CHAPTER 2

LITERATURE REVIEW
In recent years, vesicles have become the vehicle of choice in drug delivery. Lipid vesicles were found to be of value in immunology, membrane biology, diagnostic techniques, and most recently, genetic engineering (Ogihara-Umeda et al., 1997; Park et al., 1997; Kao et al., 1996) [1-3]. Vesicles can play a major role in modeling biological membranes, and in the transport and targeting of active agents. Vesicular drug delivery systems delay drug elimination of rapidly metabolizing drugs, and function as sustained release systems. This system solves the problems of drug insolubility, instability, and rapid degradation. Consequently, a number of vesicular delivery systems such as liposomes, niosomes, pharmacosomes etc, were developed.

Rigorous conditions required for handling liposomes under cryogenic atmosphere have prompted the use of non-ionic surfactant in vesicular drug delivery system, in lieu of phospholipids. Thus, the new vesicular delivery system consisting of unilamellar or multilamellar vesicles called niosomes was introduced. In this case, an aqueous solution is enclosed in a highly ordered bilayers made up of non-ionic surfactant, with or without cholesterol and dicetyl phosphate, and exhibit behavior similar to liposomes in vivo. The bilayered vesicular structure is an assembly of hydrophobic tails of surfactant monomer, shielded away from the aqueous space located in the center and hydrophilic head group, in contact with the same.

Niosomes entrap solute in a manner analogous to liposomes. They are osmotically active, and are stable on their own, as well as increase the stability of the entrapped drugs (Rogerson et al., 1988; Baille et al., 1984) [4-5]. Handling and storage of surfactants require no special conditions. Niosomes possess an infrastructure consisting of hydrophilic and hydrophobic moieties together, and as a result, can accommodate drug molecules with a wide range of solubilities (Udupa et al., 1991) [6]. They exhibit
flexibility in structural characteristics (composition, fluidity, size,), and can be designed according to the desired situation (Chitnis et al., 1984)[7].

2.1 Factors affecting characteristics of niosomes

The application of non-ionic surfactant vesicles as vectors for drug delivery has been reviewed by many researchers. Numerous studies have been undertaken till date to investigate correlation between the various component, formulation, process and physicochemical aspects with the various characteristics of resultant niosomes i.e. vesicle size and encapsulation efficiency. Following is the summary of the observation made by the various researchers.

2.1.1 Factors affecting vesicle size

Effect of Entrapped solute on the vesicles size

Hexadecylglyceryl ether based vesicles demonstrated the effect of incorporated solution on the size of vesicles. It was observed on increase in drug entrapment generally the size of niosomes increases. Yoshika et al, 1994 have explained the effect of drug entrapment on size of niosomes as drug content increase the size of niosomes increases [8].

Effect of vesicle forming components on the vesicles size

It was observed that mixed poly(glycerols), poy(oxyethylene) ether niosomes exhibited remarkable effect of solulan C24. The size of vesicles was recorded relatively smaller with increasing chain length of polyoxyethylene most probably due to reduction in radius of curvature of bilayers of surfactant I and II owing to a better interaction of polyoxyethylene ether segment of surfactant (Gtiener et al, 1993) [9].

2.1.2 Factors affecting entrapment efficiency

Effect of method on the entrapment efficiency

Entrapment efficiency depends mainly upon the preparation method. Non-ionic surfactants prepared by ether injection method demonstrated higher entrapment
efficiency as compared to those prepared by hand shaking method. Ether injection method produces conventional unilamellar vesicles more uniform in size resulting into higher drug entrapment compared to MLVs.

**Effect of surfactant and lipid levels**

The level of surfactant: lipid used to make niosomal dispersions is generally 10–30 mM (1–2.5%w:w) (Baillie et al., 1985; Lawrence et al., 1996; Santucci et al., 1996) [10-12]. 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 increases the total amount of drug encapsulated, although highly viscous systems result, if the level of surfactant: lipid is too high [13].

