CHAPTER II

LITERATURE REVIEW

1. Minoxidil

Minoxidil is a pyrimidine derivertive (2, 4–diamino-6-piperidino-pyrimidine-3-oxide, $C_9H_{15}N_5O$). Figure 1 shows chemical structure of minoxidil. The drug was initially developed as a systemic anti-hypertensive agent. Minoxidil promotes peripheral vasodilation by selectively relaxing the smooth muscle of peripheral arterioles. It has become important to dermatologists, because it has potent hypertrichotic effect and promotes regrowth of hair in male balding. This led to the development of a topical formulation of minoxidil for the treatment of androgenetic alopecia in men and in women. Rogaine® (Upjohn, USA) is the original product on the market. The products are currently available as solutions containing 2% or 5% minoxidil, in formulations composed of 60% ethanol, 20% propylene glycol, and 20% water. Recently, a new minoxidil preparation is commercially available under the trade name of Lipoxidil®. Lipoxidil® is the first product on the market that contains 1.5% or 4.5% minoxidil entrapped in liposomes (Han et al., 2004; Eller, Szpunar and Coletta, 1989; Messenger and Rundegren, 2004).

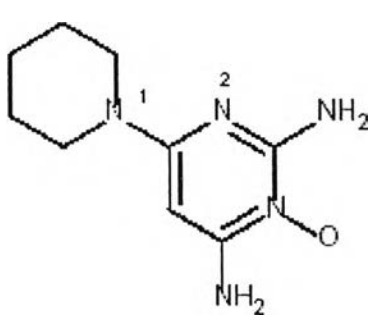


Figure 1. Chemical structure of minoxidil (Dennis, 1988)

1.1 Physicochemical properties of minoxidil (Dennis, 1988)

1.1.1 Solubility

The approximate solubilities of minoxidil in propylene glycol, methanol, 95% ethanol, 2-propanol, and water are 75, 44, 29, 6.7, and 2.2 mg/ml, respectively.

1.1.2 Stability

Minoxidil is a relatively stable substance at room temperature and shows no evidence of significant decomposition. The degradation of minoxidil in solution (20 °C, pH 7.0, phosphate buffer) follows the first-order degradation kinetics with a rate constant of 9.464×10^{-3} day^{-1} and calculated activation energy of 11.7 Kcal/mole. Degradation is acid-base dependent with the greatest stability at pH 5.0.

1.1.3 Storage

Minoxidil (tablets and topical solutions) should be stored in tightly closed containers and at controlled temperature between 15-30 °C.

1.1.4 Dissociation constant

The pK_a of minoxidil determined in buffer (0.01M ionic strength) is 4.61.

1.1.5 Partition coefficient

The octanol-water partition coefficient of minoxidil is 1.24.

1.2 Mechanism of action of minoxidil

Minoxidil's mechanism of action is important, both from the point of view to develop the drug for effective treatment of hair loss disorders and for the insights it may give into the biology of hair growth. The mechanism by which minoxidil promotes hair

growth is still not fully understood, and multiple pathways are thought to be involved. The proposed mechanisms are as follows (Median and Touitou, 2001):

1. Minoxidil, metabolized to minoxidil sulfate in the hair follicles, acts as a potassium channel agonist to reduce the cytoplasmic free calcium concentration. This prevents epidermal growth factor from inhibiting hair formation.
2. Minoxidil up-regulates the expression of vascular endothelial growth factor and its receptors, an action that subsequently stimulates angiogenesis and anagen.
3. Minoxidil is a potent activator of prostaglandin endoperoxide systhase-1, a cytoprotective enzyme that stimulates hair growth.
4. Minoxidil increases the number of DNA synthesizing cells in the dermal papilla, bulbar matrix, outer root sheath, and per follicular fibrocytic cells. These changes result in the prolongation of anagen and the conversion of vellus hairs to terminal hairs.

2. Pilosebaceous targeting by vesicles (liposomes and niosomes)

Traditionally, the prime pathway for the topical delivery of active agents across the skin was thought to be through transepidermal route of the stratum corneum. However, alternative means such as appendageal transport, i.e. follicular transport, is gaining more acceptances in the scientific community. Targeting specific sites of the hair follicle may represent a feasible therapeutic approach to skin diseases such as hair loss. The development of novel niosomal formulations for enabling the topical delivery of difficult-to-absorb agents for localized action, specifically to the hair follicle and sebaceous glands, were regarded (Ciotti and Weiner, 2002).

Entrapment of drugs in vesicles is a promising means to control transdermal drug delivery. Vesicular drug formulations are also useful for investigating the limits of drug transport, permeability, and integrity of the skin (Hofland et al., 1994). Although the use of vesicles in dermal or transdermal drug delivery has been studies, little is known about the mechanisms of the interaction between drug-loading vesicle bilayers and human

stratum corneum. There are few studies focusing on the influences of this interaction on penetration of lipophilic and hydrophilic compounds through human stratum corneum in vitro and in vivo (Hofland et al., 1994). In a previous report, interaction between vesicles and human keratinocytes was studied where vesicles were topically applied to human epidermis reconstructed in vitro (Schmid and Korting, 1994). The authors reported that the morphological correlate of the epidermal permeability barrier is an intact stratum corneum, which can be found in reconstructed human epidermis. Upon interaction with vesicle dispersion, reconstructed skin shows serial arrangement of big lipid droplets between corneocytes and keratinocytes. However, corneocytes is decreased in density and, as keratinocytes, osmophilic membrane indicating lipid transfer. Vesicles or their remnants can be found within the stratum corneum cells (Schmid and Korting, 1994). There is, however, no evidence for the penetration of intact vesicles into the stratum corneum, but there may be interaction between dispersed molecules of lipids from the vesicles and skin lipids (Schmid and Korting, 1994; Redelmeier and Kitson, 1993). Although intact vesicles do not appear to penetrate the stratum corneum, there is evidence that particles can penetrate along the hair follicles. Fluorescent liposomes were reported to penetrate into the hair follicles far below the skin surface (Redelmeier and Kitson, 1993). As indicated, this form of passive targeting appears particularly relevant to delivery to pilosebaceous glands or hair follicles.

