CHAPTER II

LITERATURE REVIEW

1. Lipid-based vesicles

Drug delivery systems using lipid-based vesicles such as phospholipid vesicles (liposomes) (Couvreur et al., 1991) or nonionic surfactant vesicles (niosomes) Schreier and Bouwstra, 1994) formed from the self-assembly of phospholipids or nonionic surfactants in aqueous media resulted in closed bilayer structures. These vesicles are characterized by the number of bilayer they contain. Multilamellar vesicles (MLVs) consist of a few to a large number of lipid bilayers, whereas unilamellar vesicles (ULVs) consist of a single bilayer. Liposomes and niosomes composed of hydrophobic parts of the molecule are shielded from the aqueous solvent and the hydrophilic head groups. Therefore, these vesicles are capable to dissolve both hydrophobic and hydrophilic substances by incorporating soluble substances into aqueous compartments and incorporating insoluble substances into hydrophobic domains. Amphiphilic substances are also solubilized in this system by partitioning substance molecules in lipid-aqueous interface of bilayer structure. The bilayer structure of a niosomes was shown in **Figure 1.**

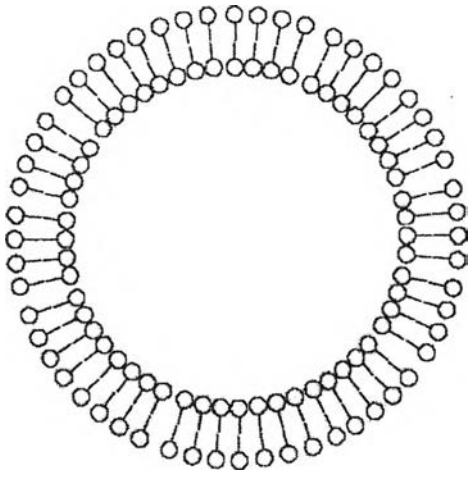


Figure 1. Schematic representation of a niosome (Uchegbu and Vyas, 1998)

2. Association structures and packing

The self-assembly of amphiphilic molecule (phospholipid or nonionic surfactant) contain hydrophobic part, which is generally readily soluble in oil but sparingly soluble or insoluble in water, and hydrophilic part which is sparingly soluble or insoluble in oil but readily soluble in water. However, ampliphiles may associate to form a range of different structures, including rods, lamellae, and bicontinuous interconnected structures (Malmsten, 2002).

Type of association structures formed in surfactant systems depends on a number of parameters, including surfactant structure, composition of the system, temperature and presence of salt, oil and cosolutes. A schematic illustration of different liquid crystalline structures is given in **Figure 2.**

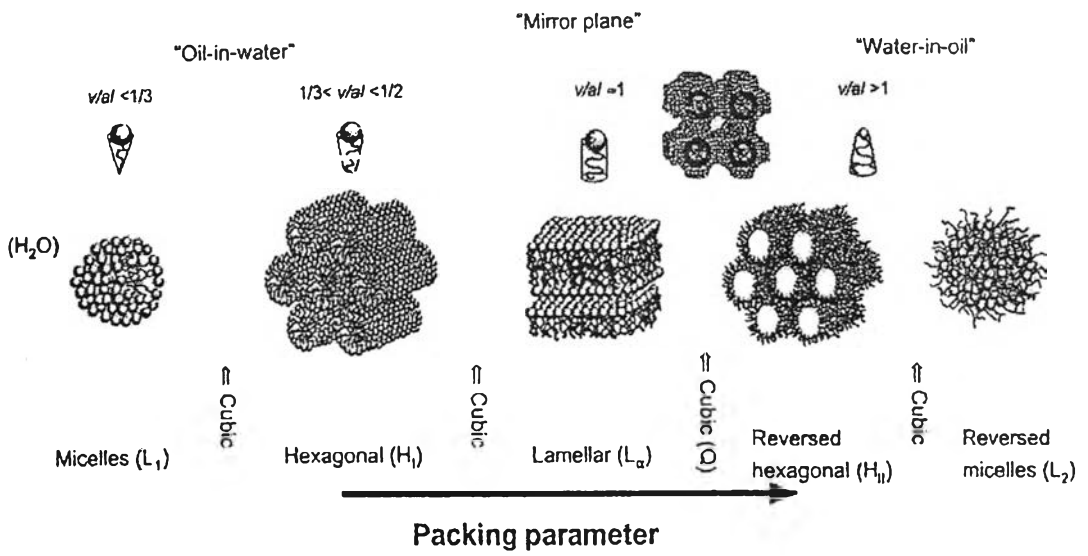


Figure 2. Schematic illustration of association structures formed in surfactant, and the packing of surfactant molecules in different association structures (Jonsson et al., 1998)

In order to understand the formation of these structures, it is helpful to consider the packing properties of amphiphile molecules in the different structures. The critical packing parameter (CPP) laid own by Israelachvili (1992) is defined as a following equation:

$$\text{CPP} = \frac{v}{a \cdot l} \qquad (1)$$

Where v is the volume of the hydrophobic tail(s), a is the polar head group area, and l is the length of the hydrophobic chain(s) of the surfactant **(Figure 3)**

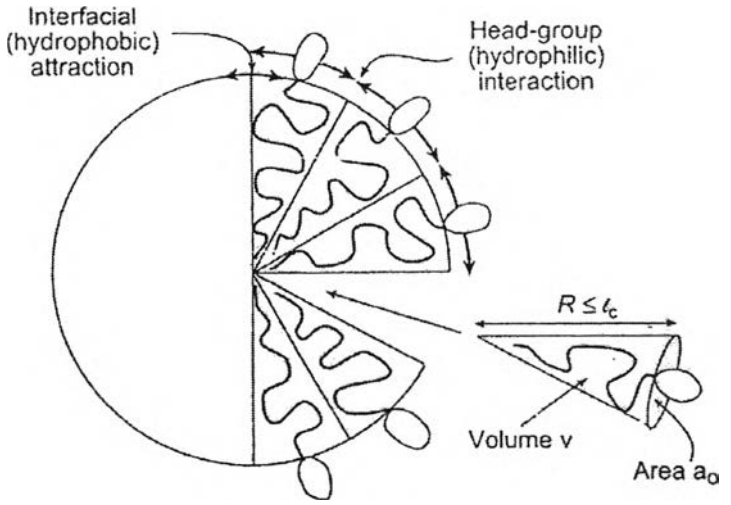


Figure 3. Schematic illustration of the parameters defining the critical packing parameter (Israelachvili, 1992)

As can be seen in **Figure 2**, there is a direct correlation between the value of the CPP and the type of aggregate formed. For example, a calculated value of CPP 1 the amphiphile molecules assemble preferentially to a lamellar phase. While, the spherical micelle would form with CPP value less than 1/3.

3. Method of preparation

The formation of vesicular assemblies usually requires the input of some form of energy (Lasic, 1990). All the experimental methods surveyed consist of the hydration of a mixture of the surfactant/lipid at elevated temperature maybe followed by optional size reduction to obtain a colloidal dispersion. Similar to the liposome types, the types of niosomes is largely divided into 3 classes, large unilamellar vesicles (LUVs), small unilamellar vesicles (SUVs) and multilamellar vesicles (MLVs) **(Table1)**.

