ABSTRACT: Delivering the drug molecule through conventional ways of drug delivery has been a challenging task for the researchers since years. Many novel technologies and systems have been developed to overcome this limitation. Vesicular carriers such as niosomes, liposomes, aquasomes, phytosomes, etc. have been developed as one of the novel ways for the delivery of drug molecules effectively. They provide various advantages such as increased bioavailability, increased pharmacological activity, decreased toxicity, enhancement of stability, sustained delivery, and protection from physical and chemical degradation that occurs inside the body over conventional delivering systems. These vesicular systems can be lipidic or non-lipidic. The composition of niosomes involves nonionic surfactants-lipids and liposome involves phospholipids (natural and synthetic). This review article describes the study of the four vesicular carriers namely niosomes, liposomes, aquasomes and phytosomes and their various aspects such as their definition, types, methods of preparation, advantages, disadvantages, evaluation and characterization. Such different systems are widely used in gene delivery, tumor targeting to brain, oral formulations, in stability and permeability problems of drugs.

INTRODUCTION

The application of vesicular system in drug delivery has changed the definitions of diagnosis and treatment in different aspects of biomedical field. The vesicular system such as liposomes, niosomes, aquasomes, and phytosomes are used to improve the therapeutic index of both existing and new drug molecules by encapsulating an active drug inside vesicular structure in one such system.

It prolongs the existence of the drug in systemic circulation and finally reduce the toxicity. Such different systems are widely used in gene delivery, tumor targeting to brain, oral formulations, in stability and permeability problems of drugs. In this review we really focused on different aspects of vesicular system in terms of its advantages, limitation, application and their characterization.

AQUASOMES

The word aquasomes was derived from Aqua-water and some-cell like body. So aquasomes are bodies of water and their water like properties protect and preserve fragile biological molecules. The word aquasomes was first derived by ‘Nir Kossovsky’. Aquasomes are three layered structure containing a core, coating layer, and a drug layer. The three-layered structures are self-assembled by various bond. The principal of self-assembly is governed by three physicochemical processes like interaction between charged groups, Hydrogen bonding and dehydration effect, Vander Waal forces.

Aquasomes are one of the most recently developed delivery systems for bioactive molecules like peptide, protein, hormones, antigens and genes to specific sites.
Aquasomes are spherical in shape with 60–300 nm particles size. These are nanoparticulate carrier systems but instead of being simple nanoparticles these are three layered self-assembled structures, comprised of a solid phase nanocrystalline core coated with oligomeric film to which biochemically active molecules are adsorbed with or without modification. These structures are self-assembled by non-covalent and ionic bonds. The solid core provides the structural stability, while the carbohydrate coating protects against dehydration and stabilizes the biochemically active molecules.

FIGURE 1 - Structure of Aquasome [1]

Materials used for the preparation of aquasomes

The following materials are used for the preparation-

1. **Core material**-Ceramic like diamond particles, brushite (calcium phosphate) and tin oxide are used. Polymers can also be used as core materials.

2. **Coating material**-Commonly used materials are cellobiose, pyridoxal-5-phosphate, sucrose, tetrahalose, chitosan, citrate, etc.

3. **Bioactive/ drug layer**-The drug is coated and has a property of interacting with coating film by means of non-covalent and ionic interactions.

Method of Preparation of aquasomes [2,3,4]

The method of preparation involves 3 steps-

A. **Formation of an inorganic core**-

1) **Tin oxide**-It can be synthesized by using a high-pressure mixture of argon and oxygen, on which 3-inch diameter of high purity tin is sputtered. As a result ultrafine particles are formed in the gas phase, which are collected on copper tubes cooled to 770k with flowing nitrogen.

2) **Brushite (calcium phosphate) core**-A solution of disodium hydrogen phosphate is reacted with calcium chloride to form calcium phosphate.

\[
2\text{Na}_2\text{HPO}_4 + 3\text{CaCl}_2 + \text{H}_2\text{O} \rightarrow \text{Ca}_3(\text{PO}_4)_2 + 4\text{NaCl} + \text{Cl}_2 + [\text{O}]
\]

(Disodium phosphae)(Calciumhydrogen phosphate)

The precipitated calcium phosphate core particles are centrifuged and washed with distilled water to remove sodium chloride. To obtain the core particles of desired size, the precipitate is resuspended in distilled water and passed through membrane filter.

3) **Diamond core**-Diamond particles are subjected to ultra-cleansing followed by sonification to form the inorganic core.

B. **Coating of core**

The coating material is added to the aqueous dispersion of core under sonication. The coated core is subjected to lyophilization, which helps to make an irreversible adsorption of coat on the core surface. Then by centrifugation, unabsorbed coating particles are removed.