**2.1.3 Factors affecting entrapment efficiency and solute release rates**

In spite of many investigations into the efficiency of encapsulation of hydrophilic or hydrophobic solutes within niosomes, there is no satisfactory theoretical approach to the topic, partly because the mode of preparation and subsequent processing of the system (which is usually varied) determines the outcome. However it can be said that non-ionic surfactant vesicle encapsulation efficiency is a product of the stability of the dispersion, the method and factors governing vesicle loading and the intrinsic properties of the vesicles. The stability of the encapsulated solute and the solute retention capability of the encapsulating membrane, together with the stability of both the surfactant and the vesicle structure, all contribute to stability of the complete formulation. Intrinsic properties of the vesicles that govern the encapsulation efficiency are vesicle size, cholesterol content and nature of the membrane components. The method of loading, which also defines the encapsulation efficiency, is governed by the nature of the solute, hydration temperature and ultimately the actual loading method used. As might be expected, larger niosomes and phospholipids vesicles, as a rule, show higher entrapment efficiencies than smaller vesicles (Uchegbu, 1994; Vemuri et al., 1990) [14-15]. The
encapsulation of water soluble solutes appears to result in increased vesicular size, which could be due to the interaction of solutes with the amphiphile head groups, thus increasing the mutual repulsion of surfactant bilayers (Stafford et al., 1988) [16]. Vesicles loaded by transmembrane ion gradients show higher entrapment efficiency than those loaded during the hydration step (Montero et al., 1993) [17]. Niosome membrane stability is important as the continued integrity of the carrier system has a direct bearing on encapsulation efficiency; stable membranes are less likely to lose intravesicular solute and hence can retain higher levels of solute. Cholesterol adds a positive component to membrane stability.

The Influence of Cholesterol

The synthetic alkyl glycosides form vesicles without the inclusion of cholesterol (Kiwada et al., 1985) although the incorporation of 29 mole% cholesterol increases the encapsulation efficiency of C_{16}-glucoside vesicles three fold [18]. The inclusion of 29 mole% cholesterol in vesicle formulations based on synthetic polyhydroxyl lipids increased the encapsulation efficiency in all the surfactants studied by between 50 and 100% (Assadullahi et al., 1991) [19]. The encapsulation efficiency of a series of C_{20} \text{a}, and C_{18} sorbitan monooesters showed an almost linear increase with increasing cholesterol content (from 0 to 50 mole% cholesterol content) (Yoshioka et al., 1994) [8]. Encapsulation efficiency is a measure of the solute retention and cholesterol has been shown by a number of authors to enhance solute retention. The release of doxorubicin from C_{16}G_{3} niosomes increased from below 5% after 6 h with niosomes bearing 50 mole % cholesterol to above 10% with cholesterol free niosomes (Rogerson et al., 1987) [20]. The initial concentration of doxorubicin in the niosomes is not given, but it is expected that the more permeable membranes (cholesterol free) would entrap a lower amount of the drug and hence concentration gradient effects cannot be used to explain these observations. Doxorubicin release from C_{10}G_{2} - stearylamine (stearylamine 5 mole %)
niosomes was approximately 2% over 6 h when the formulation contained 8.5 mole % cholesterol and a mere 0.1 % over the same time period when the formulation contained 47.5 mole % cholesterol (Florence et al., 1990) [21]. These studies demonstrate that the stabilization of the membrane observed with phospholipids membranes by cholesterol inclusion is also apparent with non-ionic surfactant vesicles.

In niosomes prepared from polyoxyethylene (20) 1,2 distearoyl ether (Chauhan et al., 1989), cholesterol had no significant influence on encapsulation efficiency [22]. When vesicles were produced by two different methods, namely the stable plurilamellar method (Gruner et al., 1985) and the hydration of lipid films to produce conventional multilamellar vesicles [23]. The presence of the polyoxyethylene hydrophilic side chain is deemed to be responsible for these similar encapsulation efficiencies as aqueous soluble drug molecules may be entrapped within the long hydrophilic polyoxyethylene side chain regions of these structures (Chauhan et al., 1989) [22].

