CHAPTER II

LITERATURE REVIEWS

1.Surfactant properties

Surfactants are amphiphilic molecules that possess hydrophilic group and hydrophobic group. These molecules can self-assemble and form structure in aqueous phase. Israelachvili et al explained that a particular aggregate is formed by determining of critical packing parameter. This parameter describes the relationship between hydrophobic chain length (L_c) hydrophobic chain volume (V) and hydrophilic head group area (a_o). The derivation of a critical packing parameter is shown in equation 1.

$$CPP = V/ a_o L_c \qquad (1)$$

Mostly there are three types of aggregation such as micelles, bilayers and inverse micelles. Generally, the amphiphilic molecules prefer to form micelles with CPP<0.5 and form bilayer with the CPP 0.5-1.0. If the CPP>1.0, inverse micelles are

formed. Estimation of the critical packing parameter in the range of 0.5-1.0 for these molecules suggests the formation of vesicles. However, several nonionic surfactants that do form vesicles, appear not to have the ideal geometric structure. In addition, not only the balance between hydrophobicity and hydrophilicity, but also the balance between geometric factor, forces of attraction and repulsion of both hydrophobic and hydrophilic groups are crucial in determining the type of aggregates formed in aqueous environment.

Many synthetic amphiphilic molecules can forms vesicular structure in aqueous media. There are wide varieties of nonionic surfactant structures that can form vesicles with the presence of cholesterol.

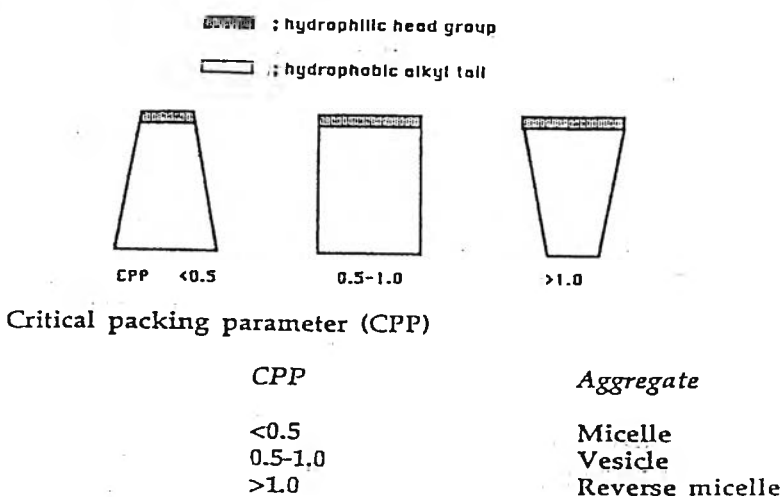


Figure 2. Critical packing parameters for micelle, vesicle and reverse micelle formation (Florence, 1993).

2. Formation of Niosomes (Uchegbu, 1998)

Nonionic surfactant based vesicles (niosomes) are formed from the self-assembly of nonionic amphiphiles in aqueous media resulting in closed bilayer structure usually with some input of energy such as physical agitation or heat. The result is 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 served as drug carriers. The lower cost, greater stability and resultant ease of storage of nonionic surfactants (Florence, 1993) has leaded to the exploitation of nonionic surfactants as alternatives to phospholipids.

The versatility of synthetic nonionic surfactants as vesicle formers allow the exploration of new structures and forms, from disc-shaped systems to multiple vesicles, all of which might have a special role in drug delivery, either as reservoirs for topical delivery or for parenteral administration. (Florence, 1993)

The ultimate identity of any niosomal system and hence its properties were determined by the factors as list in Figure 3.

