Review Article

Niosomes and Liposomes - Vesicular Approach Towards Transdermal Drug Delivery

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ABSTRACT

Drug targeting is the ability to direct a therapeutic agent specifically to desired site of action with little or no interaction with nontarget tissue. Novel drug delivery system aims to deliver the drug at a rate directed by the needs of the body during the period of treatment, and channel the active entity to the site of action. Niosomes are one of the best carriers for drug targeting. Niosomes are self assembled vesicles composed primarily of synthetic surfactants and cholesterol. They are analogous in structure to the more widely studied liposomes formed from biologically derived phospholipids. Niosomes are biodegradable, relatively nontoxic, more stable and inexpensive, an alternative to liposomes. On the other hand liposomes are vesicular structures consisting of hydrated bilalyers which form spontaneously when phospholipids are dispersed in water. They are simple microscopic vesicles in which an aqueous volume is entirely enclosed by a membrane composed of lipid bilayers. The focus of this review is to bring out the application, advantages, and drawbacks of vesicular systems i.e. niosomes and liposomes.

Keywords: Niosomes, Liposomes, vesicles, sonication.

INTRODUCTION

One of the major advances in vesicle research was the finding that some modified vesicles possessed properties that allowed them to successfully deliver drugs in deeper layers of skin. Transdermal delivery is important because it is a noninvasive procedure for drug delivery. Further, problem of drug degradation by digestive enzymes after oral administration and discomfort associated with parenteral drug administration can be avoided[1]. It is the most preferred route for systemic delivery of drugs to pediatric, geriatric and patients having dysphasia. Hence, transdermal dosage forms enjoy being the most patient compliant mode of drug delivery[2].

Despite the promise, there were many problems that researchers had to face with while attempting successful transdermal drug delivery. The skin is a multi-layered structure made up of stratum corneum (SC), the outermost layer, under which lies the epidermis and dermis. Within these layers of skin are interspersed fibroblasts, hair follicles and sweat glands that originate in the dermis blood supply. The almost unsurmountable nature of SC is a major challenge for systemic delivery of percutaneously applied drugs[3]. The Òbrick and mortarÓ arrangement of corneocytes, flattened mononucleated keratinocytes, with interspersed lipids and proteins makes the SC approximately 1000 times less permeable than other biological membranes. Furthermore, it is even more difficult for anything to penetrate to the deeper strata of skin[4].

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 bilayer made up of non- ionic

surfactant, with or without cholesterol and dicetyl phosphate, and exhibit a behaviour 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. Addition of cholesterol results in an ordered liquid phase formation which gives the rigidity to the bilayer, and results in less leaky niosomes. Dicetyl phosphate is known to increase the size of vesicles, provide charge to the vesicles, and thus shows increase entrapment efficiency. Other charge-inducers are stearylamine and diacylglycerol, that also help in electrostatic stabilization of the vesicles. Niosomes have unique advantages over liposomes. Noisomes are quite stable structures, even in the emulsified form[5]. They require no special conditions such as low temperature or inert atmosphere for protection or storage, and are chemically stable. Relatively low cost of materials makes it suitable for industrial manufacture. A number of non- ionic surfactants have been used to prepare vesicles viz. polyglycerol alkyl ether, glucosyl dialkyl ethers, crown ethers, ester linked surfactants, polyoxyethylene alkyl ether[6], Brij, and a series of spans and tweens[7]. 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[8]. 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[9]. They exhibit flexibility in structural characteristics (composition, fluidity, size,), and can be designed according to the desired situation. Niosomes improve the oral bioavailability of poorly absorbed drugs and enhance skin penetration of drugs[10].