Most of the surfactants used to make non-ionic surfactant vesicles have a low aqueous solubility but the freely soluble surfactant polysorbate 20, which usually forms micelles on hydration, has been reported by Murtas and co-workers (Murtas et al, 1994) to form vesicles but only in the presence of cholesterol in excess of 33.33 mole % [24]. As the cholesterol content of these formulations decreased, the entrapment efficiency of calcein also decreased, falling from 0.47 l mole⁻¹ in niosomes prepared from a formulation containing 50 mole % cholesterol to 0.1 l mole⁻¹ in a formulation in which the cholesterol content was 12.4 mole%. Fluorescence anisotropy measurements indicate that although a vesicular bilayer appears to be present in formulations containing 50, 42.75 and 33.33 mole% cholesterol, formulations containing less than 33.33 mole% cholesterol consisted largely of micelles. The compound C₁₂EO₇ also forms micelles in the absence of cholesterol whereas with the inclusion of 10 mole % cholesterol 100 nm vesicles were formed, the vesicles decreasing in size as the molar fraction of cholesterol increased
from 33 mole % to 50 mole %. It is clear from the above results that the inclusion of an optimum amount of cholesterol in the bilayers increases the stability of the bilayers.

The Nature of the Hydrophilic Head Group

Other factors contribute to the stability of niosomes, one of which is the nature of the hydrophilic head group. The entrapment efficiency of a series of C\textsubscript{16}-glycoside-cholesterol vesicles was determined (Kiwada et al., 1985) that while C\textsubscript{16}-glucoside and C\textsubscript{16}-mannoside gave entrapments of above 7%, C\textsubscript{16}-galactoside gave entrapment efficiencies of only 5% [18]. The sugar moiety in these glycosides differs by the orientation of the 4-hydroxy group (equatorial in glucose and mannose but axial in galactose). Vast differences in the structural transitions and stability of glycolipid model membranes have been reported with differences in sugar moiety stereochemistry (Hinz et al., 1991) [25] and although the hydration of galactoside vesicles are said to be low (Kiwada et al., 1985), the exact mechanism by which stereochemical differences affect membrane structure is not clear. However the lower hydration of the galactose moiety in relation to the glucose moiety has been attributed to the increased possibility of hydrogen bonding between the hydroxyl groups in positions 4 and 6, a situation that does not arise with the glucose moiety. The entrapment efficiency of lactoside glycolipids was as low as 3%, an effect probably, once again, due to different levels of hydration of the sugar moiety (Jousma et al., 1989) as lower levels of hydration lead to a decrease in $\mu_0$ and hence an increase in the CPP (critical packing parameter) [26].

A comparison between non-ionic surfactant based bilayers composed of monomers with identical alkyl groups but different hydrophilic groups - ethoxy versus glycerol moieties - revealed that the latter group of compounds had lower head group area measurements than those of the former. This gave an increased mobility to the polyethoxylated alkyl chains and an increased fluidity to the corresponding bilayers when compared to the alkylglycerol bilayers of similar compound hydrophobicity (Ribier et al., 1984) [27].
Ultimately these fluidity differences would lead to a less leaky membrane with the alkylglycerol derivatives.

**The Nature of the Alkyl Side Chain**

Kiwada and co-workers, 1985b have reported on the effect of alkyl side chain on percent encapsulation. They have reported the effect of alkyl chain length on the encapsulation efficiency of \(^{14}\)C sucrose [28]. They reported that longer the alkyl chain glycolipids better is the encapsulation efficiency. These workers suggested that the alkyl chain length cut off point for vesicle formation may be 14 carbon chain. They also claimed that the dialysis procedure used to separate the encapsulated material from unencapsulated material would have resulted in the loss of C\(_6\), C\(_{10}\) and C\(_{12}\) compounds.

However, vesicles purification using gel filtration technique (using Sephadex G-50) is said to prevent the destabilization of vesicles prepared from low molecular weight Sorbitan lauryl esters (C\(_{12}\)) (Yoshioka et al., 1994; Uchegbu, 1994).

One should know the effect of alkyl chain length on vesicle size. It was reported that a gradual decrease in size as the alkyl chain length increases is observed with 5(6)-carboxyfluorescein entrapped niosomes (Yoshioka et al., 1994). These vesicles are smaller than doxorubicin-loaded vesicles which show a size of approximately 6 \(\mu\)m, irrespective of the Sorbitan surfactant used (Uchegbu, 1994). The relationship observed between niosome size and Sorbitan monoester monomer hydrophobicity has been attributed to the decrease in surface free energy with increasing hydrophobicity. Uchegbu, 1994 has reported that the size of doxorubicin niosomes decreases with a decrease in hydrophilicity.