Nonionic surfactant vesicles have attracted a great deal of attention in the delivery of dermal and transdermal drugs due to many advantages of the system (Agarwal, Katare, and Vyas, 2001). They are biodegradable, amphiphilic in nature (Uchegbu and Vyas, 1998), non-toxic (Mezei and Lee, 1970; Baillie et al., 1985), able to act as penetration enhancers (Endo, Yamamoto, and Ijuin, 1996), and effective in the modulation of drug release (Carafa, Santucci, and Lucania,2002). The system is also advantageous in terms of chemical stability when compared with phospholipid liposomes (Ruckmani, Jayakar, and Ghosal, 2000; Manconi et al., 2003). Nonionic surfactants are less irritating to the skin than ionic surfactants (Korting et al., 1994). Topically applied niosomes can increase the residence time of drugs in the stratum corneum and the epidermis while reducing the systemic absorption of the drugs and thus drug toxicity (Manconi et al., 2002). Various classes of lipophilic drugs such as estradiol (Hofland et al., 1994), nimesulide (Shahiwala

and Misra, 2002), dithranol (Agarwal, Katare, and Vyas, 2001), levonorgestrel (Vora, Khopade, and Jain, 1998), and ketorolac tromethamine (Alsarra et al., 2005) as well as hydrophilic drugs such as tetanus toxoid (Gupta et al., 2005) and lidocaine hydrochloride (Carafa, Santucci, and Lucania, 2002) were successfully encapsulated in niosomes. Thus, niosomes seem to have a potential for applications in transdermal and dermal delivery of both lipophilic and hydrophilic drugs.

There is some evidence that vesicular drug carriers can direct drug molecules into the pilosebaceous unit. In one study, in vivo deposition of calcein from liposomes was compared with that from solution in mice (Meidan and Touitou, 2001). Calcein from the vesicular formulation penetrated deeply into the shafts and hair follicle cells, in contrast to the free calcein from solution, which remained on the skin surface. In addition, the vesicular system did not deliver any calcein into the systemic circulation over 24 hours, indicating that targeted follicular delivery was possible. There is also evidence that liposomes can penetrate along hair follicles in a manner similar to other particles (Gregoriadis, Florence, and Patel, 1993). However, the evidence for this relies upon fluorescence to follow the presence of the lipid, and therefore does not differentiate between the penetration of lipid monomers and intact liposomes. However, there is an observation that fluorescent liposomal beads can penetrate along hair shafts. This form of passive targeting by vesicular structures appears particularly relevant to delivery to pilosebaceous glands (Redelmeier and Kitson, 1993).

The in vivo hamster ear skin (skin model containing human-like pilosebaceous unit) was used to quantify pilosebaceous deposition of a predominantly hydrophilic peptide, α-interferon, and a predominantly hydrophobic peptide, cyclosporine (Niemec, Ramachandran, and Weiner, 1997). Deposition of the peptides from niosomes, aqueous interferon, and hydroalcoholic cyclosporin solution was assessed. The greatest drug deposition resulted after application of niosomal formulations and the authors suggested that protein penetration was probably mediated via the follicular pathway.

With phospholipid-based minoxidil liposomes, the difference in drug concentration produced by the liposomal and solution forms was found to be greatest within the dermis and subcutaneous tissues, where the hair follicles are located (Gregoriadis, Florence, and Patel, 1993). When liposomal minoxidil was evaluated in

comparison to the Upjohn's formulas (solution and suspension), the results indicated the superiority of the vesicle form over the conventional forms. The results showed that the vesicle form delivered more drug to the site of action (hair follicles) and less to the internal organs (Gregoriadis, Florence, and Patel, 1993).

3. Niosomes (Uchegbu and Vyas, 1998; Uchegbu and Florence, 1995; Florence, 1993)

Non-ionic surfactant vesicles (niosomes) are formed from the self-assembly of non-ionic amphiphiles in aqueous media resulting in closed bilayer structures (Figure 2). The assembly into closed bilayers is rarely spontaneous and usually involves some input of energy such as physical agitation or heat. The result is an assembly in which the hydrophobic parts of the molecule are shielded from the aqueous medium and the hydrophilic head groups have maximum contact with the medium. These structures are analogous to phospholipid vesicles (liposomes) and are able to encapsulate aqueous solutes and serve as drug carriers. The low cost, the greater stability, and the resultant ease of storage of non-ionic surfactants have led to the exploitation of these compounds as alternatives to phospholipids. Niosomes were first reported in the seventies as a feature of the cosmetic industry but have since been studied as drug targeting agents. Niosomes are classified by their size and number of bilayers into multilamellar vesicles (MLVs: several bilayers, size 0.1-20 μm), small unilamellar vesicles (SUVs: single bilayer, size 0.01-0.1 μm), and large unilamellar vesicles (LUVs: single bilayer, size 0.1-1 μm). Drugs with various degree of lipophilicity can be encapsulated in niosomes, either in the bilayer, in the entrapped aqueous volume, or at the bilayer interface (Figure 3).