Niosomes are lamellar structures that are microscopic in size. They constitute of non-ionic surfactant of the alkyl or dialkyl polyglycerol ether class and cholesterol with subsequent hydration in aqueous media. The surfactant molecules tend to orient themselves in such a way that the hydrophilic ends of the non-ionic surfactant point outwards, while the hydrophobic ends face each other to form the bilayer. Controlled release drug products are often formulated to permit the establishment and maintenance of any concentration at target site for longer intervals of time. One such technique of drug targeting is niosomes. Niosomes are microscopic lamellar structures formed on admixture of a nonionic surfactant, cholesterol and diethyl ether with subsequent hydration in aqueous media. They behave *in vivo* like liposomes prolonging the circulation of entrapped drug and altering its organ distribution.
Niosomal drug delivery has been studied using various methods of administration including intramuscular, intravenous, peroral and transdermal. In addition, as drug delivery vesicles, niosomes have been shown to enhance absorption of some drugs across cell membranes, to localize in targeted organs and tissues and to elude the reticuloendothelial system. Niosomes has been used to encapsulate colchicines, estradiol, tretinoin, dithranol, enoxacin and for application such as anticancer, anti-tubercular, anti-leishmanial, anti-inflammatory, hormonal drugs and oral vaccine .

Liposomes were first described by Bangham in 1965 while studying cell membranes. He found that lipsomes are vesicular structures consisting of hydrated bilalyers which form spontaneously when phospholipids are dispersed in water. Since this, further studies into liposomes and their application in various fields such as medicine and research have been explored.
Liposomes are defined as structure consisting of one or more concentric

spheres of lipid bilayers separated by water or aqueous buffer compartments.

Phospholipids are the main component of naturally occurring bilayers. These phospholipids include phosphatidylcholines (PC), phosphatidylethanolamines (PE) and phosphatidylserines (PS)[11]. The key common feature that bilayer-forming compounds share is their amphiphilicity ie., they have defined polar and non-polar regions. This is the reason the non-polar regions orientate themselves towards the interior away from the aqueous phase, the polar regions being in contact with it.

Niosomes behave in vivo like liposomes, prolonging the circulation of entrapped drug and altering its organ distribution and metabolic stability. As with liposomes, the properties of niosomes depend on the composition of the bilayer as well as method of their production. It is reported that the intercalation of cholesterol in the bilayers decreases the entrapment volume during formulation, and thus entrapment efficiency.

However, differences in characteristics exist between liposomes and niosomes, especially since niosomes are prepared from uncharged single-chain surfactant and cholesterol, whereas liposomes are prepared from double-chain phospholipids (neutral or charged). The concentration of cholesterol in liposomes is much more than that in niosomes. As a result, drug entrapment efficiency of liposomes becomes lesser than niosomes. Besides, liposomes are expensive, and its ingredients, such as phospholipids, are chemically unstable because of their predisposition to oxidative degradation; moreover, these require special storage and handling and purity of natural phospholipids is variable.

CLASSIFICATION

LIPOSOMES

I. Based on composition and mode of drug delivery

1. Conventional liposomes: Composed of neutral or negatively charged phospholipids and cholesterol. Subject to coated pit endocytosis, contents ultimately delivered to Lysosomes if they do not fuse with the endosomes, useful for E.E.S targeting; rapid and saturable uptake by R.E.S; short circulation half life, dose dependent pharmacokinetics.

2. pH sensitive liposomes : Composed of phospholipids such as phosphatidyl ethanolamine, dioleoyl phosphatidyl ethanolamine. Subjected to coated pit endocytosis at low pH, fuse with cell or endosomes membrane and release their contents in cytoplasm; suitable for intra cellular delivery of weak base and macromolecules.Biodistribution and pharmacokinetics similar to conventional liposomes.

3. Cationic Liposomes: Composed of cationic lipids. Fuse with cell or endosome membranes; suitable for delivery of negatively charged macromolecules (DNA, RNA); ease of formation, structurally unstable; toxic at high dose, mainly restricted to local administration

4. Long circulating or stealth liposomes: Composed of neutral high transition temperature lipid, cholesterol and 5-10% of PEG-DSPE. Hydrophilic surface coating, low opsonisation and thus low rate of uptake by RES long circulating half life (40 hrs); Dose independent Pharmacokinetics

5. Immuno liposomes: Conventional or stealth liposomes with attached Antibody or Recognition Sequence. Subject to receptor mediated endocytosis, cell specific binding (targeting); can release contents extra cellularly near the target tissue and drugs diffuse through plasma membrane to produce their effects.