**Nature of entrapped solute**

The effect of method of loading on retention of encapsulated doxorububicin within Sorbitan monoesterate vesicles was studied (Uchegbu et al., 1994; Cullis et al., 1987; Harrigan et al., 1993; Montero et al., 1993; Haran et al., 1993) [14,29-32]. It is found that
when vesicles are loaded using ordinary pH gradients, approximately 50% of the drug is releases over 24 h at 37 °C. However, only 20% of the drug was released over the same period from vesicles loaded using ammonium sulphate gradients (Uchegbu and Florence, 1995) [33]. This is attributed to the nature of the counter-ion and ultimately the stability of the ionic association produced by the cationic drug ions, and intravesicular sulphate counter ions, which have a major influence on solute retention. From this it can be understood that leakage of encapsulated solute due to membrane permeability can be overcome using a suitable intravesicular ionic trap. The release rate of a solute is affected by the nature of the solute, even when similar counter ions are considered. The release of 5(6)-carboxyfluorescein and doxorubicin from C_{16}G_{2}: cholesterol: solulan C24 (30: 80: 40) vesicles were 3% and 10% over a time period of 6 h at 22 °C (Uchegbu, 1994). The slow release of 5(6)-carboxyfluorescein is attributed to the reason the they are fully ionized at the working pH 7.4 and thus require a high energy process to pass through the membrane as compared to the passage of the partially ionized doxorubicin molecule through the membrane.

**Vesicle surface charge**

The presence of surface charge in vesicular dispersions is critical. It has been found that aggregation of vesicles in isotonic saline solution occurs when the vesicles are prepared without the inclusion of charged molecule in the bilayer (Haran et al., 1993). Aggregation is attributed to the shielding of the vesicle surface charge by ions in solution and there by reducing the electrostatic repulsion. However, a reduction in the formation of aggregated was observed when a charged molecule like Dicetylphosphate was incorporated in C_{n}EO_{m} bilayer vesicles (Cable, 1989) [34]. Vesicle surface charge can be estimated by measurement of particle electrophoretic mobility and is expressed as the zeta potential, which is calculated using the Henry equation (Vanhal, 1994) [35].
\[ \zeta = \frac{\mu E \pi \eta}{\Sigma} \]

Where \( \zeta \) = Zeta potential; \( \mu E \) = electrophoretic mobility; \( \eta \) = Viscosity of the medium; \( \Sigma \) = dielectric constant.

### 2.1.4 Factors affecting solute release profile

In regard to release of entrapped solute from niosomal system, cholesterol was noted to have distinct effect on release profile as well as on vesicle stability. In a study the effect of cholesterol incorporation on \( \text{C}_{16}\text{G}_{3} \) based niosomes has been well correlated with mixed monolayers consisted of surfactants and cholesterol. Cholesterol was found to retard release rate of solute and stabilize the system against degradation (Khand et al., 1987) [36].

NSVs demonstrated temperature dependency on release of solute, which could successfully be utilized in therapeutics using local hyperthermia. The characteristics recorded were similar to the liposome. In case of liposomes it was related to some lipid component that brings down phase transition temperature of lipoidal system. Cholesterol is however noted to abolish the clear temperature dependency of solute film. Similarly, incorporated drug may effect temperature dependent efflux profile of itself (Rogerson, et al., 1987).

Tanaka et al., 1990 studied and determined fluidity of bilayer using ESR spectrum of lipophilic probe 5-12 and 16 deoxystearic acid, 5Ns, 12Ns and 1.6Ns respectively). Order of parameters is function of number of ethylene oxide units; the order however decreases with increasing HLB of surfactant used in the formation of bilayer or vesicles.
2.2 Findings reported on niosomes for topical or transdermal applications

Drug delivery through niosomes is one of the approaches to achieve localized drug action, since their size and penetrability through epithelium and connective tissue keeps the drug localized at the site of administration. Localization of drug action results in enhancement of efficacy or potency of the drug and at the same time reduces its systemic toxic effects.