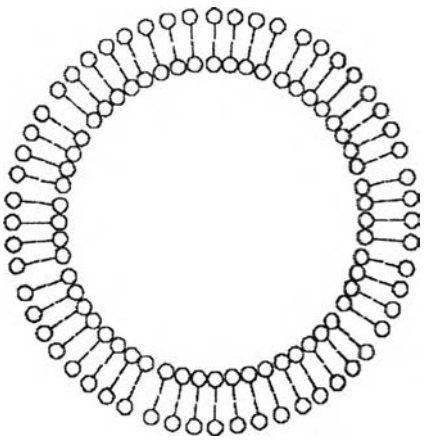


Figure 2. Schematic representation of a niosome, o = hydrophilic head group, ---------- = hydrophobic tail (Uchegbu and Vyas, 1998)

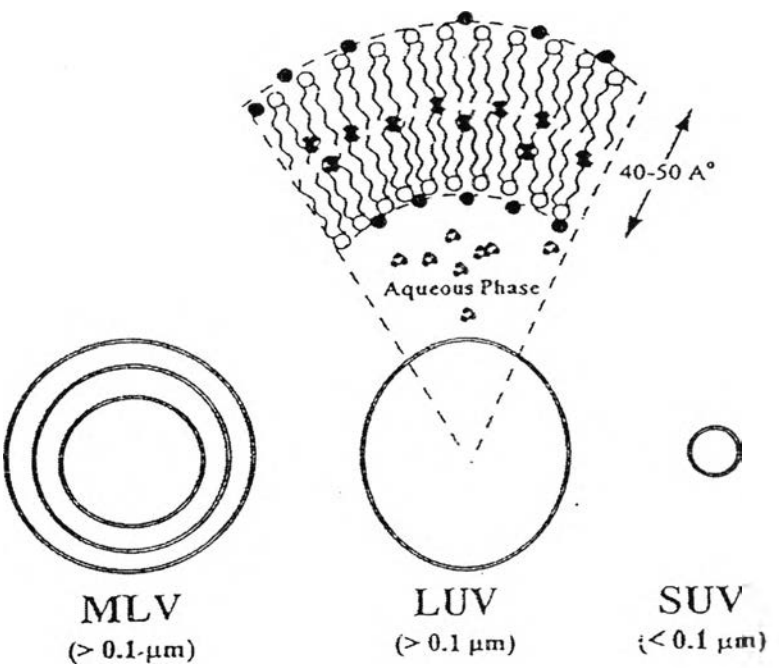


Figure 3. Types of niosomes depending on size and number of lamellae (Sharma, 1997)

3.1 The influence of molecular geometry of the surfactant monomer on vesicle formation

The geometric features of amphiphilic monomer aggregation have been analyzed by a parameter generally known as the critical packing parameter (CPP). This is essentially a dimensionless number that describes the preference of any amphiphile to

form aggregates, whether micelles or vesicle. The critical packing parameter has a simple formula as follows:

$$CPP = \frac{v}{I_c a_o}$$

where v = the hydrocarbon chain volume, I_c = the critical hydrophobic chain length (the length above which hydrocarbon chain fluidity may no longer be guaranteed), a_0 = area of the hydrophilic head group (Figure 4). A calculated value of CPP below 0.5 indicates the spherical micelle formation, values between 0.5 and 1 indicate that the surfactant monomers assemble preferentially into vesicles, while a CPP of above 1 would predispose a compound to form inverted micelles. The true CPP depends on the molecular geometry of the monomer, which is not a fixed parameter (e.g., in polyoxyethylated surfactants in which the head group dimensions vary). Factors such as solvent ionic strength, temperature, degree of hydrogen bonding between the hydrophilic head groups, and the nature of the aqueous solute will affect monomer conformation, as will partial molal volume, head group hydration, and hydrocarbon chain extension; factors which can not always be measured and therefore not always be incorporated into the equation.

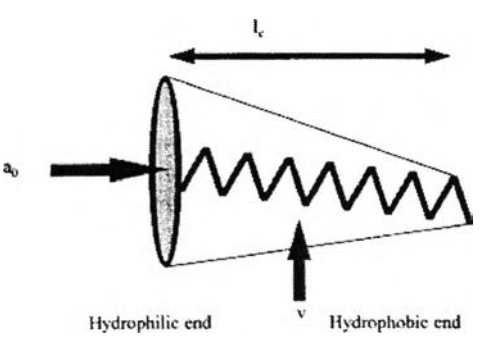


Figure 4. Schematic representation of an amphiphile, a_0 = hydrophilic head group area, v = hydrophobic chain volume, I_c = hydrophobic chain length (Uchegbu and Vyas, 1998)

3.2 Materials used in the preparation of niosomes (Uchegbu and Florence, 1995; www.uniqema.com)

It is often useful to identify the basic structural units of niosomes. While an amphiphilic nature is an inescapable prerequisite for molecules to form vesicular assemblies, variations exist in the nature of facilitating hydrophilic head groups. Freely soluble surfactants such as polysorbate 80 will not form vesicles under normal conditions, as the micellar state will be favored. These compounds may form vesicles when mixed with cholesterol, a suitably hydrophobic compound, in accordance with the observation that cone shaped (e.g., lysolecithin) plus wedge shaped (e.g., cholesterol) molecules operate co-operatively to form bilayer membranes in vivo.

Both single as well as double alkyl chain non-ionic surfactant analogues are vesicle-forming compounds. Principal among vesicle forming non-ionic compounds are the alkyl ether lipids. These can be broadly divided into two classes based on the nature of the hydrophilic head group: alkyl ethers in which the hydrophilic head group consists of repeated glycerol subunits, related isomers, or larger sugar molecules and those in which the hydrophilic head groups consist of repeated ethylene oxide subunits. Alkyl esters, amides, and fatty acid as well as amino acid compounds also form vesicles that have hydrophobic alkyl side chains and esters, amides, and fatty acids as the hydrophilic head groups, respectively. Alkyl ethers and alkyl esters are a wide variety of the non ionic-surfactants for drug delivery applications that have been explored.

