UCL SCHOOL OF PHARMACY

# LIPID-BASED NANOPARTICLES FOR TOPICAL DELIVERY OF HAIR GROWTH THERAPEUTIC MOLECULES

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*Dedicated to my husband, Mohd Husni Yusoff*

*and my children, Iman Syakirin and Nur Damia Safrina*

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# Abstract

**Introduction:** Androgenic alopecia (AA) patients usually have high levels of dihydrotestosterone on their balding scalp area. Currently, dutasteride (DST) is given orally and has systemic adverse effects; diminished sexual desire, increased depression and ejaculation disorder. Topical administration of DST is an appropriate drug-delivery strategy with the potential to reduce systemic side effect, skin irritation and cytotoxicity effects.

**Materials and method:** Chitosan oligomer (CSO) conjugated with stearic acid (SA) or lauric acid (LA) was synthesised and characterised. Dutasteride-loaded nanostructured lipid carriers (DST-NLCs) were prepared using a melt-dispersion ultrasonication method. DST-NLCs were optimised using a design of experiments approach. DST-NLCs, uncoated and coated with CSO-SA or CSO-LA were characterised for particle size distribution, surface charge and morphology. *In vitro* release and permeation studies were performed. Cytotoxicity was investigated using human hair follicle dermal papilla cells, and skin irritation was performed using an EpiDerm™ RHE model. Cou-6 loaded NLCs were prepared and characterised before proceeding with the cell and skin uptake study.

**Results:** CSO-SA and CSO-LA were successfully synthesised; confirmed using $^{1}$H NMR and FTIR. The mean size of DST-NLCs was significantly increased ($p<0.05$) when coated with 5% CSO-SA but not with 5% CSO-LA ($p>0.05$). The zeta potential changed from negative to positive charge when coating DST-NLCs with CSO-SA or CSO-LA. All formulations were physically stable over six months when stored at 4-8°C. However, DST-NLCs coated with CSO showed aggregation. All formulations exhibited rapid drug release. No dutasteride permeated through pig ear skin after 48 h for all formulations. The cytotoxicity ($IC_{50}$) for DST nanoparticles, coated and uncoated, was greater than for DST alone ($p<0.05$). The *in vitro* skin irritation study indicated no irritation for all nanoparticle preparations. For the cell and skin uptake studies, all samples showed time-dependent skin and cell uptake.

**Conclusions:** These stable, low cytotoxic and irritant, positively-charged DST-NLCs with CSO-SA or CSO-LA, represents a promising strategy for topical/ transfollicular delivery of DST.

# Research Impact Statement

Androgenic alopecia is a common disorder affecting almost 50% of men in their lifetime due to the androgen effect. Based on the Euromonitor market study predicted in 2013, it was expected that the hair loss treatment would attract up to US$100 million in sales globally by 2016. Even though hair loss is not a life-threatening disease, but it has a psychological and emotional effect, especially in young people. This study aims to prepare and characterise dutasteride-loaded nanostructured lipid carriers (DST-NLCs), coated with chitosan oligomer conjugated with stearic acid, and lauric acid to enhance local drug delivery and reduce toxicity.

This study will help in our understanding of the potential of nanoparticle formulations for topical delivery of hair growth molecules, which will benefit society especially, androgenic alopecia patients, improving their quality of life. Currently, few products are available in the markets which are prescribed for the androgenic alopecia patient. Due to their systemic adverse effects, the delivery of dutasteride for promoting hair growth using topical route would be a great advance. Phase III clinical studies on the use of dutasteride for promoting hair growth have shown significant hair growth for the people with hair loss. Unfortunately, no topical product based on dutasteride has been approved.

This research can generate good networks between researchers in the academia and people from industries whereby they can exchange the idea, skills and knowledge on the research and business areas. Also, this research can be one of the platforms for other molecules to be applied for topical or non-topical applications. A trained and skilled researcher who has cross-disciplinary generated from this study would be one of the main impacts on the academic area. In term of the economic, it would generate a knowledge transfer between researchers and industrial people who will lead to spin out companies, and the creation of new processes and products.

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# Abbreviations and symbols

| | |
|---|---|
| **ANOVA** | analysis of variance |
| **Avodart®** | dutasteride based medication |
| **BBB** | blood-brain barrier |
| **BCS** | biopharmaceutics classification system |
| **BPH** | benign prostatic hyperplasia |
| **CAGR** | compound annual growth rate |
| **Cou-6** | coumarin-6 |
| **CSO** | chitosan oligomer |
| **Da** | Dalton |
| ***df*** | degree of freedom |
| **DHT** | dihydrotestosterone |
| **DL** | drug loading |
| **DLS** | dynamic light scattering |
| **DoE** | design of experiment |
| **DS** | degree of substitution |
| **DST** | dutasteride |
| **EDC** | 1-ethyl-3-(3-(dimethylamino) propyl) carbodiimide |
| **EE** | entrapment efficiency |
| **FDA** | food and drug administration |
| **FT-IR** | Fourier Transform Infrared Spectroscopy |
| **GSK** | GlaxoSmithKline |
| **HCl** | hydrochloric acid |
| **HFs** | hair follicles |
| **HLB** | hydrophobic-lipophilic balance |
| **HPLC** | high performance liquid chromatography |
| **ICH** | International Conference on Harmonisation |
| **LA** | Lauric acid |
| **LOD** | limit of determination |
| **Log P** | partition coefficient |
| **LOQ** | limit of quantification |
| **Lutrol® micro 68** | Poloxamer 188 |

| | |
|---|---|
| **MR** | magnetic resonance |
| **MW** | molecular weight |
| **NLCs** | nanostructured lipid carriers |
| **NMR** | Nuclear Magnetic Rosonance Spectroscopy |
| **PDI** | polydispersity index |
| **PEG** | polyethylene glycol |
| **Phosal® 53 MCT** | 53% of phosphatidylcholine and medium chain triglyceride |
| **PLGA** | poly(lactic-co-glycolic acid) |
| **$R^2$** | coefficient of determination |
| **Rogaine®** | minoxidil based medication |
| **RP** | reverse phase |
| **RSD** | relative standard deviation |
| **SA** | stearic acid |
| **SC** | stratum corneum |
| **SD** | standard deviation |
| **Sephadex®** | cross-linked dextran |
| **SLNs** | solid lipid nanoparticles |
| **SMEDDS** | self-emulsifying drug delivery system |
| **T** | testosterone |
| **TEM** | transmission electron microscope |
| **TFA** | trifluoroacetic acid |
| **TNBS** | trinitrobenzene sulfonic acid |
| **UV/Vis** | ultraviolet-visible spectroscopy |
| **XPRD** | X-ray powder diffractometer |
| **ZP** | zeta potential |

# Chapter 1 Introduction

## 1.1 Skin structure and function

Skin is the largest, heaviest and most versatile organ of the human body. The vital roles of the skin are the protection of the body, regulation of body temperature and sensory perception. The skin protects the body from water loss and the possibility of access by potentially toxic compounds, allergens, irritants and microbes (Bartosova and Bajgar, 2012). To ensure these diverse functions can be fulfilled, healthy skin is needed. For an adult, the skin surface area is approximately 1.8 - 2.0 $m^2$ (Uchechi et al., 2014). Figure 1.1 shows the structure of the skin.

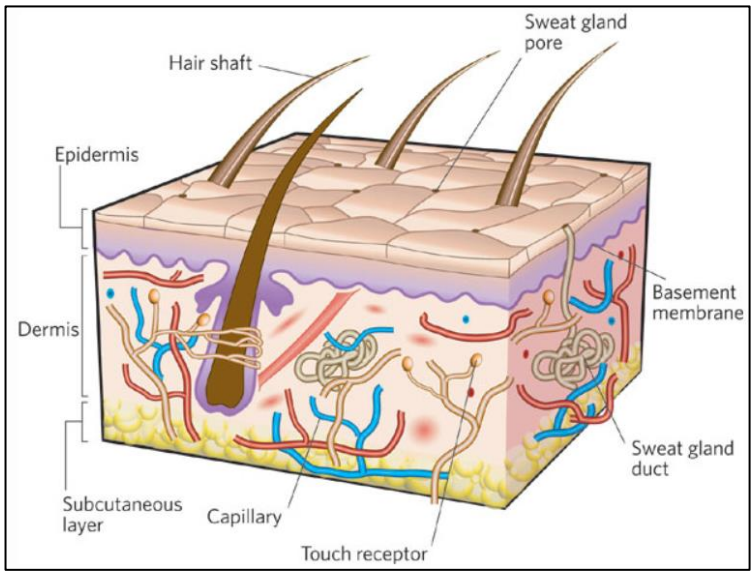



**Figure 1.1 Structure of skin** (adapted from MacNeil, 2007)

The skin consists of three main layers (Figure 1.2). The outermost layer is the epidermis which is made up of stratum corneum, stratum lucidum, stratum granulosum, stratum spinosum and stratum basal (Williams, 2018). The barrier properties of the skin are due to the stratum corneum. The stratum corneum is a very hydrophobic layer, comprising differentiated non-nucleated cells, corneocytes, filled with keratins embedded in the lipid domain (Godin and Touitou, 2007). The lipid domain is composed of equal proportions of ceramides, cholesterol and free fatty

acids (Pappas, 2009). The synthesis of these three components promotes the acidic condition of the skin.

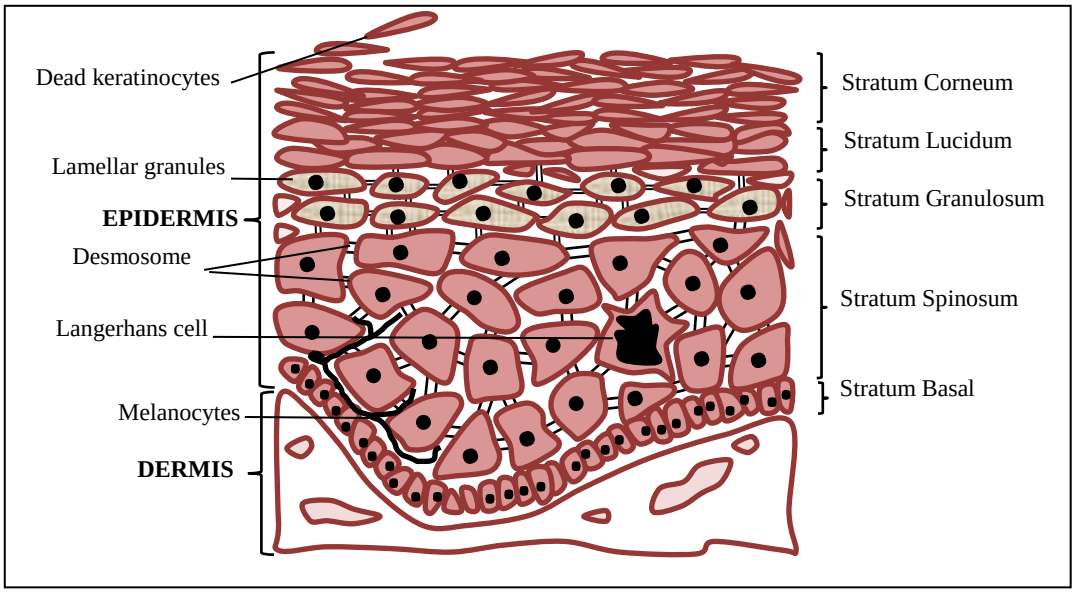



**Figure 1.2 Skin structure and layer** (redrawn from Bensouilah and Buck, 2006)

The second layer is dermis. The dermis is composed of connective tissues such as collagen fibrils and elastic tissues that mostly provide support, mechanical strength, elasticity and flexibility of the skin. The dermis is supplied with a reticulate network of blood vessels, lymphatic vessels, nerve endings, hair follicles, sebaceous glands and eccrine glands (Thakur et al., 2008).

