicular density, size and reservoir

It is commonly known that the density and size of hair follicles depend on the body site. The higher absorption rates occur in a skin area with higher follicular density. The highest hair follicle density can be found on the forehead, and the highest average hair follicle size can be found on the calf region. The hair follicle morphological structure is compared as a canal lied from the epidermis into a dermis, this providing actual area for potential absorption. The surfaces of hair follicle orifices are only initially keratinized, while in the lower infundibulum corneocytes cell are smaller and more crumbly, resulting in more penetrable of substances in this area. Moreover, hair follicles are surrounded by extensive capillary network, which made absorption of substances faster with more potential than in the stratum corneum. Only the membrane surrounding hair follicle and keratinous layer of outer root sheath are physically restricting the passage of substances into the deeper layer of the follicle.

Substances that are topically applied into the hair follicle are well protected, it did not washed away with body cleanse or subjected to skin metabolism, which provided more benefits than stratum corneum absorption. While, the barrier in follicular absorption is only sebum, but the sebum creation is a long process, thus

more absorption efficacy is obtained in follicular penetration. Moreover, the storage time of substances in the hair follicle is 10 times longer than the stratum corneum storage time. From previously mentioned, transfollicular absorption potential is greater than stratum corneum absorption.

2. Activity status of hair follicle

Hair follicles that are responsible for the transfollicular absorption of substances are active hair follicles, which refer to hair follicles that undergoing sebum secretion process and/or in anagen phase. The inactive hair follicles are usually covered with dry sebum, desquamated corneocytes, and other cell debris, these are barriers for transfollicular absorption. Fortunately, more than 70% of hair follicles are open for penetration.

3. Physicochemical properties of topically applied substances

The active substances and their vehicles also have an influence on the effectiveness of follicular penetration. The lipophilic vehicles such as ethanol, or propylene glycol have more absorption efficacy than hydrophilic vehicles, which may be a result from compatability of skin sebum.

Micro/Nanoparticles such as liposome, niosome or others have been found to enhance percutaneous absorption of several agents into the skin and may be a useful system in follicular-targeted drug delivery system. Among these, nanoparticles size less than 1μm can be found in upper of stratum corneum and in the follicular orifice, while particle 3 - 10 μm can be found in the follicular orifice only. Particles larger than 10μm totally remained on the skin surface (Schaefer and Lademann, 2001).

**2.6 Lipid nanoparticles**

There are two classess of lipid nanoparticles (LN); solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC). SLN had been developed at the beginning of 1990s as an alternative carrier system to emulsions, liposomes and polymeric nanoparticles. SLN are derived from o/w emulsion by simply replacing the liquid lipid (oil) by a solid lipid (wax) or a blend of solid lipids. SLN are composed of 0.1 – 30 % (w/w) solid lipid dispersing in an aqueous vehicle and stabilized with 0.5 – 5 % (w/w) surfactants. The particle sizes of SLN are ranged from 40 – 1000 nm, which is in submicron range (Pradeike et al., 2009).

SLN provide more benefit than traditional emulsion as following (Müller et al., 2002; Wissing and Müller, 2003),

1. SLN are able to incorporate either hydrophilic or lipophilic compounds
2. SLN provide more stability of incorporated active compounds
3. SLN have a controlled-release properties (although drug-enriched shell SLN type showed in burst release of actives)
4. SLN are considered as the safe and biocompatible vehicle
5. SLN act as an occlusive agent, they have an ability to retain moisture in skin
6. SLN have a UV-blocking ability by a mechanism same to physical sunscreen

There are four models of SLN (Figure 4): (A) homogenous matrix model (B) drug-enriched shell model (C) drug-enriched core model and mixed type

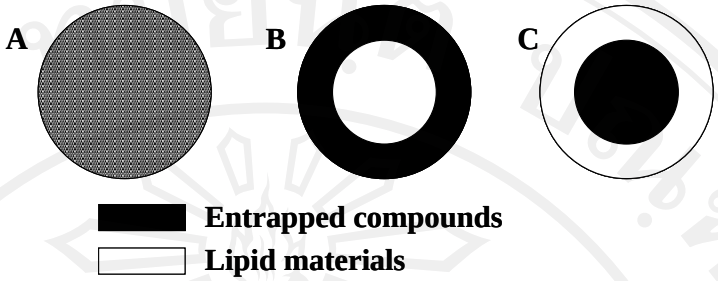


**Figure 4** Models of incorporated drugs in SLN; (A) homogenous matrix, (B) drug-enriched shell, and (C) drug-enriched core

In homogenous matrix model, drugs are molecularly dispersed or in an amorphous cluster. This model is thought to be mainly obtained via cold homogenization method and when incorporating a highly lipophilic drugs in SLN. This model gives constant drugs release.

