CHAPTER II

LITERATURE REVIEW

VESICULAR CARRIERS FOR DERMAL DRUG DELIVERY

The dermal route of administration has been utilized either to produce local effects for treating skin disorders or to produce systemic drug effects. The primary purpose of dermal application is to induce local effects close to the site of application. In such cases, cutaneous absorption is required but percutaneous absorption is not. On the other hand, for systemic effects, the use of various vehicles containing penetration promoters or drug carriers to ensure sufficient penetration of the drug has been attempted (Singh and Vyas, 1996). Various types of carriers, including liposomes and niosomes, have been studied.

LIPOSOMES

Liposomes are lipid vesicles composed mainly of phospholipids and cholesterol (CHO). Other components, such as a charge-imposing lipid and an antioxidant, are often included in the formulation. Liposomes are long known to be beneficial for topical skin delivery. It has been proposed that phospholipids can act as surface-active agents to promote drug penetration into the skin. It is also believed that liposomes can enhance skin hydration and, thus, skin penetration of various active compounds (Uchegbu and Vyas, 1998).

Topical delivery of drugs by liposomal formulations has widely been of interest to increase drug absorption through the skin for both systemically and locally active drugs. Several groups have reported increased percutaneous absorption from liposomal formulations, leading to higher systemic levels for a given topical dose (Foldvari, 1994; Yu and Liao, 1996). Others have observed increased levels of drugs in the skin combined with decreased percutaneuous absorption (Mezei, 1990; Meizei and Getsztes, 1990; Du Plessis, Weiner, and Muller, 1994). Still, more have reported that liposomes can either reduce or increase cutaneous clearance, depending on the type and composition of the liposomal

product (Imbert and Wickett, 1995). Some common mechanisms of action have been suggested (Schreier and Bouwstra, 1994). Most investigators agree that no transport of lipids takes place across whole skin. It was shown that intact liposomes were not able to penetrate into the granular layer of the epidermis (Ganesan et al., 1984; Ho et al., 1985; Komatsu et al., 1986; Knepp et al., 1988; Knepp, Szoka, and Guy, 1990; Du Plessis, Egbaria, and Weiner, 1992; Kirijavainen et al., 1996; Yu and Liao, 1996). On the contrary, Cevc and Blume (1991) reported that a special type of liposomes, transfersomes, could transfer a large amount of lipid in form of "lipid aggregates" to deeper layers of the skin. Most studies, however, are in agreement that direct contact between vesicles and the skin is essential for penetration and drug delivery. Although the mechanism of interaction of liposomes with the skin is not clear, it does appear to be strongly dependent on liposomal parameters such as the number of lamellae, surface charge, and particle size (Michel et al., 1992).

Liposomes are vesicles that can incorporate both lipophilic and hydrophilic drugs because they have both hydrophilic and hydrophobic compartments. They may serve as organic solvent for solubilization of poorly soluble drugs. They increase permeability of the skin for various entrapped drugs and, at the same time, diminish the side effects of these drugs (Mezei, 1990; Mezei and Getsztes, 1990; Du Plessis et al., 1994). They can be a local depot for sustained release of dermally active compounds and enhance the accumulation of the drugs at the site of administration (Schreier and Bouwstra, 1994). In addition, the liposomal structure allows slow osmotic diffusion of water or water soluble agents through the lipid membrane into the skin (Idson, 1975). A liposomal formulation should thus retain skin moisture and decrease skin irritation. The lipid components of liposomes are arranged in bilayers which can also prevent water evaporation from the skin (Idson, 1983).

NIOSOMES

Niosomes are non-ionic surfactant-based vesicles. They were first reported in the seventies as a feature of cosmetic industry. Niosomes are analogous to liposomes and are able to entrap solutes and serve as drug carriers. When compared with phospholipid vesicles, non-ionic surfactant vesicles have advantages of lower cost, greater stability, and thus resultant ease of preparation and storage. These benefits have lead to the exploitation of these non-ionic compounds as alternatives to phospholipids (Uchegbu and Vyas, 1998). Besides, from the property of non-ionic surfactants, solubility of the drug may be increased, and use of the organic solvent may be avoided. The interest in niosomes as drug carriers is thus expanding, and the number of studies in the literature is increasing over the years.

Niosomes are formed from the self assembly of non-ionic amphiphiles in aqueous media resulting in closed bilayer structures (Figure 1). 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 part of the molecule is shielded from the aqueous solvent and the hydrophilic head groups have maximum contact with water. There are many factors governing the self assembly of non-ionic surfactants into niosomes. In certain cases, CHO is required in the formation of vesicles. Vesicle aggregation may be prevented by inclusion of molecules that stabilize the system against the formation of aggregates by repulsive steric or electrostatic effects. An example of steric stabilization is the inclusion of Solulan®C24 (cholesteryl poly-24-oxyethylene ether). An example of electrostatic stabilization is the inclusion of dicetylphosphate (DCP).