C. **Loading of drug layer**

In this stage, a solution of known concentration of drug is prepared in a suitable pH buffer and coated particles are dispersed into it. The dispersion is kept overnight at low temperature, which governs drug loading after some time to obtain drug-loaded formulation.

Advantages of aquasomes [5,6]

1. These systems act like a reservoir to release the molecules either in a continuous or a pulsatile manner.

2. These nanoparticles offer favorable environment for proteins thereby avoiding their denaturalization.

3. Aquasomes increases the therapeutic efficacy of pharmaceutically active agents and protects the drug from degradation.

4. Multilayered aquasomes conjugated with molecules such as antibodies, nucleic acid, peptides that are known as biological labels.

5. Novel carrier for enzymes such as DNAses and pigment/dyes.

6. Aquasomes-based vaccines offer many advantages as a vaccine delivery system.

Properties of Aquasomes

1. Preserve the conformational integrity of bioactive substances.

2. Delivering of contents through a combination of specific targeting, molecular shielding and slow sustained release.

3. These carriers also protect the drug/antigen/protein from harsh pH conditions.

4. Mechanism of aquasomes is controlled by their surface chemistry and delivers their contents though the combination of specific targeting, molecular shielding, slow and sustained release process.
**Evaluation of aquasomes** [7]

a. **Size and shape** - The mean particle size and size distribution are determined by a photon correlation spectroscopy using a Autosizer II C apparatus and SEM.

b. **Glass transition temperature** - DSC studies have been extensively used to study glass transition temperature.

c. **In vitro drug release studies** - It is determined to study the release pattern of drug from the aquasomes by incubating a known quantity of drug loaded aquasomes in a buffer of suitable pH at 37°C with continuous stirring. Samples are withdrawn periodically and centrifuged at high speed for certain lengths of time. Equal volumes of medium must be replaced after each withdrawal. The supernatants are then analyzed for the amount of drug released by any suitable method.

d. **Drug loading efficiency** - This test is done to ensure the amount of drug, which is bound, on the surface of aquasomes.

e. **The Hb loading capacity** - It is estimated by the difference between the control sample (HbA solution) and the free hemoglobin contained in all fractions without nanoparticles. The spectrophotometric measurements of hemoglobin are done according to Drabkin’s method.

f. **The antigen-loading efficiency for the aquasomes** - Accurately weight antigen-loaded aquasome formulations were suspended in Triton X-100 and incubated in a wrist shaker for 1 h. Then, samples are centrifuged at and absorbance is determined using micro- BCA methods with set a blank of unloaded aquasomes formulation. Antigen loading is expressed as per unit weight of aquasomes particles (g of antigen/mg of sample).

**Characterization of Aquasomes**

a) **Size distribution** - Particle size distribution and morphological properties can be determined by SEM (Scanning electron microscope) and TEM (Transmission electron microscope). For measuring mean particle size and zeta potential of particle, photon correlation spectroscopy is used.

b) **Structural analysis** - FTIR Spectroscopy using potassium bromide pellets is performed for structural determination. IR is recorded in wave number range 4000 - 400cm-1.

c) **Crystallinity** - X-ray diffraction is used to determine crystalline/amorphous behavior of ceramic core.

d) **Coating elegance and strength** - Concanavalin A – induced aggregation method or anthrone method is used for determining coating strength and adsorption of coating on core.

e) **Glass transition temperature** - The transition from glass to rubber state as a change in temperature upon melting is recorded by DSC (Differential Scanning Calorimetry).

f) **In vitro drug release study** - The release pattern of drug from aquasomes is determined by incubating a known quantity of drug-loaded aquasomes in incubator at 370°C. The sample is withdrawn at regular intervals and centrifuged at high speed and estimated for drug content by UV Spectroscopy.

**Applications of aquasomes** [7]

1. Aquasomes are used for delivering of enzyme like DNAs and pigments/dyes.

2. Aquasomes can be used for pharmaceutical delivery like insulin.

3. They have been used for successful targeted intracellular gene therapy.

4. They are used as red blood substitutes.

5. They are used as vaccines for delivery of viral antigen i.e Epstein-Bars virus, Immunodeficiency virus antigen to evoke the correct antibody.

**PHYTOSOMES**

The term phyto means plant and some means cell like bodies. Phytosomes are patented methods developed by Indena to include phospholipids into herbal extracts. Phyto is a newly introduced patented technology developed to incorporate standardized plant extracts or water-soluble phytoconstituents into phospholipids to produce lipid compatible molecular complexes, which enhances their absorption and bioavailability.

