CHAPTER 2

LITERATURE SURVEY

- Novel drug delivery system
- Merits
- Limitations
- Classification of targeting
- Types of Niosomes
- Methods of preparation of Niosomes
- Stability of Niosomes
- Invivo behaviour of Niosomes
- Characterization of Niosomes
- Toxicity aspects
- Routes of administration
- Advantages
- Applications

2.1.1 NOVEL DRUG DELIVERY SYSTEM

In 21st century, the pharmaceutical industry is caught between downward pressure on prices and the increasing cost of successful drug discovery and development. The average development cost of a new chemical entity (approximately $ 500 million) is much higher than that required to develop a novel drug delivery system (NDDS). In the form of NDDS, an existing drug molecule can get a "new life", thereby, increasing its market value, competitiveness, and extended product life. Limited formularies, patent expiry with subsequent entry of generic competition and the vertical integration have spawned entire pharmaceutical industry (approximately 350 drug delivery companies and 1000 medical device companies) focused on designing and developing new and better methods of delivering drugs. There has been a significant increase in the approval of NDDS in the last few years and this rate is expected to gain momentum in the near future. Analysts estimate that the drug delivery market will be at $ 120 billion by 2007. For a country like India, where pharmaceutical industry has limited resources and development of new drug molecules has made a modest beginning, interest in new drug delivery technologies is an obvious direction, especially in view of the changed patent regime.

Drug delivery companies are engaged in the development of multiple platform technologies for controlled release, delivery of large-molecule, liposomes, polymeric delivery, taste-masking, oral fast

dispersing dosage forms, technology for insoluble drugs, and delivery of drugs through intranasal, pulmonary, transdermal, vaginal, colon and transmucosal route.[1]

The goal of any drug delivery system is to provide a therapeutic amount of drug to the proper site in the body to promptly achieve and then maintain the desired drug concentration. This idealized objective points to the two aspects most important to drug delivery, namely, spatial placement and temporal delivery of a drug. Spatial placement relates to targeting of a drug to a specific organ or tissue, while temporal delivery refers to controlling the rate of drug delivery to the target tissue. An appropriately designed sustained release drug delivery system can be a major advance towards solving these two problems. It is for this reason that the science and technology responsible for development of sustained release pharmaceuticals have been and continue to be the focus of a great deal of attention in both industrial and academic laboratories. There currently exist numerous products in the market formulated for both oral and parenteral routes of administration that claim sustained or controlled drug delivery. The bulk of research has been directed at oral dosage forms that satisfy the temporal aspect of drug delivery, but many of the newer approaches under investigation may allow for spatial placement as well.

Non-immediate release delivery systems may be conveniently divided into four categories

1. Delayed release
2 Sustained release
    a. Controlled release
    b. Prolonged release
3. Site-specific release
4. Receptor release

DELAYED RELEASE

Delayed-release systems are those that utilize repetitive, intermittent dosings of a drug from one or more immediate release units incorporated into a single dosage form. Examples of delayed-release systems include repeat action tablets and capsules and enteric coated tablets where timed release is achieve by a barrier coating. A delayed release dosage form does not produce or maintain uniform drug blood levels within the therapeutic range, but nonetheless is more effective for patient compliance than conventional dosage forms.

SUSTAINED-RELEASE:

Sustained-release systems include any drug delivery system that achieves slow release of drug over an extended period of time. If the system is successful at maintaining constant drug levels in the blood or target tissue, it is considered a controlled-release system. If it is

unsuccessful in this but nevertheless extends the duration of action over that achieved by conventional delivery, it is considered a prolonged release system.

SITE-SPECIFIC AND RECEPTOR RELEASE:

Site specific and receptor release refer to targeting of a drug directly to a certain biological location. In the case of site-specific release, the target is a certain organ or tissue; for receptor release, target is the particular receptor for drug within an organ or tissue Both of these systems satisfy the spatial aspect of drug delivery.

2.1.2 MERITS OF NOVEL DRUG DELIVERY SYSTEM

1. Improved treatment of many chronic illnesses where symptom break through occurs when the plasma level of drug drops below the minimum effective level (Ex. Asthma, Arthrits)

2. Increased bio availability.

3. Reduction in the incidence and severity of untoward systemic side effects related to high peak plasma drug concentration.

4. Maintenance of the therapeutic action of a drug during overnight no dose periods.

5. Reduction in the total amount of drug administered over the period of drug treatment. This contributes to the reduced incidence of systemic and local side effects.

6. Protection from first pass metabolism and gastrointestinal tract degradation.

7. Improved patient compliance resulting from the reduction in the number and frequence of doses required to maintain the desired therapeutic response.

8. Targeting the drug molecule towards the tissue (or) organ reduces the toxicity to the normal tissues.

9. Pulsatile and pH dependent systems release the drug whenever the body demands.

10. Biocompatibility.

11. Economic savings are claimed to be made from better disease management achieved with this system.

2.1.3 LIMITATIONS OF NOVEL DRUG DELIVERY SYSTEM

Though there are so many advantages in this system there are a few factors that limit its usage.

1. Variable physiological factors such as gastro intestinal pH enzyme activities, gastric and intestinal transit rates. Food and severity of patients disease which often influence drug bioavailability from conventional dosage forms may also interfere with the precision of control release and absorption of drugs from this system.

2. The products which tend to remain intact may get lodged at some sites. If this occurs slow release of drug from the dosage form may produce a high localized concentration of drug which causes local irritation.

3. Drugs having biological half-life of 1 hr. or less are difficult to be formulated as sustained release formulations.

The high rate of elimination of such drugs from the body needs an extremely large maintenance dose which provides 8-12 hrs. of continuous therapy

4. These products normally contain a large amount of drug. There is a possibility of unsafe over dosage, if the product is improperly made and the total drug contained therein is released at one time or over too short time interval.

5. If it is once administered it may be difficult to stop the therapy for reasons of toxicity or any other.

6 It may be unwise to include potent drugs in such systems

RATIONAL FOR SITE SPECIFIC DELIVERY:[4]

1. To reach previously inaccessible domains e.g. intracellular site, bacteria, viruses, parasites etc

2. Exclusive drug delivery to specific cells (or) diseased site in the body.

3. Reduction in the drug dose and side effects.

4. To control the rate and frequency of delivery at the pharmacological receptor.

5 To protect the drug and the body from one another until the desired site of action is reached.

At present the drug targeting is achieved by one of the three approaches.

I. Carrier Mediated Delivery Systems Include:

A. Macromolecular delivery systems which use carriers like

1 Proteins

2. Dextran and other polysaccharides

3. Deoxyriboneuclic acid

4. Liposomes

5. Synthetic polymeric drugs

B Particulate drug delivery systems such as:

1. Microparticles and nanoparticles

2. Emulsions and microemulsions

3. Systems with magnetic control

C. Cellular drug carriers like

1 Erythrocytes

2. Leukocytes

3. Other cellular carriers.

II. Prodrug or Chemical modification

III. Mechanical devices.

Presently research is oriented towards the development of improved and unique drug carriers which act like 'guided missiles'[5,6] or 'magic bullets'[6] that can deliver a drug to a specific site in the body.

An ideal drug carrier for site specific delivery should have the following characteristics.

1. It should be able to deliver the drug selectively to the desired site of action from the site of administration

2 It must make the drug readily accessible to tissue parenchyma in the target sites.

3. The drug carrier conjugate should remain inactive during transport but should be able to activate the drug on reaching the target site.

4 The carrier material must be non-toxic and biocompatible.

5. It must be pharmaceutically as well as clinically acceptable with regards to stability, sterilizability, cost of manufacture and ease of administration.