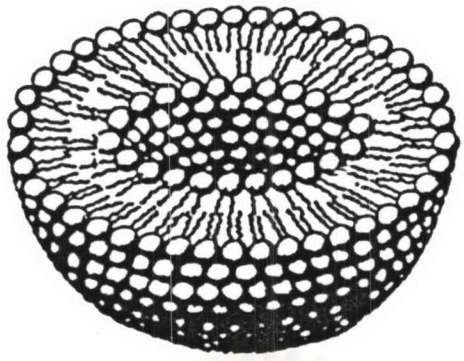

Figure 1. Closed bilayer structure of non-ionic amphiphiles (From Nacht, 1995)

Materials used in the preparation of niosomes

As stated earlier, niosomes are formed from the self assembly of non-ionic amphiphiles in aqueous media resulting in closed bilayer structures. Formulators can increase stability of these vesicles by adding CHO, DCP, and Solulan®C24 (Uchegbu and Vyas, 1998). In brief, the commonly used components of niosomes are as follows (Roson, 1989; Kibbe, 2000):

1. Non-ionic surfactants

Non-ionic surfactants with a wide variety of structures, usually in the presence of CHO, form both multilamellar and unilamellar vesicles. The balance between hydrophobicity and hydrophilicity (HLB number) and forces of attraction and repulsion of both hydrophobic and hydrophilic parts are crucial in determining the type of aggregate formed in aqueous environments, whether spherical or asymmetrical micelle, mesophase, or vesicle. Besides, the optimum of head group area, hydrocarbon chain volume, and hydrocarbon chain length of non-ionic surfactants are also important.

Polyoxyethylene alkyl ethers (PAE) and long-chain carboxylic acid esters are non-ionic surfactants most commonly used in niosome preparations. In addition, other non-ionic surfactants that form vesicles are the following: alkyl polyglycosides, alkyl methylglucamides, alkyl polyglycerol ethers, alkyl polyethylene glycol polyglycerol ethers, alkyl sucrose esters, steroidal oxyethylene ethers, steroidal lariat ethers, dialkyl polyglyceryl ethers, dialkyl polyoxyethylene ethers, dialkyl oxyethylene glycerol ethers, and hexadecyl diglycerol (Florence, 1993). The properties of some of these commonly used surfactants are

as follows:

1.1. Polyoxyethylene alkyl ethers (Kibbe, 2000)

Polyoxyethylene alkyl ethers are non-ionic surfactants widely used in topical pharmaceutical formulations and cosmetics. PAE are a series of polyoxyethylene glycol ethers of n-alcohols (lauryl, myristyl, cetyl, and stearyl alcohol). It can be produced by the polyethoxylation of linear fatty alcohols. These products tend to be mixtures of polymers of slightly varying molecular weights, and the numbers used to describe polymer lengths are average values. The most common synonyms or trade names applicable to PAE are Brij® and Steareth®. For example, polyoxyl 10 stearyl ether has the other name of Brij®76 (POE-10) or Steareth®10. The structure formula is $CH_3 (CH_2)_x(OCH_2CH_2)_yOH$, where (x+1) is the number of carbon atoms in the alkyl chain and y is the number of ethylene oxide groups in the hydrophilic chain, typically 10-60. PAE are chemically stable in strongly acidic or alkaline conditions.

1.2. Long-chain carboxylic acid esters

In this long-chain carboxylic acid ester group, polyoxyethylene sorbitan fatty acid esters and sorbitan fatty acid esters are usually used in niosome preparation.

1.2.1. Polyoxyethylene sorbitan fatty acid esters

Polyoxyethylene sorbitan fatty acid esters are a series of fatty acid esters of sorbitol, its anhydrides copolymerized with approximately 20 moles of ethylene oxide for each mole of sorbitol and its anhydrides. Their synonyms are Tween® and polysorbate. These compounds are hydrophilic non-ionic surfactants used widely in cosmetics and food products. The most commonly used derivatives to prepare niosomes are Tween®20 and Tween®80 (Parthasarathi et al., 1994; Souza et al., 1997; Carafa et al., 1998; Murdan, Gregoriadis, and Florence, 1999; Ruckmani, Jayakar, and Ghosal, 2000). Polysorbates are stable to weak acids and bases. Gradual saponification occurs with strong acids and bases.