Finally, the inner layer of the skin is subcutaneous tissue or hypodermis which contains adipose cells in and between the connective tissue. The primary function of subcutaneous tissue is to provide insulation to the body (Williams, 2018).

## 1.2 Hair

The largest appendages, which consist of hair follicles and sebaceous glands, can provide ‘short cut’ routes which drugs can pass across the stratum corneum barrier (Williams, 2018). The hair follicle is an organ and part of the skin. It starts in

the dermis and goes through the outer part of the skin to the epidermis layer (Figure 1.3). Hair consists of proteins, lipids, water, trace elements and pigments (Robbins, 2012). The hair protein is called keratin. Hair can be divided into two types. First is vellus hair, appearing on the body, or hair that changes to terminal hair at puberty. The second is called terminal hair. Terminal hair may be short (eyebrows, ears, nose) and long (head hair, beard, underarm, pubic area).

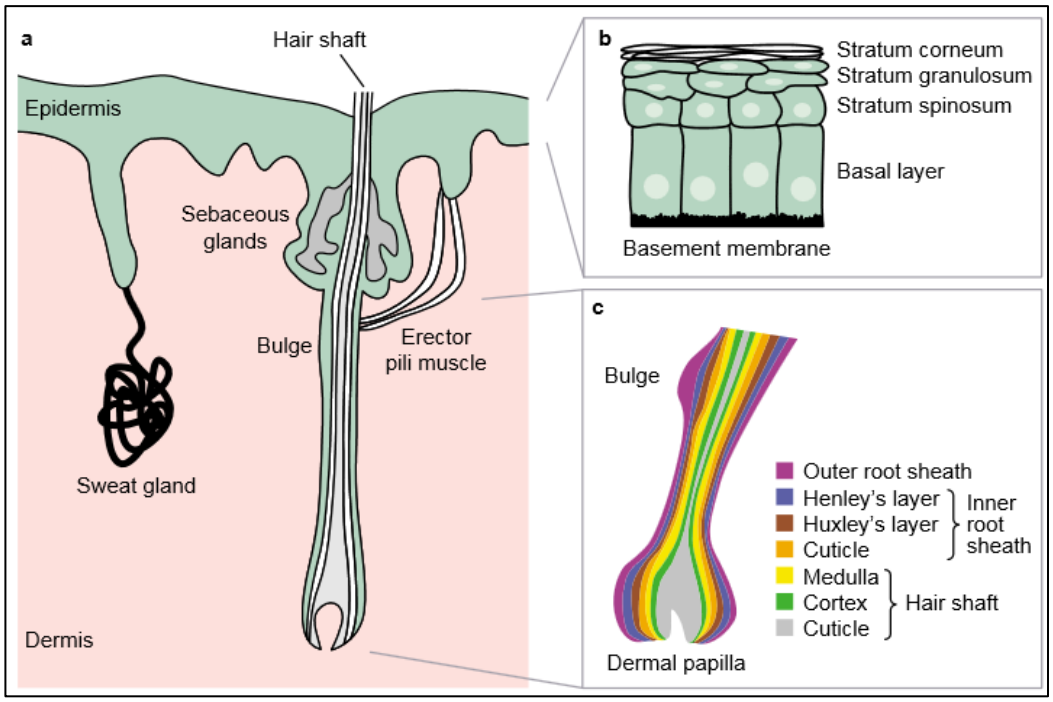



**Figure 1.3 Structure of the hair follicles** (adapted from Adolphe and Wainwright, 2005)

Hair that is visible above the skin is called the hair shaft. It consists of cuticle, cortex and medulla. Previous studies have found the hair diameter on the scalp and face differ (Tolgyesi et al., 1983). The normal adult hair shaft diameter (Caucasian) is approximately 70 µm, whereas in the beard it is 126 µm. These have made skin appendages (sweat gland and hair follicle) which is only 0.1% to 1% of the area of the skin (Schaefer and Redelmeier, 2001) used as a follicular route for targeted delivery of drugs.

Hair that is invisible or within the skin is called the hair root. The bulbous end of the hair root is called the hair bulb (Williams, 2018). All biological processes including cell division happen in this part. The hair bulb is positioned in a tubular pocket called the hair follicle. Human hair follicles are one of the organs that are affected by hormones such as androgens (Randall et al., 1991). Androgen promotes hair growth and also sometimes inhibits scalp hair growth causing androgenetic alopecia (AGA). As it is specific to the hair follicle itself, the response to androgens varies with the body site. In the case of AGA, androgens cause the miniaturisation of the scalp hair follicle (Hibberts et al., 1998).

## 1.3 Hair growth cycles

Hair is produced by the hair follicle and undergoes a cycle with different phases. It starts at the anagen phase, then passes through catagen and lastly telogen phases. All hair growth phases in the body occur at the same time; one hair might be in the anagen phase and others in the catagen or telogen phase. The original lower follicle will be destroyed, and the new follicle will regenerate to form a new hair (Randall and Botchkareva, 2008).

Figure 1.4 shows the cycles of hair growth in the normal human body. Different areas have different hair growth cycle. Hair is produced in the anagen phase or so-called growth phase. Scalp follicles have the longest anagen phases, lasting up to several years (Randall, 2008). Once hair reaches its full length in the anagen phase, then the catagen phase (regression) will take place, in which cell proliferation, differentiation, and pigmentation will stop, and extensive apoptosis occurs, and the dermal papilla shrinks (Randall, 2008). The catagen phase normally takes 1 to 2 weeks. In the catagen stage, the hair is fully keratinised and a specialised structure, the club hair is formed and moves upwards (Randall and Botchkareva, 2008). After this, the telogen phase takes over, which lasts for several months. During the telogen phase, the round-shaped dermal papilla is closely situated near to the secondary hair germ keratinocytes containing hair follicle stem cells. In an early-mid anagen phase, a new lower follicle develops inside the same dermal sheath, and the new hair grows into the original upper follicle, and the existing hair ejected (Randall and Botchkareva, 2008).

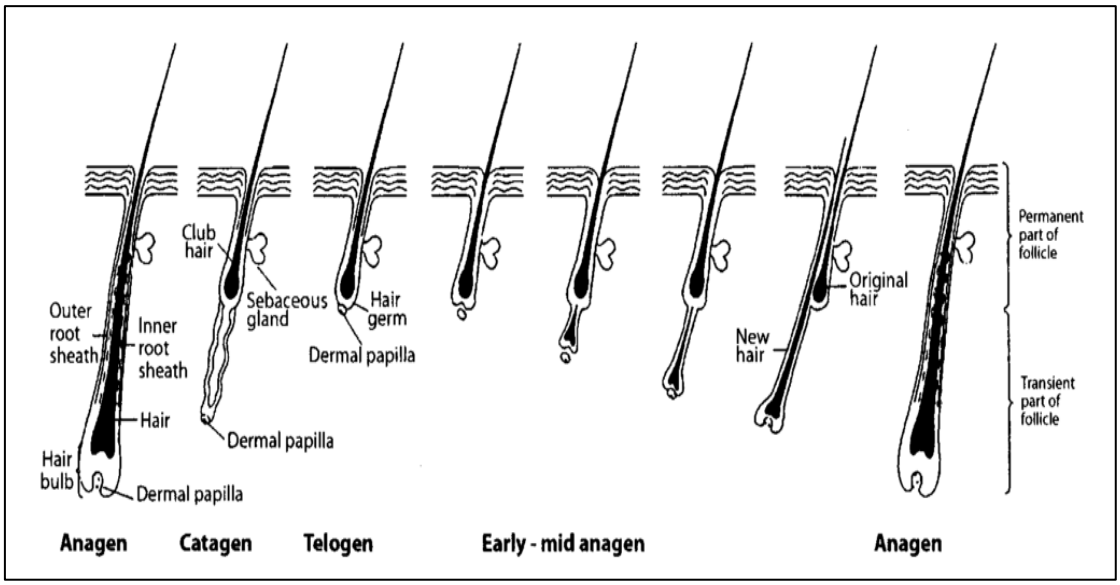



**Figure 1.4 The hair follicle growth cycle** (adapted from Randall and Botchkareva, 2009)

## 1.4 Mechanism of hair loss

Most problems associated with hair relate to either hirsutism (excessive hair growth) or alopecia. Alopecia is a common medical term for hair loss or baldness. Baldness which is affected by androgens is called AGA, also known as male pattern hair loss (MPHL) or female pattern hair loss (FPHL) (Kaufman, 2002). Testosterone and dihydrotestosterone are the major androgens that regulate the hair growth.

Dihydrotestosterone (DHT) has approximately a five-fold greater affinity for the androgen receptor than testosterone (Kaufman, 2002). In hair loss patients, the 5α-reductase enzyme acts as a catalyst that converts testosterone, which is the primary androgen, to the more potent androgen, DHT which makes the hair follicle miniaturise and shed hair (Olsen et al., 2006).

Figure 1.5 shows the hair loss cycle for a patient with hair loss. Due to the effect of the potent androgen (DHT), their normal hair starts as long, thick and pigmented, then changes to be thin, short and less pigmented at the end of the process. The new hair colour becomes less pigmented from one cycle to another, the hair shaft becomes thinner, and there is the appearance of baldness. Two isozymes

participate in androgen synthesis; namely, Type I 5α-reductase isozyme which is present in the skin, hair follicles and sebaceous glands, liver, prostate, and kidney and Type II 5α-reductase isozyme which is present in hair follicles, male genitalia and the prostate (Russell and Wilson, 1994; Kandavilli et al., 2010). Both these isozymes are involved in steroid metabolism and interact with the androgen receptors. In androgen synthesis, the 5α-reductase enzyme acts as catalyst converting testosterone to the more potent dihydrotestosterone. By introducing a 5α-reductase inhibitor, the conversion of testosterone to DHT can be decreased and reduce hair loss.