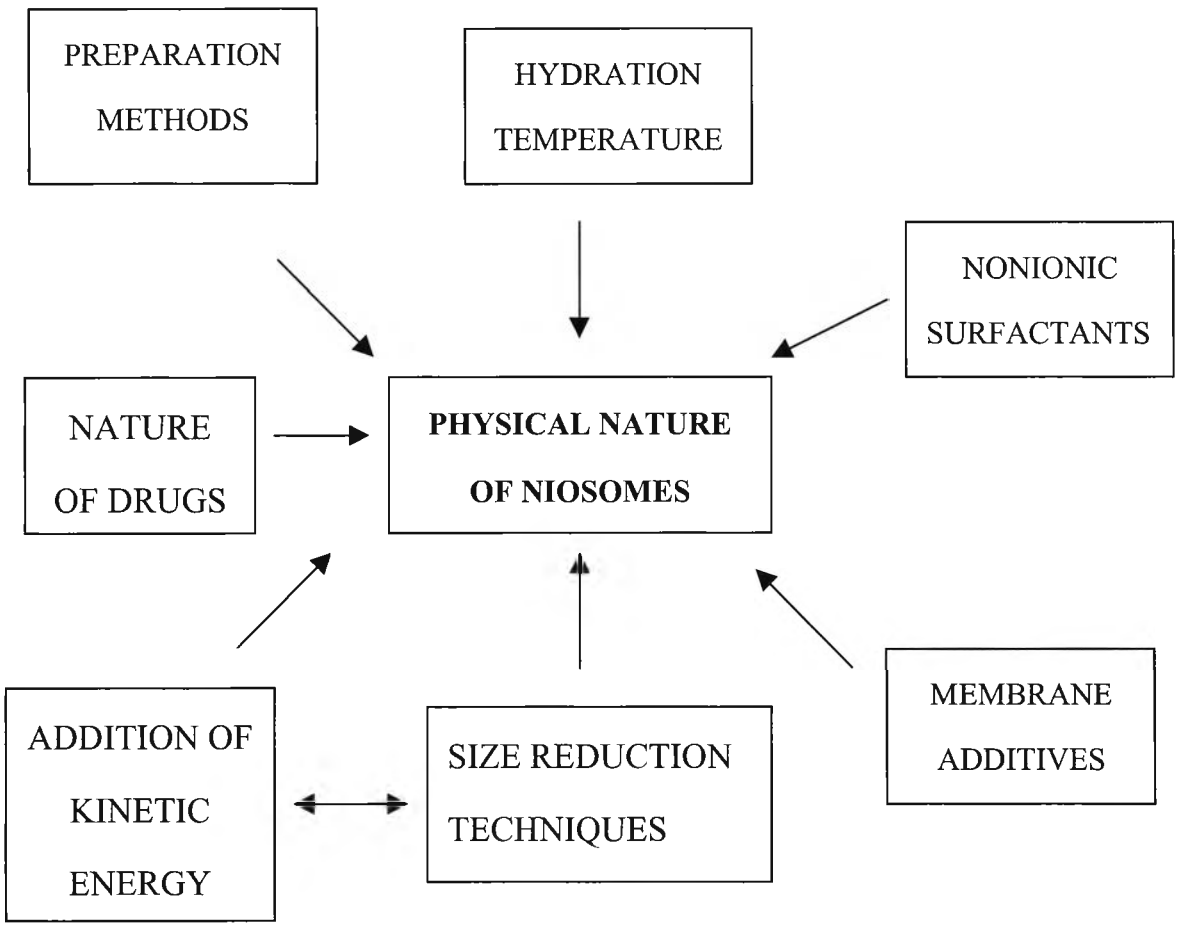


Figure 3. Factors influencing physical chemistry niosomes.(Uchegbu, 1998)

There are many physical natures of niosomes such as the shape, the size, the lemellarity, the phase transition temperature, the state of bilayer i.e.fluidity, rigidity and the entrapment. The factors influencing on the nature of niosomes were as follows:

Firstly, the preparation method has influenced on the size, the shape and the lamellarity. Secondly, the hydration temperature has influenced on the state of bilayer. Thirdly, the nonionic surfactants have influenced on the entrapment and the

state of bilayer. Lastly, the membrane additive has influenced on the phase transition temperature. It was obvious that all these variables must be carefully controlled in the design of a niosomal drug delivery system.

3.Factors governing the self-assembly of nonionic surfactants into niosomes (Uchegbu, 1998).

3.1 Nonionic surfactant structure

Theoretically, niosome formation requires the presence of particular class of amphiphile. Amphiphiles possess a hydrophilic head group as shown in figure 4 and hydrophobic tail as shown in figure 5. The amphiphilic molecules may possess one or three alkyl chains. The alkyl group chain length is usually from C_{12}-C_{18}. The two portions of the molecule may be linked via ether, amide or ester bonds.

Additionally, some of these surfactants such as the Span and Brij surfactants are already established in pharmaceutical excipients.

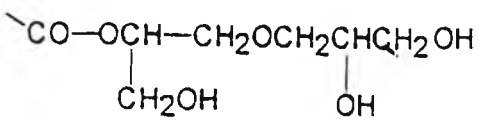
A hydrophilic lipophilic balance (HLB) is a good indicator for the ability of vesicle forming amphiphiles. A HLB number of between 4 and 8 was found to be compatible with vesicle formation (Yoshioka, 1994 and Uchegbu, 1995).

3.2 Membrane additives

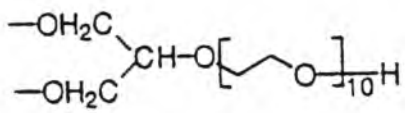
Various additives must often be included in the formulation in order to obtain stable niosomes. The most common additive found in niosomal systems is cholesterol. It is known to abolish the gel to the liquid phase transition of liposomal and niosomal systems resulting in the systems that are less leaky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

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

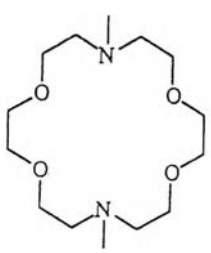
a.Glyceryl head group.



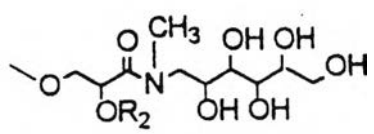
b.Ethylene Oxide head group



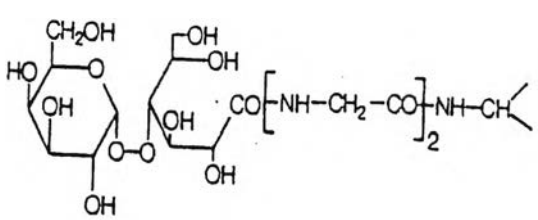
c.Crown ether head group



d. Poly hydroxy head group



e.Sugar head group + amino acid



f. Sugar head group

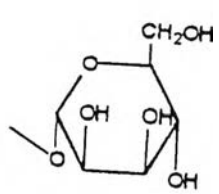
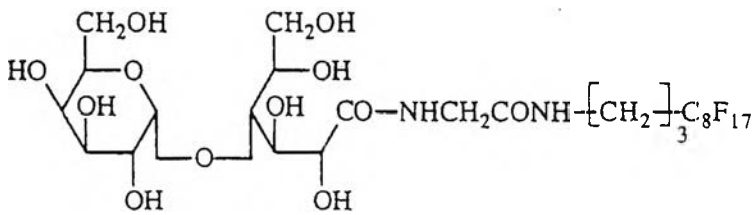


Figure 4. Hydrophilic head groups found in vesicle forming amphiphiles

(Uchegbu, 1998)

a. Perfluoroalkyl tail.



b. Single steroidal group.