6. Magnetic Liposomes: Composed of P.C, cholesterol and small amount of a linear chain aldehyde and colloidal particles of magnetic Iron oxide. These are liposomes that indigenously contain binding sites for attaching other molecules like

antibodies on their exterior surface. Can be made use by an external vibrating magnetic field on their deliberate, on site, rapture and immediate release of their components.

7. Temperature (or) heat sensitive liposomes: Composed of Dipalmitoyl P.C. These are vesicles showed maximum release at 41^{o}, the phase transition temperature of Dipalmitoyl P.C. Liposomes release the entrapped content at the target cell surface upon a brief heating to the phase transition temperature of the liposome membrane.

II Based on Size and Number of Lamellae

1. Multi lamellar vesicles (M.L.V): (Size 0.1 - 0.3 micro meter)

Have more than one bilayer; moderate aqueous volume to lipid ratio 4: 1 mole lipid. Greater encapsulation of lipophilic drug, mechanically stable upon long term storage, rapidly cleared by R.E.S, useful for targeting the cells of R.E.S, simplest to prepare by thin film hydration of lipids in presence of an organic solvent.

a) Oligo lamellar vesicles or Paucilamellar vesicles - Intermediate between L.U.V & MLV.
b) Multi vesicular liposomes - Separate compartments are present in a single M.L.V.
c) Stable Pluri lamellar vesicles - Have unique physical and biological properties due to osmotic compression.

2. Large Unilamellar Vesicles (L.U.V): (Size 0.1 - 10 micro meter)

Have single bilayer, high aqueous volume to lipid ratio (7: 1 mole lipid), useful for hydrophilic drugs, high capture of macro molecules; rapidly cleared by R.E.S. Prepared by detergent dialysis, ether injection, reverse phase evaporation or active loading methods.

3. Small Unilamellar Vesicles (S.U.V): (Size 0.1 micro meters)

Single bilayer,homogeneous in size, thermodynamically unstable, susceptible to aggregation and fusion at low or no charge, limited capture of macro molecules, low aqueous volume to lipid ratio (0.2 : 1.5 : 1 mole lipid) prepared by reducing the size of M.L.V or L.U.V using probe sonicator or gas extruder or by active loading or solvent injection technique.

NIOSOMES

Types of Niosomes Based on the vesicle size, niosomes can be divided into three groups:

1. Small Unilamellar Vesicles (SUV, Size=0.025-0.05 µm)
2. Multilamellar Vesicles (MLV, Size=>0.05 µm)
3. Large Unilamellar Vesicles (LUV, Size=>0.10 µm).

Comparison of Niosomes vs. Liposomes

1. Liposomes exhibit certain disadvantages such as: they are expensive, their ingredients like phospholipids are chemically unstable because of their predisposition to oxidative degradation, they require special storage and handling and purity of natural phospholipids is variable. Niosomes do not have any of these problems.
2. Differences in characteristics exist between liposomes and niosomes, especially since niosomes are prepared from uncharged single-chain surfactant and cholesterol whereas liposomes are prepared from double-chain phospholipids (neutral or charged).
3. Niosomes behave in-vivo like liposomes, prolonging the circulation of entrapped drug and altering its organ distribution and metabolic stability.[12] Such vesicular drug carrier systems alter the plasma clearance kinetics, tissue

distribution, metabolism and cellular interaction of the drug.[13] They can be expected to target the drug to its desired site of action and/or to control its release.[14]

4. As with liposomes, the properties of niosomes depends both on the composition of the bilayer and on method of their production.[15]
5. The entrapment efficiency increases with increase in the concentration and lipophilicity of surfactant. Chandraprakash et al made Methotrexate loaded non-ionic surfactant vesicles using lipophilic surfactants like Span 40, Span 60 and Span 80 and found that Span 60 (HLB = 4.7) gave highest percent entrapment while Span 85 (HLB = 9.8) gave least entrapment. They also observed that as HLB value of surfactant decreased, the mean size was reduced.[16]