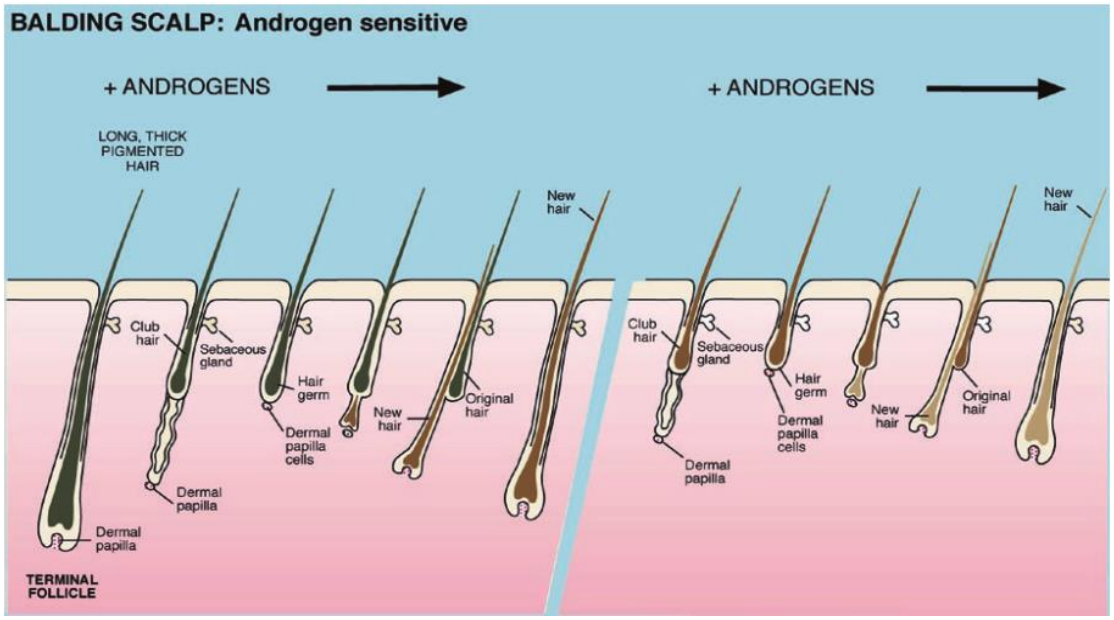



**Figure 1.5 Hair loss cycle in patients with male pattern baldness** (adapted from Randall, 2010)

## 1.5 Anti-androgenic activity for treating hair loss

Hair loss is not a life-threatening disease, but has an emotional impact and affects certain individuals, especially young people and they may experience psychological distress (Hunt and Mchale, 2005). Androgenic alopecia (male pattern baldness) is a common disorder affecting almost 50% of men in their life (Yassa et al., 2011) due to effect from androgen. Androgen is one of the prerequisites for male pattern baldness (Randall and Botchkareva, 2008). Normally, patients with androgenic alopecia have higher levels of DHT (dihydrotestosterone) and 5α-reductase enzyme activity on their balding scalp area than those with a non-balding

scalp area (Hibberts et al., 1998). Figure 1.6 shows the circulation of androgens in the hair follicles. Testosterone (T) is mainly secreted by the Leydig cells of the testes of males and a lesser amount by the ovaries of female (Brownlee et al., 2005).

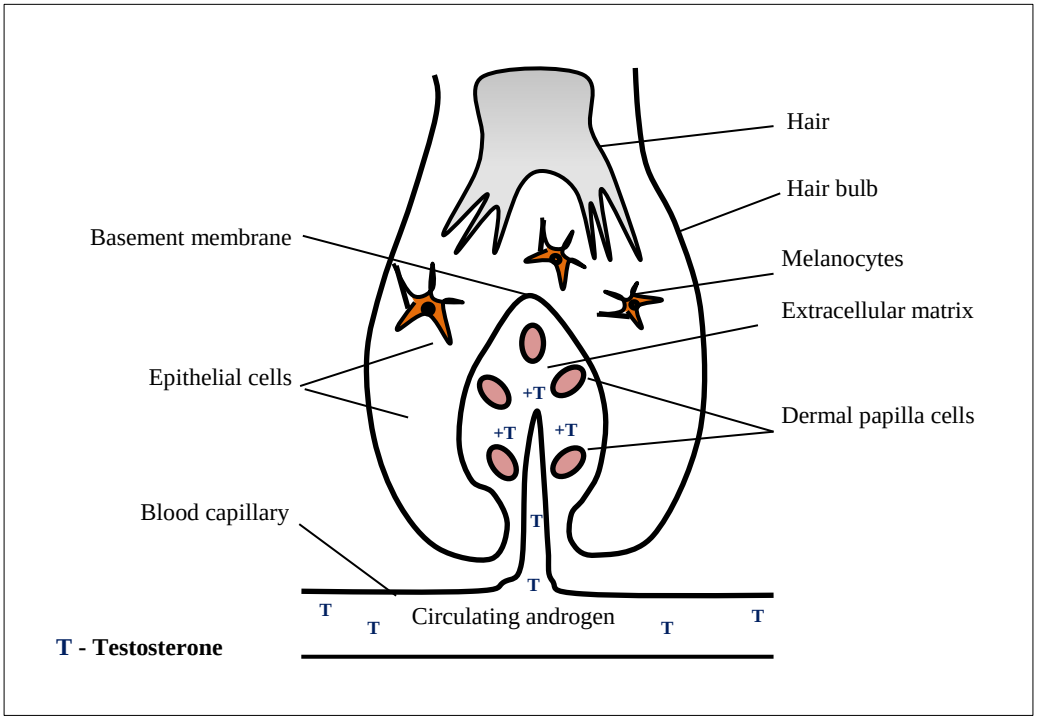



**Figure 1.6 The action of androgen in the hair follicle** (redrawn from Randall and Botchkareva, 2009)

Testosterone will circulate in the blood and enter the hair follicle through the dermal papilla's blood supply and interact with the androgen receptors in the dermal papilla cells. Several studies have focussed on the inhibition of the activity of the 5α-reductase enzyme, either by using natural extracts or drugs. One example of natural extract for 5α-reductase inhibitor was the use of fatty acids (Liu et al., 2009); those with $C_{12}$-$C_{16}$ and C=C double bond which enhanced Type II 5α-reductase inhibition activity. Finasteride which has Type II 5α-reductase inhibitor activity is approved by the FDA to treat benign prostatic hyperplasia and male pattern baldness. Dutasteride is approved for treating benign prostatic hyperplasia (BPH) and has both Types I and II 5α-reductase inhibitor. Finasteride and dutasteride are discussed in detail in Section 1.8.2 and 1.8.3 respectively.

## 1.6 Market overview of hair loss products

Figure 1.7 shows the estimated size of the global hair care market in 2013 from 2006 to 2016 (Euromonitor International, 2013). Hair care products comprise products that promote health; hair nourishment, prevention of hair damage and hair loss treatment.

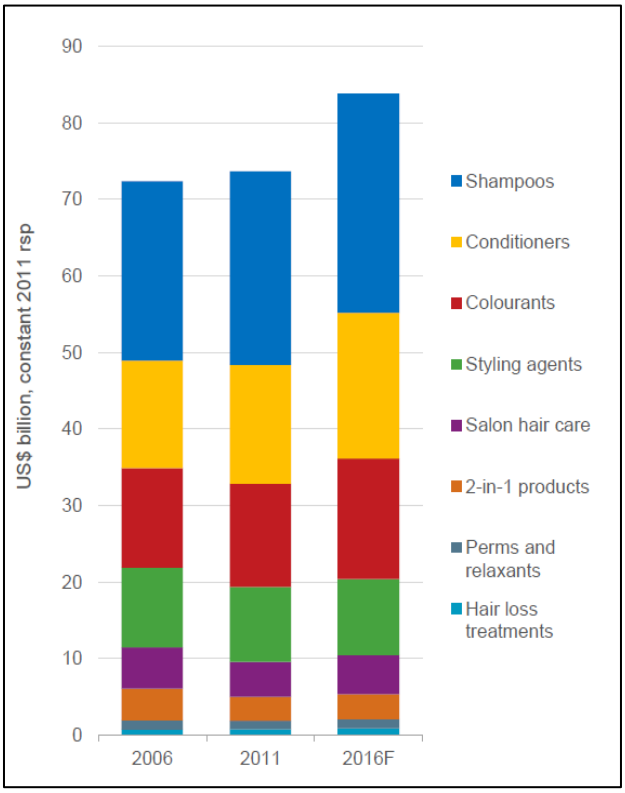



**Figure 1.7 Size of the global hair care market in 2006, 2011 and the estimated market in 2016 (in billion U. S. dollars)** (adapted from Euromonitor International, 2013)

From this data, the sales of the hair loss treatments are dominated by minoxidil (Regaine®, Johnson and Johnson) which exhibits a significant sales growth especially in Western Europe (Euromonitor International, 2013). Minoxidil is an over-the-counter drug and has been approved for treating hair loss in both men and women. However, some people use cosmetic products to treat hair loss rather

than pharmaceutical products due to their adverse effects. In this case, cosmetics are defined in Regulation (EC) No. 1223/2009 as:

"any substance or mixture intended to be placed in contact with the external parts of the human body (epidermis, hair system, nails, lips and external genital organs) or with the teeth and the mucous membranes of the oral cavity with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance, protecting them, keeping them in good condition or correcting body odours." (European Commission, 2009).

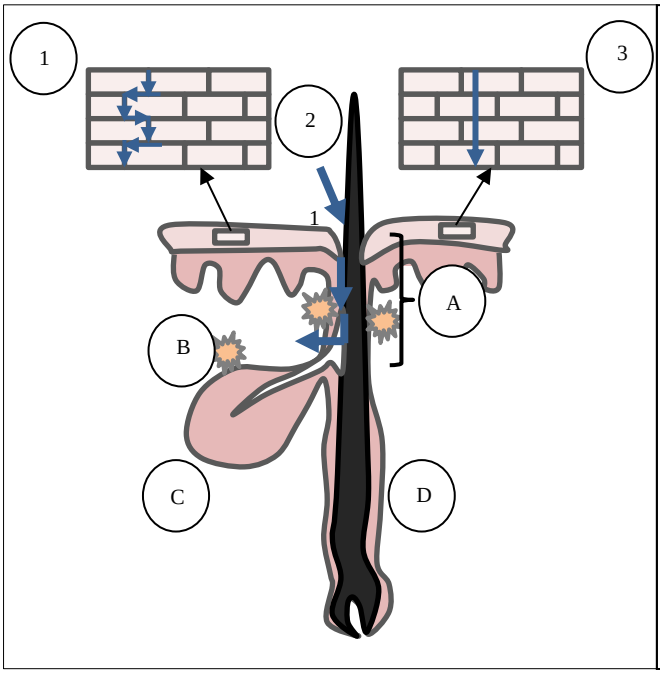
Cosmetics or drugs used for treating hair loss are not permanent and their activity is reversible, which means when someone uses the product; hair loss will decrease or stop, but when they stop using the product, then the hair loss returns. To reduce this problem, an effective delivery system for a drug acting as a potential hair growth promoter should be designed to have a long-lasting effect such as having controlled release properties which is targeted to the hair follicle area and at the same time reduce the adverse effects.

## 1.7 Delivery of drugs to the skin

Many studies have been conducted to deliver drugs to the transfollicular region (Bhatia et al., 2013; Mittal et al., 2015). Figure 1.8 shows the potential penetration pathways of a drug through the skin. The drug can pass either through the skin barrier (No. 1 - 3) or to the transfollicular area (A - D). Drug delivery through the skin offers convenience to the patient, is pain-free and allows self-administration, and may eliminate frequent administration especially when long-term treatment is needed (Paudel et al., 2010).

The transfollicular route has shown promise for delivery; particulate delivery would be ideal by allowing deep intrafollicular penetration, sustained release and selectively targeted delivery (Patzelt and Lademann, 2013). The size of particulate materials is one of the key criteria to deliver the drug to the transfollicular area. Hair follicles have a size range from 10 - 70 μm depending on the hair type, location and race (Singh et al., 2000) making it a suitable site for delivering nanoparticles to the dermal papilla cells for hair growth products.

Previous research has found that, applying 320 nm fluorescence dye-containing nanoparticles, and massaging the area enhanced the penetration for up to 10 days (Lademann et al., 2007); with nanoparticulates of dye penetrating deeper than the dye in solutions.