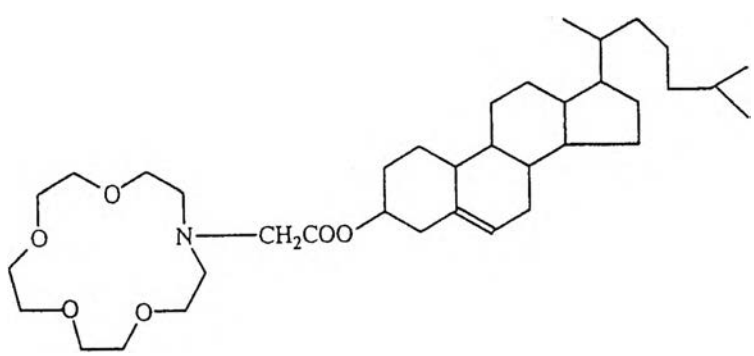
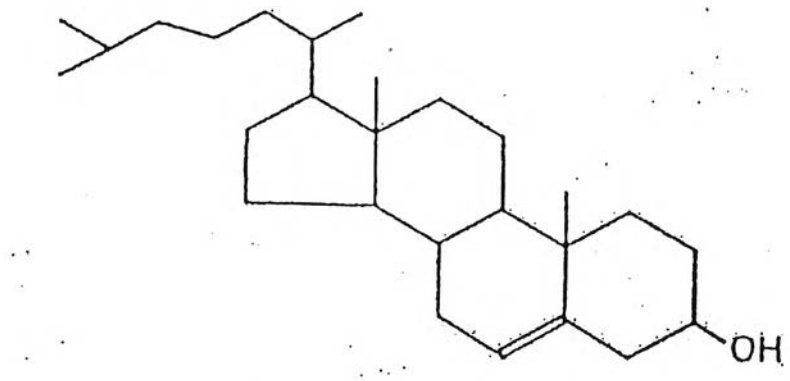
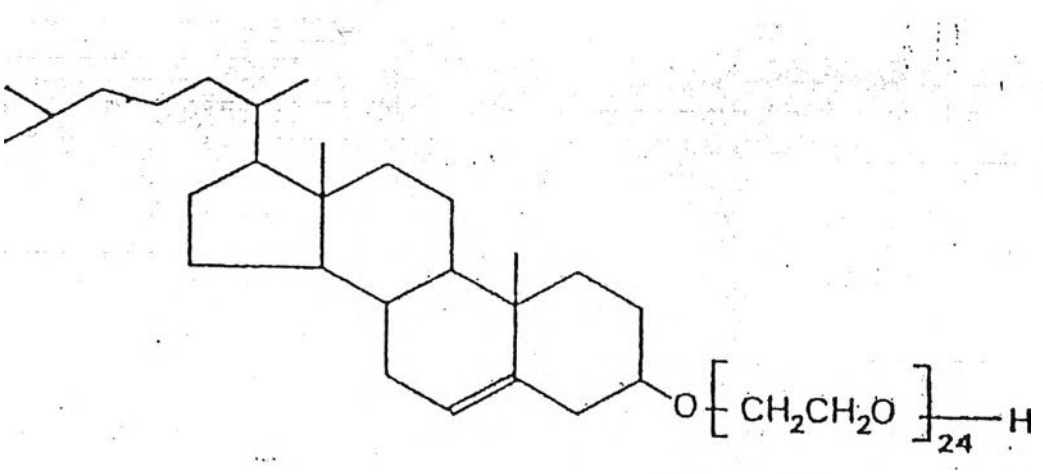


Figure 5. Hydrophobic tail found in vesicle forming amphiphiles (Uchegbu, 1998)

Often niosomes must be stabilized by the addition of a charged molecule to the bilayer such as dicetyl phosphate. A steric stabilizer, Solulan C-24 (poly-24-oxyethylene cholesteryl ether), must be added to the formulation to ensure a homogeneous formulation devoid of aggregates.(Uchegbu,1994). The structure of cholesterol and solulan C-24 were shown in figure 6.



(a) cholesterol



(b) solulan C-24.

Figure 6. The structure of (a) cholesterol (b) solulan C-24

3.3 Nature of encapsulated drugs

In choosing a suitable drug to be delivered by niosomes. Niosomes encapsulating hydrophobic drugs and macromolecules were more stable than ones encapsulating low molecular weight drugs. Also they decrease the leakage of niosome.(Uchegbu, 1998)

In contrast, hydrophilic drug can easily leak from niosomes and decreased the stability of niosomes dispersion. Amphiphilic drugs, they increased encapsulation.

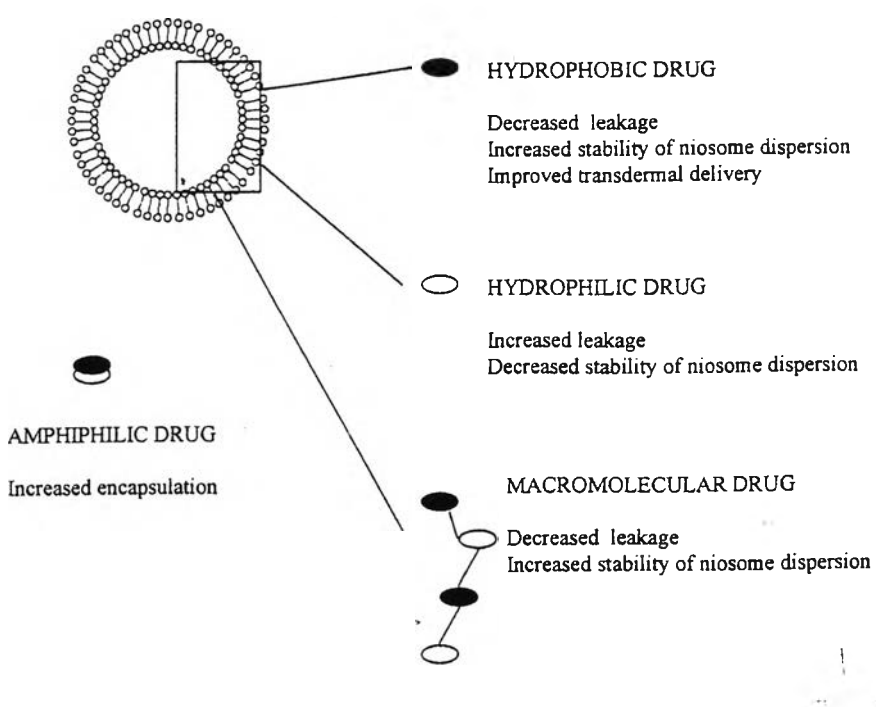


Figure 7. The effect of the nature of the encapsulated drugs on the properties of the niosome dispersions (Uchegbu, 1998)

3.4 Surfactant and lipid levels

The level of surfactant/lipid used to make niosomal dispersion was generally 10-30 mM. Altering 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.