Table 1. The types and features of niosomes (Schmid, 1994)

Type of vesicle	Size (μm)	Features of niosomes
Multilamellar vesicles (MLVs)	0.05-10	Large retention volume, good stability
Small unilamellar vesicles (SUVs)	0.025-0.05	Uniform size and shape, small retention volume, easy refusion
Large unilamellar vesicles (LUVs)	> 0.1	Large retention volume, not uniform size

Firstly, MLVs are the simplest type of niosomes to be prepared by hand-shaking method. The internal space of multilamellar vesicle is occupied by lipid bilayers separated by aqueous layers. Secondly, SUVs come from MLVs or LUVs reduced the size of vesicles by sonication or extrusion. The vesicles contain only one lipid layer and one aqueous layer. Thirdly, LUVs contain aqueous compartment at the core of vesicle with the large volume, so they are suitable for carrying hydrophilic drugs.

Liposomes and niosomes can be produced by various methods due to the different features of lamellarity. There are 5 methods of preparation as following:

3.1 Sonication method (Rental et al., 1999)

The lipophilic components are dissolved in non-polar solvent, such as a mixture of chloroform and methanol. The solvent is evaporated overnight under vacuum, resulting a surfactant film. The film is then hydrated with aqueous solution and the mixture is then sonicated at 80°C. The method results in the formation of both unilamellar and multilamellar vesicles.

3.2 Ether injection method (Baillie et al., 1986)

The oil-soluble components are dissolved in diethyl ether and injected slowly into aqueous phase at 60°C. The method results in the formation of mainly multilamellar vesicles.

3.3 Hand-shaking method (Baillie et al, 1985)

Lipid/surfactants are dissolved in diethyl ether and ether. The solvents then are evaporated under vacuum. The dried surfactant film is hydrated with aqueous phase at 50-60°C with gentle agitation. The resulting multilamellar vesicles are formed.

3.4 Dehydration-rehydration method (Arunothayanun et al, 1999a)

The surfactant film of non polar ingredients is hydrated with aqueous solution at 55°C. The vesicles was then freeze-dried overnight and rehydrated with aqueous medium. This method results in the formation of small multilamellar vesicles.

3.5 Reversed phase evaporation method (Arunothayanun et al, 1999a)

The lipophilic components are dissolved in non-polar solvent, such as a mixture of chloroform and diethyl ether. Then the aqueous phase is injected into the organic phase. The mixture is sonicated and the organic solvent is vacuum evaporated. The unilamellar vesicles are mainly formed by this method.

Hand-shaking method is selected to prepare niosomes in this research. This method produces multilamellar vesicles which are most widely used, due to their simplicity and reproducibility in preparation (Namdeo and Jain, 1999). They reported

that niosomes prepared by hand-shaking method had the least permeability compared to reversed phase evaporation method and ether injection method.

4. Characterization of vesicles

4.1 Determination of particle size and size distribution

Particle size and size distribution of vesicles can be characterized by various methods as following:

4.1.1 Microscopic method

Light microscopy

This technique is widely used for observing size and size distribution by directly look at particles. Niosomes larger than 0.5 μm are able to be seen by light microscope. The existence of unentrapped drug in niosomal dispersion can be confirmed by this method.

Electron microscopy

Electron microscopy technique provides precise analysis of vesicle size, but size distribution of niosomes could not be completely measured. This technique is able to observe the lamellarity of niosomes.

At present, there are some instruments using this technique i.e. a transmission electron a microscope, a scanning electron microscope, a cryo-scanning electron microscope and a freeze fracture electron microscope.

4.1.2 Photon correlation spectroscopy

Photon correlation spectroscopy, the analysis of the time-dependence of intensity fluctuations in scattered laser light due to the Brownian motion of particle has been utilized in measuring particle size in the range of 1nm-1μm. The particle size is calculated based on the assumption of spherical particles.

This technique is also capable to measure zeta potential value of the charged particles. The velocity of moving charged particles in electric field is detected and computed as zeta potential value.

4.1.3 Laser diffraction

Laser diffraction, called low angle laser light scattering, relies on the fact that diffraction angle is inversely proportional to particle size. Mie theory is used to completely solve the equation for interaction of light with matter. The particle size is measured based on the assumption of spherical particles. The applicable range of particle size to this method is 0.2-2000 μm.

4.2 Determination of Entrapment efficiency

To determine the amount of entrapped drug, expressed in terms of entrapment efficiency, niosome dispersion will be separated for the unentrapped drug from the entrapped drug. Drug loading value expressed as the ratio of drug to surfactant in the final formulation in ($g \times g^{-1}$) or ($mol \times mol^{-1}$) gives adequate information on the level of excipient that must be administered at each dose level.

The amount of drug entrapped in niosomes is obtained by separating free drug and entrapped drug by removal of unentrapped drug by following methods:

4.2.1 Exhausive dialysis

The unentrapped drug is removed from niosome suspension by placing the niosomal dispersion in dialysis tubing. It is exhaustively dialyzed against mobile phase. The vesicles are disrupted with organic solvent in order to measure the amount of entrapped drug.

This method is suitable for highly viscous niosomal system and the system with a large vesicle size of more than 10 μm. The niosomal suspension may be diluted by mobile phase (Baillie et al., 1985, 1986).

4.2.2 Gel filtration

The unentrapped drug in niosomal suspension is separated by size exclusion chromatography on sephadex G-50. The entrapment efficiency is determined by dissolving niosomes obtained from gel chromatography with Triton X-100.

Though, this method takes short time to remove unentrapped drug, the niosomal dispersion may be diluted by mobile phase. The highly viscous formulation is not suitable for this method (Pillai and Salim, 1999).

4.2.3 Centrifugation

Niosomal suspension is centrifuged at 7000×g for 30 min. The supernatant is drawn and the niosomes forming the pellet are disrupted to analyze the amount of entrapped drug.

This method used to concentrate the niosomal dispersion, may fail to sediment the sub-micron niosomes and lead to the destruction of fragile systems (Rogerson et al., 1987).

4.2.4 Ultracentrifugation

Niosomal suspension is centrifuged at 150000×g for 1.5 h. The supernatant is drawn and the niosomes forming the pellet are disrupted to analyze the amount of entrapped drug.

Although ultracentrifugation method can sediment all size of niosome populations, it may destroy fragile systems and lead to the formation of aggregates (Duncan et al., 1997).

In this research, ultracentrifugation was utilized for investigating the amount of entrapped drug in terms of entrapment efficiency. This method separated whole niosomal pellets from dispersing media. Therefore, the actual amount of entrapped drug could be obtained.