Penetration pathways: (1) intercellular penetration pathway around the corneocytes within the lipid layers, (2) follicular penetration pathway with transfollicular penetration option, dependent on skin condition and physicochemical properties of the applied substance, (3) intracellular penetration pathway across the corneocytes. Hair follicle morphology: (A) infundibulum region with increased permeability in the inferior part, (B) antigen presenting cells surrounding the infundibulum, (C) sebaceous gland associated with the hair follicle, (D) bulge region with stem cells

**Figure 1.8 Schematic illustration of the potential penetration pathways of drugs through the skin** (redrawn from Patzelt and Lademann, 2013)

Lipophilic vehicles rather than hydrophilic are able to improve the delivery of drug to the skin (Motwani et al., 2004), and many studies have been conducted using lipid-based vehicles for dermal delivery (Doktorovova et al., 2011; Wang et al., 2012; Uprit et al., 2013; Montenegro et al., 2016). Due to the occlusive properties of lipid nanoparticles, an increased skin hydration effect is observed (Hommoss, 2008). Previous studies found that a lipid film formed on the top of the skin, and the subsequent occlusion effect was reported for lipid nanoparticles (Müller et al., 2002; Wissing and Müller, 2003; Escobar-Chávez et al., 2012). Particles smaller than 400 nm containing at least 35% lipid of high crystallinity have been most effective for the occlusive properties (Wissing and Müller, 2003).

Figure 1.9 shows that the occlusion factor of lipid nanoparticles depends on the sizes; reducing the particle size leads to an increase in particle number, the film becomes denser (left) and therefore the occlusion factor increases. Other criteria on the occlusion factor have been considered, such as identical lipid content; increasing the lipid concentration increases particle number and density of the film (right) which also leads to a higher occlusion factor (Pardeike et al., 2009). In solid lipid nanoparticles (SLN) or nanostructured lipid carriers (NLC) system, the skin hydration after applying these nanoparticles leads to a reduction of corneocytes packing and an increase in the size of the corneocytes gaps and facilitate the percutaneous absorption and drug penetration to the deeper skin layers (Hommoss, 2008).

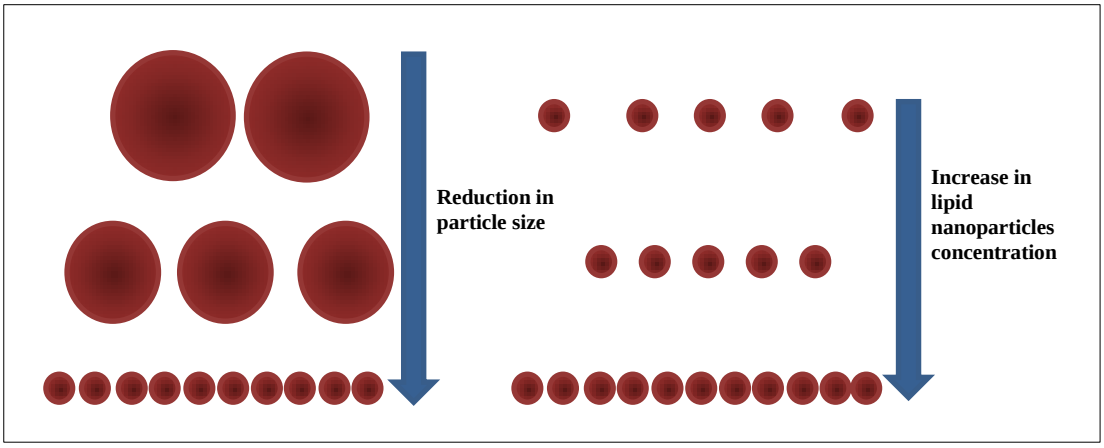



**Figure 1.9 The occlusion factor of lipid nanoparticles depends on different sizes** (redrawn from Escobar-Chávez et al., 2012)

Campbell et al., (2012) investigated the deposition of nanoparticles in mammalian skin and found that nanoparticles (mean size of 20 – 200 nm) cannot penetrate beyond the superficial layers of the barrier. Figure 1.10 shows the intensity of fluorescence nanoparticles at different sizes. Even at the smallest mean size of nanoparticles (20 nm), there is no penetration of nanoparticles to the deeper layer of the skin. This result proved that nanoparticles could not penetrate the skin barrier, but it is useful as skin surface reservoirs to control the drug release over time (Campbell et al., 2012).

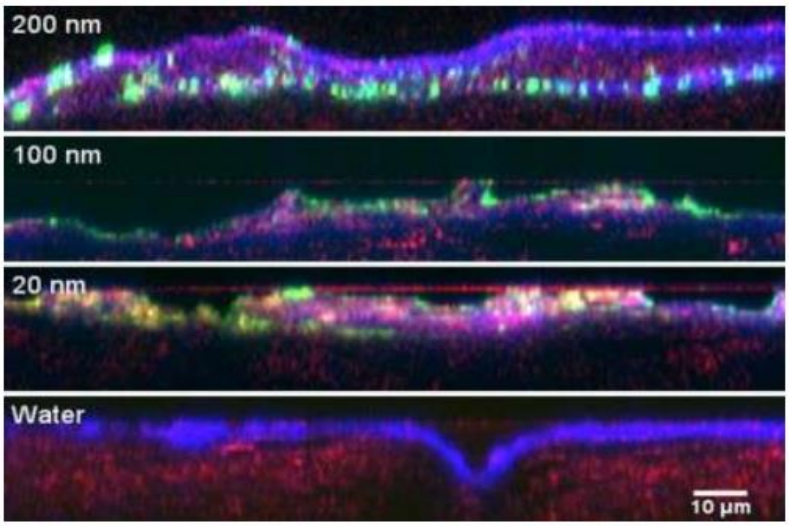



**Figure 1.10 Fluorescence intensity of different sizes of nanoparticles (without removing the stratum corneum)** (modified from Campbell et al., 2012)

Fang et al. (2014) reported the mechanism of skin permeation by drug-loaded NLCs (Figure 1.11). As mentioned before, occlusion factor which increases hydration of the stratum corneum (SC) become the main factor that can reduce the corneocytes packing and increase drug permeation (Fang et al., 2014). These findings suggest that nanoparticles could go deep in the skin and systemic circulation through transfollicular region as seen in Figure 1.11.

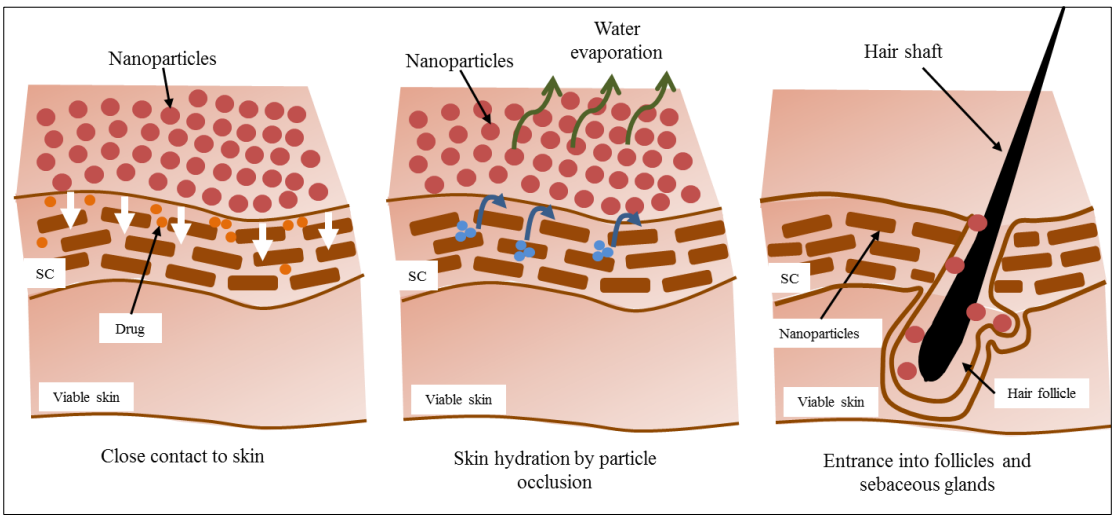



**Figure 1.11 Mechanism of drug permeation by NLCs** (redrawn from Fang et al., 2014)

## 1.8 Drugs used for treating hair loss

### 1.8.1 Minoxidil

For many years, minoxidil has been the first-choice for topically applied drug recommended by medical practitioners to treat hair loss for both men and women. Currently, Rogaine® (USA) or Regaine® (Europe and UK) is an approved hair-loss product based on minoxidil which is available on prescription and as an over-the-counter medication. Minoxidil (Figure 1.12), a pyridine derivative drug was used in the 1970s as a treatment for hypertension in patients where therapy had failed with multidrug regimens (Messenger and Rundegren, 2004; Sica, 2004). In 1988, 1% minoxidil mixed with an alcohol-based carrier was approved by the FDA to treat alopecia in men.

In a 12-month randomised double-blind trial of 150 men, 82% of the minoxidil group increased hair count (Kreindler, 1987). Sato et al., (1999) used cultured human dermal papilla cells from the balding scalp and found that minoxidil increased 17β-hydroxysteroid dehydrogenase activity by approximately 40% but had an insignificant effect on 5α-reductase activity and such as the mechanism of minoxidil action remains unknown (Silva et al., 2009). Another study (Han et al., 2004) found minoxidil prolonged the anagen phase where the dermal papilla cells proliferated and had an anti-apoptotic effect on dermal papilla cells.

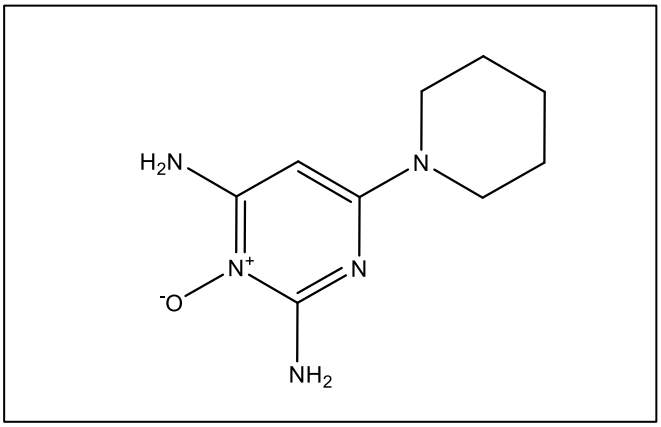



**Figure 1.12 Chemical structure of minoxidil**

#### 1.8.1.1 Nanocarrier delivery of minoxidil and minoxidil derivatives

In order to overcome the severe adverse reaction such as scalp dryness, irritation from propylene glycol-water-ethanol ingredients in the current minoxidil products, several studies have investigated the delivery of minoxidil using nanoparticle formulation. Nanostructured Lipid Carrier (NLCs) have been used for the delivery of minoxidil (Silva et al., 2009) for promoting hair growth, with a particle size of approximately 250 nm before adding to a hydrogel; particles remained below 500 nm when incorporated in the hydrogel. These formulations of minoxidil-NLCs hydrogel were a promising alternative to the conventional alcoholic solutions, as the drug is physically entrapped within the lipid matrix which can be useful to increase the bioavailability for skin delivery (Silva et al., 2009). No efficacy study on hair growth activity between the minoxidil-NLCs hydrogel and conventional minoxidil solutions was conducted. However, this formulation approach would help in reducing the risk of occurrence of adverse side effects, such as skin dryness and irritation.