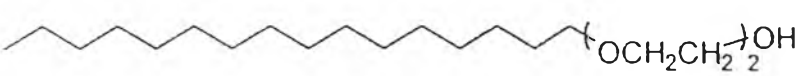
3.2.1 Polyoxyethylene alkyl ethers

Alkyl glycerol ethers were the first compounds reported to form niosomal vesicular dispersions. Alkyl glycerol ethers have also been used to prepare non-ionic surfactant vesicles for cosmetic application. Alkyl galactosides, glucosides, and mannosides have been studied as drug carriers. A group of alkyl ethers bearing polyhydroxyl head groups are also vesicle-forming compounds. The second group of alkyl ether amphiphiles with repeated oxyethylene units in the hydrophilic region has also received considerable attention.

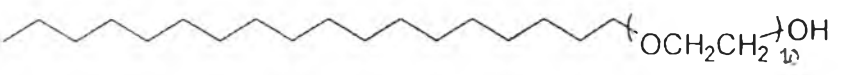
Polyoxyethylene (POE) fatty ether is in the alkyl ether groups. Brij® 52 and Brij® 76 are polyoxyethylene fatty ethers derived from cetyl and stearyl alcohol, respectively. Brij surfactants are stable to acids and alkalies beyond the pH range that ester type emulsifiers can withstand. Brij® group contains both hydrophilic and lipophilic products. Depending on the HLB, Brij surfactants can be used to form either oil-in-water (o/w) or water-in-oil (w/o) emulsions, to solubilize oils, and to improve wetting. Table 1 and Figure 5 show physical data and the structures of Brij® 52 and Brij® 76, respectively.

Table 1. Physical data of Brij® 52 and Brij® 76

Chemical composition	HLB	Color and form at 25 °C	Pour point (°C)
POE (2) cetyl ether (Brij® 52)	6.7	white solid	33
POE (10) stearyl ether (Brij® 76)	12.4	white solid	38



(a)



(b)

Figure 5. The structure of Brij® 52 (a) and Brij® 76 (b) (Plookchit Chetratanont, 2002)

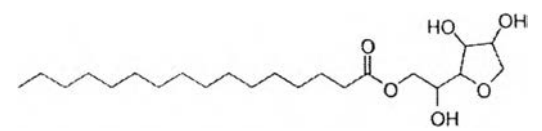
3.2.2 Sorbitan alkyl esters (www. Uniqema.com)

Alkyl esters, such as the sorbitan esters, are widely used in foodstuffs. They have been studied as the basis of nonionic surfactant vesicles. These materials contain hydrophilic and lipophilic groups. These surfactants are mixtures of partial esters of sorbitol and its anhydrides and are made from fatty acids such as palmitic and stearic acids. Span® surfactants are lipophilic. They are generally soluble or dispersible in oil, forming water-in-oil emulsions. Sorbitan esters are excellent emulsifiers and solubilizers. They offer low toxicity (food stock), have low irritancy, and have a wide range of food

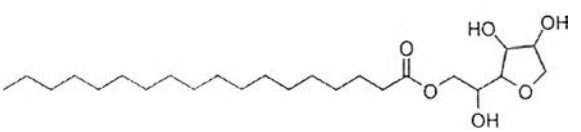
contact approvals. Table 2 and Figure 6 show physical data and the structures of Span® 40 and Span® 60, respectively.

Table 2. Physical data of Span® 40 and Span® 60

Chemical composition	HLB	Color and form at 25 °C	Pour point (°C)
Sorbitan monopalmitate (Span® 40)	6.7	tan solid	48
Sorbitan monostearate (Span® 60)	4.7	tan solid	53



(c)



(d)

Figure 6. The structure of Span 40® (c) and Span® 60 (d) (Plookchit Chetratanont, 2002)

3.2.3 Membrane additives

Membrane additives are often required in niosomal formulations for membrane and vesicle stabilization. Among these, cholesterol and Solulan® C24 are most often used.

3.2.3.1 Cholesterol

Cholesterol reduces the fluidity of membranes above the phase transition temperature (T_c) with a corresponding reduction in permeability to aqueous solutes. Consequently, inclusion of cholesterol into unsaturated membranes is often essential in order to achieve sufficient stability. On the other hand, cholesterol increases the fluidity of membranes below the phase transition temperature, so that its inclusion in saturated

membranes, which are usually in the gel phase at ambient temperature, may result in a reduction in stability. The structure of cholesterol is shown in Figure 7.

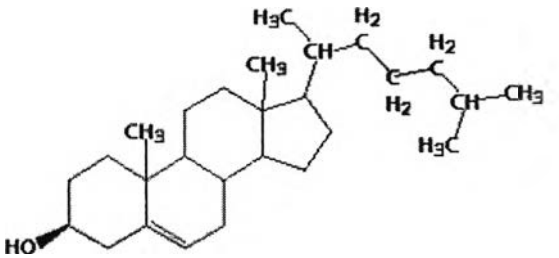


Figure 7. The structure of cholesterol (Uchegbu and Florence, 1995)

3.2.3.2 Solulan® C24

A steric stabilizer, Solulan® C24 (poly-24-oxyethylene cholesteryl ether), must be added to the formulation to ensure a homogeneous formulation devoid of aggregates. It consists of two parts of hydrophilic polyoxyethylene chains and cholesterol. The structure of Solulan® C24 is shown in Figure 8.