3.5 Temperature of hydration

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

4. 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 ($g.g^{-1}$)or($mole.mole^{-1}$). The drug loading can be affected by the chemical nature of the niosome membranes. The membranes may be manipulated to increase drug loading, by altering the nature of the hydrophilic head group and/or the hydrophobic moieties.

It appears that the more rigid the bilayer is, the higher the encapsulation efficiency is (Uchegbu, 1998). Despite the fact that cholesterol that presumably abolishes the phase transition endotherm, 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).

5. Niosome preparation

The formation of vesicular assemblies requires the input of some form of energy (Lasic, 1990) and all the experimental methods surveyed consist of the hydration of a mixture of the surfactant / lipid at elevated temperature followed by optional size reduction to obtain a colloidal dispersion. This is followed by the separation of unentrapped drug from the entrapped drug by either centrifugation, gel filtration or dialysis. 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) (Table 1)

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 prepare by hand-shaking method. The internal space of mutilamellar vesicle is occupied by lipid layers separated by aqueous layers. If the vesicles contain a few concentric lamellar. Secondly, SUVs come from MLVs or LUVs reduce the size of vesicles by sonication or extrusion. The vesicles contain only one lipid layer and one aqueous layer. Thirdly, LUVs contain aqueous aqueous comparment at the core of vesicle with the large volume. So they are suitable for carrying hydrophilic drugs.

Generally, hand-shaking method has been used to provide a large amount of multilamellar vesicles resulting in hydrophobic solutes mainly deposit in their membranes.

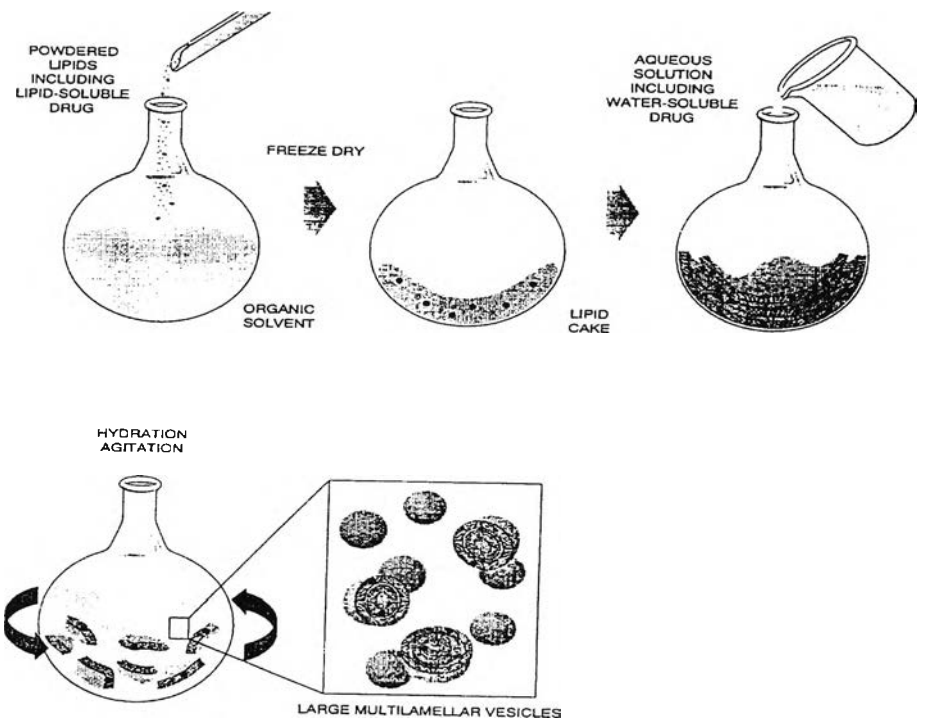


Figure 8. Hand-shaking method (Lasic, 1990)

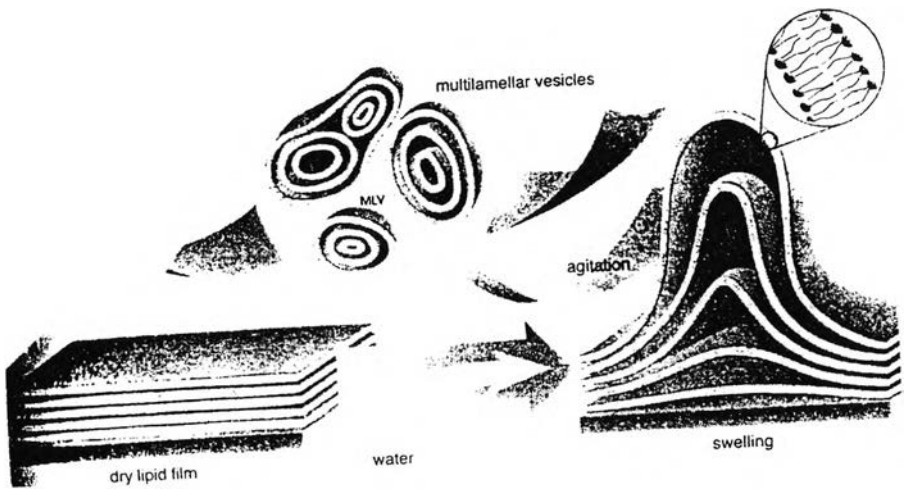


Figure 9. Multilamellar vesicle formation (Lasic, 1990)