1.2.2. Sorbitan fatty acid esters

Sorbitan fatty acid esters are a series of mixtures of partial esters of sorbitol and its mono- and di-anhydrides with fatty acids. Sorbitan esters are widely used in

cosmetics, food products, and pharmaceutical formulations as lipophilic non-ionic surfactants (Roson, 1989; Kibbe, 2000). Their synonym is Span®. The commonly used ones in niosome preparation are Span®20, Span®40, Span®60, Span®80, and Span®85.

2. Cholesterol

CHO abolishes the gel to liquid crystalline phase transition of liposomal and niosomal systems. In liposomal systems, CHO has been shown to have a remarkable effect on the release of the entrapped solutes and the stability of the systems in vitro and in vivo. Similar effects are also seen with niosomes (Philippot and Schuber, 1995; Uchegbu and Vyas, 1998). CHO can reduce the fluidity of membranes above the phase transition temperature, with a corresponding reduction in permeability to aqueous solutes. Consequently, inclusion of CHO into unsaturated membranes is often essential in order to achieve sufficient stability. On the other hand, CHO increases the fluidity of membranes below the phase transition temperature. Its inclusion in saturated membranes, which are usually in the gel phase at ambient temperature, may result in a reduction in stability (Uchegbu and Vyas, 1998). CHO is usually included at a 1:1 molar ratio in most liposomal and niosomal formulations (Philippot and Schuber, 1995).

3. Other additives

Additives are often added to liposomal and niosomal systems to control the properties of the vesicles. Additives such as those giving steric and electrostatic stabilization can improve entrapment efficiency (EE) of the vesicles by preventing flocculation, and hence fusion, resulting in less leakage of the entrapped molecules. These additives also increase the entrapment of water-soluble substances by increasing the thickness of the aqueous layers between the lipid bilayers (Rose, Ribier, and Vanlerberghe, 1993).

Solulan®C24 is one of additive substances useful in the vesicle preparation for antiaggregation by steric hindrance. It was first produced by mixing CHO and ethylene oxide in a sealed vial for some time (Holmberg and Schick, 1998). This polyoxyethylene sterol is of high purity and has been of interest in the formulation of drug delivery systems, especially niosomes (Uchegbu and Vyas, 1998). Solulan®C24 has been used as a stabilizer

at a concentration of 5% by weight (Dimitrijevic et al., 1997).

Dicetylphosphate (DCP) is one of the charged lipids often used to improve stability of the vesicles by preventing their flocculation. It has been widely used in both liposomal and niosomal systems at a concentration of 5 % by weight (Uchegbu and Vyas, 1998).

Niosome preparation methods (Uchegbu and Vyas, 1998)

There are many methods available for niosome preparation. Most methods used for liposomes may be applied to niosomes. In addition, since non-ionic surfactants are heat stable, methods devoid of organic solvents are also possible. In brief, most methods consist of hydration of a mixture of the surfactants/lipids at elevated temperature followed by optional size reduction. The hydration temperatures are usually above the gel to liquid transition temperature of the system. In case of hydrophilic drugs, separation of the free drug molecules from the entrapped molecules is usually required. This can be done by various methods such as centrifugation, gel filtration, or dialysis. From a pharmaceutical point of view, this can be wasteful if the EE is low. For hydrophobic molecules, the amount of drug in the formulation is usually predetermined, without free drug left in the final preparation. Some of the preparation methods commonly used for niosomes are as follows:

1. Ether injection (Baillie et al., 1985)

An organic solution of surfactants/lipids is injected in an aqueous phase, which is heated above the boiling point of the organic solvent. The drug can be in either the organic phase or the aqueous phase, depending on the nature of the drug.

2. Film hydration method (Baillie et al., 1985)

A surfactant/lipid film is formed on a smooth surface by evaporation of an organic solution of the surfactants/lipids under vacuum. Hydrophobic drugs can be included in the film. The film is then hydrated, with appropriate agitation, with preheated aqueous phase. If the drug to be entrapped is hydrophilic, it can be dissolved in the aqueous phase prior to hydration. This method is particularly suitable for water-soluble drugs that are sensitive to organic solvents since direct contact with the organic solvent can be avoided.

3. Reverse phase evaporation

An oil-in-water emulsion is formed from an organic solution of surfactants/lipids and an aqueous phase. The organic solvent is evaporated under vacuum to yield niosome dispersion. Szoka and Papahadjopoulos first described this method for preparation of liposomes with high EE (Kiwada et al., 1985a).

4. Mixing of melted surfactants/lipids with the aqueous phase

Niosomes can be formed by injecting melted surfactants/lipids into a highly agitated preheated aqueous phase (Wallach and Philippot, 1993). Alternatively, the preheated aqueous phase can be added to the melted surfactants/lipids (Niemiec et al., 1994). In some instances, the warmed aqueous phase can be added to a mixture of the solid surfactants/lipids to form niosomes (Handjani-vila et al., 1979).