Niosomes In Lieu Of Liposomes – Reasons

One of the most significant problems associated with the use of liposomes as adjuvant is the susceptibility of phospholipids to oxidative degradation in air. This requires that purified phospholipids and liposomes have to be stored and handled in an inert (e.g. nitrogen) atmosphere36. Phospholipid raw materials are naturally occurring substances and as such require extensive purification thus making them costly[17]. Alternatively, phospholipids can be synthesised de novo, however this approach tends to be even more costly than using naturally occurring lipids.Because of liposomes above mentionioned drawbacks, and alternative nonionic surfactants have been investigated. This involves formation of liposome-like vesicles from the hydrated mixtures of cholesterol and nonionic surfactant such as monoalkyl or dialkyl polyoxyethylene ether[18]. Niosomes are unilamellar or multilamellar vesicles capable of entrapping hydrophilic and hydrophobic solutes38. From a technical point of view, niosomes are promising drug carriers as they possess greater stability and lack of many disadvantages associated with liposomes, such as high cost and the variable purity problems of phospholipids[19]. Another advantage is the simple method for the routine and large-scale production of niosomes without the use of unacceptable solvents. Cholesterol, 5-cholesten-3β-ol is used in combination with nonionic surfactant for the formation of niosomes.

Characterization of Niosomes

1. Size

Shape of niosomal vesicles is assumed to be spherical, and their mean diameter can be determined by using laser light scattering method.[20] Also, diameter of these vesicles can be determined by using electron microscopy, molecular sieve chromatography, ultracentrifugation, photon correlation microscopy, optical microscopy and freeze fracture electron microscopy. Freeze thawing (keeping vesicles suspension at -20°C for 24 hrs and then heating to ambient temperature) of niosomes increases the vesicle diameter, which might be attributed to fusion of vesicles during the cycle.[21]

2. Bilayer Formation

Assembly of non-ionic surfactants to form a bilayer vesicle is characterized by an X-cross formation under light polarization microscopy.[22]

3. Number of Lamellae

This is determined by using nuclear magnetic resonance (NMR) spectroscopy, small angle X-ray scattering and electron microscopy.

4. Membrane Rigidity

Membrane rigidity can be measured by means of mobility of fluorescence probe as a function of temperature.

5. Entrapment Efficiency

After preparing niosomal dispersion, unentrapped drug is separated by dialysis, centrifugation, or gel filtration as described above and the drug remained entrapped in niosomes is determined by complete vesicle disruption using 50% n-propanol or 0.1% Triton X-100 and analyzing the resultant solution by appropriate assay method for the drug.[23]

Entrapment efficiency = (Amount entrapped / total amount) x 100

Characterization of Liposomes

1. Particle Size and Surface Charge

The droplet size and zeta potential of the liposomes was determined by a Laser Scattering Particle Size Distribution Analyzer and Zeta Potential Analyzer at room temperature. One mL of the liposome suspensions was diluted with 14 mL and 2 mL deionized water, respectively.

2. Transmission Electron Microscopy

Transmission Electron Microscopy (TEM) was used to visualize the liposomal vesicles. The vesicles were dried on a copper grid and adsorbed with filter paper. After drying, the sample was viewed under the microscope at 10–100 k magnification at an accelerating voltage of 100 kV.

3. Entrapment Efficiency (%EE) and Loading Efficiency

The concentration of MX in the formulation was determined by HPLC analysis after disruption of the vesicles (liposomes) with Triton X-100 (0.1% w/v) at a 1 : 1 volume ratio and appropriate dilution with PBS (pH 7.4). The vesicle/Triton X-100 solution was centrifuged at 10,000 rpm at C for 10 min. The supernatant was filtered with a 0.45 m nylon syringe filter. The entrapment efficiencies and the loading efficiencies of the MX-loaded formulation were calculated by (1) and (2), respectively. (1) where is the concentration of MX loaded in the formulation as described in the above methods, and is the initial concentration of MX added into the formulation (2) where is the total amount of MX in the formulation and is the total amount of PC added into the formulation.