The Globally Harmonized System of Classification and Labeling of Chemicals (GHS) defines skin irritation as “the production of reversible damage to the skin following the application of a test substance for up to 4 hours” and skin corrosion as “the production of irreversible damage to the skin; namely, visible necrosis through the epidermis and into the dermis, following the application of a test substance for up to 4 hours” (United Nations, 2013). Padois et al., (2011) used solid lipid nanoparticles (SLNs) as a carrier for minoxidil and found SLNs suspensions which was approximately 190 nm of particle size proved efficient as commercial solutions for skin penetration; and were non-corrosive while commercial solutions presented a corrosive potential.

Some examples of minoxidil-loaded nanoparticulate carriers include research by Aljuffali et al. (2014), which used squalene-based NLCs for targeted drug delivery, known as “squarticles” and delivered minoxidil together with diphencyprone. The size was approximately 177 nm for the NLCs-based carrier. The encapsulation efficiency and zeta potential for minoxidil-loaded squalene NLCs were 63.3% and -54.0 mV respectively. They found that compared to the free control (drugs in 30% propylene glycol in water), squarticles-NLCs reduced minoxidil

penetration through the skin, indicating minimised absorption in the systemic circulation. The result also showed an improvement of drug deposition by 2-fold in the skin, using an *in vitro* skin absorption test and good tolerability of squarticles to skin based on the *in vitro* papilla cell viability and *in vivo* skin irritancy tests in nude mice.

Matos et al. (2015) formulated minoxidil sulphate-loaded chitosan nanoparticles (MXS-NP) which demonstrated sustained drug release (5-fold) compared to drugs in solutions. The MXS-NP formulation (chitosan/MXS; 1:1 w/w) had mean diameter 236 nm and positive zeta potential. They found that the drug permeation studies through the skin *in vitro* showed that MXS-NP application resulted in a 2-fold increase in MXS in uptake hair follicles after 6 h in comparison to the control solution.

### 1.8.2 Finasteride

Finasteride, (Figure 1.13) an anti-androgen steroidal drug has been used widely to treat patients with benign prostatic hyperplasia. A clinical open, randomised, parallel-group study for 12 months on 100 male patients with androgenic alopecia to investigate the efficacy of oral finasteride (1 mg per day), topical 2% minoxidil solution and topical 2% ketoconazole shampoo alone and in combination was conducted (Khandpur et al., 2002). The results demonstrated a significant increase in hair growth between a combination group having finasteride orally (1 mg) and 2% minoxidil (topically) and finasteride (orally) alone (Khandpur et al., 2002).

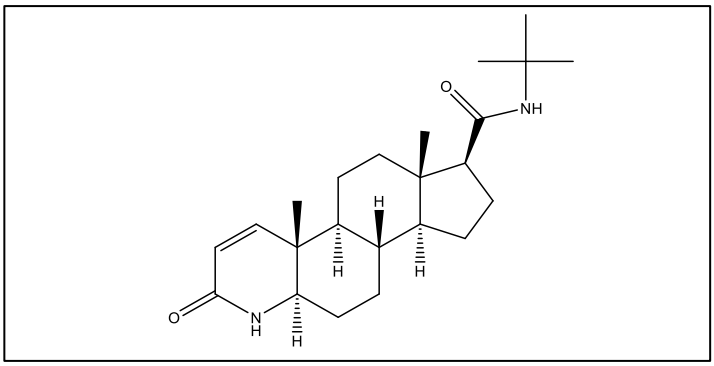



**Figure 1.13 Chemical structure of finasteride**

### 1.8.2.1 Nanocarrier delivery of finasteride

Several studies have been conducted using nanoparticle formulations of finasteride for topical delivery. Madheswaran et al., (2013) prepared liquid crystalline nanoparticles from monoolein, with size 154 – 170 nm which showed a potential for topical delivery of finasteride. The addition of different types of additives (glycerol, propylene glycol, and polyethylene glycol 400) had little or no influence on the size. The formulations produced slow release profiles and high permeation to the dermis. The release profile was significantly altered with the addition of different additives. Formulation with monoolein exhibited skin permeation which increased significantly with the inclusion of glycerol, propylene glycol, and polyethylene glycol 400, while it decreased with the addition of oleic acid. The release rate of finasteride increased when glycerol, propylene glycol, or polyethylene glycol 400 was added and decreased with the addition oleic acid.

Gomes et al. (2014) formulated lipid nanoparticles of finasteride with mean size around 200 nm and stable up to 28 days. Penetration studies using pig ear skin found that only a small amount of finasteride crossed the skin, suggesting the suitability of this formulation for dermal delivery of anti-alopecia active compounds.

Caon et al. (2014) found chitosan coated polymersomes of finasteride interacted more strongly with the skin components than non-coated formulations due to the positive surface charge. It was observed that the addition of chitosan contributed to an increase in the accumulation of finasteride in the epidermis. It was proposed that the particles (mean diameter of 180 - 404 nm) were preferentially accumulated in the follicular openings and that follicular localization was favoured by the smaller particle size, which would be more easily transported via the follicular route than the larger size.

### 1.8.3 Dutasteride

Dutasteride, approved for treating benign prostatic hyperplasia also affects hair growth. Due to its androgenic activity, dutasteride can only be taken by male patients. Dutasteride (MW = 528.5 g/mol) (Figure 1.14) is classified as Class II/IV in the Biopharmaceutics Classification System (BCS) (Tiwari et al., 2014). It has very

low water solubility (0.038 ng/mL, Log P = 5.09 and $pK_a$ = 13.5) and high solubility in ethanol (44 mg/ml) and methanol (64 mg/ml) at 25° C and has a half-life of approximately 3-5 weeks (GlaxoSmithKline Inc., 2013).

In 2009, dutasteride (0.5 mg daily orally intake) was approved in Korea for treating hair loss in men (Harcha et al., 2014), but until now no dutasteride-based product been approved for androgenic alopecia in Europe or USA. However, it is commonly prescribed as an off-label for hair loss treatment (oral administration) in the USA and Europe. This product is swallowed without chewing, crushing, or opening the capsule because it might irritate the lips, mouth, and throat. The prescribed dosage for treating benign prostatic hyperplasia (BPH) is 0.5 mg per day (GlaxoSmithKline Inc., 2013).

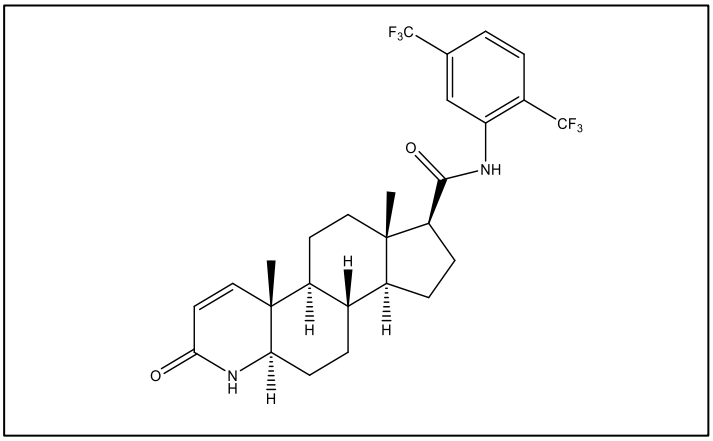



**Figure 1.14 Chemical structure of dutasteride**

Olsen et al. (2006) found dutasteride increased scalp hair growth in men with hair loss, at 2.5 mg of dutasteride (orally), it and was superior to finasteride (5 mg) (orally) at 12 and 24 weeks. Eun et al. (2010) used dutasteride for treating hair loss in male patients at 0.5 mg daily orally and reported that the dutasteride group had significant hair growth compared to the placebo group within six months. Another study (Stough, 2007) reported evidence that dutasteride significantly reduced hair loss progression in men with male pattern hair loss when tested in a randomised study in 17 pairs of identical twin males for 1 year period. They found that treatment with dutasteride (0.5 mg/day orally) slowed the progression of hair loss and enhanced hair growth compared to treatment with placebo. In 2014, a randomised, active- and

placebo-controlled study of dutasteride versus placebo and finasteride in the treatment of 917 male subjects with androgenetic alopecia was conducted by Harcha et al. (2014). They found that dutasteride 0.5 mg significantly increased the hair count, width diameter and improved hair growth at week 24 compared with finasteride and placebo.

#### 1.8.3.1 Nanocarrier delivery of dutasteride

Based on the summary of product characteristics for oral dutasteride (Avodart®), the side effects of taking oral dutasteride are: it may increase the risk of development of high-grade prostate cancer, decrease libido, and may cause breast enlargement and ejaculation disorders (GlaxoSmithKline Inc., 2013). Dutasteride has a toxicity effect on the skin, with multiple red areas produced in the skin (animals at 40 mg/kg) suggesting that dutasteride is a dermal irritant (GlaxoSmithKline Inc., 2013). In order to reduce these side effects, drug delivery with sustained release should preferably target the skin, especially to the hair follicle. Delivery of dutasteride using nanocarriers has been studied by the oral route with little research of topical delivery (Table 1.1).

Previous research on topical delivery has used a liposome system (Sharma et al., 2011). The liposomes produced significantly higher skin permeation of dutasteride through excised abdominal mouse skin compared to a hydro-alcoholic solution and conventional gels. Ansari et al. (2013) prepared different ratios of oleic acid and eucalyptus oil to prepare nanoemulsion to deliver dutasteride to the skin. The mean size range was around 18 - 213 nm, no measurements were undertaken on zeta potential, entrapment efficiency, and drug loading. Madheswaran et al. (2015) used monoolein to produce dutasteride nanoparticles, with their surface modified using different concentrations of chitosan (low molecular weight) to give a positive charge. The particle size was 239 - 259 nm, with mean zeta potential of +19.8 to +48.5 mV. The surface modified liquid crystalline nanoparticles enhanced transdermal delivery of dutasteride and increased the permeation of dutasteride using a porcine skin (700 – 800 μm thickness). Release studies on this formulation produced the cumulative amount of dutasteride released only about 5% after 24 h. The highly lipophilic nature of dutasteride, which had a stronger interaction with

lipid inside the nanoparticles produced slower release compared to finasteride (Madheswaran et al., 2015).

**Table 1.1 Published studies on the delivery of dutasteride using different types of nanocarriers**

| Delivery system | Particle Size | Application | Authors |
|---|---|---|---|
| Liposome | 1.82 ± 0.15 μm | Topical for dermal delivery | Sharma et al. (2011) |
| Eudragit E® nanoparticles | 62.2 – 180.6 nm | Oral | Park et al. (2013) |
| Hydroxypropyl-β-cyclodextrin nanostructures | <160 nm | Oral | Kim, (2013) |
| Self-microemulsifying drug delivery system (SMEDDS) | 43.9 nm | Oral | Kim et al. (2015) |
| Self-microemulsifying drug delivery system (SMEDDS) | 35.3 nm | Oral | Choo et al. (2013) |
| Nanoemulsion | 58.8 – 88.7 nm | Topical for systemic delivery | Sajid et al. (2014) |
| Liquid crystalline nanoparticles | 197.9 ± 2.5 nm | Topical for dermal delivery | Madheswaran et al. (2015) |

## 1.9 Positively-charged nanoparticles

Nanoparticles having a positive surface charged have received great interest in drug delivery, especially for topical and transfollicular delivery, where hair and the lipid layer in the SC contain high ratio of negatively-charged lipids (Bhushan, 2010; Madheswaran et al., 2015). For instance, the anionic surfactant is often added in shampoos to remove grease from the hair. The surfactant has two different regions; one region is soluble in water (hydrophilic) and the other region is soluble in the greasy material (lipophilic). The lipophilic/hydrophobic part will encircle the greasy

matter, and the other part (negative-charged) will repel the fibres because hair fibres are negatively-charged, and remove the greasy material easily.