These simple mixing methods do not require the use of organic solvents. Formation of vesicles can be facilitated by applying some energy to the system. Sonication has been employed for this purpose (Florence, 1993).

5. Enzymatic conversion (Chopineau, Lesieur, and Ollivon, 1994).

Niosomes may be formed from a mixed micellar solution with the use of enzymes. Polyoxyethylene cholesteryl sebacetate diester (PCSD) can be cleaved by esterases to yield polyoxyethylene, sebacic acid, and CHO. CHO in combination with a non-ionic surfactant ($C_{16}G_2$) and DCP can subsequently form vesicles. The mixed micellar solution of PCSD/ $C_{16}G_2$/DCP was reported to convert into a niosomal suspension upon incubation with esterases.

6. The "bubble" method (Talsma et al., 1994)

This is another method where the use of organic solvents is avoided. In this method, a surfactant/lipid mixture is homogenized, followed by the bubbling of nitrogen gas through the mixture. The homogenization step may be omitted from the procedure if a longer bubbling time is allowed.

Reduction of niosome size

Niosomes prepared as described above are usually in the micron size range although some of the methods produce niosomes in the sub-micron (approximately 300 nm) size range. Reduction of vesicle size is often desirable since vesicle size is known to have an impact on biodistribution. Reduction in vesicle size may be achieved by a number of methods (Uchegbu and Vyas, 1998).

1. Probe sonication

This method has been shown to yield $C_{16}G_3$ niosomes in the 100-140 nm size range (Azmin et al., 1985; Baillie et al., 1985).

2. Extrusion through 100 nm Nuclepore® filters

This method has been applied to sodium stibogluconate $C_{10}G_3$ niosomes. The resultant preparation was in the 140 nm size range (Stafford, Baillie, and Florence, 1988).

3. Combination of sonication and filtration

When doxorubicin loaded Span®60 niosomes were processed by a combination of sonication and filtration through 200 nm Millipore® filters, the resultant vesicles were in the 200 nm size range (Uchegbu and Florence, 1995).

4. Use of a microfluidizer

The microfluidizer is a machine that pumps fluid at a very high pressure through a filter. The fluid is then forced along microchannels which then direct the two streams of fluid to collide at right-angles at a very high velocity in an interaction chamber (New, 1990). The fluid collected is reprocessed through the pump and the interaction chamber until vesicles of the required size are obtained. The size below 50 nm is possible with the use of a microfluidizer. The method has been proved to be efficient with $C_{16}G_3$/CHO/DCP niosomes (Uchegbu and Vyas, 1998).

5. High-pressure homogenization

This method has been shown to also yield vesicles of below 100 nm in diameter. It has been used successfully with doxorubicin loaded niosomes (Uchegbu and Vyas, 1998).

Separation of entrapped materials

The hydration of surfactant/lipid mixtures rarely leads to the entire drug being entrapped. This is especially true with water-soluble drug molecules. The methods that have been used for the removal of free drug molecules from the entrapped drug include exhaustive dialysis, separation by gel filtration, centrifugation, and ultracentrifugation (150,000xg for 1.5 h). All these methods have their advantages and disadvantages (see Table 1). The choice of method must take all these factors into account. From an industrial point of view, it may be more practical to concentrate efforts and resources on the achievement of high levels of drug loading to avoid these separation steps. Alternatively, the unentrapped drug may be designed to serve as a specific priming dose. The feasibility of this latter approach depends largely on pharmacology and therapeutic aspect of the drug.

Table 1. Advantages and disadvantages of the different methods used for the removal of the entrapped from the unentrapped drug (From Uchegu and Vyas, 1998)

Separation method	Advantage	Disadvantage
Exhaustive dialysis	-Suitable for large vesicles (> 10 μ) -Suitable for highly viscous systems -Inexpensive	-Extremely slow (5-24 h) -Large volumes of dialysate required (may not be suitable for drugs requiring specialized disposal) -Dilutes the niosomal dispersion
Centrifugation (below 7,000xg)	-Quick (~30 min) -Inexpensive -Concentrates the niosomal dispersion	-Fails to sediment the sub-micron niosomes -May lead to the destruction of fragile systems

Ultracentrifugation (150,000xg)	-Sediments all size populations -Concentrates the niosomal 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 Sepahrose®2B/4B for macromolecule separation) -Gels are expensive if not reused -Dilutes the niosomal dispersion -Not suitable for highly viscous formulations -Not suitable for formulation with a large particle size (>10-20 μm)

Factors governing self-assembly of non-ionic surfactants into niosomes (Uchegbu and Vyas, 1998).