Hofland et al., 1989 [37] have demonstrated the appearance of structural changes deeper in the stratum comeum, resembling multilamellar vesicular structures following exposure of liquid state niosomes to skin. The authors speculate that either intact niosomes migrated into the stratum corneum, or that molecularly dispersed high local concentrations of non-ionic surfactants could form curved lamellar structures within the lipid interstitial spaces of the stratum corneum.

Hofland et al., 1991, [38] have employed inhibition of cell proliferation of SV-40-transformed human keratinocytes to study dermal toxicity of nonionic surfactant vesicles (niosomes) A 10-fold stronger inhibition of proliferation was found with polyoxyethylene alkyl chains linked with ester bonds, compared to those linked with ether bonds, while the presence of cholesterol appeared to have no effect on cell proliferation. Freeze-fracture electron micrographs revealed clusters of vesicular lipid lamellae, which were clearly different from endogenous lipids found within the intercellular lipid lamellae. The authors speculate that indeed intact surfactant vesicles migrate into the skin lipid layers, or that such vesicular structures form following molecular dispersion and diffusion of surfactants into the skin lipid lamellae.

Reddy et al., 1993, [39] have reported niosomes, microspheres, and beta-cyclodextrin formulations exhibited significant improvement in bioavailability and anti-inflammatory activity over the drug in ointment base.
Raja Naresh et al., 1993 [40] have reported that the anti-inflammatory activity of diclofenac sodium was higher when niosome encapsulated preparation was administered intraperitonially and transdermally to rats, compared to a plain drug solution.

Hofland et al., 1994 [41] shown that gel-state (C\textsubscript{18}EO\textsubscript{3}) niosomes did not increase the penetration of estradiol through human skin after pretreatment, while pretreatment with liquid crystalline (C\textsubscript{12}EO\textsubscript{3} or C\textsubscript{9}EO\textsubscript{10}) vesicles resulted in a significant increase in estradiol transport. Estradiol encapsulated in liquid-crystalline vesicles resulted in much higher estradiol fluxes than when applied in a buffer solution. Authors also, reported that although pretreatment with vesicles resulted in higher estradiol fluxes compared to untreated stratum corneum, the fluxes were significantly lower than when estradiol was encapsulated in vesicles.

Popli and Nair, 1995 [42] have studied the encapsulation of tenoxicam in niosomes for local and oral therapy and investigated that by varying the vesicle composition, stable vesicles with optimum size range, entrapment efficiency and drug release properties can be prepared resulting in a formulation with optimum pharmacokinetic and therapeutic characteristics.

Jayraman et al., 1996 [43] have studied topical delivery of erythromycin from niosomal formulation and investigated that this system offer advantages over phospholipids because of lower cost, better stability and ease of preparation. It has been reported that non-ionic surfactant vesicles facilitate the entry of drugs into the skin more efficiently than phospholipids liposomes.

Fang et al., 2001 [44] have reported ability of liposomes and niosomes topical formulations of enoxacin to modulate drug delivery without significant toxicity. Liposomes and niosomes were prepared by a combination of ethanol injection and freeze-drying techniques. The influence of vesicles on the physicochemical property and stability of
the formulations were measured. The enhanced delivery across the skin of liposome and
niosome encapsulated enoxacin had been observed. Authors reported both permeation
enhancer effect and direct vesicle fusion with stratum corneum may contribute to the
permeation of enoxacin across skin. Formulation with niosomes demonstrated a higher
stability compared to liposomes.

Shahiwala et al., 2002 [45] have also reported a topical application of niosomally
entrapped nimesulide, prepared by lipid film hydration technique using tweens and
spans, which was then optimized for highest percent drug entrapment (PDE). The
prepared niosomes were evaluated for drug diffusion across human cadaver skin, using
modified validated diffusion cell. Developed nimesulide niosomal gel formulation has
also demonstrated enhanced anti-inflammatory activity compared to plain drug gel and
marked formulation using acute rat paw edema method.