2.1.4 Classification of Targeting:

Targeted drug delivery may be achieved by using carrier systems, when reliance is placed on exploiting both: the intrinsic pathway(s) that these carriers follow and the bioprotection that they can offer to drugs during transit through the body. The various approaches of vectoring the drug to the target site can be broadly classified as:[7]

1. Passive targeting

2. Active targeting

3. Inverse targeting

4. Physical targeting

5 Dual targeting

PASSIVE TARGETING:

It is a sort of passive process that utilizes the natural course of (attributed to inherent characteristics) 'homing' of the carrier system, through which it finally identifies and eventually approaches the intended cell lines. The ability of some colloids to be taken up by the RES especially in liver and spleen has made them as ideal vectors for passive hepatic targeting of drugs to these compartments. Passive target ability of the colloidal carriers provides therapeutic opportunities for the delivery of anti-infective for disease conditions that involve macrophage cells of the RES, e.g., leishmaniasis, brucellosis and candidiasis. Delivery into lysosomal compartment can also be affected for the treatment of certain lysosomal storage diseases as well as for macrophage neoplasms and macrophage activation.[8]

ACTIVE TARGETING:

The process of active targeting exploits modification or manipulation of the natural distribution pattern of the drug carrier using some exogenous means, so that a particular cell lines can identify it. It is generally achieved by using specific uptake mechanisms such as

receptor dependent uptake of natural low density lipoproteins (LDL) particles and synthetic lipid microemulsions from partially reconstituted LDL particles, coated with the apoproteins. The apoprotein coat serves as a ligand for the receptors. The apility of an immunoglobulin coated carrier to promote its accelerated interception by the liver and spleen, possibly via pathways involving Fc or C3B receptor mediated uptake, is one of the approach that exemplifies and signifies active targeting. It can be classified into three different levels of targeting; first order targeting (organ compartmentalization), second order targeting (cellular targeting) and third order targeting (intracellular targeting).

First order targeting:

It refers to restricted distribution of the drug-carrier system to the capillary bed of a predetermined target site, organ or tissue. Compartmental targeting in lymphatics, peritoneal cavity, plural cavity, cerebral ventricles, lungs, joints, eyes, etc., represents first order targeting.

Second order targeting:

The selective delivery of drugs to a specific cell type such as tumor cells and not to the normal cells is referred as second order drug targeting. The selective drug delivery to the Kupffer cells in the liver exemplifies this approach.

Third order targeting:

The third order targeting implies to drug delivery specifically to the internal (intracellular) site of target cells. An example of third order targeting is the receptor mediated entry of a drug complex into a cell by endocytosis, lysosomal degradation of carrier followed by the release of drug intracellularly.

INVERSE TARGETING:

It is essentially based on successful attempts to circumvent and avoid passive uptake of colloidal carriers by reticuloendothelial system(RES). This effectively implies for reversion of bio-homing trend of the carrier, hence the process is referred to as inverse targeting. One strategy applied to achieve inverse targeting is to suppress the function of RES by a preinjection of a large amount of blank colloidal carriers or macromolecules like dextran sulphate This approach can lead to RES blockade and as a consequence impairment of host defence system. Alternative strategies include modification of the size, surface charge, composition, surface rigidity and hydrophilicity of the carriers.

Recently available literature suggests modification of the surface by imparting distinctive hydrophilicity to the carrier particles, as an effective mode of targeting of drug(s) to non RES organs. Phospholipid microspheres emulsified with Polaxamer 338 showed the lowest RES uptake in mouse peritoneal macrophages in vitro. Poloxamine 98, is another hydrophilic nonionic surfactant which diverts normal RES

uptake of coated emulsion and coated nanoparticles (polystyrene microsphere) to inflammatory sites in rabbits. Recently, it is proved that inverse targeting of drugs to the sites other than RES rich organs by coating the lipid micro-emulsion (L.M.) with polaxamer 308. It has been suggested that surface hydrophilicity may reduce (or) even eliminate the adhesion of opsonin materials/HDL on to the surface of LM, which is believed to be an essential component involved in phagocytosis and sequential uptake of LM by RES system.

PHYSICAL TARGETING:

The selective drug delivery programmed and monitored at the external level (ex-vivo) with the help of physical means is referred to as physical targeting. In this mode of targeting, some characteristics of the environment are used either to direct the carrier to a particular location or to cause selective release of its contents. The first approach is represented by the temperature sensitive liposomes, which were developed and applied to tumor[165]. The release of drug from temperature sensitive liposomes in the vicinity of a tumor, that has temperature status higher or equal to the phase transition temperature of constitutive lipid(s), is brought about by serum components mostly the lipoproteins, which at phase transition induce temperature released from rapid release of entrapped drug.

It has been suggested that weakly anionic drugs. e.g. methotrexate, released from liposomes preferentially at low pH regions

of tumors. It is also reported[166] that pH sensitive liposomes for selective release at low pH In another physical approach, the application of external magnetic field has been exploited for localization of magno-responsive liposomes and microspheres within a pre-selected capillary bed.

DUAL TARGETING:

This classical approach of the drug targeting employs carrier molecules having their own intrinsic antiviral effect thus synergise the antiviral effect of the loaded active drug. Based on this approach, drug conjugates can be prepared with a fortified activity profile against the viral replication process[167]. A major advantage is that the virus replication process can be attacked at multiple points, excluding the possibilities of resistant viral strain development.

NIOSOMES AS TARGETED DRUG CARRIERS:

The concept of carriers to deliver drugs to target organs has been widely discussed.[8] In the early 1960, Bangham et al[9] and Bangham Horne[10] described liposomes, the phospholipid vesicles as the drug carriers. The liposomes exhibit certain disadvantages such as chemical instability, high cost and variable purity of lipids used, which militates against their adoption as drug delivery vehicle. Alternatives to phospholipids are thus of interest from the technical view point and could also allow a wider study of the influence of chemical composition on the biological fate of vesicles.

Many synthetic amphiphiles form vesicles[11] but as most of them are ionic and relatively toxic, they are generally unsuitable for use as drug carriers. Niosomes are vesicles mainly consisting of non ionic surfactants. This class of vesicles was introduced by Handjani-vila et al[12] in 1979.

One of the reasons for preparing niosomes is the assumed higher chemical stability of the surfactant than that of phospholipids, which are used in the preparation of liposomes. Due to the presence of ester bonds, phospholipids are easily hydrolyzed.[13] This can lead to phosphoryl migration at low pH.

Another type of degradation is the peroxidation of unsaturated phospholipids.[9] In order to avoid perodixation process, vesicles are often stored under nitrogen atmosphere. Unreliable reproducibility arising from the use of lecithins in liposomes leads to additional problem and has led scientists to search vesicles prepared from other material, such as nonionic surfactants.

Azmin and Florence et al[14] used nonionic surfactant vesicles using lipophilic surfactants like Span-40, Span-60 and Span-80 entrapped with methotrexate was improved after entrapping with niosomes. These vesicles were also found to be osmotically stable.

Chandra Prakash et al[15] made nonionic surfactant vesicles using lipophilic surfactants like Span-40, Span-60 and Span-80 entrapped with Methotrexate. The tissue distribution of methotrexate was improved after entraping with niosomes. These vesicles were also found to be osmotically stable.

2.2 NIOSOMES

Lipids capable of forming niosomes exhibit a dual chemical nature[26] Their head groups are hydrophilic and their fatty acylchains are hydrophobic. It has been estimated that each zwitterionic head groups in phosphatidyl choline has on the order of 15 molecules of water weakly bound to it, which explains its preferences for their water phase. The hydrocarbon fatty acid chain on the other hand, vastly prefer each other company to that of water. These phenomenon can be understood in quantitative terms by considering the critical micelle concentration of phosphatidyl choline in water. The critical micelle concentration is defined as the concentration of the lipid in water above which lipid forms either micelles or bilayer structure rather that remaining in solutions as monomers. The large free energy change between water and hydrophobic environment explains the over-whelming preference of typical lipids to assemble in bilayer structures excluding water as much as possible from the hydrophobic core in order to achieve the lowest free energy level and hence the highest stability for the aggregate structure. It is clear from the thermodynamic consideration that bilayer structures do not exist as such in the absence of water because it is water that provides the driving force for lipid molecules to assume a bilayer configuration.