On the other hand, cationic surfactants are normally added to hair conditioners to neutralise the charge of hair after washing. Based on this, many studies on positively-charged nanoparticles have been undertaken in order to promote interaction with the negative target site, especially for dermal/transdermal and transfollicular delivery (Şenyiğit et al., 2010; Gelfuso et al., 2011; Ridolfi et al., 2012; Özcan et al., 2013; Madheswaran et al., 2015).

## 1.10 Chitosan

Chitosan is a natural polymeric material being used increasingly by the pharmaceutical industry. It contains free amine groups, and this makes it is insoluble in water (water-soluble only at pH<6) (Sogias et al., 2010). Chitosan oligomers or chitosan oligosaccharide (Figure 1.15) are the hydrolysates of chitosan, mainly made up of -1,4 linked D-glucosamine and partially of -1,4 linked N-acetyl-D-glucosamine (Ibrahim et al., 2016). Chitosan oligomer has been used widely for different bioactivity such as antibacterial (No et al., 2002; Merchant et al., 2014; Yildirim-Aksoy and Beck, 2017), antitumour activity (Jeon and Kim, 2002; Hu et al., 2009; Xie et al., 2012), anti-cancer activity (Nam et al., 2007a; 2007b), wound healing activity (Kang et al., 2016) and antioxidant activity (Sun et al., 2007).

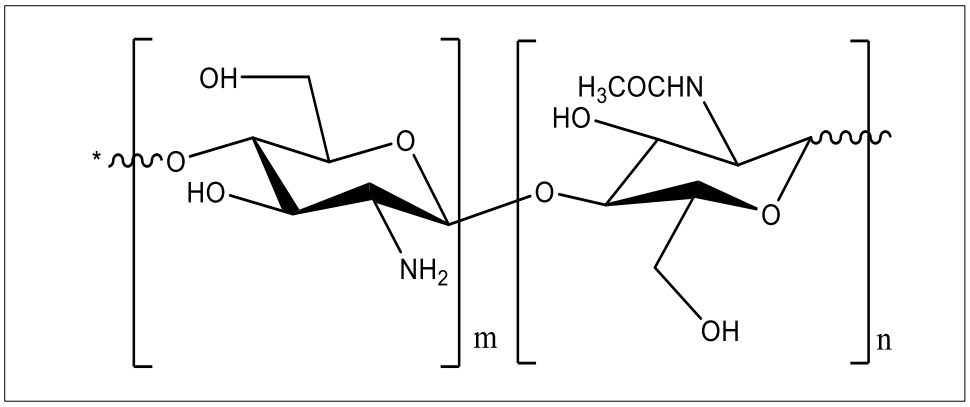



**Figure 1.15 Structure of chitosan oligomer**

In this project, chitosan oligomer (Carbosynth Ltd, United Kingdom) with molecular weight less than 3 kDa and 85% deacetylation degree was chosen. This chitosan is positively charged, which is likely to be advantageous for skin and hair, such skin and hair are negatively charged and will be attracted to positively charged moieties (Bhushan, 2010). Mittal et al. (2015) demonstrated that a nanoparticle formulation of antigen ovalbumin with chitosan increased follicular uptake when compared to a nanoparticle formulation without chitosan.

### 1.10.1 Hydrophobic derivatives of chitosan

Table 1.2 shows the potential delivery of drugs by using different types of chitosan conjugated with a hydrophobic chain (fatty acid). From Table 1.2, it can be seen that chitosan conjugated with fatty acid has been employed in many areas using nanoparticles and micellar systems.

**Table 1.2 Chitosan conjugation with different type of fatty acids and their application**

| Delivery system | Chitosan and Fatty acid | Application | Authors |
|---|---|---|---|
| **Micelles** | Chitosan 18 kDa and stearic acid | Brain targeting | Xie et al. (2012) |
| **Immobilization** | Chitosan LMW and lauric acid | Osteoblast proliferation and antibacterial | Zhao et al. (2014) |
| **Micelles** | Chitosan 5 kDa and stearic acid | Oral delivery | Li et al. (2010) |
| **Micelles** | Chitosan 9.2 kDa and stearic acid | Anti-tumour activity | Hu et al. (2009) |
| **Nanoparticles** | Chitosan 9.2 kDa and oleic acid | Optical MR/Imaging | Lee et al. (2011) |
| **Micelles** | Glycol chitosan different MWs with palmitic acid (GCPQ) | Oral, ocular, parenteral | Uchegbu et al. (2014) |

The surface activity of chitosan (non-conjugated) is low as it does not possess any hydrophobic portions and can be improved by chemical modifications at its glucosidic group with a hydrophobic substituent (Cheung et al., 2015). Szymańska and Winnicka (2015) reported chemical crosslinking with chitosan as a strategy to increase the stability of chitosan, whereby the stability of modified chitosan was based on the covalent bonds, and also interactions—hydrogen or hydrophobic bonds.

Xie et al. (2012) prepared chitosan oligosaccharide (MW 18 kDa) conjugated with stearic acid (CSO-SA) for brain targeting. The blood-brain barrier (BBB), makes it difficult for the drug to penetrate, however, the delivery of doxorubicin in a CSO-SA micellar system was beneficial. The micellar system (22 nm sizes; zeta potential +36 mV) demonstrated high drug loading and a slow release pattern. High amounts of doxorubicin were found in the brain and low amounts accumulated in the heart. This result was due to the ability of micelles to transport across the blood-brain barrier and into the brain (Xie et al., 2012). The lower toxicity of CSO-SA/doxorubicin micelles than doxorubicin in solutions might be relevant with a slow release of doxorubicin from micelles (Xie et al., 2012).

## 1.11 Nanoparticulate dermal drug delivery

Topical application of drugs has many advantages especially reducing systemic effects and targeting affected local areas, such as for skin diseases. The stratum corneum (SC) provides the main barrier function of skin, limiting the loss of essential substances from inside the body and reducing chemically or toxic materials entering the body (Trommer and Neubert, 2006). Even though the drug in the formulation for hair loss therapy should ideally target the transfollicular region, drugs will also likely permeate through the dermal or transdermal regions which represent the main permeation pathways. Due to the limited permeability of the SC (the drug should be in low molecular weight and moderate lipophilicity), several methods have been employed to enhance the delivery of the drugs to the skin. These include chemical permeation enhancers such as fatty acids, urea, phospholipids, alcohols, amide, and sulfoxides and physical permeation enhancement such as iontophoresis, sonophoresis, ultrasound and microneedles (Finnin and Morgan, 1999; Pathan and Setty, 2009; Akhtar et al., 2011; Shaji and Varkey, 2012).

Other skin penetration enhancers, more recently investigated are micro or nanoscale size drug formulations, such as liposomes, polymeric nanoparticles, nanoemulsions, solid lipid nanoparticles and nanostructured lipid carriers (Müller et al., 2002; Sintov and Shapiro, 2004; Guterres et al., 2007; Escobar-Chávez et al., 2012; Wang et al., 2012; Gomes et al., 2014). Mahe et al., (2009) found the particles of approximately 200 nm mean sizes applied to the skin were found around the hair follicles. Further studies reported that particles with a size approximately 300 nm were found in the transfollicular region (Mittal et al., 2015). These studies suggest to deliver the drug into the hair follicle regions, a size of particles in the range of 200-300 nm is appropriate.

### 1.11.1 Liposomes

Liposomes, sphere-shaped vesicles with mean size 30 nm to several micrometres, produced by self-forming enclosed lipid bilayers upon hydration consisting of one or more phospholipid bilayers, were first described in the mid-60s (Akbarzadeh et al., 2013). Liposome formulations consist of one or more non-toxic natural phospholipids such as soy phosphatidylcholine and egg phosphatidylcholine or a synthetic phospholipid such as distearoyl-phosphatidylcholine (Figure 1.16).

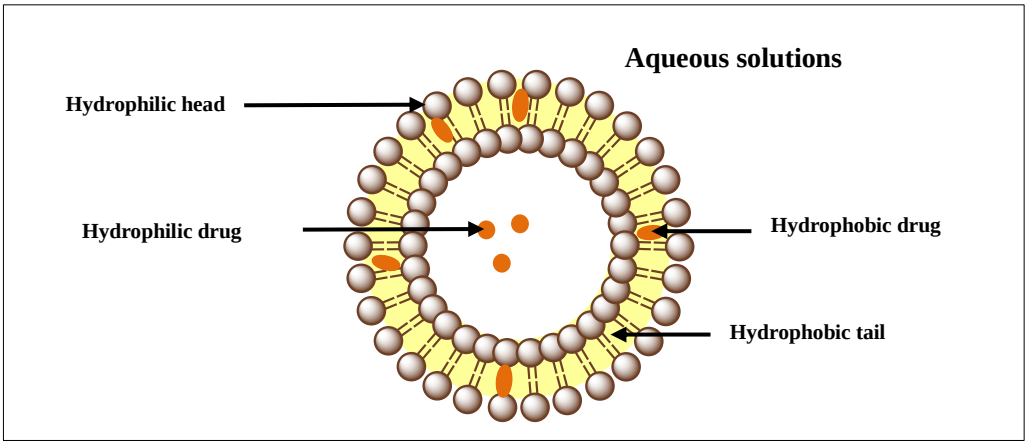



**Figure 1.16 Schematic representation of a liposome, showing the location of entrapped drugs** (redrawn from Lembo and Cavalli, 2010)

Liposomes differ based on the different type of phospholipid used, lipid composition, method of preparation, surface charge, lamellarity and size (Du Plessis et al., 1994). Liposomes have a characteristic $T_c$ (phase transition temperature), at which the liposome membranes transit from the gel phase to liquid crystalline phase, and the encapsulated drugs are released from the vesicles (Li et al., 2015). The $T_c$ depends on the nature of the polar head group, the length of the hydrocarbon chains, the degree of saturation of the hydrocarbon chains and the purity of phospholipids (Li et al., 2015). In liposomes system, hydrophobic drugs will be encapsulated in the hydrophobic region of the phospholipid bilayers whereas hydrophilic drugs will be entrapped in the aqueous core and between bilayers. The 'rigidity' or 'fluidity' and the charge of the bilayer of the liposome are dependent on the bilayer components (Akbarzadeh et al., 2013).

Liposomes have been widely used for dermal and transfollicular delivery of drugs. Du Plessis et al. (1994) found that the particle size influenced the deposition of drugs in the skin, for a liposome preparation containing ciclosporin as a model drug. They found the intermediate particle size (0.3 μm) studied resulted in the highest amount of drug in the deeper skin strata and the receiver chamber for both hamster and hairless mouse skin except the pig skin after 24 h using Franz diffusion cells. The same was not seen for pig skin because of the lipophilic nature of both ciclosporin and the pigskin with the release of ciclosporin to the receiver retarded (Du Plessis et al., 1994).