Carafa et al. 2002 [46] studied the in vitro permeation through mouse abdominal skin of
non-ionic surfactant vesicles (NSV) formulation entrapping lidocaine in the form of a free
base and a hydrochloride. NSVs were prepared from polyoxyethylene sorbitan
monolaurate (Tween20) and cholesterol. Vesicles were also prepared in the presence of
dicetylphosphate and N-cetyl pyridinium chloride to obtain negatively and positively
charged vesicles respectively. Permeation through mouse abdominal skin of lidocaine
HCl loaded vesicles showed a higher flux and a shorter lag time with respect to classical
liposome formulations, while lidocaine base permeation rate was quite similar for NSV
and liposome formulations. Vesicles with the charge showed the entrapment efficiency
of the drug was negligible.
2.3 Findings reported on dry proniosomes

Many researchers have prepared a dry proniosomes with a view to develop a stable formulation by using simple methods that are easy to scale up.

Hu et al, 1999 [47] first described the preparation of proniosomes, a dry free-flowing surfactant coated carrier particles which may be hydrated immediately before yield aqueous niosome dispersions similar to those produced by conventional methods. Proniosomes were prepared by spraying the surfactant solution in organic solvent on sorbitol particles followed by evaporation of organic solvent until sorbitol appeared to be dry and free flowing. Conventional and proniosome-derived niosomes were compared for their morphology, particle size, particle size distribution, and drug release performance in synthetic gastric or intestinal fluid. They reported that the in all comparisons, proniosome-derived niosomes are as good or better than conventional niosomes.

Rhodes et al, 2001 [48] described a novel method for the preparation of proniosomes. A slurry method has been developed to produce proniosomes using maltodextrin as the carrier. Maltodextrin-based proniosomes were studied for the effect of surfactant loading on the encapsulation. They reported efficient encapsulation of amphiphilic drug into the niosomes was achieved for a wide range of surfactant loadings, time required to produce proniosomes by this method is independent of the ratio of surfactant solution to carrier material.

Rhodes et al, 2001 [49] studied SEM imaging for prediction of quality of niosomes from maltodextrin-based proniosomes. Proniosomes with different surfactant loading were prepared by the slurry method using maltodextrin as the carrier and examined for surface characteristics by scanning electron microscopy. They have observed inefficient rehydration and occurrence of aggregation and precipitate in the final niosome suspension for proniosomes with coarse and broken surface.
2.4 Finding reported on proniosomes for transdermal applications

Many researches were carried out studies on formulation and evaluation of proniosomes for transdermal application of various drugs.

**Vora et al, 1998** [50] have first reported proniosomal gel based transdermal delivery of levonorgestrel for effective contraception. The proniosomal structure was liquid crystalline-compact niosomes hybrid, which could be converted into niosomes upon hydration. The system was evaluated in vitro for drug loading, rate of hydration (spontaneity), vesicle size, polydispersity, entrapment efficiency and drug diffusion across rat skin. The effect of composition of formulation, amount of drug, type of Spans, alcohols and sonication time on transdermal permeation profile was observed. The study demonstrated the utility of proniosomal transdermal patch bearing levonorgestrel for effective contraception.

**Fang et al, 2001** [51] have reported the skin permeation of estradiol form various proniosome gel formulations across excised rat skin. Proniosome gels were prepared by using two types of surfactant span and tween. The encapsulation efficiency and size of niosomal vesicles formed from proniosomes upon hydration were characterized. In order to verify the possible mechanism of estradiol permeating across skin from proniosomes, a series of permeation studies were performed i.e. Estradiol release rate across cellulose membrane. Estradiol permeation across skin pretreated with solutions of lecithin, span and tween. Estradiol permeation from reconstituted niosome suspension. They have reported that only proniosome gel not niosome suspension appears to efficiently deliver estradiol by the transdermal route in this study.

**Kumhar et al. 2003** [52] were prepared proniosomal gel formulations by coacervation phase separation technique for transdermal drug delivery of ethinylestradiol and levonorgestrel. Proniosomal gels were prepared from different nonionic surfactants, egg lecithin, Brij 58, dicetyl phosphate, soya lecithin and cholesterol. The authors reported
the formulation prepared using span 20 and span 40 have shown better in vitro and in vivo performance.

Alsarra et al, 2004 [53] studied the in vitro permeation of ketorolac from different Proniosomal gel formulations. Proniosomes were prepared by using span or tween, lecithin and cholesterol and characterized for encapsulation efficiency and vesicle size after dilution with buffer. Proniosomes was mixed with polymeric gels. They reported each of the prepared Proniosomal gel significantly improved drug permeation and reduced the lag time.
2.4 References


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