5. Advantages and disadvantages of vesicular systems

Phospholipid vesicles (liposomes) prepared with a variety of phospholipids were introduced in 1965 and have been intensively studied as drug carriers and drug delivery. Liposomes have been investigated for their potential application in pharmaceutics such as drug delivery (Gregoriadis et al., 1993 ; Iwanaga et al., 1999) for drug targeting (Booser et al., 1994), for controlled release (Barber et al., 1993), for increasing solubility (Gregoriadis et al., 1996) and for promoting uptake of entrapped drugs into target site while drug toxicity is diminished (Mayer et al., 1990). However, their widespread use of liposomes is still constrained by higher cost and intrinsic chemical instability of phospholipids which is caused by hydrolysis (Frfkjaer et al., 1984) and oxidation (Gebicki et al., 2000). Therefore, niosomes are now widely

studied as an alternative to liposomes because of the lower cost, greater stability and resultant ease of storage nonionic surfactants (Ruckmani et al, 2000).

6. Niosomes

Nonionic surfactant based vesicles (niosomes) have presented as the alternatives to liposomes. Niosomes have been investigated for drug delivery through the most common routes of administration, such as intramuscular (Arunothayanun et al., 1999a), intravenous (Pillai and Salim, 1999), subcutaneous (Oommen et al., 1999 ; Medda et al, 1999), ocular (Saettone et al., 1996), oral (Rentel et al., 1999 ; Varshosaz et al., 2003) and transdermal (Shahiwala and Misra, 2002 ; Fang et al., 2001; Reddy and Udupa, 1993).

Niosomes are the assemblages of non-ionic amphiphiles into closed bilayer structures. In general, surfactants are classified according to their polar head group, i.e. surfactants with negatively charged head group are referred to as anionic surfactants, whereas cationic surfactants contain polar head groups with a positive charge. Zwitterionic surfactants contain both a negatively charged and positively charged group whereas uncharged surfactants are generally referred to as nonionic. The later surfactants, the ones used most frequently in drug delivery applications, are used to prepare niosomes.

6.1 Formation of Niosomes

Non-ionic surfactant based vesicles (niosomes) are formed from the self-assembly of the nonionic amphiphiles in aqueous media resulting in closed bilayer structure usually with input of energy such as physical agitation or heat (Uchegbu, 1998). This results in an assembly in which the hydrophobic parts of the amphiphilic molecules are shielded from the aqueous medium and the hydrophilic head groups enjoy maximum contact with the same. These structures are analogous to phospholipid vesicles (liposomes) and are able to encapsulate both hydrophilic and hydrophobic solutes and surved as drug carriers. The lower cost, greater stability and

resultant ease of storage nonionic surfactants (Florence, 1993) has leaded to the exploitation of nonionic surfactants as alternatives to phospholipids.

The versatility of synthetic nonionic surfactant as vesicles formers allows the exploration of new structures and forms, from disc-shapes systems to multiple vesicles. These vesicles might have a special role in drug delivery system, gather as reservoirs for topical delivery or for parenteral administration (Florence, 1993).

There are many physical natures of niosomes such as shape, size, lamellarity, phase transition temperature, state of bilayer and entrapment. The ultimate identity of any niosomal system and hence its properties are determined by many factors including type of nonionic surfactant, nature of membrane additives, preparation method, hydration temperature and others (Uchegbu and Florence, 1995). These factors influence the physical natures of niosomes will be discussed in the following (section 6.2). It is very important that all these variables must be carefully controlled in the design of a niosomal drug delivery system.

6.2 Factors governing the self-assembly of nonionic surfactants into niosomes

6.2.1 Nonionic surfactant structure

Theoretically, niosome formation requires the presence of particular class of amphiphile. Amphiphiles possess various kinds of hydrophilic head group and hydrophobic tail as shown in **Figure 4** and **Figure 5.** The amphiphilic molecules may possess one or two alkyl chains. The alkyl group chain length usually ranges from C_{12}-C_{18} (Arunothayanun et al., 1999b; Yoshida et al., 1992). The two portions of the molecule may be linked via ether, amide or ester bonds.

A hydrophilic lipophilic balance (HLB) is a good indicator for the ability of vesicle forming amphiphiles. A HLB number of 8.6 was found to be compatible with formation of sorbitan monooleate (Span®80) niosomes with the highest entrapment, whereas a HLB number between 14-17 did not allow niosome formation (Shahiwala and Misra, 2002).

Nonionic surfactants are categorized in to 3 groups as the following: (Rieger, 1988)

1.1 Nonionic esters

The nonionic ester surfactants are usually derived from fatty acids by esterification with polyhydric alcohols or polyethylene glycol. These fatty acid esters are relative stable under neutral conditions but can be hydrolyzed in acidic or alkaline media. Examples of this group are ethylene glycol ethers, propylene glycol esters, glycerol esters, polyglycerol esters, sorbitan esters, sucrose esters, ethoxylated esters.

1.2 Nonionic ethers

Nonionic ethers are generally derived from polyethylene glycol or polypropylene glycol. Examples of this group are as the followings:

Fatty alcohol ethoxylates (polyoxyethylene ethers)

: This fatty alcohol ethoxylates can resist to chemical reaction, concentrated acidic/ basidic condition

Propoxylated alcohols

: The propoxylated alcohol group has the hydrophobic property resulted low HLB value.

Ethoxylated/ Propoxylated block polymers

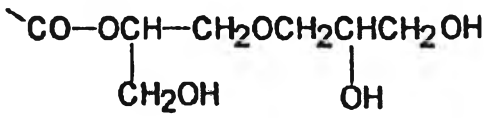
: These surfactants, known as poloxamers, possess a wide range of hydrophobic and hydrophilic properties, depending on the ratio of the building blocks. They are widely used as emulsifiers and solubilizers.

1.3 Nonionic amide

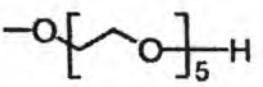
The amides prepared from the reaction of a fatty acid or fatty acid esters with mono-or diethanolamine. Nonionic amides are not common used in pharmaceuticals, they widely used for cleansing products.

According to fatty alcohol ethoxylates (polyoxyethylene ethers) are such a kind of surfactants which can resist to chemical reaction with concentrated acidic or

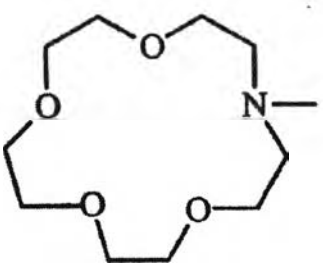
basidic condition. The property of this surfactant group is suitable for studying the feasibility of niosomes as oral drug delivery. Polyoxyethylene alkyl ethers are selected to study the feasibility to form niosomes in 0.1N hydrochloric acid and phosphate buffer pH 6.8 which are similar to the physiological pH.