4. Stability Evaluation of Liposomes

Liposomes were stored at C and C (room temperature, RT) for 30 days. Both the physical and the chemical stability of MX were evaluated. The physical stability was assessed by visual observation for sedimentation and particle size determination. The chemical stability was determined by measuring the MX content by HPLC on days 0, 1, 7, 14, and 30.

Advantages of Niosomes[24]

- Since the structure of the niosome offers place to accommodate hydrophilic, lipophilic as well as ampiphilic drug moieties, they can be used for a variety of drugs.
- Niosomes exhibits flexibility in their structural characteristics (composition, fluidity and size) and can be designed according to the desired situation.
- They improve the therapeutic performance of the drug by protecting it from the biological environment and restricting effects to target cells, thereby reducing the clearance of the drug.
- Niosomes can act as a depot to release the drug slowly and offer a controlled release.
- They can increase the oral bioavailability of drugs.
- They are osmotically active and stable.
- They increase the stability of the entrapped drug.
- They can enhance the skin penetration of drugs.
- They can be made to reach the site of action by oral, parenteral as well as topical routes.
- The surfactants are biodegradable, biocompatible, and non-immunogenic.

- The niosomal dispersions in an aqueous phase can be emulsified in a non-aqueous phase to control the release rate of the drug and administer normal vesicles in external non-aqueous phase.
- Handling and storage of surfactants do not require any special conditions.
- The vesicle suspension being water based offers greater patient compliance over oily dosage forms.

ADVANTAGES OF LIPOSOMES

- Provide controlled drug delivery
- Biodegradable, biocompatible, flexible
- Non ionic
- Can carry both water and lipid soluble drugs
- Drugs can be stabilized from oxidation
- Improve protein stabilization
- Controlled hydration
- Provide sustained release
- Targeted drug delivery or site specific drug delivery
- Stabilization of entrapped drug from hostile environment
- Alter pharmacokinetics and pharmacodynamics of drugs
- Can be administered through various routes
- Can incorporate micro and macro molecules
- Act as reservoir of drugs
- Therapeutic index of drugs is increased
- Site avoidance therapy
- Can modulate the distribution of drug

Methods of preparation of Niosomes

Preparation of vesicles: The preparation methods should be chosen according to the use of niosomes, since the preparation methods influence the numbers of bilayers, size, size distribution and entrapment efficiency of the aqueous phase and the membrane permeability of the vesicles.

1. Ether injection method[25]

This method provides a means of making niosomes by slowly introducing a solution of surfactant dissolved in diethyl ether into warm water maintained at 60°C. The surfactant mixture in ether is injected through 14-gauge needle into an aqueous solution of material. Vaporization of ether leads to formation of single layered vesicles. Depending upon the conditions used, the diameter of the vesicle range from 50 to 1000 nm.

2. Hand shaking method (Thin film hydration technique)

The mixture of vesicles forming ingredients like surfactant and cholesterol are dissolved in a volatile organic solvent (diethyl ether, chloroform or methanol) in a round bottom flask. The organic solvent is removed at room temperature (20°C) using rotary evaporator leaving a thin layer of solid mixture deposited on the wall of the flask. The dried surfactant film can be rehydrated with aqueous phase at 0-60°C with gentle agitation. This process forms typical multilamellar niosomes.

Thermosensitive niosomes were prepared by Raja Naresh et al /i> by evaporating the organic solvent at 60°C and leaving a thin film of lipid on the wall of rotary flash evaporator. The aqueous phase containing drug was added slowly with intermittent shaking of flask at room temperature followed by sonication.