1. Non-ionic surfactant structure

Vesicle formation can be predicted by the critical packing parameter (CPP) proposed by Israelachvili (Uchegbu and Vyas, 1998).

$$CPP = v/(l_c a_0)$$

Where v = hydrophobic group volume

l_c = the critical hydrophobic group length

a_0 = the area of the hydrophilic head group

A CPP between 0.5 and 1 indicates that the surfactant is likely to form vesicles. A CPP below 0.5 is expected to give spherical micelles, and a CPP above 1 should produce inverted micelles. The latter presumably occurs only in an oil phase; otherwise, precipitation would occur.

Another parameter like the hydrophile-lipophile balance (HLB) may also be a good indicator for vesicle formation in certain cases (Uchegbu and Vyas, 1998). An HLB number of 4-8 was found to be compatible with vesicle formation with the sorbitan (Span®) surfactants. Surfactants with high HLB numbers may also form vesicles, provided that other suitable membrane additives are present. For example, polysorbate 20 forms niosomes in the presence of CHO though the HLB number of this compound is as high as 16.7 (Santucci et al., 1996).

Non-ionic surfactants are composed of a hydrophilic head group and a hydrophobic tail. These molecules are amphiphilic in nature. Niosome formation requires at least the presence of an amphiphile and aqueous solvent. In certain cases, CHO is also required in forming niosome vesicles. Non-ionic surfactant molecules may possess one, two, or three alkyl chains. The two portions of the molecule may be linked via ether, amide, or ester bonds. The hydrophobic part may consist of one or two alkyl or perfluoroalkyl groups or a single steroidal group. The alkyl chain length usually contains 12 to 18 carbon atoms. Perfluoralkyl surfactants that can form vesicles may have chain lengths as short as 10 carbon atoms. Crown ether amphiphiles with a steroidal or C_{16} alkyl chain have been shown to form vesicles. On the other hand, there is a wide variety of the hydrophilic head group in vesicle-forming non-ionic surfactants.

2. Membrane additives

Various additives are often included in the formulation to form stable niosomes. Some surfactants do not naturally form vesicles unless an additive is added to the system. For example, the water soluble surfactant polysorbate 20 forms niosomes in the presence of CHO (Uchegbu and Vyas, 1998).

The most common additive for both liposomes and niosomes is CHO. Sterols are important constituents of most natural membranes. CHO by itself does not form bilayer structures, but it can be incorporated into membranes in very high concentrations. Incorporation of sterols into vesicular bilayers can bring about major changes in the properties of these membranes. The bilayer membrane is an ordered structure and may exist in the gel state or the liquid crystalline state, which is sometimes called the lamellar phase. The difference between these two phases is the degree of order of the membrane with the gel state being the most ordered state. In the liquid crystalline state, there is lateral diffusion of bilayer materials, whereas in the gel state the alkyl chains are crystallized or otherwise less mobile. The liquid crystalline state exists at a temperature above the phase transition temperature of the surfactants/lipids. CHO is known to abolish the gel to liquid phase transition of liposomes and niosomes, usually resulting in vesicles that are less leaky. It is thus included in a 1:1 molar ratio in most formulations. To prevent the vesicles from aggregation, a charged molecule, such as DCP or stearylamine, is often added to the bilayer of the vesicles. The quantity of these non vesicle-forming additives must be carefully adjusted. Otherwise, structures other than liposomes or niosomes may result (Uchegbu and Vyas, 1998).

3. Nature of the entrapped molecule

The nature of the entrapped drug may affect vesicle formation ability of non-ionic surfactants. Hydrophobic or amphiphilic drugs can affect the CPP value of the system since these molecules will be incorporated into the vesicle membrane. Sorbitan monostearate (Span®60) niosomes containing DCP formed homogenous dispersion when the entrapped chemical was a water-soluble dye, carboxyfluorescein. However, the same niosomal system formed an aggregated dispersion with an amphipathic drug, doxorubicin (Uchegbu, 1994). Doxorubicin is thought to be incorporated in the vesicle membrane since it has been shown to alter the electrophoretic mobility of hexadecyl diglycerol ether ($C_{16}G_2$) niosomes in a pH dependent manner (Cable, 1989).

4. Surfactant and lipid levels (Uchegbu and Vyas, 1998)

The level of surfactants/lipids used to make niosomal dispersions is generally 10-30

mM (1-2.5 % w/w). Altering the surfactant to water ratio during the hydration step may affect microstructure and hence properties of the system. As mentioned earlier, highly viscous systems may result if the level of surfactants/lipids is too high.