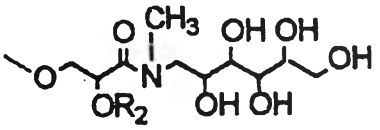
a. Glycerol head group



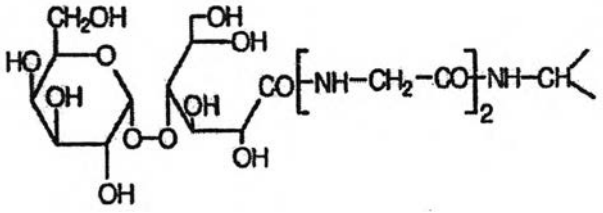
b. Ethylene Oxide head group



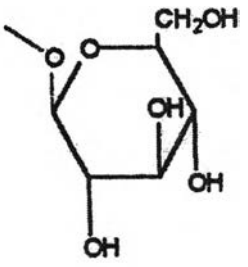
c. Crown ether head group



d. Polyhydroxyl head group

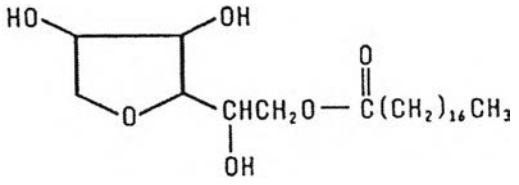


e. Sugar head group + amino acid

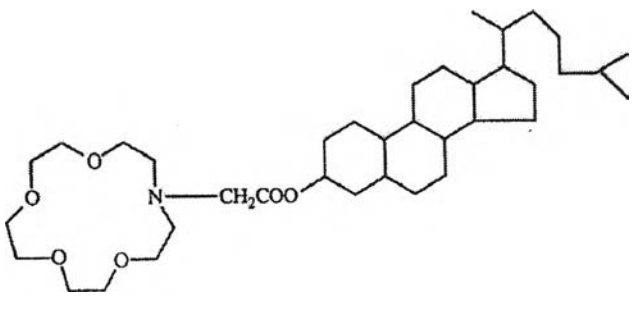


f. Sugar head group

Figure 4. Hydrophilic head groups found in vesicle forming amphiphiles (Uchegbu and Vyas, 1998)



a. C_{18} alkyl chain.



b. Single steroidal group.

Figure 5. Hydrophobic tail found in vesicle forming amphiphiles (Uchegbu and Vyas, 1998)

6.2.2 Membrane additives

Various additives must often be included in the formulation in order to obtain stable niosomes. The most common addition found in niosomal systems is cholesterol. It is known to abolish to gel to the liquid phase transition of liposomal and niosomal systems resulting in the systems that are less leaky and stable (Rogerson et al., 1987). Therefore, cholesterol is usually included in 1:1 molar ratio in most formulations (Uchegbu, 1998).

However even after the addition of cholesterol, the intrinsic phase transition behavior of vesicle forming surfactants still influences the properties of the dispersions: notably the membrane permeability, encapsulation efficiency bilayer rigidity.

The stable niosome dispersions have to exhibit a constant particle size and a constant level of entrapped drug. There must be no precipitation of the membrane components, which are to a large extent not insoluble in aqueous media.

Normally, niosomes are stabilized by the addition of stabilizers, steric stabilizers and electrostatic stabilizers. The steric stabilizers, polyoxyethylene derivatives, are used to prevent the niosome aggregation by providing stealth properties, for example, Solulan®C-24 (poly-24-oxyethylene cholesteryl ether) (Uchegbu et al., 1996) and polyethylene glycol 2000 (Iwanaga, et al., 1999). An electrostatic stabilizer, a charged molecule included in the bilayer, such as dicetyl phosphate (Yoshida et al., 1992) and stearylamine (Erdogan et al., 1996), is added to the formulation to ensure a homogeneous formulation devoid of aggregates by electrostatic repulsion.

In this study, polyoxyethylene stearate (Simulsol®M52), the polyethylene glycol ester of stearic acid, was introduced to the formulations to function as a stearic stabilizer.

6.2.3 Nature of encapsulated drugs

Niosomes encapsulating hydrophobic drugs and macromolecules were more stable than ones encapsulating low molecular weight drugs. Also, they decrease the leakage of niosomes (Uchegbu, 1998). In contrast, hydrophilic drug can easily leak from niosomes and decrease the stability of niosome dispersion. Amphiphilic drug can increase the drug encapsulation. The macromolecular drug could decrease leakage and increase stability of niosome dispersion. The effect of the nature of encapsulated drugs on niosome properties are illustrates in **Figure 6.**

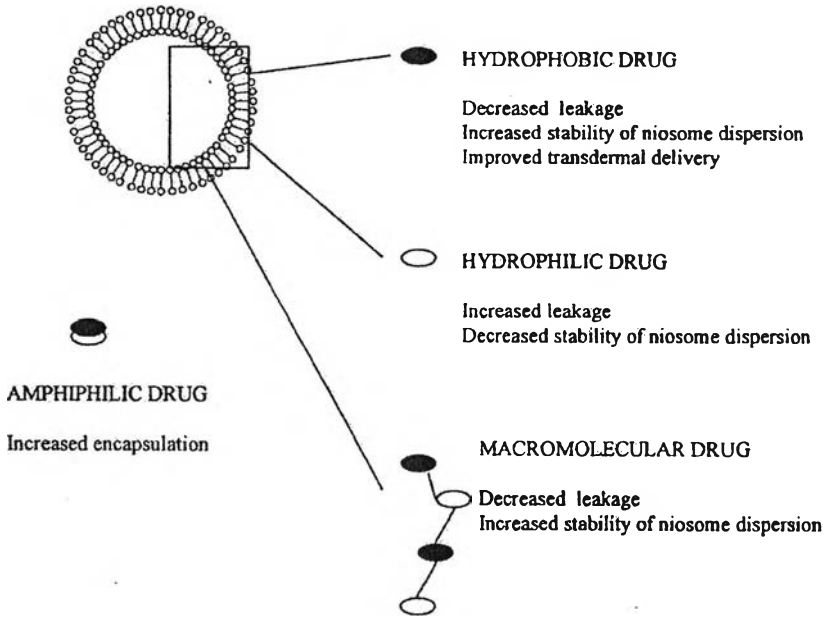


Figure 6. The effect of the nature of encapsulated drugs on the properties of the niosomal dispersions (Uchegbu and Vyas, 1998)

6.2.4 Surfactant and lipid levels

The level of surfactant/lipid used to make niosomal dispersion was varied generally 10-60 mM. Alternating the surfactant: water ratio during the hydration step may affect the system's microstructure (Tanaka, 1990) and hence the system's properties. However increasing the surfactant/lipid level also increased the total amount of encapsulated drugs (Yoshioka et al., 1994).

6.2.5 Temperature of hydration

The hydrating temperatures used to prepare niosomes should usually be above the gel to liquid phase transition temperature (Tc) of the system. At above the phase transition temperature, the membrane is liquid state. So the drugs or solutes can be entrapped more easily.

6.2.6 Drug loading optimization

It was important to emphasize that due care and attention must be paid to the units used to quote drug-loading values. Drug-loading values are often quoted as the percentage of drug encapsulated. However, for these values must be

qualified with details on the initial drug and surfactant/lipid ratio. Sometimes, it may be useful to state the ratio of drug to surfactant in the final formulation in $g \times g^{-1}$ or $mole \times mole^{-1}$. The drug loading can be affected by the chemical nature of the niosome membranes. The membranes may be manipulated to increase drug loading, by alternating the nature of the hydrophilic head group and/or the hydrophobic moieties.