3. Sonication

A typical method of production of the vesicles is by sonication of solution as described by Cable[26]. In this method an aliquot of drug solution in buffer is added to the surfactant/cholesterol mixture in a 10-ml glass vial. The mixture is probe sonicated at 60°C for 3 minutes using a sonicator with a titanium probe to yield niosomes.

4. Micro fluidization[27]

Micro fluidization is a recent technique used to prepare unilamellar vesicles of defined size distribution. This method is based on

submerged jet principle in which two fluidized streams interact at ultra high velocities, in precisely defined micro channels within the interaction chamber. The impingement of thin liquid sheet along a common front is arranged such that the energy supplied to the system remains within the area of niosomes formation. The result is a greater uniformity, smaller size and better reproducibility of niosomes formed.

5. Multiple membrane extrusion method

Mixture of surfactant, cholesterol and dicetyl phosphate in chloroform is made into thin film by evaporation. The film is hydrated with aqueous drug solution and the resultant suspension extruded through polycarbonate membranes, which are placed in series for upto 8 passages. It is a good method for controlling niosome size.

6. Reverse Phase Evaporation Technique (REV)[28]

Cholesterol and surfactant (1:1) are dissolved in a mixture of ether and chloroform. An aqueous phase containing drug is added to this and the resulting two phases are sonicated at 4-5°C. The clear gel formed is further sonicated after the addition of a small amount of phosphate buffered saline (PBS). The organic phase is removed at 40°C under low pressure. The resulting viscous niosome suspension is diluted with PBS and heated on a water bath at 60°C for 10 min to yield niosomes.

Raja Naresh et al have reported the preparation of Diclofenac Sodium niosomes using Tween 85 by this method.

7. Trans membrane pH gradient (inside acidic) Drug Uptake Process (remote Loading)[29]

Surfactant and cholesterol are dissolved in chloroform. The solvent is then evaporated under reduced pressure to get a thin film on the wall of the round bottom flask. The film is hydrated with 300 mM citric acid (pH 4.0) by vortex mixing. The multilamellar vesicles are frozen and thawed 3 times and later sonicated. To this niosomal suspension, aqueous solution containing 10 mg/ml of drug is added and vortexed. The pH of the sample is then raised to 7.0-7.2 with 1M disodium phosphate. This mixture is later heated at 60°C for 10 minutes to give niosomes.

8. The "Bubble" Method

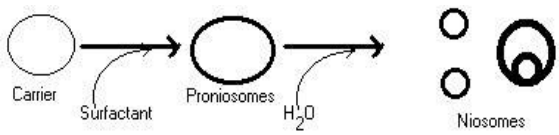
It is novel technique for the one step preparation of liposomes and niosomes without the use of organic solvents. The bubbling unit consists of round-bottomed flask with three necks positioned in water bath to control the temperature. Water-cooled reflux and thermometer is positioned in the first and second neck and nitrogen supply through the third neck. Cholesterol and surfactant are dispersed together in this buffer (pH 7.4) at 70°C, the dispersion mixed for 15 seconds with high shear homogenizer and immediately afterwards "bubbled" at 70°C using nitrogen gas.

9. Formation of niosomes from proniosomes

Another method of producing niosomes is to coat a water-soluble carrier such as sorbitol with surfactant. The result of the coating process is a dry formulation. In which each water-soluble particle is covered with a thin film of dry surfactant. This preparation is termed "Proniosomes". The niosomes are recognized by the addition of aqueous phase at T > Tm and brief agitation.

T = Temperature.

Tm = mean phase transition temperature.



Formation of Niosomes

Blazek-Walsh A.I. et al have reported the formulation of niosomes from maltodextrin based proniosomes. This provides rapid reconstitution of niosomes with minimal residual carrier. Slurry of maltodextrin and surfactant was dried to form a free flowing

powder, which could be rehydrated by addition of warm water.