NONIONIC SURFACTANTS USED FOR THE PREPARATION OF VESICLES

Nonionic Surfactant	Structural formula
Hexadecylpoly(3)glycerol	$C_{16}H_{33}(CH_2CH_2O)_3H$ | CH_2OH
Cholesterolpoly (24) oxyethyiene ether	$C_{16}H_{33}$ - CH - $O(CH_2CHO)_7H$ | | CH_2 CH_2OH /
Dialkylpoly (7) glycerol ether	$C_{12}H_{25}O$
Alkylglucoside	$H(CH_2)_2 - C_6H_{11}O_6$ n=8, 10, 12,14, 16, 18
Cetyl mannoside	$C_{16}H_{33} - C_6H_{11}O_6$
Cetyl lactoside	$C_{16}H_{33} - C_6H_{11}O_6$
Alkyl galactoside	$H(CH_2)_n - C_6H_{11}O_6$
Polyoxyethylenealkyl ether	$H(CH_2)_n - (OC_2H_4)_M OH$ n = 10,12,14,16,18 m = 3,4,5,6,7,8
Neutral crown ethers Polyoxyethylene glycerol-α α - diether	$(H(CH_2)_n - OCH_2)_2CHO(C_2H_4O)_xH$ N - 12, 14, 16, 18 x = 6 to 30
n-Decyloxyethyleneoctadecyl - myricylamine	$H(CH_2)_{18}$ - N - $(C_2H_4O)_{10}H$ | $H(CH_2)_{13}$ C = O
Cetyldiglycerolester	$C_{15}H_{31}CO -(OCHCH_2)OCH_2CHOHCH_2OH$ | CH_2OH

2.2.1 TYPES OF NIOSOMES:

The niosomes have been classified as a function of the number of bilayer (eg. MLV, SUV) or as a function of size.(eg. LUV, SUV) or as a function of the method of preparation(e g. REV, DRV). The types of niosomes[27] are given below and the following are frequently used nomenclature.

i) Multi lamellar vesicles (MLV)

ii) Large unilamellar vesicles (LUV)

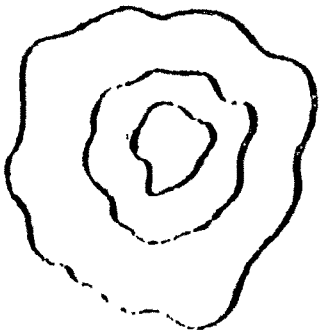
iii) Small Unilamelar vesicles (SUV)

MULTILAMELLAR VESICLES:

It consist of a number of bilayer surrounding the aqueous lipid compartment separately. The approximate size of this vesicles are 0 05-10mcm diameter. Multilamellar vesicles are the most widely studied type of niosomes, as pointed out by Bangham et al in 1974[28]. It is simple to make and they are mechanically stable upon storage for long periods of time. These vesicles are highly suited as drug carrier for lipophilic compounds.

LARGE UNILAMELLAR VESICLES

Niosomes of this type have a high aqueous lipid compartment ratio, so that larger volumes of bio-active materials can be entrapped



MLV (1-5 μm)

SUV (25-500 nm)

LUV (0.1-1 μm)

TYPES OF NIOSOMES

with a very economical use of membrane lipids. These provide a number of advantages as compared to multilamellar vesicles, including high encapsulation of water-soluble drugs, economy of lipid and reproducible drug release rates.

The term large Unilamellar usually means any unilamellar structure larger than 100mcm. Because of the larger size of the vesicles attainable a high percentage capture can often be achieved. A number of techniques for producing large unilamellar vesicles have been used including freeze-thaw cycling[29], Reverse phase evaporation method, Ether injection method and calcium induced fusion method.

SMALL UNILAMELLAR VESICLES:

These small Unilamellar vesicles are mostly prepared from multilamellar vesicles by sonication method, french press extrusion method (or) homogenization method. The approximate size of small Unilamellar vesicles are 0 025-0.05mcm diameter. They are thermodynamically unstable and are susceptible to aggregation and fusion. Their entrapped volume is small and percentage entrapment of aqueous solute is correspondingly low.

2.2.2 SPECIFIC METHODS OF PREPARATION FOR NIOSOMES

The methodology for niosome preparation has been evolved rapidly during the last few years as a response to the need to prepare well defined niosomes for specific applications.

MULTI LAMELLAR VESICIES:

Bangosomes popularly known as multilamellar vesicles are prepared according to the method of Bangham et al[28]. Lipids are dissolved in an organic solvent in round bottom flask. A thin lipid layer is formed on the inside wall of the flask by removal of the organic solvent by rotatory evaporation at reduced pressure.

Multilamellar vesicles form spontaneously when an excess volume of aqueous buffer is added to the dry lipid and shaking (by hand or vortex mixer) yields a dispersion of multilamellar vesicles. Duration and intensity of shaking, the presence of charge inducing agents in the bilayer, ionic strength of the aqueous medium and lipid concentration are important parameters influencing the size and the encapsulating efficiency of multilamellar vesicles. The lipid formed are quite heterogeneous both in size and in the number of lamella.

SMALL UNILAMELLAR VESICLES:

A) SONICATION:

Methods for the preparation of sonicated small lamellar vesicles have been reviewed by Bangham[28] and others. The usual multilamellar vesicles and large unilamellar vesicles are sonicated either with a bath type sonicator or a probe sonicator, under an inert atmosphere (usually nitrogen or argon) to get the small unilamellar vesicles. During sonications the multilamellar vesicles structure is broken down and small unilamellar vesicles with high radius of curvatures are formed

B) FRENCH PRESS METHOD:-

Dispersions of MLV's can be converted to small unilamellar vesicles by passage through a small orifice under high pressure[30]. A French pressure cell was used by Hamiltion and Guo for this purpose[31]. Multilamellar vesicles dispersion is placed in the French press and extruded at about 20000 psi at 4°C[31]. One pass through the cell produces a heterogeneous population of vesicles ranging from several micrometers in diameter to small unilamellar vesicle size. Multilple extrusions results in; a progressive decrease in the mean particle diameter (30-80nm in diameter depending upon the pressure used). These niosomes are more stable than sonicated ones and can be used advantageously as drug delivery carriers.

C) ETHANOL INJECTION METHOD:-

An alternative method for producting small niosomes that avoids both sonication and high pressure is the ethanol injection method, first described by Batzri and Korn[32] Lipids dissolved in ethanol are rapidly injected into an excess of buffer solution or other aqueous medium though a needle. The force of the injection is usually sufficient to achieve complete mixing, so that the ethanol is diluted almost instantaneously in water and phospholipid molecules are dispersed evenly through out the medium.

This procedure can yield a high proportion of small unilamellar vesicles. This method has the advantage of extreme simplicity and a very low risk of bringing about degradative changes in sensitive lipids. Its major short comings are the limitation of solubility of lipids in ethanol and on the volume of ethanol that can be introduced into the medium (7.5% V/V maximum). The percentage of encapsulation is low if the materials to be entrapped are dissolved in the aqueous phase. Another drawback is the difficulty of removing ethanol from phospholipid memberane.