It appears that the more rigid the biayer, the higher encapsulation efficiency is (Uchegbu, 1998). Despite the fact that cholesterol presumably abolishes the phase transition endotherm, it is included in a 1:1 molar ratio in all formulations. The intrinsic membrane gel to liquid phase transition temperature has a fundamental influence on the encapsulation efficiency (Uchegbu, 1998).

6.3 The applications of niosomes

Vesicular systems, i.e. liposomes and niosomes are intensively studied and reported as drug delivery systems (Iwanaga et al., 1999; Siegal et al., 1995; Lu et al., 2003; Balasubramaniam et al., 2002; Arunothayanun et al., 1999a: 1999b; Rental et al., 1999; Baillie et al., 1986). The lipid-based vesicle systems are reported for the efficiency of increasing the solubility of hydrophilic (Hao et al., 2002) and lipophilic drugs (Chen, et al., 2003; Pillai and Salim, 1999). The systems were also exploited as drug carriers with the appropriate properties, such as sustained release (Arunothayanun, 1999a; Pillai and Salim 1999; Vyas and Venkatesan 1999; Baillie et al., 1986; Takada et al.,1984), targeting (Namdeo and Jain, 1999; Siegal et al., 1995; Baillie et al., 1986), improved therapeutic effect (Lu et al., 2003; Shahiwala and Misra, 2002) and reduced toxicity (Mayer et al., 1990). In addition, these systems were able to enhance drug absorption enhance via skin (Fang et al., 2001; Ludewig et al., 2001) and gastrointestinal tract (Rental et al., 1999; Tomizawa et al., 1993; Reddy and Udupa, 1993). Unfortunately, liposomal system has the problems of chemical stability caused by phospholipids. With regard to noisome delivery systems such as topical (Fang et al. 2001), intramuscular (Arunothayanun et al., 1999a), intravenous (Lu et al., 2003) and oral delivery (Varshosaz et al., 2003; Rental et al., 1999; Reddy and Udupa, 1993; Vyas and Venkatesan, 1999) have been investigated.

Niosomes are vesicles in which an aqueous phase is enclosed to high ordered bilayer made up of nonionic surfactant with or without cholesterol. Niosomes forming nonionic surfactants are chemically stable, available by synthesis in pure form at lower cost compared to liposomes forming phospholipids which are prone to degradation by hydrolysis (Frfkjaer et al., 1984) and oxidation (Gebicki et al., 2000) of phospholipids molecules, thereby requiring handling and storage in a nitrogen atmosphere and oxidation. Niosomes were first reported for the formation of vesicles on hydration of a mixture of cholesterol and a single alkyl chain nonionic surfactant (Handjani-Vila et al., 1979). Later, a number of nonionic surfactants for example, polyglycerol alkyl ether, glucosyl dialkyl ethers (Kiwada et al., 1985), crown ethers (Echeogyen et al., 1988), ester linked surfactant (Hunter et al., 1988), polyoxyethylene alkyl ethers (Arunothayanun, P., 1999b and Rajanaresh et al., 1993) and series of spans (Uchegbu et al., 1995) have been used to prepared niosomes.

As stated above, niosomes are able to encapsulate hydrophilic and lipophilic drugs in their bilayer structures. Hydrophilic drug will be entrapped in the inner compartment of niosome where is the hydrophilic area. The example of the increase in solubility of hydrophilic drug is the development of a high encapsulation of colchicine by sorbitan stearate (Span®60) niosomes dispersed in water (Hao et al., 2002). The colchicine niosomes showed a high entrapment efficiency of 99.0%. This niosomal system also displayed a good stability at ambient temperature at least 40 days with no difference in encapsulated efficiency from that of freshly prepared niosomes. On the contrary, niosomes were reported to increase the solubility of lipophilic drug. Pillai and Salim (1999) were successfully prepared polyoxyethylene 20 sorbitan monostearate (Tween®60) niosome encapsulated indomethacin.

One of the appropriate properties of niosomal system is sustained release. In order to achieve sustained drug release, drug may be incorporated in to niosomes. The encapsulation of drug in niosomes would provide some protection from rapid release. The study of luteinising hormone releasing hormone (LHRH) loading hexadexyl diglycerol ether ($C_{16}G_2$) niosomes as drug delivery system was reported by Arunothayanun et al. (1999a). Niosomes were prepared from $C_{16}G_2$ and Solulan® C24 (poly-24-oxyethylene cholesteryl ether), with or without cholesterol. They found

that LHRH exhibited slow release from niosomes comprised $C_{16}G_2$, Solulan®C24 (poly-24-oxyethylene cholesteryl ether compared to free drug solution. Pillai and Salim (1999) studied the sustained antiplatelet effect of polyoxyethylene 20 sorbitan monostearate (Tween®60) niosome encapsulated indomethacin. They found that niosomal indomethacin showed significant higher percent inhibition of aggregation induced by the agonist adenosine 5 -diphosphate (ADP) than that of free drug. ATP release study showed that 100% inhibition was achieved by encapsulating drug, while inhibition by free drug was 40%. Vyas and Venkatesan (1999) reported the successfully controlled drug delivery by poly(phthaloyl-L-lysine)-coated niosomes. Niosomes composed of sorbitan stearate (Span®60), cholesterol and dicetyl phosphate were coated with *p*-phthaloyl dichloride. The polymer-coated niosomes showed the lower drug release about 2 folds compared to uncoated-niosomes. This may be attributed to the effective diffusion double barrier consisted of polymeric coats. The preparation of sodium stibogluconate entrapped in hexadecyl triglycerol ether ($C_{16}G_3$) niosomes and dipalmitoyl phosphatidylcholine (DPPC) liposomes were studied by Baillie et al. (1986). It was found that both vesicular formulations were attained high drug concentration in liver compared to free drug solution. The drug in vesicular form was sustained high liver antimony level up to 24 h at least.

Niosomes were extensively investigated for the targeting property. There were many reports indicating that niosomes could deliver drug to site specific. For example, the delivery of 5-fluorouracil entrapped sorbitan monopalmitate (Span®40) niosomes in rats was studied by Namdeo and Jain (1999). They found that the intravenous administration of niosomal drug gave higher concentration of drug in organs, i.e. liver, lung and kidney, for a greater duration of time compared to free drug solution. The higher concentration of niosome entrapped drug in organs showed preferential phagocytic uptake of vesicles by these organ. Moreover, it was found that the plasma drug level profile of niosomal drug displayed higher and sustained compared to free drug solution. Pharmacokinetic parameters revealed an increase in half-life, area under the curve and decrease in volume of distribution of drug on encapsulation. Baillie et al. (1986) studied the absorption of sodium stibogluconate entrapped in hexadecyl triglycerol ether ($C_{16}G_3$) niosomes and dipalmitoyl

phosphatidylcholine (DPPC) in mice. Both vesicular formulations were attained high drug concentration in liver compared to free drug solution.