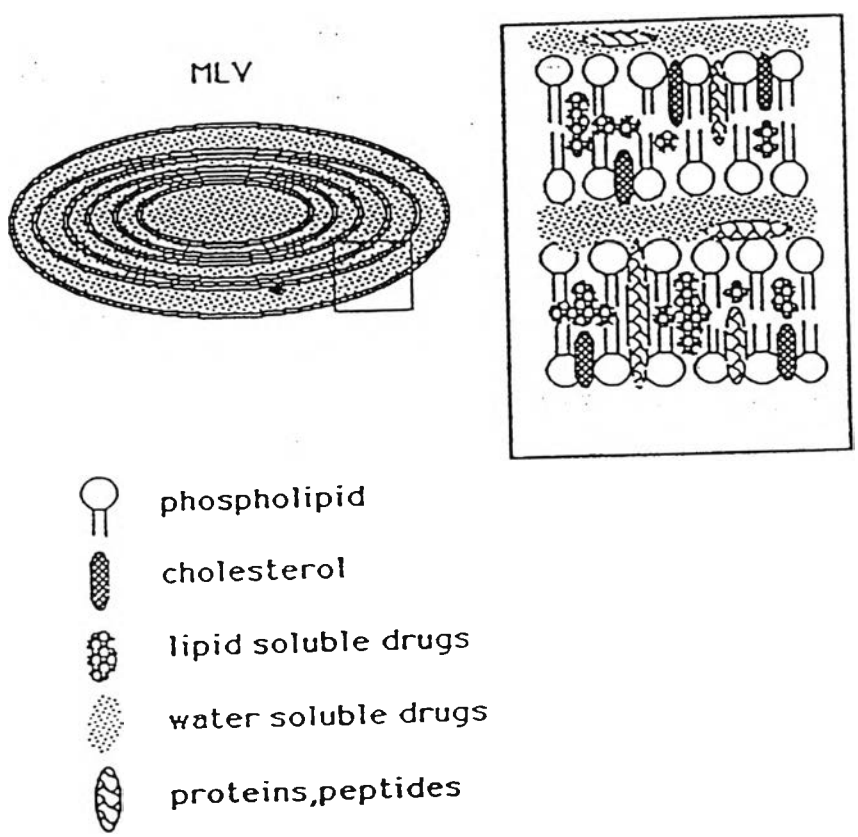


Figure 10. Multilamellar vesicle with entrapped drugs (Mezei, 1994)

6. Effects of nonionic surfactants on biological membrane (French 1993)

Nonionic surfactants could have a profound effect on the permeability characteristics of several biological membranes. The effect of nonionic surfactants on the rate of permeation of drug compounds in the formulation through membranes from a formulation was highly dependent upon the physical state of the surfactant and its concentration in the locality of the membranes. Many publications concerning the biological effects of surfactants had noted that there was a concentration-dependent biphasic action on the rate of drug permeation through membranes. The enhancement of membrane transport occured at low concentration of surfactant, but this was seen to decrease at higher concentrations, generally above the critical micelle concentration of the surfactant. The increase in transmembrane flux at low surfactant concentrations was normally attributed to the ability of the surfactant molecule to penetrate the membrane and increase its permeability.

Surfactants were known to both interact with and affect the keratin of the stratum corneum. The involvement of surfactant-protein interaction in many of the studies describing can not be rule out. However the close relation observed in several studies would suggest that the lipid lamellar of the stratum corneum and not the keratin of corneocytes was the major site of action for nonionic surfactants.

The penetration of the surfactant molecule into the lipid lamellae of the stratum corneum was strongly dependent on the partition behaviour and solubilities of the surfactant. This would initially suggest that the more lipophilic surfactant, the greater its ability to penetrate into the lipid membrane. This will depend on the vehicle in which the enhancer and drug were applied to the skin.

It could be seen that there were many factors that could affect and were complicate the use of surfactants as penetration enhancers. In order to achieve the maximum effect of the surfactants on the absorption, the delivery of both surfactant and drug to the skin should be optimized. The simplest formulation, but probably one of the most effective, would be to produce a saturated solution or dispersion of the drug to be administered in a pure monodisperse surfactant that has high membrane-fluidizing activity. It is unlikely that a single surfactant would be the optimum enhancer for all drug molecules, the choice of enhancer would have to be tailored to the specific drug.

Some nonionic surfactants have been investigated for penetration enhancing activity as follows:(French,1993)

Polyoxyethylene Sorbitan Fatty Acid Esters.

Nonionic surfactants in this group are different in alkyl chain lengths.

1.TWEEN 20® POE(20)sorbitan monolaurate

2. TWEEN 40® POE(20)sorbitan monopalmitate

3. TWEEN 60® POE(20)sorbitan monostearate

4. TWEEN 80® POE(20)sorbitan monooleate

5. TWEEN 85® POE(20)sorbitan monotrioleate

Polyoxyethylene Alkyl Ethers

Nonionic surfactants in this group are different in alkyl chain lengths and hydrophilic head grouups

1.BRIJ 30®	$C_{12}E_4$	7. BRIJ 72®	$C_{18}E_2$
2. BRIJ 36T®	$C_{12}E_{10}$	8. BRIJ 76®	$C_{18}E_{10}$
3. BRIJ 35®	$C_{12}E_{23}$	9. BRIJ 78®	$C_{18}E_{20}$
4. BRIJ 52®	$C_{16}E_2$	10. BRIJ 92®	$C_{18}=E_2$
5. BRIJ 56®	$C_{16}E_{10}$	11. BRIJ 96®	$C_{18}=E_{10}$
6. BRIJ 58®	$C_{16}E_{20}$	12. BRIJ 98®	$C_{18}=E_{20}$

Sorbitan Esters

Nonionic surfactants in this group are difference in alkyl chain lengths.

1.SPAN 20® Sorbitan monolaurate

2. SPAN 40® Sorbitan monopalmitate

3. SPAN 85® Sorbitan trioleate

The permeability constant depends on surfactant alkyl chain length, hydrophilic-lipophilic balance and concentration (French et. al, 1993).