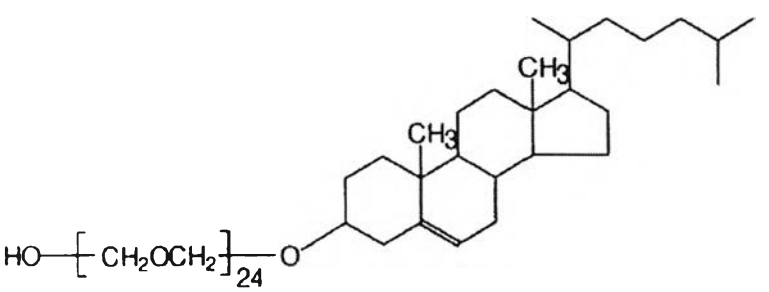


Figure 8. The structure of Solulan® C24 (Uchegbu and Florence, 1995)

3.3 Characterization of niosomes (Florence, 1993)

Characterization of niosomes is necessary to confirm that the properties of the structure formed are suitable for their intended uses. Niosomal preparations are usually characterized in terms of morphology, size and size distribution, entrapment efficiency, lamellarity, as well as physical stability. Some of these parameters such as size and size distribution are routinely used for batch-to-batch quality control.

3.3.1 Morphology

The vesicle formulations can be examined by freeze fracture electron microscopy (Carafa, Santucci, and Lucania, 2002), transmission electron microscopy (TEM) (Arunothayanum, 2000; Palozza et al., 2005), and scanning microscopy (SEM) (Touitou et al., 2000) to characterize the microstructure.

3.3.2 Size and size distribution

The particle size and size distribution of vesicular formations are measured by photon correlation spectroscopy or by dynamic light scattering (Agarwal, Katare, and Vyas, 2001; Uchegbu and Duncan, 1997) and electron microscopy.

3.3.3 Entrapment efficiency

Entrapment efficiency can be determined by removing unentrapped solute from the vesicles before determining the quantity of drug in the vesicles. The methods that have been used for the removal of unentrapped materials include:

- Exhaustive dialysis (Udupa et al., 1993; Hao et al., 2002)
- Gel filtration (Yoshioka, Sternberg, and Florence, 1994)
- Centrifugation (Uchegbu and Vyas, 1998)
- Ultracentrifugation (Agarwal, Katare, and Vyas, 2001; Shahiwala and Isra, 2002).

All these methods have their advantages and disadvantages as given in Table 3.

3.3.4 Lamellarity

Lamellarity is the number of layers making up the shell of the vesicles. It can be detemined by electron microscopy (Chrai, Murari, and Ahmed, 2001) or ^{31}P-nuclear magnetic resonance spectroscopy (Touitou et al., 2000).

3.3.5 Physical stability

Physical stability, expressed as the resistance to an osmotic stress, can be verified by monitoring reductions in vesicular mean hydrodynamic diameter (Baillie et al., 1995; Carafa, 1998), size distribution, and amount of the material encapsulated.

Table 3. The advantages and disadvantages of the different methods of separation of the entrapped from the unentrapped drug (Uchegubu and Vyas, 1998)

Separation method	Advantages	Disadvantages
Exhaustive dialysis	- Suitable for large vesicles (>10 μm)	- Extremely slow (5-24 h)
	- Suitable for highly viscous systems	- Large volumes of dialysate required (may not be suitable for drugs requiring specialized disposal)
	- Inexpensive	- Dilutes the niosome dispersion
Centrifugation (below 7000 x *g*)	- Quick (~30 min)	- Fails to sediment the sub-micron niosomes
	- Inexpensive instrumentation	- May lead to the destruction of fragile systems
	- Concentrates the niosome dispersion	
Ultracentrifugation (150,000 x *g*)	- Sediments all size populations	- Expensive Instrumentation
	- Concentrates the niosome dispersion	- Long centrifugation times (1–1.5 h)
		- May lead to the destruction of fragile systems
		- May lead to the formation of aggregates
Gel filtration	- Quick (4–5 min with Sephadex® G50)	- Slow (1–2 h when using Sepharose® 2B/4B for macromolecule separation)
		- Gels are expensive if not reused
		- Dilutes the niosome dispersion
		- Not suitable for highly viscous formulations
		- Not suitable for formulations with a large particle size (>10–20 mm)

4. Stability of minoxidil niosomes

4.1 Minoxidil stability

The photostability profile of minoxidil solutions exposed to fluorescent light shows that photodegradation follows first order reaction. Sodium thiosulfate acts as an antioxidant and has a photostabilizing effect for minoxidil solutions (Chinnian and Asker, 1996). Hydroalcoholic solution and hydroalcoholic gel of topical minoxidil were formulated and investigated for the color change at room temperature (30 °C) with naked eyes. Minoxidil preparations containing propylene glycol turned into yellow color, which resulted form the oxidation reaction between minoxidil and propylene glycol. This oxidation reaction is catalyzed by heavy metal impurities in propylene glycol and minoxidil themselves. However, the reaction can be inhibited by adding a chelating agent (0.01% EDTA sodium) or an antioxidant (sodium bisulfite, more than 0.5%) (Pimolpan Pithayanukul, 1988). This finding has prompted a search for a more photostable formulation for minoxidil.

The study on the degradation of drug substances under light is important because the photodegradation products may be toxic and might appear in the formulation during storage (Nema, Washkuhm, and Beussink, 1995). Light exposure can induce chemical degradation in susceptible molecules. For light to induce a chemical reaction, the light must be absorbed. Since transmission of ambient light (solar, fluorescent) through glass is minimal or nil at wavelengths shorter than about 320 nm, one generally is most concerned with drug chromophores having relatively long ultraviolet to visible absorptions (Waterman and Adami, 2005). Although various mechanisms are involved in photochemical reactions, such reactions can be divided broadly into processes that depend on oxygen (photooxidations) and those independent of oxygen (such as dehydrogenations, rearrangements, and dimerizations) (Waterman and Adami, 2005).

Clarified reaction paths indicate that photodegradation chiefly follows zero, first, or apparent first order kinetics (Tonnesen, 1996). Investigations of the photostability of ubidecarenone in the solid state show the degree of degradation as a function of the light absorption properties of the yellow-colored substrate. The photolytic degradation may

follow apparent first order kinetics, which is promoted by irradiation wavelength. Short wavelengths are more deteriorating than longer wavelengths. (Tonnesen, 1996). However, photodegradation pathways of minoxidil are still not known.