The phospholipid used commonly is phosphatidylcholine. Phosphatidylcholine is a bifunctional compound in which the phosphatidyl moiety is lipophilic and choline moiety is hydrophilic in nature. Lipophilic moieties forms the tail and hydrophilic moiety (choline) forms the polar head.

The drug is incorporated as an integral part of membrane into the polar head part of phospholipid (phosphatidylcholine) by means of chemical bonds.

The Phytosome technology produces a little cell, better able to transit from a hydrophilic environment into the lipid-friendly environment of the enterocyte cell membrane and from there into the cell, thus protecting the valuable components of the herbal extract from destruction by digestive secretions and gut bacteria. [8,9,10]
Differences between liposomes and phytosomes

Phytosomes differ from liposomes in the fact that, drug in phytosomes remains chemical bonded to the polar head part of membrane, while in liposomes drug is dissolved within the cavity or may remain in membrane. [11]

Method of preparation of phytosomes [12]

1. **Solvent evaporation method** - A fixed quantity of drug, polymer and phospholipid (phosphatidylcholine) is taken in a round bottom flask (RBF). It is refluxed with specific solvent at 50-60°C for 2 hours. Then the mixture is concentrated to 5-10 ml to get the precipitate, which is filtered and collected. The dried precipitate is phytosome loaded with drug.

2. **Rotary evaporation technique** - Quantity of drug, polymer, and solvent is taken in a rotary flask and stirred for 3 hours below 40°C. As a result, thin film of sample is obtained to which n-Hexane is added and continuously stirred using magnetic stirrer. As a result, phytosomes loaded with drug are obtained, that are collected and stored at room temperature.

3. **Antisolvent precipitation technique** - Known quantity of drug, phospholipids and polymer is taken in round bottom flask (RBF) and refluxed with specific solvent not exceeding 60°C for 2 hours. The mixture is concentrated to 5-10ml and n-Hexane is carefully added to it with continuous stirring to get the precipitate, which is filtered and kept in vacuum desiccator overnight. The dried precipitate is crushed in mortar and sieved through 100-mesh size. As a result, phytosomes loaded with drug are obtained, which are placed in amber coloured glass bottles at room temperature.

Properties of phytosomes [13,14]

4. **Physico Chemical properties** - Phytosomes is a complex between a natural product and natural phospholipids, like soy phospholipids. Such a complex is obtained by reaction of stoichiometric amounts of phospholipids and the substrate in an appropriate solvent. The main phospholipids-substrate interaction is due to the formation of hydrogen bonds between the polar head of phospholipids (i.e. phosphate and ammonium groups) and the polar functionalities of the substrate. When treated with water, phytosomes assumes a micellar shape forming liposomal-like structures.

5. **Biological properties** - Phytosomes are better absorbed, utilized and as a result produce better results than conventional herbal extracts. The increased bioavailability of the phytosome over the non-complexed botanical derivatives has been demonstrated by pharmacokinetic studies or by pharmacodynamic tests.

Advantages of phytosomes

- Phosphatidylcholine, component of phytosome acts a carrier and gives hepatoprotective effect.
- The composition of phytosome is safe.
- The absorption and bioavailability of water-soluble phytoconstituents is increased. This results in better therapeutic effects.
- Due to increased bioavailability of phytoconstituents the dosage required to produce desirable effect is reduced.
- The phytosomes have a better stability than liposomes.
- Phospholipids add to the nutritional value of the plant extract.
- High market demand for products.
- The process of manufacturing phytosomes is relatively simple.
- Phytosomes have the ability to permeate through skin.

Disadvantages of phytosomes

- Phytoconstituent is rapidly eliminated from phytosomes.
- The duration of action is short.

Method of evaluation of phytosomes

1) **Percentage yield** - It is calculated by the following formula –

\[
\% \text{ Yield} = \frac{\text{Practical Yield} \times 100}{\text{Theoretical Yield}}
\]

2) **Differential scanning calorimetry (DSC)** - Glass transition temperature is noted by DSC.

3) **FTIR** - For structural analysis, FTIR is performed using potassium bromide (KBr) pellets.

4) **Particle size** - It is determined by Zetasizer Zen 3600 at a fixed scattering angle of 90 degree at 25oC.
5) **Entrapment efficiency**—It is determined by following formula:

\[
\text{Entrapment efficiency (\%)} = \frac{(\text{Total amount of drug} - \text{Amount of free drug})}{\text{Total amount of drug}} \times 100
\]

To determine total amount of drug, 0.1 ml phytosome loaded suspension is diluted upto 10ml and estimated with UV Visible Spectrophotometer.