D) ETHER INJECTION METHOD:-

This method is very similar in concept to the ethanol injection method. This is introduced by Deamer and Bangham in 1976[33] This method provides a means of making small lamellar vesicles by slowly introducing a solution of lipids dissolved in diethyl

ether into a warm aqueous medium. The lipid mixture is injected into an aqueous solution of the material to be encapsulated (using a syringe-type infusion pump) at 55-65°C or under reduced pressure, subsequent removal of residual ether under vaccum leads to the formation of single layer vesicles.

Ether injection is a method which treats sensitive lipids very gently and runs very little risk of causing oxidative degradation. Since the solvent is removed at the same rate as it is introduced. There is no limit to the final concentration of lipid which can be achieved, since, the process can be run continuously for a long period of time, giving rise to a high percentage of the aqueous medium encapsulated with in the vesicles. The disadvantages of this techniques are the long time taken to produce a batch of niosomes and careful control needed for introduction of lipid solution, requiring a mechanically operated infusion pump.

E) HOMOGENIZATION:

Homogenization of multilamellar vesicles or other lipid dispersions by commercially available high sheer homogenizer like the microfluidizer produces unilamellar vesicles. The small unilamellar vesicles formed are longer than the minimal size formed by sonication and significant amounts of larger particles are also present. The size of

the vesicles produced by microfluidizer depends on the pressure used and on the number of passes of the preparation through this device and on the niosomal lipid composition.

F) DRIED RECONSTITUTED VESICLES:-

This method[34,35] is another one where a way has been sought to disperse the solid lipid into a finely divided form before contact with the aqueous fluid, which will form the medium for the final suspension. Freeze drying has been used instead of drying the lipids down from any organic solution and subsequently the suspension of empty small unilamellar vesicles are frozen and lyophilized. The small unilamellar vesicles dried liplid is already very highly organized into membrane structures which on addition of water can be rehydrated, fused and resealed to form vesicles with high capture efficiency.

LARGE UNILAMELLAR VESICLES:

Large unilamellar vesicles provide a number of important advantages as compared to multilamellar vesicles including high encapsulation of water soluble drugs economy of lipid and reprodcible drug release rates. However large unilamellar vesicles are perhaps the most difficult type of niosomes to produce

A) REVERSE PHASE EVAPORATION METHOD:

Large Unilamellar vesicles can be prepared by forming a water in oil emulsion of phospholipids and buffer in the excess organic

phase followed by removal of the organic phase under reduced pressure The two phases are usually emulsified by sonication. Removal of the organic solvent under vaccum causes the phospholipid coated droplets of water to cool and eventually form a viscous gel. The next step is to bring about the collapse of certain proportion of water droplets.

In these circumstances the lipid monolayer which enclosed the collapsed vesicle is contributed to adjacent intact vesicles to form the outer leaflet of the bilayer of a large unilamellar niosomes. The aqueous content of the collapsed droplet provides the medium required for suspension of these newly formed niosomes. After conversion of the gel to a homogeneious free flowing fluid the suspension is dialysed in order to remove the last traces of solvent. This method has gained widespread use for applications that require high encapsulation of water soluble drug[36]. Entrapment efficiency up to 65% can be obtained with this method. This method has been used to encapsulate both small and large molecules. Biologically active macromolecules such as RNA and various enzymes have been encapsulated without loss of activity. The principle disadvantage of this method is exposure of the material to be encapsulated to organic solvents and mechanical agitation which lead to the denaturation of some proteins or breakage of DNA strands.

B) CALCIUM INDUCED METHOD:

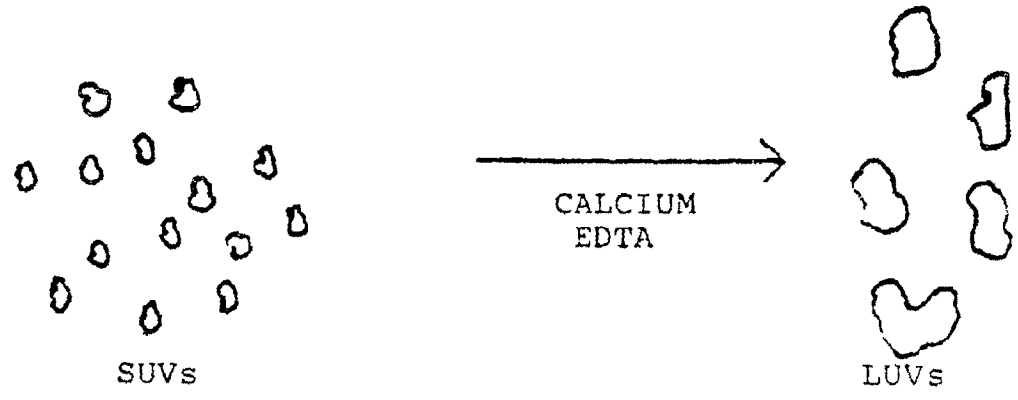
This method is used to produce unilamellar vesicles and it is of high interest for the present investigation. This method takes advantages of the fact that small vesicles aggregate in the presence of calcium and subsequently fuse.

In this method[37] the percentage of encapsulation depends on the lipid concentration used and generally about 30% of encapsulation of the drug is expected. The vesicles obtained will be generally in the size range of 0.2 - 1 mcm diameter.

The flocculent precipitate obtained is formed as a result of aggregation of the negatively charged vesicles by calcium cations. After incubation, the membranes fuse to give extended sheets of phospholipild lamella which are said to roll up into long cochleate cylinders with a swill-roll cross section, presumable again with calcium ions as a driving force, bringing in different parts of the same membrane sheet, together being pulled in upon itself. On addition of EDTA these lamella are unravel and released subsequently forming large unilamellar vesicles.

This technique has the advantage that it does not expose lipids or entrapped materials to deleterious chemicals or physical condition.

FORMATION OF LUVs - CALCIUM INDUCED FUSION METHOD



DEHYDRATION/REHYDRATION OF SMALL UNILAMELLAR VESICLES:-

In this method[38] sonicated vesicles are mixed in an aqueous solutions with the solute desired to be encapsulated and the mixture is dried under a stream of nitrogen. As the sample is dehydrated, the small vesicles fuse to form a multilamellar film that effectively sandwichs the solute molecules between successive layers. Upon rehydration, large vesicles are produced which have encapsulated a significant proportion of the solute. The optimal mass ratio of lipid to solute was reported to be approximately 1:2 to 1:3. This method has potential application to large scale production, since it depends only on controlled drying and rehydration processes and does not require extensive use of organic solvents, detergents or dialysis system.

DETERGENT REMOVAL METHOD:

Removal of detergent from mixed micelles formed by solubilization of dried lipid mixtures or performed niosomes with a detergent containing aqueous phase results in the formation of unilamellar vesicles.

This is a gentle method where no strong mechanical forces and no high temperature are applied. The preparation procedures should include a step to minimize residual detergent levels after niosomes formation. The techniques reported for the removal of detergents include dialysis[39], column chromatography[40], and Biobeads[41] method.

2.2.3 FACTORS AFFECTING VESICLES SIZE, ENTRAPMENT EFFICIENCY AND RELEASE CHARACTERISTICS

DRUG:

Entrapment of drug in niosomes increases vesicle size, probably by interaction of solute with surfactant head groups, increasing the charge and mutual repulsion of the surfactant bilayer thereby increasing vesicle size[42]. In polyoxyethylene (PEG) coated vesicles, some drug is entrapped in long PEG chain thus reducing the tendency to increase the size[43].

Degree of entrapment is affected by the hydrophilic-lipophilic balance of a drug. For a series of Spans and Tweens, Raja Naresh et al[44] reported maximum entrapment of water-soluble drug Diclofenac sodium in hydrophilic surfactant, Tween60 and Chandraprakash et al[45] reported maximum entrapment of slightly water soluble drug, Methotrexate in lipophilic surfactant Span 60.