In drug-enriched shell model, drugs are undergoing phase separation during the cooled down process from the liquid oil droplet into the formation of SLN. This model has a fast release profile, when applied to the skin, an increasing in drug penetration should be observed, especially when occlusive effect of SLN are used at the same time.

Drug-enriched core model can be achieved by active compounds tend to precipitate before the cooling of lipid matrix. In this model, the shell have distinctively amount of drugs, which lead to a membrane controlled release followed by Fick's law of diffusion.

It have been found that SLN have some drawbacks when using in the formulation as following,

1. Water contents of SLN is very high (70 – 99.9 % w/w), thus it is difficult in incorporating SLN into some kinds of products
2. When SLN are being kept, they will rearrange themselves into perfect crystalline form that may lead to a drug expulsion, resulting degradation of drugs or loss of controlled release properties (Figure 5)

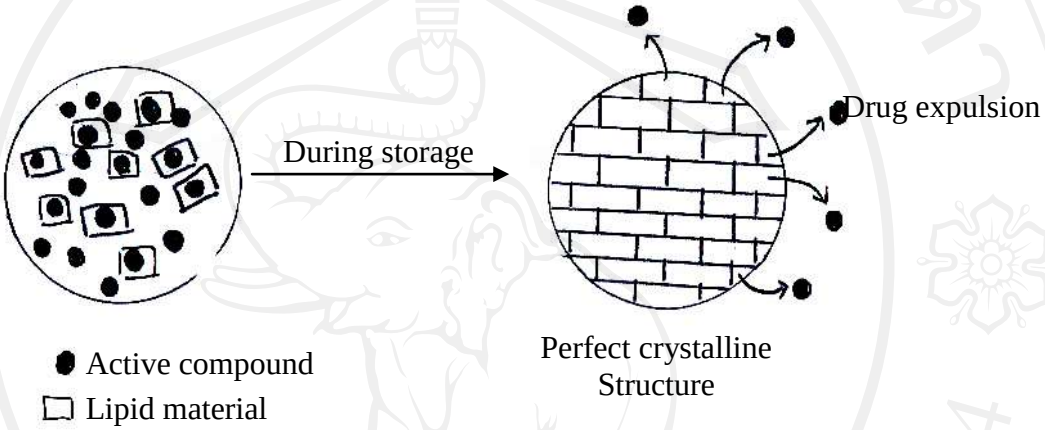


**Figure 5** Mechanism of drug expulsion during storage of SLN

The next generation of lipid nanoparticles, NLC were developed to overcome some limitations seen in SLN. NLC show higher loading capacity for numerous active compounds, a lower water content, and lower drugs expulsion by using mixed lipid, thus NLC can avoid perfect crystal rearrangement. NLC can be summarized into 3 models (Figure 6).

1. The imperfect type
2. The amorphous type
3. The multiple type

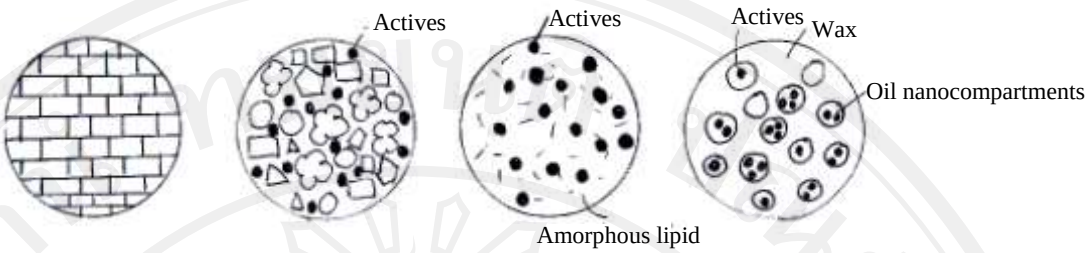


**Figure 6** The three types of NLC compared to the SLN (left), imperfect NLC (middle left), amorphous NLC (middle right), multiple NLC (right)

The first imperfect type NLC is using multiple solid lipids combination to avoid perfect crystal rearrangement. The amorphous type NLC is using amorphous lipid, which has no crystalline form. Multiple-type NLC is comparable to multiple emulsion; they contained drug dissolved or suspended in liquid lipid as an oil-in-solid lipid-in-water dispersion. NLC are produced using the same method as SLN production.