5. Temperature of hydration (Uchegbu and Vyas, 1998)

As a rule, the hydrating temperatures used to make liposomes or niosomes should be above the gel to liquid phase transition temperature of the system.

Entrapment efficiency

(Namdeo and Jain, 1996; Urchegbu and Vyas, 1998; Namdeo and Jain, 1999; Gaikwad et al., 2000)

Entrapment efficiency describes drug loading in niosomes and is thus crucial in application of niosomes as delivery systems. There are several ways of describing drug loading in liposomes and niosomes. For example, drug loading can be described as the percentage of drug entrapped. In this case, the initial drug to surfactant/lipid ratio must be specified. Another way to describe drug loading is the volume of drug solution, usually in liter, entrapped per mole of surfactants/lipids. This method assumes no change in the concentration of the drug in the aqueous phase throughout the process of preparation and analysis steps. The ratio of drug to surfactant/lipid by weight (g/g) or on molar basis (mol/mol) can also be used to describe drug loading. This latter term gives adequate information on the level of excipient that must be concomitantly administered at each dose level of the drug. It is probably the most useful description to formulators.

As mentioned earlier, many factors may govern the vesicle formation and drug entrapment in vesicular carriers. These factors may also interact and thus making systematic prediction of vesicle forming behavior very complicated. It is envisaged that an Expert System will emerge that will hold details on the optimum conditions for vesicle formation, predicted vesicle size, drug-loading capability, and expected pharmacology (Uchegbu and Vyas, 1998). Meanwhile, studies involving niosome formation and drug entrapment in niosomes are accumulating.

The chemical nature of the niosomal membrane may be manipulated to increase drug loading. This can be achieved by altering the nature of either the hydrophilic head group or the hydrophobic part of the molecule. For example, the inclusion of DCP in C_{16} glucoside niosomes was found to increase the entrapment of [^{14}C]-sucrose (Kiwada, Niimura, Kato, 1985b). The entrapment of doxorubicin N-(2-hydroxypropyl) methacrylamide copolymer (pK1) in $C_{16}G_2$ niosomes was twice as much as that in hexadecyl poly-5-oxyethylene niosomes under identical conditions (Uchegbu, and Duncan, 1997). The increased entrapment was thought to be due to the difference in fluidity between the two types of niosomal membrane. In general, the less fluid the membrane bilayer is, the higher the EE is achieved. The surfactant with higher phase transition temperature usually forms less leaky vesicles, and thus resulting in higher EE. Similarly, unsaturation in the alkyl side chain can lead to a more leaky membrane and lower drug loading. Namdeo and Jain (1999) also reported that entrapment of 5-fluorouracil (5-FU) in niosomes prepared by film hydration method varied with vesicle membrane composition. They found that for Span®40 niosomes, inclusion of CHO or DCP into the bilayer membrane reduced 5-FU loading in the vesicles. In another study, niosomes prepared from Tween®80, Span®60, or Tween®20 gave comparable cytarabine hydrochloride entrapment, whereas a much lower entrapment was seen with Span®80 (Ruckmani et al., 2000). However, Rogerson, Cummings, and Florence (1987) studied entrapment of adriamycin in niosomes and found that the EE of the drug was apparently independent of vesicle composition and method of preparation.

Inclusion of CHO may alter the physical structure of niosomes as well as the EE of the system (Arunothayanun et al., 2000). Polyhedral niosomes were formed from a system containing $C_{16}G_2$ and Solulan®C24. When CHO was added to the system, spherical vesicles mixed with tubular structures resulted. The polyhedral systems were reported to have a larger particle size and higher EE.

The method of drug loading can also alter EE of vesicular drug carriers. Various techniques may be used to optimize drug loading. The dehydration-rehydration vesicle technique described by Kirby and Gregoriadis (1984) dramatically increased the EE of PK1 in $C_{16}G_2$ niosomes (Uchegbu and Duncan, 1997). Other methods that have been reported to

increase drug loading in vesicular carriers include the use of pH gradients and ammonium sulfate to trap the drugs inside the vesicles (Haran et al., 1993). This intravesicular trap has been used successfully with doxorubicin Span®60 niosomes (Uchegbu and Florence, 1995). Luteinizing hormone releasing hormone displayed high entrapment in polyhedral niosomes when the remote-loading methods using pH or ammonium sulfate gradients were applied. This was in contrast with the other methods used, including the direct hydration at pH 7.4 or pH 4.3, dehydration-rehydration, and reverse phase evaporation (Arunothayanun et al., 1998).