To determine amount of free drug, 0.1 ml phytosome loaded suspension is diluted upto 10ml solvent and centrifuged at 18000 rpm for 30 min at -40°C using cooling centrifuge machine. The supernatant is isolated, and quantity of free drug is estimated by UV Visible Spectrophotometer.

6) **Scanning electron microscopy (SEM)**—SEM is used to confirm particle size distribution and surface morphology of phytosomes at various magnifications (like 1000, 5000, 10000, 30000 magnifications)

### Characterization of Phytosomes

1. **Transition temperature**: The transition temperature of the vesicular lipid system can be settled via differential scanning calorimetry.

2. **Entrapment efficiency**: The entrapment efficiency of a phytosomal preparation can be determined by exposing the preparation to ultracentrifugation method.

3. **Vesicle size and zeta potential**: The particle size and zeta potential of phytosomes can be confirmed by dynamic light scattering, which usage a computerized examination system and photon correlation spectroscopy.

4. **Surface tension activity measurement**: The surface tension activity of the drug in aqueous solution can be determined by the Du Nouy ring tensiometer.

5. **Visualization**: Visualization of phytosomes can be accomplished using Scanning Electron Microscopy (SEM) and by Transmission Electron Microscopy (TEM).

6. **Vesicle stability**: The steadiness of vesicles can be measured by calculating the size and structure of the vesicles over time. The mean size is calculated by DLS and structural changes are monitored by TEM.

### Therapeutic benefits and applications of phytosomes

1) Phytosomes can be used for targeting drug to organs, like hepatic targeting. They can also be used for targeting drugs to tumor cells.

2) They can be used for providing sustained drug action

3) They can enhance bioavailability of certain drugs by improving solubility and facilitating membrane permeation of the drugs

4) They increase systemic absorption of drugs

5) They are nontoxic and non mutogenic, so better accepted

6) Phytosomes are used for loading a large number of drugs Silymarin, curcumin, green tea, grape seed, rutin, mitomycin, lutoolin, quercetin, flavonoids, terpenes, polyphenol, silybin, ginseng, ginkgo, etc.

7) Recent literature survey reveals various research updates on phytosomes—

- Kidd.et al. prepared 4 polyphenol preparations like Silybin, curcumin, green tea, and grape seed, but they had poor bioavailability. So, they prepared phytosomes to improve their affectivity.

- Maley et al. investigated that the oral bioavailability of rutin is very low, so it was designed as phytosome to enhance its skin uptake to treat inflammatory disease, rheumatism, athletic aches, etc.

- Mehti et al. prepared phytosomes of luteolin to enhance the bioavailability of luteolin and improve passive targeting in carcinoma cells. They also advice that phytosome technology can improve the effectiveness of chemotherapy by overcoming resistance and enhancing porosity of cancer cells to chemical agents (drugs).

- Zhang et al. developed a curcumin phytosome loaded chitosan microsphere by encapsulating curcumin phytosome in chitosan microspheres and reported that this system combined benefits of phytosomes as well as chitosan microspheres. So, it had higher effects of promoting oral absorption and retention time of curcumin, so it may be used for sustained delivery of curcumin.

- ZhenquingHov et al developed phytosomes loaded with mytomycin, which showed initial burst release of mytomycin followed by substituent sustained release. Moreover, mytomycin loaded phytosomes had very high dose dependent anti-tumor activity.

### LIPOSOMES

Liposomes are the vesicles having concentric phospholipids (phosphatidycholine, phosphatidylethanolamine) bilayers, which encloses an aqueous compartment. In place of phospholipid liposomes can also have bilayer of sphingolipid, glycolipid or sterols.

The phospholipids have two portions-
Hydrophilic head
Lipophilic tails

So phospholipid is an amphiphilic molecule.

When large numbers of phospholipids are placed together they will arrange themselves spontaneously to match their heads together on one side and tails on the other side. Under specific physical conditions concentric bilayered vesicles are formed (means 2 layers of phospholipids make concentric vesicles). As a result a hollow sphere is formed in which aqueous molecules can be entrapped.

Method of Preparation of liposomes [16,17,18,19,20,21,22,23]

1) Lipid Hydration Method (Used mainly for preparing MLVs and OLVs)- In this method lipids are dissolved in suitable organic solvent. This organic solution of lipid is placed in a flask or beaker and dried to form a thin film at the bottom of vessel. The thin film is hydrated by adding an aqueous buffer (containing water soluble drug). Heat liposomal dispersion at 60°C for 10 min to obtain liposome. It is agitated to encapsulate the drug in lipid bilayer film.