AMOUNT AND TYPE OF SURFACTANT:

The mean size of niosomes increases regularly with increase in the hydrophilic-lipophilic balance (HLB) from Span 85 (HLB) 1.8) to Span 20 (HLB 8.6) because surface free energy decreases on increasing hydrophobicity of surfactant.[46-47]

Yoshioka et al[6] reported linear correlation between concentration of lipid and entrapment efficiency phase transition temperature (T_c) of surfactant also effects entrapment efficiency i.e. Span 60 having higher T_c provides the highest entrapment[48].

CHOLESTEROL CONTENT AND CHARGE:

Inclusion of cholesterol in niosomes increases its hydrodynamic diameter and entrapment efficiency[47]. Presence of charge tends to increase the inter-lamellar distance between successive bilayers in multilameller vesicle structure and leads to greater overall entrapped volume. Vesicle size is slightly decreased as charge might increase the membrane curvature[48].

Presence of cholesterol in bilayer composition due to its membrane stabilizing activity[49], reduces permeability and improves retention of solute. Baillie et al[50] reported that incorporation of 50% cholesterol in surfactant composition reduces vesicle permeability to 5(6) - carboxyfluorescein (CF) by a factor of 10. Cholesterol has decreasing effect on gel-liquid transition temperature, at which rapid efflux of vesicle content occurs. It converts one well defined gel-liquid transition temperature of a pure surfactant to gel-liquid transition range[51].

METHOD OF PREPARATION:

Methods of preparation of niosmes such as hand shaking, ether injection and sonication, developed on the basis of liposome production technique have been reviewed by Khandare et al[52]. Hand

shaking method form vesicles with greater diameter (0.35[45,53] - 13μm) compared to those prepared by ether injection method (50-1000nm)[52]. Sonication of MLV prepared by above methods, either with probe sonicator or bath type sonicator forms unilamellar vesicles with considerably reduced diameter. Increase in sonication time results in hydration help to reduce the size of vesicles prepared by hand shaking method.

Stafford et al[42] extruded the niosomal dispersion prepared by hand shaking method through 0.1μm, nuclepore polycarbonate membranes in series. After 8 such extrusions average diameter of vesicles was 135-340μm. Small sized niosomes can be produced by Reverse Phase evaporation (REV) method. In this, emulsion of aqueous phase in organic solvent containing lipid is prepared by sonication followed by evaporation of organic solvent, resulting in formation of vesicles[54-55]. Microfluidization method which gives greater uniformity and smaller size vesicles is based on submerged jet principle where in two fluidized streams interact at ultra high velocities (upto 1700 ft/sec) in precisely defined microchannels with in the interaction chamber to form niosomes.

Parthasarathy et al[54] prepared niosomes by Transmembrane pH gradient (inside acidic) drug uptake process (Tm pH) or remote loading, in which multilameller niosomes were prepared at acidic pH by hand shaking method and then subjected to freeze-thaw cycle and later sonicated. Remote loading of drug was done by adding aqueous

solution of drug, pH was adjucted to 7.0-7.2 and then mixture heated. Niosomes so formed showed greater entrapment efficiency and better retention of drug.

. Niosomes bearing 5(6) - carboxyfluorescein prepared by ether infection method show entrapment efficiency significantly higher than those prepared by Hand Shaking method or sonication[50] compared to hand shaking and reverse phase evaporation methods, niosomes prepared by remote loading method show greater entrapment efficiency and slower release of drug[54].

OSMOTIC EFFECT:

Addition of hypertonic salt solution to suspension of niosomes brings about reduction in vesicle diameter with concomitant water efflux which may be due to pumping out of vesicle content[50] whereas in hypotonic salt solution, there is initial slow release with slight swelling of vesicles probably due to inhibition of eluting fluid from vesicle, followed by faster release, which may be due to mechanical loosening of vesicles under osmotic stress.

2.2.4 METHODS FOR CONTROLLING NIOSOME SIZE:

The vesicle size can have dramatic effects on the invivo behaviour of niosomes[56]. Therefore before niosome drug carrier systems can be taken seriously for pharmaceutical applications, their size will have to be controlled within reasonable and verifiable limits.

Three possible approaches have been explored for achieving defined particle size distributions of niosomes.

a) Fractionation

b) Homogenization

c) Extrusion

A) FRACTIONATION

Two methods have become popular for fractionating defined sized niosomes from a heterogenous population viz, centrifugation; and size extrusion chromatography. Both can be used to enrich the product with the desired particle size but are limited in terms of the volumes that can be easily handled.

CENTRIFUGATION:

Niosomes sediment in a centrifugal field at a rate that is related to their size and density. Large niosomes composed of neutral lipids such as phosphatidyl choline can easily be pelleted at fairly low gravitational forces in conventional centrifuge. Under proper conditions the smaller niosomes will remains in the supernatant.

SIZE EXTRUSION CHROMATOGRAPHY:

Column chromatography has been used for many years as an analytical method to measure the particle size of niosomes. Preparative scale chromatography has also been applied to isolate niosomes of fairly homogenous sizes. This method is particularly useful for separating small unilamellar vesicles from larger structure. Typically a

column of sepharose 48 is used[57]. As the column is washed with buffer, larger niosomes elute in the void volume.

Larger pore size chromatographic media have been used in a similar fashion to fractionate populations of larger particles[58]. In general such chromatographic separations are quite limited in terms of volumes and through put must be carried out in batches, resulting in significant dilution of the product and require strict controls to ensure that no microbial contamination of gel beds occur.

B) HOMOGENIZATION:

When fairly small particles are desirable homogenization has proven to be a useful approach The average particle size of niosome dispersions can be reduced by passage under high pressure through a homogenizer.

Methew et al [59], used microfluidizer to generate vesicles in the 50-200nm size range. Conventional homogenizers and high sheer mixers have also been reported to be useful for down sizing niosomes[60,61].

C) CAPILLARY PORE MEMBRANE EXTRUSTION

A technique that has gained widespread acceptance for the production of niosomes of defined size and narrow size distribution, introduced by Olson et al[62] is based on the extrusion of a heterogenous population of fairly large niosomes through polycarbonate membranes under pressure Polycarbanate membranes have uniform straight

through capillary pores of defined size and normally do not bind niosomes.

This simple techniques can reduce a heterogenous population of multilamellar vesicles and reverse phase evaporator vesicles to a much more homogenous suspension of vesicles exhibiting a mean particle size that approaches the diameter of the pores through which they are extruded.

D) CERAMIC EXTRUSION:

If the size of the niosome is greater than the membrane pore diameter, the pores of the membrane tend to clog. The clogged membranes cannot easily be cleared because the filter housing configuration does not allow back-flushing.

One approach that overcomes the above mentioned limitations makes use of a ceramic membrane[63].

The suspension may be alternately passed through the membrane in a outside to inside direction to maintain the membrane in an unclogged conditon. The average size of niosomes may further be reduced by passage through similar types of ceramic filters that have been rated at smaller inner surface pore diameters.

2.2.5 STABILITY OF NIOSOMES:-

The factors affecting the stability of niosomes can be classified into the three types. They are

a. Physical Stability

b. Chemical Stability

c. Stability in Biological fluids.

A. PHYSICAL STABILITY:

Niosomes can change their physical characteristics in several ways.

1. Particle size can change because of aggregate formation and fusion.
2. Phase separation of bilayer components, upon storage can occur.
3. Leakage of encapsulated material from niosomes.

Particle size changes upon storage of phosphatidyl choline containing niosomes over pharmaceutically relevant time intervals, can be, in general, minimized by selection of proper charge inducing agents. Mostly negatively charged phospholipids (phosphatidyl glycerol) are used to successfully stabilize the niosomes.