Preparation of liposomes

An important parameter to consider when addressing the formation process of liposomes is the rigidity of the bilayer. Hydrated-single component phospholipid bilayers can be in a liquid-crystalline ('fluid') state or in a gel state. By increasing the temperature, the gel state bilayer melts and is converted into the liquid state. This occurs at a temperature known as the transition temperature (Tc). The Tc of a bilayer depends on:

1. Acyl chain length.
2. Degree of saturation.
3. Polar head group.

The Tc can vary between 15°C for egg yolk phosphatidylcholine (high degree of unsaturation) to over 50°C for fully saturated distearolylphosphatidylcholine (DSPC)[30].

The raw material for liposome formation depends on the intended use of the liposome. Several companies supply reasonable grade and priced lipids which usually contain at least 98% phospholipid and less than 1% lysophospholipid, low endotoxin and microbial load and trace metals. It is up to the individual investigator to purify the lipid to acceptable standards.

There are five main groups of phospholipids that are available that can be used for liposome preparation.

1. Phospholipid from natural sources.
2. Phospholipid modified from natural sources.
3. Semi-synthetic phospholipid.
4. Fully-synthetic phospholipid,
5. Phospholipid with non-natural headgroups[31].

Phosphatidylcholine (PC), phosphatidylenolamine (PE) and phosphatidyserine (PS) are commonly used phospholipids for liposome preparation. Cholesterol can be added to the bilayer mixture to reduce the permeability of fluid crystalline state bilayers.

There are many different strategies for the preparation of liposomes, which can be classified into 3 main groups.

1. Mechanical methods

A. Film method.

The original method of Bangham et al. is still the simplest procedure for the liposome formation but is limited because of its low encapsulation efficiency. This technique produces liposomes by hydrating thin lipid films deposited from an organic solution on a glass wall by shaking at temperatures above the Tc. The solvent is removed at reduced pressure in a rotary evaporator. The dry film of lipids which has been deposited onto the wall of a round-bottom flask is hydrated by adding a buffer with a water soluble marker. As the lipid becomes hydrated and starts to form into closed vesicles only a small amount of the solute becomes entrapped. This method yields a heterogeneous sized population of MLVs over 1μm in diameter. Further procedures must be employed to achieve a homogeneous population, which will be discussed later[32].

B. Ultrasonication method.

Ultrasonication of an aqueous dispersion of phospholipids with a strong bath sonicator or a probe sonicator will usually yield SUVs with diameters down to 15-25nm.

2. Methods based on replacement of organic solvent.

A. Reverse-phase evaporation

In this method, several phospholipids (pure/mixed with cholesterol) can be used. The lipid mixture is added to a round bottom flask and the solvent is removed under reduced pressure by a rotary evaporator. The system is purged with nitrogen and the lipids are re-dissolved in the organic phase. This is the phase that the reverse phase vesicles will form. Diethly ether and isopropyl ether are the usual solvents of choice.

After the lipids are re-dissolved in this phase the aqueous phase (contains compound to be encapsulated) is added. The system is kept under continuous nitrogen and the two-phase system is sonicated until the mixture becomes clear one-phase dispersion. The mixture is then placed on the rotary evaporator and the organic solvent removed until a gel is formed. Non-encapsulated material is removed. The resulting liposomes are are called reverse-phase evaporation vesicles (REV). The large unilamellar and oligolamellar vesicles formed have the ability to encapsulate large macromolecular vesicles with high efficiency[33].

B: Ether vaporisation method.

In this method a mixture of lipids in an organic solvent (diethyl ether, ethanol, etc.) is slowly injected into a warm aqueous solution. This results in osmotically active, unilamellar vesicles with a well defined size distribution and high volume trapping efficiency (about ten times that of sonicated and hand shaken preparations.

3. Methods based on size transformation or fusion of preformed vesicles.

A: Freeze-thaw extrusion method.

Liposomes formed by the film method are vortexed with the solute to be entrapped until the entire film is suspended and the resulting MLVs are frozen in a dry ice/acetone bath, thawed in lukewarm water and vortexed again. After two additional cycles of freeze-thaw and vortexing the sample is extruded three times. This is followed by six freeze-thaw cycles and an additional eight extrusions. The resulting liposomes are called large unilamellar vesicles by extrusion technique (LUVET) and they typically contain internal solute concentrations which are much higher than external solute concentrations ie. , they have entrapment ratios greater than one . Proteins can be effectively encapsulated using this technique[34].