2) Sonication Method: (It is used to obtain SUVs from MLVs and OLVs) - MLVs are sonicated either with a bath type sonicator or a probe sonicator under an inert atmosphere in an inert atmosphere to obtain SUV. The main drawbacks of this method are very low internal volume/ encapsulation efficiency (means less amount of drug can be encapsulated), possibly degradation of phospholipids and compounds to be encapsulated, exclusion of large molecules, metal contamination from probe tip and presence of MLV along with SUV.

3) French Pressure Cell Method: (It is used to obtain SUVs from MLVs and OLVs) - The method involves the extrusion of MLV by using a pressure cell having very small orifice at 20,000 psi at 4°C. The method has several advantages over sonication method. The method is simple rapid, reproducible and involves gentle handling of unstable materials. So less chances of degradation of phospholipids. The resulting liposomes are somewhat larger than sonicated SUVs. The drawbacks of the method are that the temperature is difficult to achieve, and the working volumes are relatively small about 50 ml maximum.

4) Solvent Injection Methods. (It is used for the preparation of LUVs and MUVs)-

(a) Ether Infusion Method: A solution of lipid is dissolved in diethyl ether or ethanol. This solution is slowly injected to an aqueous solution of the material to be encapsulated at 55-65°C or under reduced pressure. The subsequent removal of ether under vacuum leads to the formation of LUVs or MUVs

(b) Ethanol Injection Method: A lipid solution of ethanol is rapidly injected to a buffer. The MLVs are immediately formed. The drawbacks of the method are that the LUVs or MUVs are heterogeneous (30-110 nm, non-uniform size), liposomes are very dilute, it is difficult to remove all ethanol because it forms azeotropic mixture with water

5) Reverse Phase Evaporation Method: First water in oil emulsion is formed by a brief sonication of a two-phase system containing phospholipids in organic solvent (diethylether or isopropylether or mixture of isopropyl ether and chloroform) and aqueous buffer containing

Classification of liposomes

a) MLV (Multilamellar vesicles Liposomes)- These liposomes are made of a series of concentric bilayers of lipids enclosing the internal volume. Size- 500 to 10,000nm

b) OLV (Oligolamellar vesicles Liposomes)- These are made of 2 to 10 bilayers of lipids surrounding the internal volume. Size- 100- 500 nm

c) ULV (Unilamellar vesicles Liposomes)- These are made of single bilayer of lipids enclosing the internal compartment. They are further divided into –

- SUV (Small Unilamellar vesicles Liposomes) Size- 20- 40 nm
- MUV (Medium Unilamellar vesicles Liposomes) Size- 40- 80 nm
- LUV (Large Unilamellar vesicles Liposomes) Size-100- 1000 nm
- GUV (Giant Unilamellar vesicles Liposomes) Size- More than 1000 nm

FIGURE 3: Structure of Liposome [15]
drug. The organic solvents are removed under reduced pressure, resulting in the formation of a viscous gel. The liposomes are formed when residual solvent is removed by continued rotary evaporation under reduced pressure. With this method high encapsulation efficiency up to 65% can be obtained in a medium of low ionic strength for example 0.01 M NaCl. The method has been used to encapsulate small, large and macromolecules. Mainly LUVs and MUVs are obtained. The main disadvantage of the method is the exposure of the materials to be encapsulated to organic solvents and to brief periods of sonication. These conditions may possibly result in the denaturation of some proteins or breakage of DNA strands. We get a heterogeneous sized dispersion of vesicles by this method.

6) **Microfluidization Method:** The technique of microfluidization/microemulsification/homogenization for the large-scale manufacture of liposomes. In this method MLVs are prepared by lipid hydration method. The dispersion of MLVs is passed through a microfluidizer at very high velocity. They reach in the precisely defined microchannels present in the interaction chamber of microfluidizer. Here pressure is very high upto 10,000 psi. Thus LUVs are obtained. The reduction in the size range can be achieved by recycling of the sample. The process is reproducible and yields liposomes with good aqueous phase encapsulation.

7) **Freeze-Thaw Method:** The method of freezing and thawing is introduced for increasing the trapped volume of liposomal preparations. The freeze-thaw method is dependent on the ionic strength of the medium and the phospholipid concentration. It influences to a physical disruption of lamellar structure leading to formation of unilamellar vesicles. The unilamellar vesicles are rapidly frozen followed by slow thawing, while the freeze and thawing cycles are repeated. The preparation of MLV propranolol liposomes by freeze-thaw method is described in the literature. The liposomal propranolol formulation is prepared by using distearoylphosphatidylcholine and dimyristoylphosphatidylcholine as phospholipids in phosphate buffered saline buffer, followed by six freeze-thaw cycles.