Phase separation can occur when the bilayer composition changes because of chemical degradation reactions or when the bilayer goes through temperature cycles[64]. Proper selection of bilayer components can avoid these problems. Sometimes, phase separation occurs invivo. when bilayer components are selectively drawn from the bilayer plasma components[65]. If this effect is undersired, components that form more rigid bilayers are to be preferred. In other cases one might wish to deliberately destabilise the niosomes invivo so that a rapid

release of the encapsulated drug is induced. An example of plasma destabilized niosomes are niosomes composed of phosphatidyl ethanolamine and oleic acid [66].

The permeability for bilayers is highly dependent on the physico-chemical properties of the bilayer, drug and the temperature[66]. Three categories of drugs can be discerned[67].

1. highly hydrophilic, non-bilayer interacting drugs.
2. drugs with some lipophilicity
3. Strongly lipophilic drugs.

In category first the presence of cholesterol in the bilayer of the egg phosphatidyl choline niosomes dramatically reduces the permeability[68]. For gel state bilayers permeability is low with or without cholesterol. It is clear that if invivo performance allows 'gel state' bilayers to be used, shelf life of the niosomes in aqueous media with the proper pH might easily meet industrial demands. The second category of drugs tends to be difficult to keep entrapped over periods of months as long as outside sink conditions prevail. The third category of strongly lipophilic drugs has a high affinity to the bilayer and these drugs stay there over a long period of time, independently of the state of the bilayer.

As the final remark, the presence of hydrolysis or oxidation reaction products can affect bilayer properties Although lysophosphatidyl choline is known to be lipid bilayer solubilizer, the

solubilizing effect of lysophosphatidyl choline in degrading niosomes is neutralized by the simultaneous appearance of fatty acids in the bilayer[69].

NIOSOMES STORED IN FREEZE DRIED FORM:

Niosomes stored in freeze dried form is preferred for the proper invivo performance of niosomes with sufficient longterm stability. To maintain the same particle size distribution after the freeze drying-rehydration cycle, a cryoprotectant needs to be added Different types of cryoprotectants and their possible mechanism of action have been discussed by Crowe et al and Ozer et al[70,71], for niosome stabilization usually sugars are used as cryoprotectant, although other type of excipients also have been reported to exert cryoprotective effects[68,72]. A number of effects may contribute to the cryoprotective action.

1) The formation of amorphous glass structures during the freeze drying process might avoid mechanical damage inflicted by ice crystals. It is recommended to store these cakes below the glass transition temperature.

2) The sugars may interact with the polar head groups of the phospholipids and stabilize the membranes when the bilayer stabilizing water is removed by sublimation.

THE PROLIPOSOME CONCEPT:

In proliposome formulations, lipsomes are formed by hydrating lipids at the bed side. The dry lipids (coated as a film on the glass

vessel wall in the form of a freeze dried cake) are hydrated by shaking with an aqueous medium just before injection[73-76].

Another way to improve stability of lipids is by covalently cross linking the membrane using methods such as glutaraldehyde fixation or polymerization[77]. The membrane stability be increased by imparting charge to the niosomes[78].

CHEMICAL STABILITY:

The stability of niosomes depend on the chemical stability of the lipid components. The main bilayer components of niosomes, designed for carrying a drug or phospholipids. Commonly, hydrolysis and peroxidation are the two degradation process which occur with phospholipids[79]. Different analytical technique to monitor hydrolyis and oxidation reactions were reviewed before[80-82].

LIPID HYDROLYSIS:

Grid et al[83-86] described in number of articles different variables that influence the hydrolysis reactions of phosphatidyl choline, the major phospholipids in the most niosome preparations and the charge inducing phospholipids phosphatidyl glycerol. Apart from pH, other experimental conditions like temperature, ionic strength, buffer species, ultra sonification were reported to influence hydrolysis reactions. Many investigators choose the formation of lysophosphatidyl choline as a standard measure for the chemical stability of phospholipids. Since the presence of lysophosphatidyl choline in lipid bilayer greatly enhances

the permeability of niosomes, the most important method for minimizing this problem is by the proper sourcing of the phospholipid to be used. They should be essentially free of any lyso-phosphatidyl choline to start with and free of any lipases.

LIPID PEROXIDATION:

Most of the phospholipid niosomal dispersions used will contain unsaturated acyl chains as part of their molecular structures. These chains are vulunerable to oxidative degradation (lipid peroxidation)[87].

The peroxidation can occur during preparation, storage or actual use. Peroxidation of phospholipids produces the formation of cyclic peroxides and hydroperoxides. Peroxidation of the phospholipids may be minimized by a number of ways.

i) minimum use of unsaturated phospholipids.

ii) use of nitrogen or argon minimize exposure to oxygen.

iii) use of light resistant container

iv) removal of heavy metals (EDTA)

v) use of antioxidants such as α-tocopherol or BHT.

Recently it was reported that niosome of different lipid composition could be steam sterilized without substantial hydrolytic or oxidative degradation[88].

Stability in Biological fluids

The inability of niosomes to retain entrapped substances when incubated in blood or plasma has been known for about a decade. The

instability of niosomes in plasma appears to be the result of the transfer of bilayer lipids to albumin and high density lipo proteins[89]. Both lecithin and cholesterol also exchanges with the membrane of red blood corpuscle. Niosomes are most susceptible to high density lipoprotein attack at their gel to liquid crystalline phase transition temperature. The susceptibility of niosomal phospholipids to lipoprotein and phospholipase attack is strongly dependent on niosome size and type. Generally multilamellar vesicles are most stable and small lamellar vesicles are least stable.

The bile salts will also destabilize the bilayer membrane structure, thereby leading to the release of the entrapped material.

2.2.6 INVIVO BEHAVIOUR OF NIOSOMES

Invivo niosomes have been found equiactive to liposomes in improving the therapeutic performance of drug[90] and their distribution in body follows the pattern of their colloidal drug delivery systems. Although tissues of extravasation: liver, lung, spleen and bone marrow are responsible for disposition of a major part of niosomes yet their level in liver is always significantly higher due to the natural vectoring process[90,91]. Variation in size also influences the pattern of niosome disposal from blood, large size niosomes may reside in lung due to alveolar and effect of alveolar phagocytic cells, which small sized vesicles, which can pass through fenestrations in liver sinusoidal epithelium, have better access to spleen [53,92].

It appears that, like liposomes, niosomes are also taken up intact by liver and break down substantially to release the free drug which eventually re-enters the circulation and maintains the plasma drug level[91]. The effect of two doses of niosomal sodium stibogluconate given on successive days was additive, indicating that liver might act as depot of drugs.

Parthasarathi et al[54] found niosomes to be stable in plasma. However non-ionic surfactants in higher concentration delipidize the low density lipoproteins[93].

Moser et al[94,95] found niosomes bearing heamoglobin to be physically stable with plasma protein component. Albumin and transferrin were identified and determined to absorb on vesicles without destabilizing them. Erythrocytes donate cholesterol to niosomes particularly to cholesterol-free and cholesterol poor niosomes, maintaining their integrity in body as well as keeping them less vulnerable to destabilisation[96].