The nature of the drug to be entrapped also governs the apparent EE in niosomes. It is challenging to predict drug loading in niosomal preparations since interaction between the drug and the membrane can exist in various manners. For hydrophobic drugs, lipid packing seems to be a major determinant. On the other hand, ionic interaction seems to play a major role for ionizable drugs (Philippot and Schuber, 1995). The extent of drug entrapment varies between drugs and membrane compositions as well as methods of preparation. All these factors have to be simultaneously considered. When pentoxifylline niosomes were prepared by lipid film hydration method, the EE was only 9.4% (Gaikwad et al., 2000). On the other hand, rifampicin niosomes prepared by the same technique had an EE of 30.5% (Kamath et al., 2000). As mentioned earlier, it is often difficult to compare results from different laboratories, especially when percent EE are reported.

In a recent study, various combinations of Span® and Tween® surfactants were used to optimize entrapment of all-trans retinoic acid in niosomes. Inclusion of a positively charged lipid, stearylamine, gave high EE due to the anionic nature of the drug (Desai and Finlay, 2002). The EE of the unionizable tretinoin in niosomes prepared from Span®40, Span®60, Brij®30 or Triton®CG 110 with CHO and DCP were invariably very high, especially for MLVs and extruded vesicles when compared to those of SUVs (Manconi et al., 2002). In addition, EE of Span®40 SUVs was much lower than that of Span®60 SUVs. The authors suggested that this finding could be due partly to the difference in the vesicle size between the two types of SUVs since Span®40 SUVs were smaller than Span®60 counterparts. For an ionizable drug, chemical form of the drug (acidic, basic, or salt form), vesicle composition,

and environment pH conditions all seem to be major determinants. This has been shown with lidocaine niosomes. Lidocaine has a pKa of 7.86. At pH 5.5, most of lidocaine is positively charged. At pH 8.6, most of the drug is uncharged. Niosomes were prepared with various compositions. In niosomes prepared from Tween®20 and CHO, amount of lidocaine entrapped at pH 8.6 was negligible for all formulations, whereas the best EE was obtained at pH 5.5 (Carafa, Santucci, and Lucania, 2002). For charged vesicles containing DCP or N-cetylpyridinium chloride, however, remarkably low EE was obtained in all cases, even at pH 5.5 regardless of the chemical form of lidocaine.

Niosomes as topical drug delivery carriers

As with liposomes, niosomes have been studied as potential carriers for topical skin delivery. Estradiol permeation from n-alkyl polyoxyethylene ether niosomes was studied (Hofland et al., 1994). It was concluded that direct contact between liquid-state MLVs niosome formulations and skin was imperative to exert the highest effect on drug transport. Niemiec et al. (1995) reported that non-ionic vesicles enhanced the topical delivery of peptide drugs into pilosebaceous units in the hamster ear model. Other drugs studied include flurbiprofen (Reddy and Udupa, 1993), erythromycin (Jayaramann, Ramachandran, and Weiner, 1996), cimetidine (Lieb, Flynn, and Weiner, 1994), α-interferon, and cyclosporin-A (Waranuch, Ramachandran, and Weiner, 1998). In conclusion, the effects of niosomes on drug transport through the skin can be either impairment or enhancement, depending on the drug and the vesicle composition. Thus, niosomes appear to have potential as a drug carrier system for both dermal and transdermal delivery of drugs (Hofland et al., 1994).

Niosome stability

A stable niosomal dispersion must exhibit a constant particle size and a constant level of entrapped drug. No precipitation of any of the membrane components should be observed in the preparation. There are many methods to stabilize niosomes. Incorporation of CHO into niosomal systems can decrease the leakiness of the membrane. The addition of polymerized surfactants to the formulation, the use of membrane spanning lipids, and the

interfacial polymerization of surfactant monomers in situ have been used to stabilize nisomes. The inclusion of a charged molecule in the bilayer also prevents niosome aggregation (Uchegbu and Vyas, 1998).

Hydrated bilayer systems such as liposomes and niosomes are not thermodynamically stable. To produce a system with sufficient stability requires that the transformation be slowed down to produce a product with a reasonable shelf life. Decreasing the air-water interface may prevent crystallization of the surfactant monomers (Engberts and Hoekstra, 1995). This can be one measure to increase stability of the system.

The choice of membrane surfactant determines the nature of the membrane and thus also affects stability of the system. Leakage of carboxyfluorescein from Span® surfactant niosomes was found to be in the order of Span®80 < Span®20 < Span®40 < Span®60 (Yoshioka, Strenberg, and Florence, 1994), which was determined by the degree of fluidity of the membrane.

The encapsulated drug could be the major determinant of niosome stability in terms of drug leakage. The drug polymer conjugate, PK1, remained entrapped within the vesicles for a relatively long period of time. The entrapped polymer was thought to lead to a more stable system since the membrane was sufficiently impermeable to the macromolecule (Gianasi et al., 1997).