8) **Calcium-Induced Fusion Method:** The calcium-induced method is based on adding of calcium to SUV. On adding calcium to a dispersion containing SUVs, the individual SUVs get fused to form vesicles inspiral configuration. When EDTA is injected to this, it results in formation of LUVs.

**Advantages of liposomes**

1) Liposomes encapsulated drugs are delivered intact to cells and tissues and can be released when liposome bilayer is destroyed. Thus, it enables site specific and targeted drug delivery.

2) Liposomes can be used for both hydrophilic and lipophilic drugs without chemical modification.

3) Other tissues and cells of body are protected from the drug until it is released by liposome, thus toxicity does not occur.

4) The size of liposome can be altered depending on the nature and dose of drug as well as an intended use of product.

5) They are biocompatible, have high stability and high diffusivity in the skin.

**Disadvantages of liposomes**

- Less stability
- Low solubility
- Short half life
- Phospholipids undergoes oxidation, hydrolysis
- Leakage and fusion
- High production cost
- Allergic reactions may occur to liposomal constituents

**Applications of liposomes**

Liposomes are used for delivery of both hydrophilic and hydrophobic drugs to target sites. Various drugs have been administered in the form of liposomes like-

1) **Targeting of anti-fungal drug.** Amphotericin B is an anti-fungal agent produced from Streptomyces nodosus. It is produced in the form of liposome by using different phospholipids.

   Example-
   - Ambisome-
     It is Amphotericin B liposome in which amphotericin is entrapped in a liposomal membrane made of mixture of various lipids. It is sterile, non-pyrogenic, lyophilized product meant for intravenous Infusion.
   
   Amphotec-
     It is a sterile lyophilized powder meant for intravenous administration that is to be reconstituted before use.

2) **Targeting of anti-cancer drugs.** Anti cancer drugs like Dauxorubicin and daunorubicin can be prepared in form of liposomes to increase their targeting to tumor cells. Example-

   Daunoxome-
     It is liposomal vesicles composed of an aqueous solution of daunorubicin citrate encapsulated with membrane of
distearoylphosphatidylcholine and cholesterol (2:1). This liposomal formulation helps to protect Daunorubicin from chemical and enzymatic degradation, minimizes protein binding and decreases its uptake by normal tissues.

Doxil-

It is a liposome made of dauxorubicin-HCl encapsulated in lipid membrane made of phosphoethanolamine sodium, phosphatidylcholine, cholesterol lipids and ammonium sulphate with histidine as buffer. Methotrexate has also shown increased effectiveness in form of liposomes.

3) **Targeting of antiviral drugs** - Ganciclovir and Foscarnet are the antiviral drugs that are used in CMV retinitis. CMV retinitis is a disease of retina caused due to Cytomegalovirus. It leads to blindness. These drugs are given by IV injection but have narrow therapeutic index and low safety margin. Ganciclovir causes bone- marrow depression and Foscarnet causes renal (kidney) damage. To reduce these adverse effects, Ganciclovir and Foscarnet are prepared as liposomes with phosphatidyl ethanolamine-cholesterol lipids

4) **Site specific delivery of antibiotics** - Gentamycin is an aminoglycoside antibiotic obtained from micromonosporapurpurea. It is used in respiratory tract infections, pneumonia, lung abscess, but it causes ototoxicity and nephrotoxicity, which can be reduced by preparing its liposomes using phosphatidylcholine.

5) **Transdermal drug delivery** - Liposomes are useful in enhancing the uptake of drugs through the skin. This technique has been utilized for topical immunization using tetanus toxoid. Liposomes are proved to increases penetration of transdermal patches through skin.

6) **Treatment therapy of Leishmaniasis** - Leishmaniasis (Kala azar) is a parasite disease caused by Leishmania donovani and transmitted by female sandflyphlebotomus. The most commonly used drugs for this are antimonials like Sodium stibogluconate and Meglumine antimonate, but these drugs can cause cardiac and liver damage. It can be reduced if these drugs are administered in the form of liposomes.

**Evaluation parameter for liposomal drug delivery**-

1) **Entrapment efficiency** - Drug entrapped is determined by complete description using 50 % N propanol or 0.1% triton 100 and analyzing the sample in U.V.

\[ EE = \text{(Total amount of drug entrapped)} * 100 \]

2) **Vesicle diameter** - It can be determined by optical microscopy or electron microscopy and TEM.

3) **In-vitro release study** - It is performed by using dialysis tube. A dialysis sac is washed and soaked in distilled water. The vesicle suspension is placed into dialysis bag and sealed. It is placed in 200 ml phosphate buffer in a 250 ml beaker with constant shaking at 370C. At various time interval 5ml buffer is removed and analyzed for drug content by UV.