Niosomes interactions with the cells

Niosomes can interact with cells in many ways to cause niosome components to be one associated with those cells[97,98]. Five mechanisms are generally known for the niosome cell interactions and they are discussed below.

i) Intermembrane transfer

Intermembrane transfer of lipid components can take place upon close approach of the two phospholipid bilayers without the need of disruption of the niosome or prejudicial of the membrane integrity. Interactions can take place between niosomes and lipoproteins and in certain circumstances proceed to the extent of destroying the niosomes altogether.

ii) Contact release

Contact release of aqueous contents of niosomes occurs in which contact with the cells causes an increase in permeability of the niosome membrane. This leads to release of water soluble solutes in high concentration in the close vicinity of the cell membrane through which these solutes may under certain circumstances, then pass. This phenomenon can provide very effective means for introducing materials into specific cells without the need for ingestion of the whole niosome.

iii) Adsorption

Adsorption of niosomes to the cell surface occur with little or no internalization of either aqueous or lipid components. It may take place either as a result of physical attractive forces or as a result of binding by specific receptors to ligands on the vesicle membrane.

iv) Fusion

Close approach of niosomes and cell membrane can lead to fusion of the two resulting in complete mixing of niosomal contents into the cytoplasm.

v) Endocytosis

The niosome is engulfed by the cell. The lysozyme present in the cytoplasm degrades or digests the membrane structure of niosomes thereby releasing the entrapped material into the medium.

2.2.7 CHARACTERIZATION OF NIOSOMES:

i) Vesicle diameter

Niosomes, similar to liposomes assume spherical shape, its diameter can be determined using light microscope[54], photon correlation microscopy[14] and freeze-fracture electron microscopy[50].

ii) Entrapment efficiency

After preparing niosomal dispersion, unentrapped drug is separated by dialysis[14], centrifugation or gel chromatography[50]. The drug remaining entrapped in niosomes is determined by complete vesicle disruption using 50% n-propanol or 0.1% Triton X-100[44] and entrapment efficiency (EE) is expressed as (amount entrapped/Total amount added) x 100.

The intercalation of cholesterol in the bilayers decreases the entrapment volume and thus entrapment efficiency. As the concentration of cholesterol increases, entrapment efficiency decreases[99].

The entrapment efficiency increases with increase in the concentration[100] and lipophilicity of surfactant[101,102].

iii) Osmotic shrinkage

Osmotic shrinkage of vesicles can be determined by monitoring reductions in vesicle diameter, initiated by addition of hypertonic salt solution to suspension of niosomes.

Niosomes prepared from pure surfactant are osmotically more sensitive in contrast to vesicles containing cholesterol, confirming the membrane stabilizing activity of the lipid.

Release of Drug From Niosomes:

Release of drug can be monitored by dialising diluted niosome suspension against buffer at definite temperature and determining the drug content of dialysate[103]. Inclusion of cholesterol improves drug retention and thus reduces permeability[99,104]. On incorporation of cholesterol the release was decreased to 4.6% and on inclusion of dicetyl phosphate it was further reduced to 2.1%.

2.2.8 TOXICITY ASPECTS

There is an enormous amount of literature available about the toxicity of surfactant molecules, but only a few studies are available in which the toxicity of niosomes has been investigated. The toxicity of C_nEO_m (n is the number of carbon atoms in the alkyl chain, while m is the number of oxyethylene) units surfactants studied with two different models. These models are (a) the ciliary beat frequency (CBF) of

trachea, which is important for intranasal administration, and (b) the cell proliferation of keratinocytes, which is important for the transdermal application of vesicles. A decrease in CBF was considered to be a measure for the toxicity of the formulation.

The CBF of chicken embryo trachea was measured at 31°C, which is the physiological temperature of mucosa nasal tissue. The vesicles were prepared from 60 mol % surfactant and 40 mol % cholesterol. Application of gel state niosomes did not cause a decrease in CBF, whereas liquid state niosomes did result in a significant decrease in ciliary beat frequencies. Only in the case of niosomes prepared from $C_{12}EO_3$ was the decrease in CBF irreversible. No relationship was found between the potential of surfactants to form micelles in equilibrium with the niosomes (e.g. $C_{12}EO_7$) and the decrease in ciliary beat frequency.

As a toxicity model for topical application of niosomes, the cell proliferation of keratinocytes was measured after 3 days incubation with niosomes. In this study, ester and ether type surfactants were compared. It appeared that ester linked surfactants were significantly less toxic than the ether linked surfactants, probably due to the degradation of the ester bond by enzymes. No difference in toxicity was observed between gel and liquid state vesicles, which is different from the results obtained by measuring the CBF. The authors suggest that this is probably due to a difference in incubation times. In this case of CBF measurements the incubation was only 1 hour, while 3 days'

incubation time was used in the cell proliferation experiments. In the latter case the kinetic aspects, such as exchange of surfactants from the bi-layers to the environment, does not affect the toxicity, while in the case of the CBF the kinetic aspects might be very important.

2.2.9 ROUTES OF ADMINISTRATION

Usually niosomes during experimentations have been administered to the laboratory animals intravenously through caudal vein. Azmin et al[105] investigated niosomal delivery of methotrexate to mice by oral and intravenous administration and reported that on oral administration, absorbtion of methotrexate increased but seemed methotrexate concentration dependent. Hofland et al[106] studied in-vitro transdermal delivery of estradiol entrapped within niosomes. Studies on adjuvant activity of niosomes were performed on the BALB/C humoral response to bovine serum albumin after intraperitioneal and subcutaneous administration.

2.2.10 ADVANTAGES :

1) Niosomes entrap solute in manner analogous to liposomes.

2) Niosomes are osmotically active and stable as well as they increase the stability of entrapped drug.

3) Handling and storage of surfactants require no special conditions.

4) Niosomes possess and intrastructure consisting of hydrophobic and hydrophilic moieties together and as a result can accommodate drug molecules with a wide range of solubilities.

5) Niosomes exhibit flexibility in their structural characteristic (Compositon, fluidity, size) and can be designed according to the desired situation.

6) Niosomes improve oral bioavailability of poorly absorbed drugs and enhance skin penetration of drugs.

7) They can be made to reach the site of action by oral, parenteral as well as topical routes.

8) They allow their surface for attachment of hydrophilic group and can incorporate hydrophilic moieties in bilayer to bring about changes in the invivo behaviour of niosomes.

9) Niosomal dispersion in aqueous phase can be emulsified in non aqueous phase to regulate delivery rate of drug and administer normal vesicles in external non-aqueous phase.

10) Niosomal surfactants are biodegradable, bio compatible and non-immunogenic.

11) Niosomes improve the therapeutic performance of the drug molecules by delayed clearnce from the circulation, protecting the drug from biological environment and restricting effects to target cells.

2.2.11 APPLICATIONS:

Niosomal drug delivery is potentially applicable to many pharmacological agents for their action against diseases. Some of its therapeutic applications are discussed below.

1. TARGETING OF BIO ACTIVE AGENTS:

a) To reticulo-endothelial system

The vesicles are preferentially taken by the cells of reticuloendothelial system. The uptake of niosomes by the cells is also by circulating serum factors known as opsonins which mark them for clearance. Such localized drug accumulation has, however been exploited in treatment of animal tumors known to matestasize to the liver and spleen and in parasitic infestation of the liver.

b) To organs other than reticulo-endothelial system

It has been suggested that carrier systems can be directed to specific sites in the body by use of antibodies[57,55]. Immunoglobulins seem to bind quite readily to the lipid surface thus offering a convenient means for targeting of drug carrier[74]. Many cells possess the intrinsic ability to recognize and bind particular carbohydrate determinates and this can be exploited to direct carrier systems to particular cells[107,108,109].

2. NEOPLASIA:

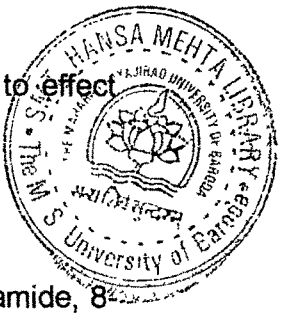
Doxorubicin, the anthracycline antibiotic with broad spectrum anti tumor activity shows a dose dependent irreversible cardiotoxic effect. Niosomal delivery of this drug to mice bearing S-180 tumor increased

their life span and decreased the rate of proliferation of Sarcoma. Cummings et al[110] investigation showed rapid distribution of doxorubicin and rapid attainment of equilibrium in liver and heart tissue after intravenous injection, followed by slow-phase plasma drug clearance. Niosomal entrapment increased the half life of the drug, prolonged its circulation and altered its metabolism. Intravenous administration of methotrexate entrapped in niosomes to s-180 tumor bearing mice resulted in total regression of tumor and also higher plasma levels and slower elimlination[111].