Esposito et al. (1998) found the permeability of a liposome preparation of methyl nicotinate through a synthetic membrane was influenced by the charged particles, a higher amount of phosphatidylcholine, the smaller size of particles and also the higher viscosity of the samples. In the case of the non-extruded sample, the permeability was affected by the vesicle size, where smaller size increased the permeability rather than the viscosity. However, for extruded samples, the permeability was affected by the viscosity of the formulation.

Flexible liposomes, which are mixtures of lipids and surfactants have been studied in order to penetrate the SC (Cevc et al., 1998; Ogunsola et al., 2012). Cevc et al. (1998) prepared flexible liposomes, called as Transferosomes® containing

soybean phosphatidylcholine, sodium cholate and biocompatible surfactants produced liposome dispersion that passed through a filter of much smaller pore size.

Another study conducted by Ogunsola et al. (2012) prepared flexible liposomes with egg phosphatidylcholine and Tween 80 or sodium cholate or ethanol with the mean size range from 74 – 110 nm that passed through the filter with 50 nm pore size. *In vitro* penetration studies found that fluorescent-tagged lipid to liposomes with a higher amount of Tween 80 (60 or 68%) showed penetration of fluorescent-tagged lipid flexible liposomes into the epidermis of hairless guinea pig and excised human skin.

Although liposomes have been used for drug delivery to reduce the toxicity of drugs, increase efficacy and stability and site-specific drug delivery, there are also some disadvantages. Conventional liposomes have poor drug loading capacity, poor stability, production costs are high, and the use of volatile solvents are required in their preparation.

### 1.11.2 Polymer-based carrier for drug delivery

Figure 1.17 shows different types of the polymer-based carrier for drug delivery. Hydrogels are produced by a group of polymeric materials having a hydrophilic structure which is capable of holding large amounts of water in their three-dimensional networks (Ahmed, 2015). Drugs can be loaded into the gel matrix and the drug release rate is dependent on diffusion of the small molecule or macromolecule through the gel network (Hoare and Kohane, 2008). Many studies have reported incorporation of nanoparticles or microparticles into a hydrogel in order to improve dermal delivery. Bhaskar et al. (2009) produced flurbiprofen loaded SLNs or NLCs (average particles sizes of less than 300 nm) incorporated with a hydrogel and found that sustained drug release over a period of 24 h was higher with the SLNs and NLCs hydrogel compared to SLNs and NLCs without hydrogel.

Biodegradable polymers such as poly(D,L-lactic acid) (PLA), poly(D,L-lactic-co-glycolic acid) (PLGA), and poly (ε-caprolactone) (PCL) and their copolymers diblock or multiblock with poly(ethylene glycol) (PEG) have been commonly used to form polymeric nanocarriers (polymeric micelles, capsules,

spheres) in order to encapsulate a variety of therapeutic compounds (Chan et al., 2010). Polymeric nanocarriers such as micelles are formulated by self-assembly of block copolymers consisting of two or more polymer chains with different hydrophobicities (Chan et al., 2010) such amphiphilic polymers which have both hydrophilic blocks and hydrophobic blocks can be used for drug delivery where normally the hydrophobic blocks form the core to minimize their exposure to aqueous surroundings (Chan et al., 2010; Trivedi and Kompella, 2010).

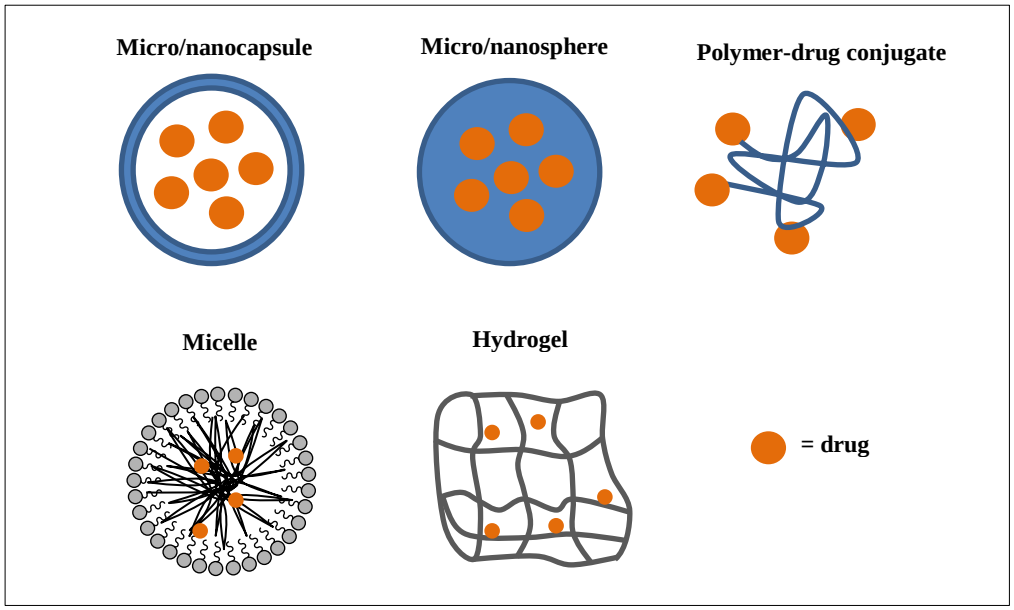



**Figure 1.17 Different structures of nanocarriers** (redrawn from Janssen et al., 2014)

Nanospheres (matrix system) or nanocapsules (reservoir system) are both in the polymeric nanoparticle group. Nanocapsules are polymeric nanoparticles containing either an oily or aqueous core surrounded by a polymeric shell (combination with a mixture of lipophilic and hydrophilic surfactants), whereas nanospheres are polymer-only matrix systems (Elmowafy et al., 2017). Nanospheres and nanocapsules are able to modify the activity of drugs, sustain and control drug release, and increase the drug adhesivity in the skin (Guterres et al., 2007).

Elmowafy et al. (2017) reported indomethacin loaded into polymeric nanocapsules and nanospheres produced a higher cumulative amount of drug in human skin compared to a marketed product (indomethacin in gel) at 24 h. They

found significantly higher skin permeation from nanocapsules of indomethacin compared to nanospheres. Even though nanocapsules had a larger particle size (186 – 193 nm) than nanospheres (138 - 142 nm), higher permeability was attributed to higher nanocapsules deformability than nanospheres (rigid matrix system).

Biodegradable, natural polymers are the first choice materials for producing polymeric nanoparticles, in order to minimise toxicity. In some studies, investigators have developed new compounds for nanoparticle production by conjugating the polymer with drugs especially for tumour targeting (Dragojevic et al., 2015). Ringsdorf (1975) introduced a model for pharmacologically active polymers, consisting of a biocompatible polymer backbone bound to three components; solubilizer, drug which is bound to the polymeric backbone via a linker, and a targeting moiety whose function is to provide transport to a desired physiological destination or to bind to a particular biological target (Larson and Ghandeharia, 2012). Castleberry et al. (2017) prepared all-trans retinoic acid conjugated with polyvinyl alcohol and produced sustained controlled delivery of active up to 10 days and significantly increased dermal accumulation of the all-trans retinoic acid in the pig skin. Polymer-conjugated drugs generally exhibit prolonged half-life, higher stability, water solubility, lower immunogenicity and antigenicity and often also specific targeting to tissues or cells (Pasut and Veronese, 2007).

The disadvantages of polymeric nanoparticles are the need for the approval by regulatory authorities on the safety issues, and also their high production costs (Bala et al., 2004).

### 1.11.3 Lipid nanocarriers

There are different types of lipid nanocarriers used for drug delivery system. Nanoemulsions comprise liquid mixtures of oil, water, surfactant and sometimes a co-surfactant having a droplet size in the range of 50 - 200 nm (Kong et al., 2011). In terms of production, the concentration of surfactant used is much lower (3-10%) compared to microemulsion preparation (more than 20%) (Bouchemal et al., 2004). Borges et al. (2013) found a dapsone-loaded nanoemulsion with isopropyl myristate as the oil phase promoted high *in vitro* epidermal permeation using Franz cells on

porcine ear epidermis. Hussain et al. (2016) formulated an amphotericin B-loaded nanoemulsion (mean droplet size 76 nm) and found a higher skin percutaneous permeation flux rate through rat skin as compared to drug solution using Franz diffusion cell. Amphotericin B in solution showed the highest release at 2 h, while amphotericin B-loaded nanoemulsion gel showed slower release compared to amphotericin B loaded-nanoemulsion alone. However, there are some disadvantages of nanoemulsions such as limited controlled release properties due to the liquid state of the carrier (Martins et al., 2007).

A second type of lipid-based nanocarriers is solid lipid nanoparticles (SLNs). In the 1990s, these were introduced as drug carrier system (Müller et al., 2002), and produced by replacing the liquid lipid (oil) of an emulsion by solid lipids or a blend of solid lipids (Müller et al., 2007). SLNs have a mean particle size of between 50 and 1000 nm (nanoparticles) (Müller et al., 2000). SLNs have been produced for pharmaceutical, traditional Chinese medicine and cosmetic applications. SLNs have been used to deliver bioactive ingredients such as vitamin A (Jenning et al., 2000), rifampicin, isoniazid and pyrazinamide (Pandey and Khuller, 2005), oridonin (Zhang et al., 2006), isotretinoin (Liu et al., 2007), *Artemisia arborescens* essential oil (Lai et al., 2007), repaglinide (Vijayan et al., 2010), virgin coconut oil (Noor et al., 2013), docetaxel (Naguib et al., 2014) and olanzapine (Iqbal et al., 2017). Different methods have been proposed in order to produce SLNs including high-pressure homogeniser, solvent evaporation, ultrasonication or melt dispersion techniques (Gasco, 1993; Müller et al., 2000; Trotta et al., 2003).

The second generation of SLNs is called as nanostructured lipid carriers (NLCs). NLCs are produced by incorporating blends of solid lipids and liquid lipids (oils) (Pardeike et al., 2009). Some examples of liquid lipid used in NLCs production are medium chain triglyceride such as oleic acid. Figure 1.18 shows the structural differences between SLNs and NLCs. Due to the main ingredients in SLNs being solid lipid (at room temperature), SLNs are in a highly crystalline form, limiting drug loading, whereas NLCs (combination of solid and liquid lipid) have a less crystalline structure, increasing drug loading. NLCs were introduced in order to overcome some of the problems associated with SLNs by increasing drug loading and reducing the burst release of drugs (Wissing and Müller, 2002; Hommoss, 2008; Silva et al., 2009). The mean particle size for NLCs is usually less than 1000 nm.