**Characterization of liposome**

a) **Physical properties**

- Size and its distribution - Microscopy, Laser light scattering
- Surface charge - Gel electrophoresis
- Entrapped volume – NMR
- Lamellarity - Freeze electron microscopy, P-NMR
- Phase behavior of liposomes – DSC
- Drug release - In vitro diffusion cell
- Encapsulation efficiency (% capture) - Mini column centrifugation, protamine aggregation

b) **Chemical properties**

- Quantitative determination of phospholipids - Barlett assay, Stewart assay, TLC
- Phospholipid hydrolysis –HPLC
- Phospholipid oxidation –UV, GLC, TBA

**Niosomes**

Niosomes are non-ionic surfactant vesicles obtained on hydration of non-ionic surfactants, with or without incorporation of cholesterol. Niosomes are vehicle for drug delivery and are non-ionic. Niosomes are biodegradable, biocompatible, non-immunogenic and exhibit flexibility in their structural characterization. The particle size ranges from 10nm-100nm. A niosome vesicle consists of a vesicle forming amphiphile i.e. a non-ionic surfactant (Span-60), which is stabilized by the addition of cholesterol and a small amount of anionic surfactant (dicetyl phosphate), also helps in stabilizing the vesicle [24].

**Structure of niosomes**

![Structure of Niosome](image)
Niosomes are microscopic lamellar structures. They have a bilayer made up of non-ionic surfactant. The bilayer has its hydrophilic ends exposed on the outside and inside of the vesicle, while their hydrophobic tails face each other within the bilayer. Thus, the vesicles are formed where polar drugs can be entrapped, while hydrophobic drugs can be incorporated in the bilayer tail region itself.

Advantages of niosomes [26]

1) Niosomes are highly stable structure. They require no special conditions such as low temperature or inert atmosphere for storage.
2) They can entrap both hydrophilic and hydrophobic drugs.
3) They are non-toxic, biodegradable, biocompatible, and non-immunogenic.
4) They provide controlled drug delivery at desired site.
5) Relatively low cost of material makes them economical than other controlled delivery routes.
6) Provide a prolonged and sustained release of drugs.
7) They can be administered by oral, parenteral, as well as topical route.
8) They improve therapeutic performance of drug by delayed clearance from the circulation and protect drug from biological environment and restrict the drug action to target cells.

Types of niosomes

a. Multilamellar vesicles (MLV): (Size-0.05 m) - It consists of a number of bilayers surrounding the aqueous lipid compartment separately. The approximate size of these vesicles is 0.5-10 µm. Multilamellar vesicles are the most widely used niosomes. These vesicles are highly suited as drug carrier for lipophilic compounds.

b. Large Unilamellar vesicles (LUV): (Size-0.10 m) - Niosomes of this type have a high aqueous/lipid compartment ratio, so that larger volumes of bio-active materials can be entrapped with a very economical use of membrane lipids.

c. Small Unilamellar vesicles (SUV): (Size-0.025-0.05 m) - These small unilamellar vesicles are mostly prepared from multilamellar vesicles by sonication method. French press extrusion electrostatic stabilization is the inclusion of dicetyl phosphate in 5(6)-carboxyfluorescein (CF) loaded Span 60 based niosomes.

Disadvantages of niosome:

1. Physical instability
2. Aggregation
3. Fusion
4. Leaking of entrapped drug
5. Hydrolysis of encapsulated drugs which limiting the shelf life of the dispersion.

Method of preparation of niosome [27,28,29,30,31,32,33]

1. Ether injection method - Nonionic surfactant with or without a small amount of cholesterol to improve stability is dissolved in diethyl ether. This solution is injected slowly into an aqueous drug solution at 60°C through a 14-gauze needle. Ether is vaporized by rotatory evaporator to form SUVs.

2. Hand shaking method (thin film hydration technique) - The mixture of vesicles forming ingredients like nonionic surfactant and cholesterol are dissolved in a volatile organic solvent (diethyl ether, chloroform or methanol) in a round bottom flask. The organic solvent is evaporated 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 drug phase at 0-60°C with gentle agitation. This process forms typical multilamellar niosomes.

3. Sonication Method - 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 Method - Drug dissolved in water is added slowly to surfactant dissolved in organic solvent. The dispersion is extruded through microfluidizer at very high velocity. The dispersion impinges to specific microchannels in the interaction chamber under high pressure resulting in formation of uniform sized LUVs.