7.Structure and function of skin (Mezei, 1994;Barry, 1983)

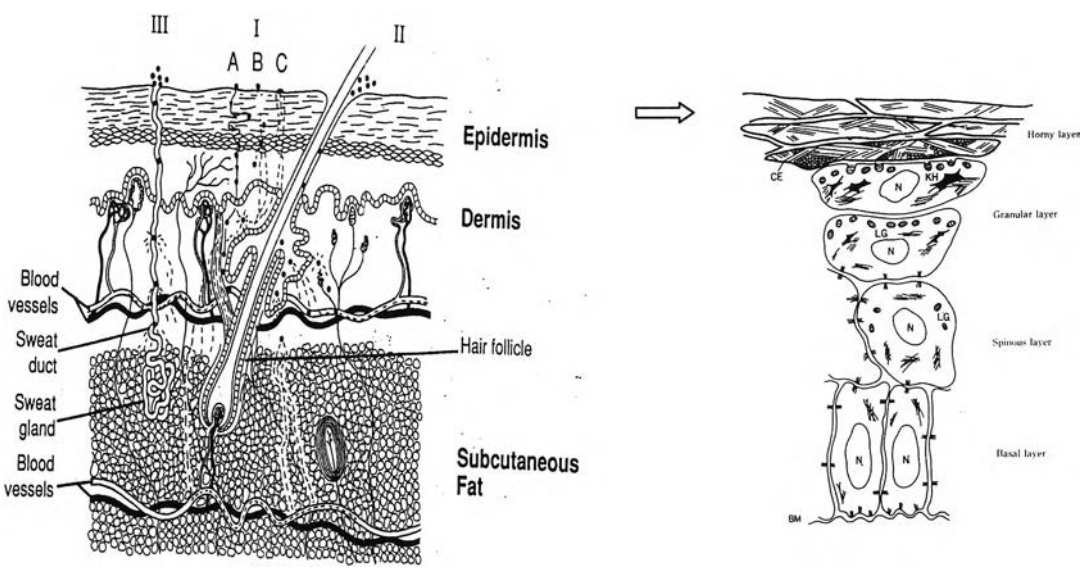


Figure 11. The cross-section of the skin structure and possible route of penetration (Patel, 1993)

The skin is the largest and most heterogeneous organ of the body. It is composed of tissue that grows, differentiates and renews itself constantly. The skin is divided into three major layers: epidermis, dermis and subcutaneous tissue.

The epidermis

The multilayered enveloped of the epidermis varies in thickness, depending on cell size and the number of cell layer. The epidermis is composed of four strata. It ascended from the proliferative layer of basal cells: the stratum germinativum , the stratum granulosum (the malpighian layer), the stratum lucidum (the granular layer) and stratum corneum (the horney layer).The epidermis changed in an ordered fashion from metabolically active and dividing cells to dense, dead, keratinized protein.

The stratum corneum is the superficial layer of the epidermis. This region is considered as a nonviable epidermis providing 10-15 layers of much flattened, keratinized dead cells, stacking them in highly organized units of vertical columns. It is approximately 10-20 micron thick. From previous studies, they indicated that the stratum corneum was not uniformly homogeneous, that it continuously evolved from below to the skin surface, and that the layers represented various stages of corneocyte and intercellular lipid maturation. Each stratum corneum cell contained mainly of keratin(~70%) and lipid (~20%)

The dermis

The dermis interfaces with the epidermis at the epidermal-dermal junction. It is ten to forty times thicker than the epidermis, depending on the area of the body. It makes up the bulk of the skin. The dermis is metabolically less active than that the epidermis; it is a matrix of loose connective tissue composed of polysaccharides and protein (collagen and elastin) that embeded in a ground substance containing a variety of lipid, protein and carbohydrate. This matrix contained nerves, blood vessels, hair follicles, sebaceous and sweat glands. The function of the dermis is to nourish the epidermis and to anchor it to the subcutaneous tissue.

The subcutaneous tissue

This served as a receptacle for the formation and storage of fat. The subcutaneous tissue was a place for dynamic lipid metabolism; it supported the nerve and blood vessels that passed to the dermis. The subcutaneous fat spreaded all over the body. Its thickness varied with the age, sex, endocrine and nutritional status of the individual.

Consequently, the skin was an effective barrier to the penetration of a whole variety of substances including drugs. The skin was also an important organ with respect to metabolism and immunology. Due to its heterogeneous structure and its

dynamic nature, which could not be maintained in in-vitro conditions, only in-vivo studies could provide reliable data related to the permeation, metabolism and generally clearance of the drug within and from the skin.

8. The Mechanism of Niosomes as topical drug carriers

The rationale for the use of vesicles as topical drug carriers.

Firstly, the vesicles are served as the solvent for drugs to obtain the higher local drug concentrations leading to the higher thermodynamic activity. Secondly, the vesicles are served as a local depot for sustained release of drugs.Thirdly, the nonionic surfactant vesicles are served as penetration enhancer and these vesicles facilitate dermal drug delivery. Lastly, the vesicles are served as a rate-limiting membrane for controlled transdermal drug delivery systems (Schreier, 1994).

There are two main objectives for topical application, systemic effect and local effect. For local effect, it is desirable that the drug penetrate through the skin and localized at its size of action without being taken up by the blood circulation and distributed to systemic sites. For systemic effect, the drug has to penetrate through the skin and should have adequate uptake by the blood circulation to have action. The

principle factors involving cutaneous or percutaneous absorption are drugs, the tissues of applied sites and the vehicle (Mezei, 1993).

Niosomes are microscopic vesicles composed of membrane-like lipid layers surrounded by aqueous compartments (Mezei, 1993). Hofland et al had demonstrated the appearance of structural changes deeper in the stratum corneum, resembling multilamellar vesicular structures. Schreier et al (1989) speculated that either intact niosomes migrated into the stratum corneum, or that molecularly dispersed high local concentrations of nonionic surfactants could form curved lamellar structures within the intercellular lipid of the stratum corneum(Scherier,1994).

Some common mechanisms that had been reported for niosomes were fusion of vesicles on the surface of the skin that might lead to high thermodynamic activity of drug that is the driving force for the permeation of lipophilic drugs (Fang, 2001).