Therapeutic effectiveness of conventionally well established drug was improved by controlled and sustained delivery upon encapsulation in niosomes. Carrier drug delivery system using niosomes enhanced therapeutic effect and reduced toxicity has been intensively researched for many decades in pharmaceutical. The therapeutic efficacy and toxicity of drug entrapped niosomes were intensively investigated for many years. Lu et al. (2003) reported the study of the biodistibution and therapeutic effect of carboplatin encapsulated nonphospholipid vesicles in mice. Carboplatin niosomes expressed high affinity for the target site and drug release rate, the slowing drug release rate may reduce the toxicity of drug. The therapeutic effect of topical nimesulide entrapped sorbitan monolaurate (Span®20) niosomes was investigated in rats by Shahiwala and Misra (2002). The experiment revealed that niosomally entrapped nimesulide gel increased the therapeutic effect by exhibiting the highest mean percentage edema inhibition of 66.8% after 24 h compared to plain drug gel (12.57%) and marketed drug formulation (20.49%).

Niosome formulations have also been extensively investigated for improving the absorption of the drug through skin for topical drug delivery and small intestine for oral delivery.

For the topical application, niosomes are being considered as permeation enhancers which serve local depot for sustain release of dermally active compounds via the skin. Fang et al. (2001) studied the permeation of enoxain encapsulated liposomes and niosomes across mouse skin. Drug encapsulated sorbitan stearate (Span®60) niosomes or soybean phosphatidylcholine liposomes were studied the transdermal permeation and skin partitioning through the nude mouse skin. It was found that the permeation of enoxacin niosomes was much higher than that from the free form and from liposomes. This may caused by the surfactant in formulation always acts as a permeation enhancer which party contributed to the enhancement of enoxacin permeation from niosomes. The example for the attempts of intramuscular delivery reported by Arunothayanun et al. (1999a) was luteinising hormone releasing

hormone (LHRH) loading hexadexyl diglycerol ether ($C_{16}G_2$) niosomes as drug delivery system. They found that niosomes were able to protect LHRH from being cleared immediately after intramuscular administration in rats compared to free drug solution. Lu et al. (2003) studied biodistibution and therapeutic effect of carboplatin encapsulated nonphospholipid vesicles in mice. Niosomally entrapped drug showed the largest value of AUC (area under the curve) and intake rate for lung after intravenous administration for 24 h compared to free drug solution. Antitumor effect of carboplatin niosomes was increased and differed significantly compared with the original drug.

Since niosomes are the particulate system, they could be rapid cleared from bloodstream circulation by reticuloendothelial system (RES) and accumulated in RES-related tissues, i.e. live, spleen, bone marrow (Torchilin, 2005). Uptake of carrier by RES, governed by macrophages through a process called phagocytosis, is initiated by adsorption of opsonins (serum proteins) at carrier surface (Olivier, J.-C., 2005). According to the serum protein absorption, carriers are cleared rapidly in bloodstream. The factors of uptake carriers are size (Eldridge et al., 1990) and surface properties (Tomizawa et al., 1993) of carriers. Increase in uptake is affected by increase in size of carriers, hydrophobicity and charge on surface. The way of reducing serum protein absorption at carriers is to modify the surface of carriers. The different types of coating materials were introduced to protect the vesicles in order to provide the prolonged blood circulation.

One of the greatest challenges in non-ionic surfactant vesicles (niosomes) delivery is the oral administration. Niosomes, resulted from the self-assembly of sufficiently insoluble surfactants in aqueous media, have attracted great attention as possible oral dosage forms. The feasibility to develop oral drug delivery based on that niosomes was investigated in vivo to determine their susceptibility to degradation during gastric and intestinal passage. The ability of niosomes entrapped insulin (Varshosaz et al., 2003), ovalbumin (Rental et al., 1999), flurbiprofen (Reddy and Udupa, 1993) and 9-desglycinamide 8-srginine vasopressin (DGAVP) (Yoshida et al., 1992) for oral delivery was investigated. Varshosaz et al. (2003) reported the study of physical property of insulin encapsulated niosomes of sorbitan monoesters (Span®20, 40, 60 and 80) for oral delivery. These niosomes comprised nonionic

surfactant, cholesterol and dicetylphosphate prepared in phosphate buffer at 55°C by film hydration method. They found that size of niosomes depended on the amount of cholesterol and charge incorporation. The diameter of niosomes increased with increasing the amount of cholesterol content from 10 to 50 %mole, while their size decreased when negatively charged dicetyl phosphate was incorporated. The amount of insulin released in simulated intestinal fluid from Span®40 and 60 was lower than Span® 20 and 80 niosomes because the molecules of Span®40 and Span® 60 in bilayer structures were in the ordered gel state at the room temperature, but those of Span®20 and 80 in the disordered liquid-crystalline state. This agreed with the report of Hao et al. (2002) was about the release study of colchicine from sorbitan stearate (Span®60) niosomes in simulated gastric fluid or simulated intestinal fluid at 37°C for 24 h. They found that Span®60 niosomes exhibited a prolonged release compared to free drug solution. In addition, the high gel to liquid crystal transition temperature and rigidity of bilayers of Span®60 niosomes affected the highest protection of insulin against proteolytic enzymes, stability in the presence of sodium desoxycholate (bile salt) and storage temperature.

Rental et al. (1999) reported that ovalbumin encapsulated niosomes comprised Wasag®7 or Wasag®15: cholesterol: dicetyl phosphate (4:5:1 mole ratio) could improve therapeutic effect of drug. Wasag®7 consists of 70% stearate sucrose ester and 30% palmitate sucrose ester possessed a HLB value of 7. Wasag®15 consists of 30% stearate sucrose ester and 70% palmitate sucrose ester possessed a HLB value of 15. Both 2 types of niosomes were prepared by sonication method. Niosomes were hydrated in water 80°C and then sonication and dehydration-rehydration method; niosomes were lyophilized and resuspended with phosphate buffer saline pH 7.4. The results showed that niosome sizes in all formulation clearly below 0.5 μm which could be uptake via Peyer's patches (Eldridge et al., 1990), indicating different methods had no effect on size of these formulations. Only encapsulation of ovalbumin into Wasag®7 niosomes resulted in a significant increase in antibody titres in mice compared to free drug, plain niosomes and physical mixture of drug and niosomes. In contrast to ovalbumin loaded Wasag®7 niosomes, application of more hydrophilic Wasag®15 niosomes did not resulted in an increase in antibody titres.

The study of the therapeutic efficacy of flurbiprofen incorporated sorbitan stearate (Span®60) niosomes in rat was reported by Reddy and Udupa (1993). Niosomes were prepared with the given mole ratio 47.5:47.5:5 of Span®60: cholesterol: dicetyl phosphate in water by hand-shaking method. They found that oral administration of flurbiprofen niosomes showed a significant decrease in paw oedema in rat compared to flurbiprofen incorporated in albumin microspheres and beta-cyclodextrin. This study revealed that flurbiprofen can be improved therapeutic efficacy by incorporating in niosomes.