4.2 Niosome stability (Uchegbu and Vyas, 1998)

Stable niosomal dispersions must exhibit a constant particle size and a constant level of entrapped drug. There must be no precipitation of the membrane components, which are not usually soluble in aqueous media. Various factors can affect niosome stability. These include the following:

4.2.1 The surfactant/lipid nature

The choice of membrane surfactant determines the nature of the membrane and ultimately affects the stability of the system. The incorporation of cholesterol into niosomal systems also decreases the leakiness of the membrane. In making a choice of surfactant, the higher phase transition surfactants appear to yield more desirable stability and toxicity profiles. The details are as displayed in Figure 9.

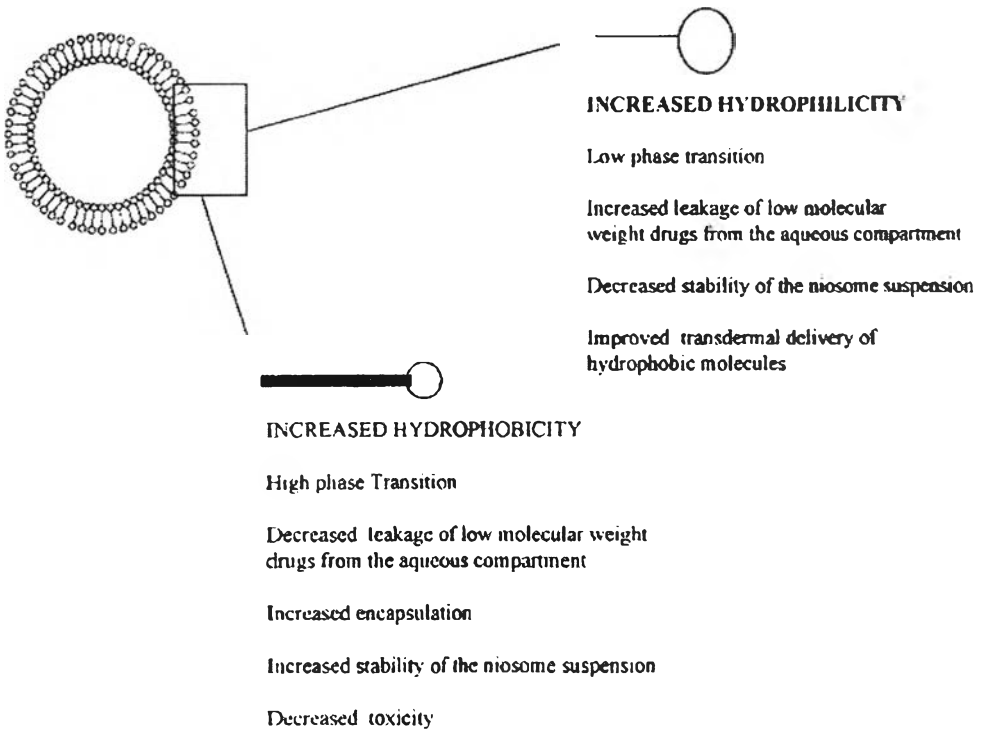


Figure 9. The effect of the choice of niosome-forming surfactant on the properties of the niosomal dispersion (Uchegbu and Vyas, 1998)

4.2.2 The encapsulated drug

The encapsulated drug can also be the major determinant of the fate of any niosomal system. In choosing a suitable drug to be delivered by niosomes, it should be born in mind that niosomes encapsulating hydrophobic drugs and macromolecules are more stable than niosomes encapsulating low molecular weight drugs. In addition, transdermal drug delivery appears possible with hydrophobic or amphiphilic molecules as described in Figure 10. In contrast, hydrophilic drugs can easily leak from niosomes and decrease the stability of niosome dispersion. Encapsulation usually increases with amphiphilic drugs.

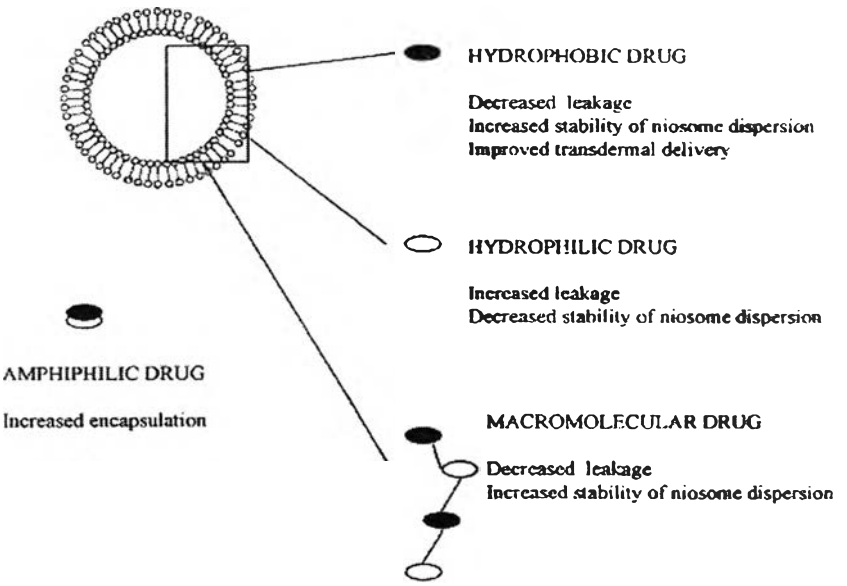


Figure 10. The effect of the nature of the encapsulated drug on the properties of the niosomal dispersion (Uchegbu and Vyas, 1998)

4.2.3 Temperature of storage

Niosomal dispersions should be stored under controlled temperature. A change in the temperature of the system often leads to a change in the fundamental nature of the system or an increase in the release of an encapsulated solute, a property which may be exploited to construct a thermoresponsive system.