Most studies were in agreement that direct contact between vesicles and skin was essential for penetration and drug delivery. If direct contact was obstructed, drug transport was decreased. Hofland and Komatsu et al (1986) showed that a penetration enhancement of the molecular components could not fully account for the increase in drug transport observed when drugs were encapsulated in vesicles. It appeared that vesicles had a more pronounce effect on transport of lipophilic drugs than on that of hydrophilic drugs (Scherier, 1994).

Transcellular and Intercellular pathways (Schaefer, 1999)

The rate-limiting step for permeation included a hydrophobic barrier, intercellular lipid. It was suggested that the only continuous domain within the stratum corneum be formed by the intercellular lipid space. This suggests that the majority of compounds penetrating the stratum corneum must pass through intercellular lipid.

There was additional evidence that other compounds could penetrate the corneocytes or altered their water-binding capacity that was found in low molecular weight moisturizers.

The protein in the stratum corneum was greatly composed of intracellular keratin filaments that were crosslinked together. Also the intercellular lipid mainly found in the stratum corneum was cholesterol and ceramide.

It was clear that the major route of permeation across the stratum corneum was via the tortuous but continuous intercellular lipid. The rate at which permeation occur was largely dependent on the physicochemical characteristics of the penetrant. (Roberts,1998)

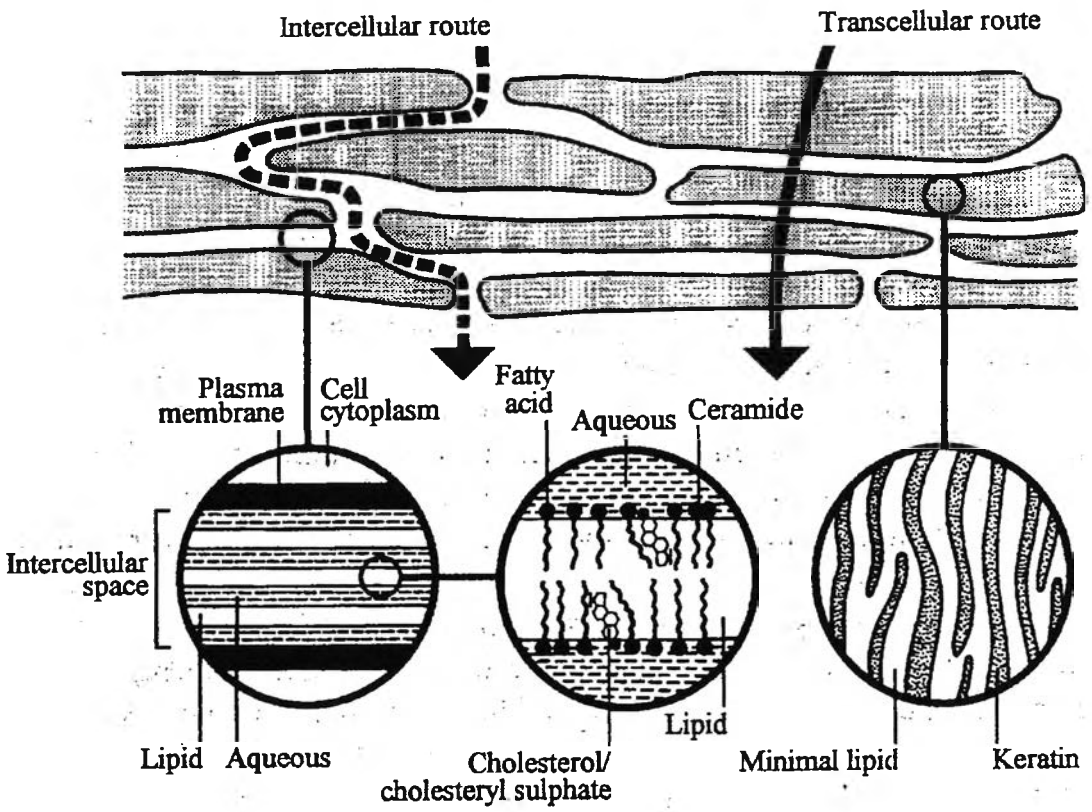


Figure 12. The model of the stratum corneum and penetration pathway

(Schaefer, 1999)