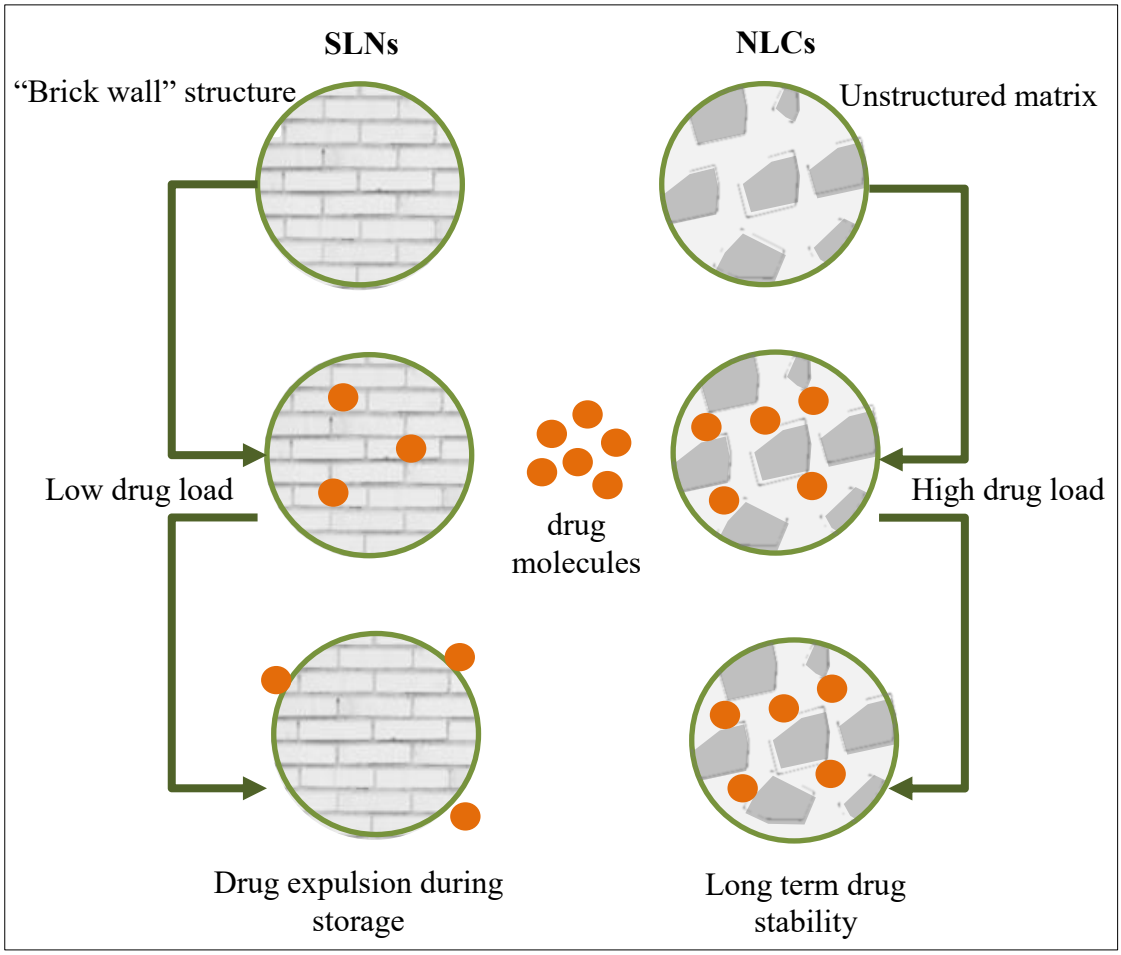



**Figure 1.18 Schematic illustration of SLN and NLC structures** (modified from Beloqui et al., 2016)

Several different types of solid lipid can be used in the production of SLNs and NLCs. The term 'solid lipid' includes fatty acids (e.g. myristic, stearic and palmitic acid), triglyceride (e.g. tripalmitin and tristearin), diglyceride (e.g. glyceryl behenate), monoglyceride (e.g. glyceryl monostearate), steroid (e.g. cholesterol) and waxes (e.g. cetyl palmitate and beeswax). Lipids used in SLNs or NLCs are generally regarded as safe (GRAS) (Attama et al., 2012). Different types of solid lipid have various degrees of crystallisation that may impact the drug entrapment efficiency and loading, size and charge of the resulted carriers. The lipid particle matrix is solid at both room and body temperatures (Müller et al., 2014).

## 1.12 Aim of the study

The aim of this project is to prepare and characterise dutasteride-loaded nanostructured lipid carriers (DST-NLCs), coated with chitosan oligomer conjugated with stearic acid, and lauric acid to enhance local drug delivery and reduce toxicity (Figure 1.19).

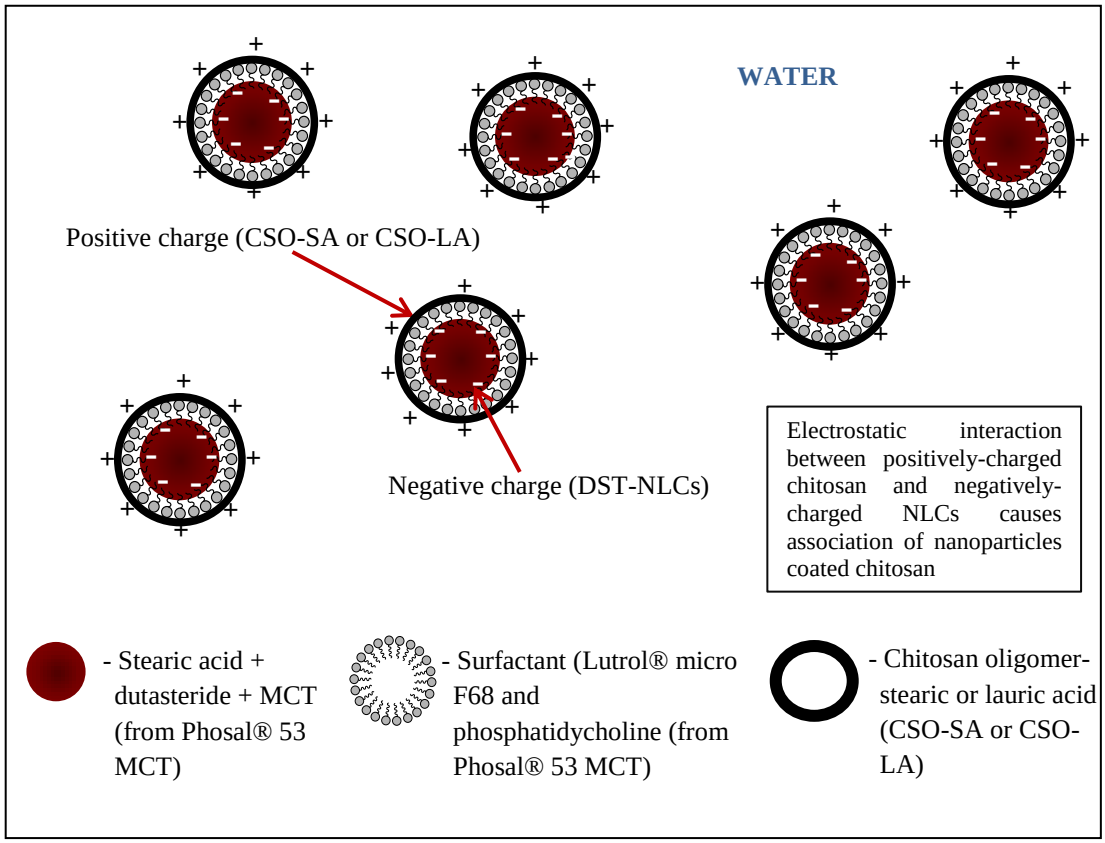



**Figure 1.19 Schematic representation of DST-NLCs coated with CSO-SA or CSO-LA to be prepared and characterised in this project**

Figure 1.20 shows the flow of the project outlined in this thesis. The project began with the conjugation and characterisation of CSO-SA and CSO-LA (Chapter 2). This was followed by optimisation of the formulation and manufacture of nanoparticles containing dutasteride and their characterisation (Chapter 3) and the *in vitro* studies (Chapter 4). Dutasteride was chosen as a drug having anti-androgenic activity, exhibiting Type I and Type II 5α-reductase inhibitions.

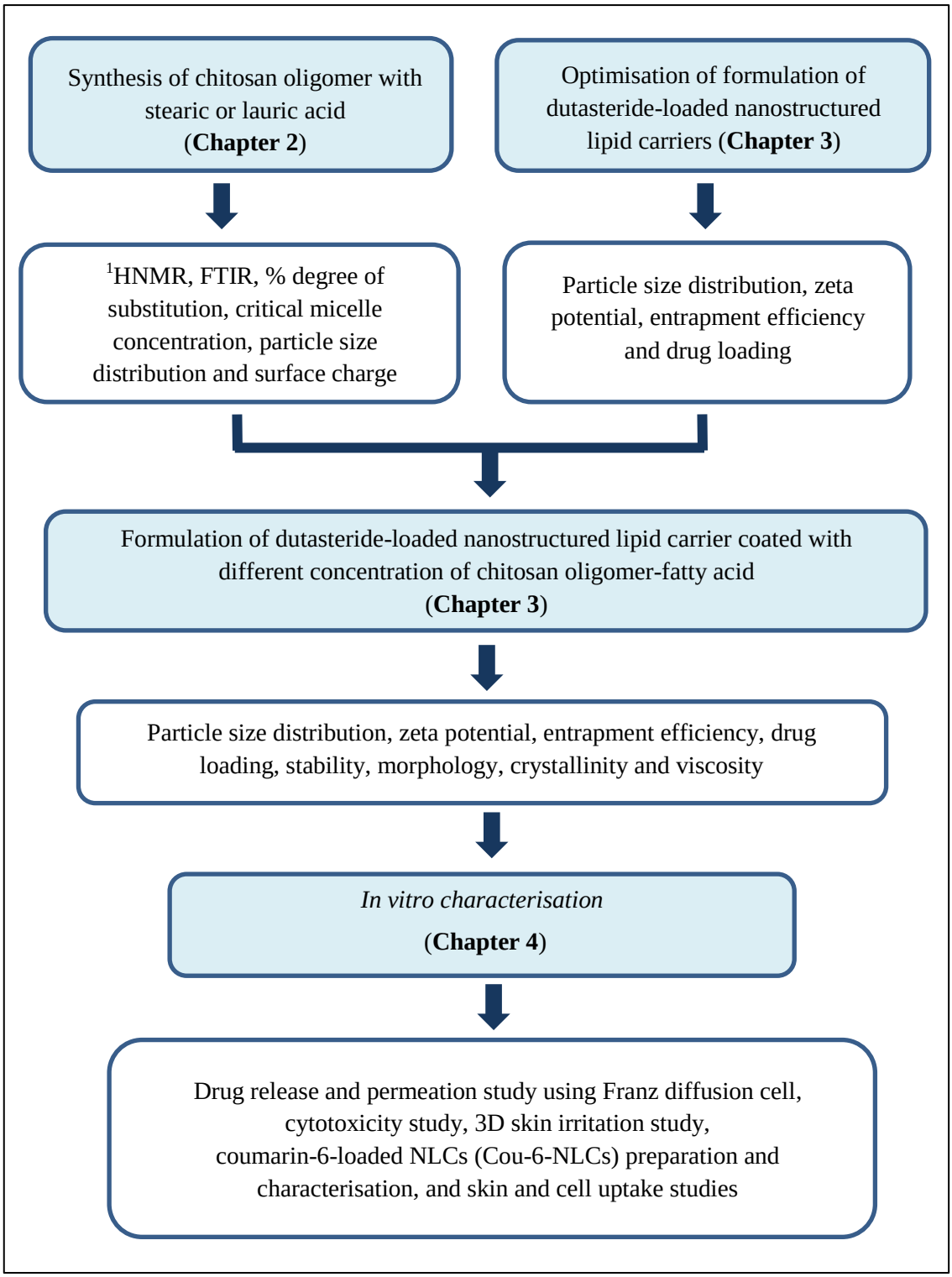



**Figure 1.20 Schematic diagram of overall preparation, characterisation and *in vitro* study of dutasteride-loaded nanostructured lipid carrier coated with chitosan oligomer-stearic or lauric acid**

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