B: The dehydration-rehydration method.

This method begins with empty buffer containing SUVs (handshaken MLVs can be also be used but are usually not preferred). These are mixed with the component to be entrapped, after which they are dried. Freeze-drying is often the method of choice but other methods such as by vacumn or under a stream of nitrogen can be used. The vesicles are then rehydrated. A mechanism has been proposed whereby as the vesicles become more concentrated during dehydration, they flatten and fuse forming multilamellar planes where the solute is sandwiched. Therefore on hydration, larger vesicles are formed. This technique is mild and simple, the main limitation being the heterogeneity of the size of the size of the liposomes.

Therapeutic Applications of Niosomes

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

Targeting of bioactive agents

To reticulo-endothelial system (RES)

The cells of RES preferentially take up the vesicles. 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 metastasize to the liver and spleen and in parasitic infestation of liver.

To organs other than RES

It has been suggested that carrier system can be directed to specific sites in the body by use of antibodies. Immunoglobulins seem to bind quite readily to the lipid surface, thus offering a convenient means for targeting of drug carrier. Many cells possess the intrinsicability to recognize and bind particular carbohydrate determinants and this can be exploited to direct carriers system to particular cells.

Neoplasia
Doxorubicin, the anthracyclic antibiotic with broad spectrumanti tumor activity, shows a dose dependant irreversible cardio toxic effect. Niosomal delivery of this drug to mice bearing S-180 tumor increased their life span and decreased the rate of proliferation of sarcoma. 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 level and slower elimination.

Leishmaniasis
Niosomes can be used for targeting of drug in the treatment of diseases in which the infecting organism resides in the organ of reticuloendothelial system. Leishmaniasis is such a disease in which parasite invades 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.
The study of antimony distribution in mice, performed by Hunter *et al* showed high liver level after intravenous administration of the carriers forms of the drug.

Delivery of peptide drugs
Yoshida *et al* investigated oral delivery of 9-desglycinamide, 8- arginine vasopressin entrapped in niosomes in an in-vitro intestinal loop model and reported that stability of peptide increased significantly.

Immunological application of niosomes
Niosomes have been used for studying the nature of the immune response provoked by antigens. Brewer and Alexander have reported niosomes as potent adjuvant in terms of immunological selectivity, low toxicity and stability.

Niosomes as carriers for Hemoglobin
Niosomes can be used as a carrier for hemoglobin. Niosomal suspension shows a visible spectrum superimposable onto that of free hemoglobin. Vesicles are permeable to oxygen and hemoglobin dissociation curve can be modified similarly to non-encapsulated hemoglobin.

Transdermal delivery of drugs by niosomes
Slow penetration of drug 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. Jayraman *et al* has studied the topical delivery of erythromycin from various formulations including niosomes or hairless mouse. From the studies, and confocal microscopy, it was seen that non-ionic vesicles could be formulated to target pilosebaceous glands.

APPLICATIONS OF LIPOSOMES
The following are some properties which make liposomes applicable in various fields.

1. Cell -liposome interaction
2. Localized drug effect
3. Enhanced drug uptake
4. Molecules with wide range of solubility and molecular weight can be accommodated
5. Cancer chemotherapy

CONCLUSION
The concept of incorporating the drug into liposomes or niosomes for a better targeting of the drug at appropriate tissue destination is widely accepted by researchers and academicians. Niosomes appeared to be a well preferred drug delivery system over liposome as niosomes being stable and economic. They presents a structure similar to liposome and hence they can represent alternative vesicular systems with respect to liposomes, due to the niosome ability to encapsulate different type of drugs within their multienvironmental structure. Niosomes are thoughts to be better candidates drug delivery as compared to liposomes due to various factors like cost, stability etc.

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