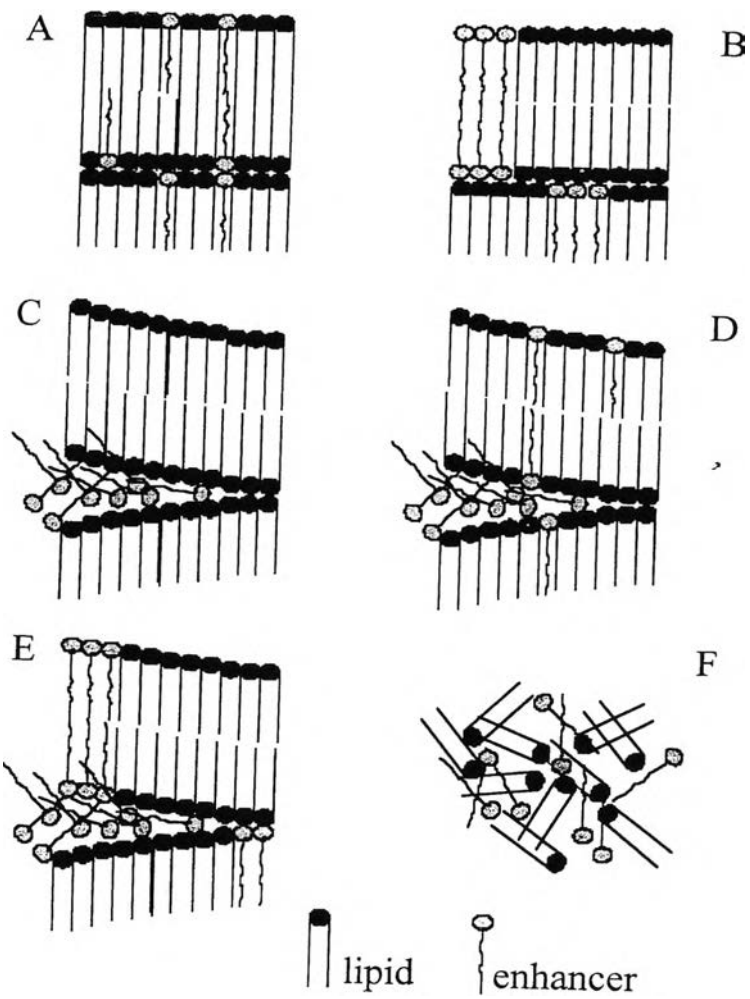


Figure 13. The possible mechanisms involved in the effect of penetration enhancers (A) Intercalation of the enhancer in the lipid lamellae. (B) Phase separation between enhancer and skin lipids in the lamellae. (C) Phase separation between lipid lamellae and an enhancer-rich phase. (D) Intercalation of the enhancer in the lipidlamellae and simultaneous phase separation between lipid lamellae and enhancer. (E) Phase separation within the lamellae and separation between an enhancer-rich phase and the lamellar phase. (F) Disappearance of the lamellar phase (Brouwstra, 2001).

9. Snake skin

Typically, shed snake skin penetration studies provided conservative estimate for human skin penetration since it was less permeable than human skin for most compound (Itoh, 1990). Recently, there had been considered interest in the use of shed snake skin as membrane model for in vitro diffusion studies.

It is particularly difficult to obtain human skin for in vitro experimentation and it is therefore important to have alternate biological or synthetic membranes that mimic human skin membranes for diffusion experiments. The epidermis was shed as a large intact sheet, thus a single snake skin could provide multiple samples. Since snakes skin lacked hair follicles, the problems associated with the transfollicular route of penetration, which might be significant in mammalian skins, could be avoided.

However shed snake skin consisted of three distinctive layers.These were the beta-keratin-rich outermost beta layer, alpha-keratin- and lipid-rich innermost alpha layer. Furthermore, the mesos layer showed three to five layers of multilayer structure with cornified cells surrounded by intercellular lipids, which was similar to human stratum corneum. This mesos layer was also a major depot of lipids, and the mesos layer and alpha layer were considered to be the main barrier to water penetration through the skin. Furthermore, water permeability had been compared

between shed snake skins from normal snakes and scaleless skin. This indicated that the existence of scales might not affect significantly the permeability of compounds through shed snake skin.

However different species displayed different permeation characteristics. It was important that if shed snake skin is used as a membrane, the species and skin site should be reported. The integrity of shed snake skin, as verified by electron microscopy, indicated that it behaved as a diffusion membrane. It appeared that shed snake skin might well be a useful membrane for comparing the diffusion of specific drugs from different formulations or the effects of different formulations or the effects of different enhancers but care must be exercised when extrapolating to the in vivo situation. (Haigh, 1998).

Fick's Laws of Diffusion (Flynn, G.L., 1990; Chien, 1987)

The amount dQ of substance diffusing in time dt across a plane of area A is directly proportional to the change of concentration dC with distance travel dX

Fick's law is written as follows:

$$dQ = -DA \frac{dC}{dX} dt \quad (1)$$

D is known as diffusion coefficient and is seen to give the amount of material diffusing per unit time across a unit area when $\frac{dC}{dX}$, called the concentration gradient that is unity. D has the dimension of area per unit time.

$$\frac{dQ}{dt} = -DA \frac{dC}{dX} \quad (2)$$

$\frac{dQ}{dt}$ is the rate of permeation

$$dQ = P(C_d - C_r) \quad (3)$$

C_d and C_r are the concentrations of a skin penetrant in the donor compartment and in the receptor compartment, respectively. P is the permeability coefficient of the skin tissue.

$$P = \frac{KD}{h} \quad (4)$$

K is the partition coefficient

D is the apparent diffusivity

h is the thickness of the skin tissue

The rate of permeation of component across a thin unit area is the flux

$$J_{\text{unit area}} = \frac{J_T}{A} = -D\left(\frac{dC}{dX}\right) \quad (5)$$

$J_{\text{unit area}}$ is the flux of component across a thin unit area

J_T is the net amount of substances crossing through the skin membrane.

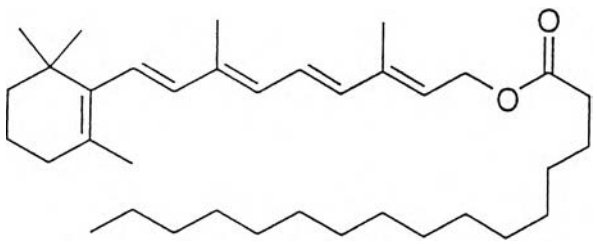
A is the area of membrane that substance can pass, cm^2

D is diffusion coefficient, $\frac{cm^2}{hr}$

(dC/dX) is the difference of concentration that cross the membrane with time.

J was proportional to the difference in concentration across the plane (dC/dX). The term D was a proportionality constant called either the diffusion coefficient or the diffusivity. The negative sign is the mathematical means of permeants at high concentrations. Penetrants were on occasion also able to induce time and concentration dependent alterations of the structures of the membrane they were crossing, with accompanying changes in diffusivity. D was thus a coefficient, not a constant. It was a differential diffusion coefficient. So Fick's first law contains three principal variables, J, C and X.

10. All-Trans-Retinyl Palmitate



Chemical Identification

The following synonyms are used for retinyl palmitate:

Aquasol A

Arovit

Axerophtol palmitate

Optovit – A

Retinol hexadecanoate

Retinol palmitate

Retinyl palmitate

Vitamin A palmitate

Physicochemical properties of all-trans retinyl palmitate

Formula	$C_{36}H_{60}O_2$
Formula weight	524.88
Melting point	28-29 °C
UV Absorption	
λmax	325-328
$E^{1\%}_{1cm}$	960

ε	50,390
Fluorescence	
Excitation λmax	325
Emission λmax	470
Solubility	
Water	insoluble.
Glycerol	insoluble.
Ethanol	soluble.
Chloroform	soluble.
Ether	soluble.