4.3 Mechanisms of drug degradation (Vichuda Savanananda, 1995; Waterman and Adami, 2005; Connors, Amidon, and Stella, 1986)

Chemical decomposition of drug can be divided into 4 categories:

4.3.1 Hydrolysis

Hydrolytic reactions are among the most common processes for drug degradation. In addition to rate dependencies on the temperature and moisture, hydrolysis rates can depend on the concentration of catalytic species, usually acids or bases. Many hydrolytic reactions involve esters and aryl carbamates. The chemical behavior of acyl compound depends greatly on the nature of the remaining atom or group, that is x in RCOX.

Carboxylic acid, ester, amide, thiol ester, acid chloride, acid anhydride, imide, lactam, or lactone as the X substitute will make the molecule more prone to hydrolysis.

Hydrolysis involves the decomposition of the active drug though reaction with the solvent present, which is moisture in most instances. Moisture is perhaps the single most important factor affecting stability of dosage forms. Direct adsorption of water molecules onto the drug surface might easily induce hydrolytic decomposition. The effect that moisture exerts on stability depends on its strength of association, whether it is free or bound water.

For drug in suspension and emulsion, the situation is complicated by the nature of a two-phase (solid drug and solution or drug in oil solution and aqueous suspending fluid) system. In many cases, the hydrolysis rate of the drug in aqueous solution will be orders of magnitude higher than that in the solid or oil phase. For this reason, the amount of drug in aqueous solution can be the major factor in determining the rate of hydrolysis.

4.3.2 Oxidation

Oxidative degradation of pharmaceuticals can broadly be divided into two types: reaction with molecular oxygen and reaction with other oxidizing agents present in the formulation. These can, in general, be distinguished by the dependence of the degradation on the presence of oxygen. In some cases, oxidizing agents are generated from oxygen-derived decomposition of excipients.

Oxidation reaction is a complementary one; its partner is reduction. Oxidation/reduction (redox) reactions involve the transfer of one or more oxygen or hydrogen atoms or the transfer of electrons (Equation 1).

$$\text{Reduced form} \Longleftrightarrow \text{Oxidized form} + e^- \qquad (1)$$

In pharmaceutical dosage forms, oxidation is usually mediated through reaction with atmospheric oxygen under ambient conditions. Oxidations that take place spontaneously are often called auto-oxidation. The mechanisms of oxidation reactions are usually complex, involving multiple pathways for initiation, propagation, branching, and termination of free radicals. Many drugs; especially those containing carboxylic acid, thiol, ether, amine, or aldehyde functionalities, or centers of unsaturation; are subjected to oxidative degradation on exposure to dry air.

Oxidative breakdown of drugs generally proceeds through the sequence of initiation, propagation (and may be chain branching), and termination. As mentioned earlier, a triggering force that may promote oxidation is "light" (namely, certain components of the electromagnetic spectrum). Not all photolytic reactions are oxidative in nature and not all oxidative reactions require light either as an initiator or as an integral component of the propagation steps. However, if light does initiate or promote an oxidative breakdown of a drug, the exclusion of the particular wavelength range of light responsible for the catalysis will often suppress the oxidation. This can be achieved by the total exclusion of light using an opaque container or the use of pigmented glass capable of excluding the damaging wavelengths.

4.3.3 Photolysis

The light energy is inversely related to wavelength so that UV > Visible > IR and is independent of temperature. When molecules are exposed to electromagnetic radiation, they absorb the light at characteristic wavelength, which causes an increase in the energy state of the compound. This can cause decomposition of drugs.

Photolysis is a consequence of the absorption of "light" or radiation energy, allowing for quantum restrictions, by a molecule A to produce an unstable excited-state species, A*, as in Equation 2. The absorbed energy can be lost either by a radiative mechanism in which the energy is given off in the form of fluorescence or phosphorescence (Equation 3) or by a radiationless mechanism (Equations 4-6). The radiationless mechanism can be physical or chemical in nature. The physical decay results in the loss of energy as heat (Equation 4) or by collision with other molecules or quenching (Equation 5). The net effect of the chemical decay is that molecule chemically decomposes (or rearranges) into a new species (Equation 6). This whole process can be defined by Equations 3-6. The photolysis process is thus represented by Equations 2-6 that follows.

$$A \xrightarrow{h\nu} A^* \qquad (2)$$

$$A^* \xrightarrow{k1} A + h\nu' \qquad (3)$$

$$A^* \xrightarrow{k2} A + \text{heat} \quad (4)$$

$$A^* + A \xrightarrow{k3} 2A \quad (5)$$

$$A^* \xrightarrow{k4} \text{product(s)} \quad (6)$$

4.4 Photostability of drug in vesicular structures

The vesicular formulation is a two-phase system and the drug stability profile is analogous to that of suspensions and emulsions. Photostability of drug in vesicular formulation relates the stability of drug in vesicular system to in vitro drug release. The drug inside the vesicular structure is, in some degree, protected from light, whereas the drug that is released from the vesicle into the medium is not. Thus, the portion of drug in the medium degrades faster than that inside the vesicles. The in vitro drug release can be modulated by varying the vesicular structure and/or bilayer composition, which in turn regulates the drug photodegradation. In vitro drug release from semisolid topical dosage forms has been extensively investigated using the Franz cell diffusion system (Siewert et al., 2003). In vitro drug release through a synthetic membrane is often performed both to check stability and to serve as a prerequisite to the investigation of topical application (Manconi et al., 2003). Drug in vesicular formulation represents one of the most studied photo-protective carriers and successful results have been reported in many studies. Vesicular structures have also been proposed as systems capable of improving the stability of photosensitive drugs (Manconi et al., 2003). Riboflavin (Habib and Asker, 1991) and doxorubicin (Uchegbu and Florence, 1995) were found to be more stable in liposomes and niosomes, respectively, than in solution. Tretinoin has a higher stability in liposomes than in methanol or in castor oil (Brisaert et al., 2001). Better stability of tretinoin in liposome complex was also reported (Ioele et al., 2005). Manconi et al. (2003) reported that tretinoin could be incorporated in high yield in niosomal structures, giving rise to very stable formulations.