The controlled drug delivery in vesicular oral administration was also interested by some researchers. Niosomes coated with polymer were introduced by Vyas and Venkatesan (1999). Poly(phthaloyl-L-lysine)-coated niosomes of sorbitan stearate (Span®60), cholesterol and dicetyl phosphate (47.5:47.5:5.5 mole ratio) successfully controlled drug delivery. The study revealed that polymeric coating acted as an effective secondary barrier in retarding the release of the vesicular content. The C_{max} (maximum drug concentration) of polymer-coated niosomes was low compared to niosomes and plain drug solution. The t_{max} (time at maximum drug concentration), $t_{1/2}$ half-life and AUC (area under the curve) of polymer-coated niosomes were higher than those of niosomes and plain drug solution.

7 Proniosomes

Although lipid-based vesicle system, liposomes and niosomes has many advantages in drug delivery system, the drawback of these systems is the physical instability in dispersions (Fang et al, 2001; Anssen et al., 1985; Wong et al., 1982). Liposomes and niosomes are aqueous suspensions may exhibit aggregation, fusion, leaking of entrapped drugs or hydrolysis of encapsulated drugs, thus limiting the shelf life of the dispersions. However, since niosomes exhibit better chemical stability during storage and the low cost of raw materials, niosomes are introduced to be an alternative of liposomes for decades.

The interesting novel approach of liposomes and niosomes is the preparation of these vesicular dispersions to be a dry product which could be hydrated immediately and transform to be vesicles before used. Proniosomes required hydration with medium at a temperature above the main phase transition temperature of the surfactant composition (Hu and Rhodes, 1996). This granular product would avoid many of the problems associated with physical stability i.e. aggregation, fusion, leaking of vesicular system could be minimized. The additional convenience of the transportation, distribution, storage and dosing would make dry granular of liposomes or niosomes, namely, proliposomes or proniosomes, respectively, more applicable.

Gerneral compositions of proliposomes and proniosomes are vesicle forming agents i.e. phospholipids or nonionic surfactants, cholesterol, stabilizers and carriers (Blazek-Welsh and Rhodes, 2001; Jung et al., 2000; Hu and Rhodes, 1996, Katare et al., 1991; Payne et al., 1986). Payne et al. (1986) gave a first description for a novel method to produce liposomes which involved coating sorbitol powder with lipids dissolved in organic solvent. The powder could then be dissolved in hot water to form multilamellar liposomal suspension. Hu and Rhodes (1996) first reported the preparation of proniosomes using the slow spray-coating method similar to that of Payne et al. (1986). A surfactant mixture of sorbitan stearate (Span®60), cholesterol and dicetyl phosphate and sorbitol was used as the substrate, the surfactant solution was introduced onto the surface of sorbitol in the round-bottom flask on the rotary evaporator by sequential spraying of surfactant mixture. It was shown that a dry free-flowing product could be produced and then be quickly hydrated with water 80°C to form a niosome dispersion with the release both in gastric fluid and simulated intestinal fluid similar to those of conventional niosomes. The average particle size of proniosome-derived niosomes is smaller than that of conventional niosomes. (Hu and Rhodes, 1999).

During the past 10 years, many preparation methods of proniosomes or proniosomes were developed, including spray-drying method (Payne et al., 1986 and Hu and Rhodes, 1999), drying method (Chung, 1999 and Ahn et al., 1995), slurry method (Blazek-Welsh and Rhodes, 2001), mixed micellar proliposomes method (Hayat and Son, 1992 and Supersaxo et al., 1991) and fluidized drying method (Katare et al., 1991: 1990). The carriers, for example, sorbitol (Jung et al., 2000),

maltodextrin (Blazek-Welsh and Rhodes, 2001) and effervescent granules, i.e. dried sodium phosphate, monohydrate citric acid, tartaric acid and sorbitol (Katare et al., 1991), etc. The optimum weight ratio of carrier to lipid/ surfactants is based on physical properties of resultant product. For example, a marked increase in the loading of lipid onto carrier material resulted in a sticky product with a marked tendency to agglomerate (Payne et al., 1986) used in proniosome/ proliposome preparation, must easily soluble in aqueous media in order to enhance to vesicle formation.

7.1 Application of proniosomes

According to the good stability of niosomes and the advantages of proniosomes such as physical stability and chemical stability, some of researchers will be intensively study proniosome preparations and characterized their properties as a carrier drug delivery. Rhodes and Hu (1999) reported the study of physical morphology and entrapment efficiency of ibuprofen proniosomes. Proniosomes composed the total concentration of lipid/surfactants of 100 mM were used with molar ratio of Span®60 to cholesterol over a range of 65:30 to 30:65 in the present of 5 mM dicetyl phosphate. Then the mixture of lipid/surfactants in chloroform was introduced to sorbitol powder and dried by spray-drying method. Proniosome-derived niosomes were prepared by hydrating proniosomes with 80°C distilled water and vortex mixing for 2 min, then their size was compared to conventional niosomes which were prepared by hydrating dried film with buffer at 60°C by hand-shaking method. They found that size and size distribution of proniosome-derived niosomes were more uniform than conventional niosomes. There was a little variation (91-94%) in entrapment efficiency of proniosome-derived niosomes when varied molar ratio of lipid/surfactant. The release of drug from conventional niosomes and proniosome-derived niosomes was slower than drug solution both in simulated gastric fluid and intestinal fluid.

Other study of proniosomes was the preparation of maltodextrin-based proniosomes loading alprenolol (Blazek-Welsh and Rhodes, 2001). Proniosmes prepared by introducing the surfactant solution directly to maltodextrin to provide enough solvent to form a slurry. Then the mixture was solvent evaporated to obtain

proniosomes. Proniosome-derived niosomes were produced by the addition of water at 80°C and agitated with vortex mixture for 2 min. The entrapment efficiency was depended on the amount of maltodextin. Proniosome-derived niosomes were suggested to be as effective carrier for amphiphilic drugs due to multilamellar structure which allowed amphiphilic molecules partition to the interfacial environment on either the inner or outer leaflet of bilayer.

Recently it appears no evidence of proniosomes in oral applications. However, it is possible to prepare proniosomes because the mixture of lipid/ surfactant and carrier could form proniosomes. In this study, water soluble carriers as lactose, dextrose and sorbitol were investigated to prepared proniosomes by oven drying.

7.2 The observation of proniosome-derived niosomes

The reconstitution of niosomes from proniosomes is observed using different type of equipments to confirm the observation by an optical microscopy at 1000× magnification (Katare et al., 1990), a transmission electron microscope (Ahn et al., 1995 and Payne et al., 1986, a coulter Counter Analysis (Payne et. al, 1986) and a laser diffraction microscopy (Hu and Rhodes, 1999).

8. **Polyoxyethylene alkyl ethers** (Kibbe, 2000)

The polyoxyethylene ether surfactants are different in alkyl chain lengths and hydrophilic head group. The series of these nonionic surfactants used are presented in **Table 2**.