The physical nature of the entrapped drug also affects stability of the system. Doxorubicin loaded vesicles using an ammonium sulfate gradient led to gel formation within the vesicles, resulting in less leaky niosomes (Haran et al., 1993).

The temperature of storage may affect niosome stability. Storage temperature must then be controlled. Changes in the temperature of the system often leads to a change in fundamental nature of the system, including solute leakage. Phospholipid-based liposomes are known to be most leaky at their phase transition temperatures (Tanaka, 1990).

Other additives in the final formulation may also affect stability of niosomes. High concentrations of detergents (soluble surfactants) are incompatible with niosomal systems. Eventually, they can cause solubilization of the vesicles to form mixed micellles. The destruction of $C_{16}G_2$ niosomes by octyl glucoside appears to proceed via the build up of a

localized concentration of the detergent within the niosome membrane followed by micellization of the system (Saras et al., 1994). Similarly, $C_{16}G_2$ niosomes were solubilized by Solulan®C24 and converted into mixed micelles (Uchegbu, Bouwstra, and Florence, 1992).

Toxicity studies of niosomes

In vitro studies on a ciliotoxicity model to estimate the toxicity of alkyl polyoxyethylene (C_nEO_x) niosomes on the nasal mucosa revealed that an increase in alkyl chain length was accompanied by a decrease in toxicity, whereas an increase in the polyoxyethylene chain length caused an increase in ciliotoxicity. In general an increase in chain length increases the gel to liquid transition while an increase in the length of the polyoxyethylene chain decrease the gel to liquid phase transition. This study concluded that gel state niosomes were less ciliotoxic than the liquid state vesicles. In addition, the more hydrophobic compounds seemed less toxic (Hofland et al., 1992).

Neither the length of the polyoxyethylene chain or the alkyl chain had any influence on the skin toxicity of alkyl polyoxyethylene niosomes as assessed by cell proliferation of human keratinocytes in vitro (Hofland et al., 1991, 1992). However, the nature of the linkage in the surfactant molecule was a determining factor in this model, and the more labile ester bond was found to be more toxic than the ether bond.

Haemocompatibility studies are often used to evaluate toxicity of parenteral preparations. $C_{16}G_2$ and Span®60 niosomes containing 10 mol% Solulan®C24 caused less than 5% haemolysis of rat erythrocytes after 5 hours of incubation. This level of haemolysis is not considered significant since both $C_{16}G_2$ and Span®60 niosomes were rapidly cleared from the plasma after dosing (Uchegbu and Duncan, 1997). Solulan®C24 was found to be toxic to Caco-2 cells in vitro. However, when incorporated into niosomes at 10 mol%, its toxicity was dramatically reduced. There was an increase in toxicity when the level of Solulan®C24 in niosomes was increased above 10 mol%. At above 10 mol%, Solulan®C24 is not completely incorporated into the membrane of $C_{16}G_2$ niosomes and is thus present in solution as monomers or micelles (Uchegbu and Vyas, 1998).

Minoxidil

Minoxidil (2, 4-diamino-6-piperidinopyrimidine-3-oxide), a pyrimidine derivative, was the first drug to become available for treating scalp hair loss. The mechanisms by which minoxidil promotes hair growth are still not fully understood. Some proposed mechanisms are the following:

1. Minoxidil, metabolized to minoxidil sulfate in 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. Minoxodil up-regulates the expression of vascular endothelial growth factor and its receptor, an action which subsequently stimulates angiogenesis and anagen.

3. Minoxidil is a potent activator of prostaglandin endoperoxide synthase-1. This enzyme is 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 perifollicular fibrocytic cells.

These physiological changes result in prolongation of anagen and the conversion of vellus hair to terminal hair. In clinical trials, application of 2% minoxidil solution twice daily over one year produced moderate to dense regrowth in approximately 30 to 35% of patients (Meidan and Touitou, 2001).

The product is currently available as a solution containing 2% minoxidil. The formulation is composed of 60% ethanol, 20% propylene glycol, and 20% water. A higher concentration (5% minoxidil) is also available from the innovator. 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. According to the Upjohn company, liposomes-based minoxidil accumulates over time in the deeper skin strata, targets the sebaceous glands, and provides a constant drug delivery to the hair roots. Lipoxidil® is claimed to be 10 times more effective than regular 2% or 5% minoxidil in reaching the subcutaneous area. Retin-A liposomes have been added to minoxidil liposomes under the trade name of Lipoxidil®Plus. The product is produced by a European pharmacy with ingredients from a well-known pharmaceutical company and

shipped directly to customers worldwide, the cost being $ 49.95 per 50 ml (The Revivogen Shop-Hairloss Pharmacy, 2001).