3. LEISHMANIASIS:

Niosomes can be used for targeting of drugs in the tratment of diseases in which the infecting organism resides in the organs of reticulo endothelial system Leishmaniasis is such a disease in which parasite invade cells of liver and spleen. The commonly prescribed drugs are antimonials which are related to arsenic and at high concentration they damage the heart, liver and kidney. Encapsulating such drugs in niosomes targets the drug to the infected organs and reduces the dose needed to treat the infection.

The studies of antimony distribution in mice, performed by Hunter et al[112], showed high level in liver after intravenous administration of the carrier forms of the drug. Carter et al[113] concluded that only multiple dosing with drug loaded sonicated vesicles was effective against parasites in liver, spleen and bone marrow, while other



treatments reduced parasite burdens in liver but either failed to effect spleen and bone marrow or were effective but toxic.

4. DELIVERY OF PEPTIDE DRUGS:

Yoshida et al[48] investigated oral delivery of 9-desglycinamide, 8-arginine vasopressin entrapped in niosomes in an invitro intestinal loop model and reported that stability of peptide increased significantly.

5. IMMUNOLOGICAL APPLICATION OF NIOSOMES:

Niosomes have been used for studying the nature of the immune response provoked by antigens. Brewer and Alexander[114] have reported niosomes as potent adjuvants for protein antigens. Niosomes offer many advantages over other adjuvants in terms of immunological selectivity, low toxicity and stability.

6. NIOSOMES AS CARRIER FOR HEAMOGLOBIN:

Niosomes can be used as a carrier for heamoglobin. Niosomal suspension shows a visible spectra super imposable on to that of free heamoglobin. Vesicles are permeable to oxygen and the heamoglobin dissociation curve can be modified similiarly to non encapsulated hemoglobin[115,116].

7. TRANSDERMAL DELIVERY OF DRUGS BY NIOSOMES:

Slow penetration of drugs through skin is the major drawback of transdermal route of delivery. An increase in the penetration rate has been achieved by transdermal delivery of drug incorporated in niosomes[117].

OTHER APPLICATIONS:

a) Sustained release:

Drugs that are the rapidly excreted or metabolized possess "Sawtooth" drug kinetics. This is undesirable especially for agents which have low therapeutic index. Experiments have suggested that niosomes can provide relatively constant and sustained blood stream levels of drug concentration. Many anti tumor drugs are cleared from the blood stream very quickly, while the same agents, entrapped in niosomes, persist in the blood for hours. In contrast to other sustained release formulations, niosomes can be injected into circulation and thus can serve as an intra vascular drug depot.

Azmin et al[91] suggested the rule of liver as a depot for the drug after niosomes are taken up by the liver cells. Sustained release action of niosomes could be applied to drugs with low therapeutic index and low water solubility. Since these could be maintained in the circulation via niosomal encapsulation.

b) Localized drug action:

Drug delivery through niosomes is one of the approaches to achieve localized drug action, since their size and low 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. The Leishmaniasis causing organism is an intra cellular parasite residing primarily in endocytotic vacuoles in

phagocytic mononuclear cells, this being the primary site of niosomes uptake in-vivo. Antimonials encapsulated within niosomes are taken up by mononuclear cells resulting in localization of the drug causing an increase in potency and hence decrease in dose and toxicity[168,169, 170].

The evolution of niosomal drug delivery technology is still at an infancy stage, but this type of drug delivery system has shown promise in cancer chemotherapy and Anti-Leishmanial thereapy. Niosomes may prove to be a convenient drug delivery system for other diseases. Niosomes are a promising vehicle for drug delivery and being non ionic, it is less toxic and improves the thereapeutic index of drugs by restricting its action to target cells.

2.2.12 MODIFIED NON-IONIC SURFACTANT VESICLES:

DISCOMES

These are disc shaped structures formed on mixing cholesteryl polyoxyethylene (Solulans) and vesicular dispersion. Uchegbu et al[118] observed them under the light microscope, existing under certain conditions of the phase diagram of non-ionic surfactant vesicles (NSVs) prepared from a hexadecyl diglycerol ether, cholesterol and dicetyl phosphate (DCP) (69:29:2) by hand shaking and sonication followed by incubation with various proportions of Solulan C24 at 74°C. Under these conditions, four different phases were distinct: a lamellar phase, a micellar phase, an uncharacterized coexistence phase and a novel phase called discome phase.

Dispersion in the discome phase consist of large "Discomes" (30-60 μm mean volume diameter).

Lipids in the proportion (i.e. Surfactant, cholesterol, Solulan C24, DCP, 49 : 19.5 : 29.5 : 2) produce discomes on sonication.

Discomes entrap water-soluble solute. Entrapment of 5(6)-carboxyfluorescein showed aqueous entrapment value of 3.603±2.916% and release of 50% solute after 24 hour at room temperature. Large volume carrying capacity and minimal capacity allow them to be used in ophthamology.

POLYMERIZED NON-IONIC SURFACTANT VESICLES:

Since vesicle systems are more or less thermodynamically unstable, proximity and regular orientation of surface active molecules at interface has been exploited to increase stability by controlled polymerization of vesicle forming non-ionic surfactant bearing a polymerisable residue[120,121]. Polymerisable surfactants used were:

i. Dimethyl n-hexadecyl [{1-isocyanoethyl} carbonyloxy methyl] ammonium bromide.

ii. N.N. (dihexadecanoyloxyethyl) maleyl amide.

iii. Dihexadecyl N.Methyl N.Maleyl ammonium bromide.

The vesicles prepared from these surfactants were polymerized by radiation or radical initiation. Polymerization restricts mobility of hydrocarbon core and hence improves the stability of niosomes. Size of the vesicles on polymerization remains unchanged while change in appearance depends upon location of polymerisable group.

Thus they combine advantages of polymers and membranes. They have stability and intriguing structural properties like polymers while retaining beneficial fluidities and organizational abilities of membranes. In terms of drug delivery they might serve as unique polydisperse, time release carriers.

EMULSIFIED NIOSOMAL DISPERSION:

Yoshioka and Florence[121] formulated a range of vesicle-in-water-in-oil (V/W/O) emulsion from niosomes lmade from Spans (20,40,60,80) in the size range 600nm. 3.4 µm, dispersed in water droplets of around 5.25µm, themselves dispersed in an oil (octane, hexadecane, isopropyl myristate). This system showed release of CF slower than vesicles suspension and W/O emulsion. The release was affected by the HLB of surfactant, nature of oil and temperature of dialysis media. Thus by their appropriate choice, delivery rate of drug could be regulated.

This system allowed administration or application of vesicle in an external non-aqueous phase while maintaining normal vesicular structure in aqueous phase and can be of potential use in drug delivery or as vaccine vehicle. Albert et al[122] have patented a similar system for cosmetic application.

NIOSOMALLY ENTRAPPED BIOACTIVE AGENTS:

Various bioactive agents entrapped in niosomes are Sodium stibogluconate[123,124] Methotrexate[101,102,125], Vincristine[126], Doxorubicin[127,104], Diclofenac sodium[128], bovine serum albumin[125] 9-desglycinamide, 8-arginine vasopressin[100], Insulin[129], Estradiol[106], Antipyrine[130], Haemoglobin[115,116], Plumbagin [131], Metronidazole [132].