Table 2. The physical properties of series of polyoxyethylene ether surfactants

Chemical name	Molecular weight	HLB	Melting point
Polyoxyl 4 lauryl ether ($C_{12}EO_4$)	362	9.7	-
Polyoxyl 2 cetyl ether ($C_{16}EO_2$)	330	5.3	33
Polyoxyl 2 stearyl ether ($C_{18}EO_2$)	359	4.9	38
Polyoxyl 20 oleyl ether ($C_{18}EO_{20}$)	1150	15.3	33

The chemical structure of polyoxyethylene alkyl ethers:

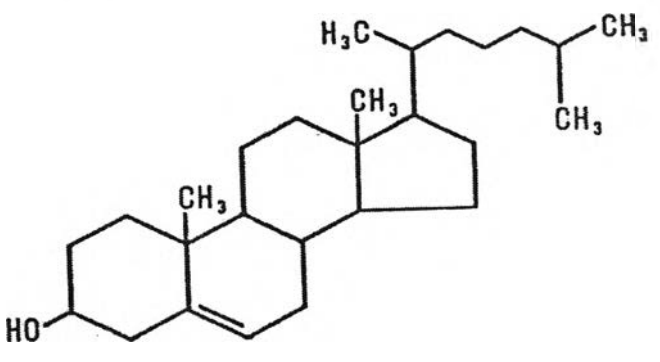
$$CH_3 (CH_2)_x COCH_2 (CH_2)_y OH$$

x = C atom in alkyl chain

y = Ethylene oxide group in hydrophilic chain

Function : Emulsifier

9. **Cholesterol** (Wenninger and McEwen, 1992)



Cholesterol is a mono-unnsaturated, secondary alcohol of the cyclopentenophenanthrene system.

Molecular weight : 387

Function : Emulsion stabilizer, skin-conditioning agent, viscosity increasing agent

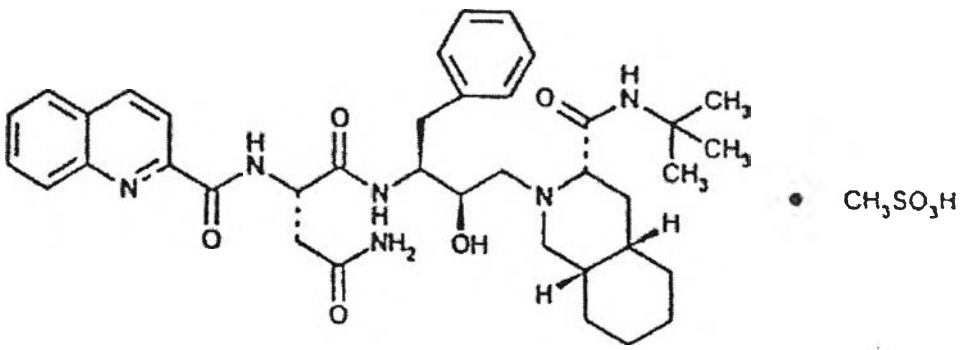
10. Polyoxyethylene stearate (POE stearate) (Wenninger and McEwen, 1992)

$$CH_3(CH_2)_{16}CO(OCH_2CH_2)_{40}OH$$

Polyoxyl 40 stearate, so-called PEG-40 stearate, is the polyethylene glycol ester of stearic acid.

Molecular weight	:	2044
HLB	:	16.9
Function	:	Cleansing agent, solubilizing agent

11. Saquinavir mesylate (McEvoy, 2004)



$C_{38}H_{50}N_6O_5 \cdot CH_4O_3S$

Molecular weight : 766.96

Saquinavir mesylate (SQV), a very fine, white to off-white crystalline powder, has an aqueous solubility of 2.22 mg/ml at 25□C. The logarithm of the octanol-water partition coefficient (log P) is 4.1 (François et al., 2004). The drug has a pKa of 7.01. SQV, synthetic peptide analog and inhibitor of HIV-1 and HIV–2 proteases, should always administered orally in conjugation with other antiviral agents for treatment of human immunodeficiency virus (HIV) This drug is a highly specific inhibitor of HIV protease with IC_{50} or IC_{90} (concentration of the drug

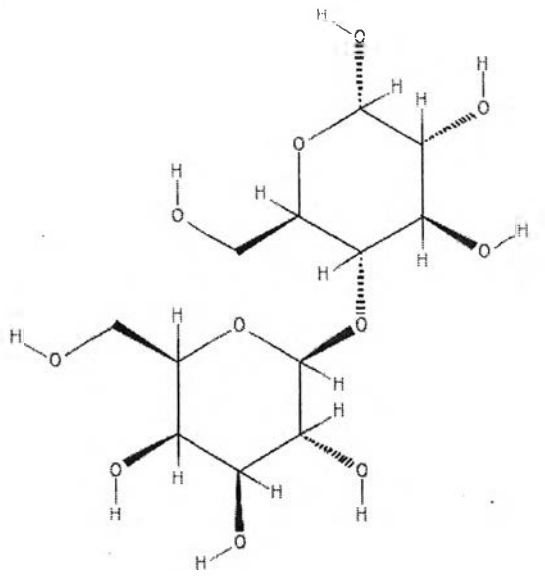
required to inhibit 50 or 90% of detective HIV replication) for HIV-1 has ranged from 1-30 or 5-80 nM, respectively.

There is only one commercially available product of SQV, Invirase®, Roache 200 mg (of SQV). Saquinavir mesylate is filled in hard gelatin capsules. The recommended dosage of Invirase® capsules for the treatment of HIV infection in adults and adolescents 16 years of age or older who are not receiving concomitant ritonavir is 600 mg 3 times daily. When Invirase® capsules are used in the conjuction with low-dose ritonavir for the treatment of HIV infection in adults and adolescents 16 years of age or older, Invirase® capsules can be given in a dosage of 1 g twice daily with ritronavir 100 mg twice daily. Alternatively, Invirase® capsules can be given in a dosage of 400 mg twice daily in conjunction with ritonavir 400 mg twice daily.

SQV, administered orally, has low oral bioavailability (4%), therefore drug should be taken within 2 h of a full meal because presence of food in the gastrointestinal tract can increase bioavailability of drug. SQV hard gelatin capsules should be taken within 2 h after a meal to optimized GI absorption. Metabolism of SQV is mediated by the cytochrome P-450 (CYP) and the isoenzyme 3A4 is involved more than 90% of mrthabolism.

Rarely the serious adverse effects considered possibly related to drug use have been reported in clinical in patients receiving SQV hard gelatin capsules. The principal adverse effects associated with saquinavir mesylate therapy involve the GI tract which more reported patients receiving the combination of SQV hard gelatin capsules with other antiretroviral agents than that of SQV hard gelatin capsules alone.

12. Anhydrous lactose (Wenninger and McEwen, 1992)



$C_{12}H_{22}O_{11}$

Anhydrous lactose is a white to off-white crystalline particle. It is a disaccharide of glucose and galactose in human and cow milk. It is commonly used in pharmacy for tablets, in medicine as a nutrient, and in industry.

Molecular weight	:	342.30
Water content (%)	:	≤ 1.0
Solubility	:	1 in 4.63 (water, 25°C) Practically insoluble in chloroform, ethanol and ether
Function	:	Filler or diluent in tablets, capsules Carrier or diluent for inhalation products and in lyophilized products