5. Toxicity and irritation studies

An important aspect of the safety assessment process for any topical product is determination of their capacity to produce adverse skin effects such as irritation. An assessment of skin irritation is required during the product development process for skin care products, and prior to manufacturing and marketing of new products, in order to help ensure worker and consumer safety (Osborne and Perkin, 1994). Non-ionic surfactants are weak irritants (Endo, Yamamoto, and Ijuin, 1996; Dimitrijevic et al., 1997). The toxic effect of two niosomal preparations (Span® 80:CHO:Solulan® C24 and hexadecyl diglyceryl ether ($C_{16}G_2$):CHO:Solulan® C24) were thought to be principally a result of the amount of free surfactants present in the niosomal suspensions. In a previous report, free Solulan® C24 at a level of 0.1% w/v was very toxic to Caco-2 cells, whereas the same concentrations of Solulan® C24 in niosomes had no effect on the cell viability, using MTT test (Dimitrijevic et al., 1997). However, some toxicity was observed at higher level of Solulan® C24 in niosomal form (Dimitrijevic et al., 1997). Degree of toxicity varies, depending on individual surfactants. In one research report using rabbit skin tests, polysorbate caused more irritation than sorbitans, and polyoxyethylene ethers caused the greatest irritation (Mezei et al., 1966). The cholesterol content does not appear to have any effect on the proliferation of the keratinocytes, implying lack of toxicity of the lipid (Hofland et al, 1991; Hofland et al., 1992). In one study, the degree of hemolysis depended on the concentration and type of surfactants or phospholipids (Stensrud, Monkkonen, and Karlsen, 1999).

From a skin irritation standpoint, most regulatory test protocols are based on methods originally developed by Draize et al. (1944). The original Draize skin irritation test involves application of test material to rabbit skin in a patch for 24 hr, removal of the patch, and subjective grading of the extent of skin erythema and edema on a 0 to 4 scales. Other evaluations of rabbit test have been used for surfactant toxicity: gross examination, microscopical examination, measurement of the respiratory metabolic activity, and changes in phospholipid (Mezei and Lee, 1970; Mezei and Sager 1967; Mezei et al., 1966). However, there is a limitation of extrapolation of animal test methods for prediction of human toxicity as well as an ethical issue. While in vitro methods have been

widely proposed as an alternative approach to the use of animals, there has been no consensus on the broad applicability of specific test methods.

In vitro skin irritation test methods can address two key needs: (1) to provide initial screening or confirmatory data prior to human skin exposures to a new ingredient or formulation, within classes of materials for which the in vitro methods are validated; and (2) to meet specific regulatory requirements for skin safety data (Osborne and Perkins, 1994). There are several in vitro tests for skin irritation using various models such as human epidermal keratinocytes (Osborne and Perkin, 1994; Hofland et al., 1991; Hofland et al., 1992), fibroblast cell lines (Benavides et al., 2004; Korting et al., 1994), macrophage cell lines (Stensrud, Monkkonen, and Karisen, 1999), lactate dehydrogenase (LDH) assay, MTT assay, and neutral red assay. Irritation test using red blood cells has also been introduced as another convenient model (Kumari and Rao, 1991; Moreau et al., 2000; Uchegbu and Duncan, 1997).

An in vitro red blood cell assay allows the estimation of the irritation potential of surfactants and surfactant-containing materials. The estimation is based on the fact that surfactants interact strongly with cellular membranes. This effect is measured photometrically via the use of an inherent native dye, oxyhemoglobin. Though the procedure was not designed primarily for skin irritation, it should be able to rank the irritation potentials of the test products, if a suitable positive control is present.

The protocol of the in vitro red blood cell assay describes an approach based on the use of red blood cells to quantify adverse effects of surfactants and detergent products on the cytoplasmic membrane (hemolysis). This can be sensitively detected by following changes in the photometrical absorbance of oxyhemoglobin, an indicator of the process. Generally, safety testing of topical products is primarily related to the injury of accidental applications, in particular to mucous membranes. The predominant interest is therefore directed to the first step of its elicitation, which is known to be the damage of cellular membranes. The red blood cell assay can be used routinely to assess irritancy in safety evaluation of surfactants and tensidoactive consumer goods. The test system is simple and characterized by a defined objective end-point. The assay is inexpensive, does not require special equipment, and needs only one hour per sample. The test can also be used

as a rapid screening assay in a first-order in vitro test battery for the assessment of acute eye irritation potential.

Hemoglobin release is an excellent end-point of cytoplasmic membrane integrity. Oxyhemoglobin is also denatured by surfactants, therefore, to take this into account, measurements are made at 575 and 540 nm to monitor (or quantify) the spectral changes of the protein as a result of tenside denaturation. The red blood cell test is not proposed as a global alternative to the Draize test, but as part of a practical in vitro test battery. It is far less expensive than other cell culture tests and commercial systems. On the contrary, the hemolysis has been designed as a test for chemicals, for which lysis of membranes constitutes their principal mechanism of action. Thus, red blood cell test is proposed as a bioassay for predicting the lytic and damaging effects of tensides or surfactants at large on the plasma membrane. The hemolytic potency alone may not be sufficient to characterize fully the irritation potential of tensides. However, it can sufficiently rank the chemical of interest among the known tenside entities if a suitable standard is also included in the test.