5. Reverse Phase Evaporation Technique (REV) - In this, cholesterol and non-ionic 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 for a few min. The clear gel formed is further sonicated after the addition of a small amount of phosphate buffered saline (PBS). The organic phase is evaporated at 40°C under low pressure. The resulting viscous noisome suspension is diluted with PBS and heated on a water bath at 60°C for 10 min to yield niosomes.

6. Trans membranes PH gradient (inside acidic) Drug Uptake Process (Remote Loading Technique) - 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 citric acid buffer (pH 4.00) and mixed with vortex mixer to form MLVs. The dispersion of MLVs is frozen and thawed three times and then sonicated. Now aqueous solution of drug is added to this suspension and vortexes. 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 get niosomes.

7. The Bubble Method - It is novel technique for the one step preparation 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. In one neck reflux condenser is attached. Thermometer is placed in second neck and a nitrogen supply unit is attached in third neck. Drug dissolved in buffer (Phosphate buffer pH 7.4) is placed in round bottom flask.

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.

Characterization of niosome [34,35,36,37,38]

\[
\text{Percentage entrapment} = \left( \frac{\text{Total drug} - \text{Diffused drug}}{\text{Total drug}} \right) \times 100
\]

- Osmotic shock – Changes in the size of vesicles are viewed under optical microscopy
- Stability studies – To determine the stability of niosomes, the optimized batch was stored in air tight sealed vials at different temperatures. The niosomes were sample at regular intervals of time (0,1,2, and 3 months), observed for color change, surface characteristics and tested for the percentage drug retained.
- Zeta potential analysis- Zeta potential analyzer based on electrophoretic light scattering and laser Doppler velocimetry method.
- Scanning electron microscopy - Particle size
- Optical Microscopy - viewed under a microscope
- Measurement of vesicle size - Laser diffraction particle size analyzer
- Entrapment efficiency- Drug entrapped in niosome is determined by complete disruption using 50% n – propanol or 0.1% Triton-X-100 and analyzing by UV.

Entrapment efficiency (EF) = (Total amount of drug entrapped) * 100

Separating the unentrapped drug by dialysis centrifugation or gel filtration, drug remained entrapped in niosomes is determined by complete vesicle disruption.

- Vesicle diameter- It can be determined by electron microscope (or optical microscope)
- In vitro release- It is performed using dialysis tube. A dialysis sac is washed and soaked in distilled water. The vesicle suspension is placed into dialysis bag and sealed. It is placed in 200ml Phosphate buffer in a 250 ml beaker with constant shaking at 37 degree Celsius. At continuous interval 5 ml sample is withdrawn and analyzed for drug content by UV.

Applications of niosome [39]

1. Delivery of peptide drugs- Peptide drugs when given orally as such are broken down by GIT enzymes resulting in loss of drug. This has been substantially reduced by using niosomal vesicles of peptide drug. For example, oral delivery of vasopressin derivative in niosomal vesicle significantly improved its stability.

2. Niosomes as a carrier for Hemoglobin- Niosomal suspension can be used as a carrier for hemoglobin. Niosomal vesicles are also permeable to oxygen. So, act as a carrier for hemoglobin in anemic patient.

3. Transdermal delivery of drugs by niosomes- Niosomes enhances the penetration of drugs through the skin. The niosomal technology has been successfully used in topical administration of antibiotics for treatment of skin diseases.

4. Antineoplastic Treatment- Antineoplastic drugs like Doxorubicin and Daunorubicin causes severe adverse effect like cardiotoxicity and methotrexate causes bone marrow depression and megaloblastic anaemia but niosomal entrapment of these drugs decreases tumor proliferation rate and enhances the plasma drug conc. in tumor cells only, thus reducing the toxicity on normal cells.

5. Ophthalmic drug delivery- Bio adhesive-coated niosomal formulation of acetazolamide prepared from span 60, cholesterol stearylamine or dicetyl phosphate exhibits more tendencies for reduction of intraocular pressure.

6. Localized Drug Action- Due to their size and low penetrability through epithelium and connective tissue keeps the drug localized at the site of administration. Localized drug action results in enhancement of efficacy, potency of the drug and at the same time reduce its toxic effects.

Evaluation parameters of niosome

a. Morphology- SEM, TEM, freeze fracture technique
b. Size distribution, polydispersity index- Dynamic light scattering particle size analyzer
c. Viscosity - Ostwald viscometer
d. Membrane thickness -X-ray scattering analysis
e. Thermal analysis - DSC
f. Turbidity- UV-Visible diode array spectrophotometer
g. Entrapment efficacy - Centrifugation, dialysis, gel chromatography
h. In-vitro release study- Dialysis membrane
i. Permeation study- Franz diffusion cell
Separation of unentrapped drug from liposome/niosome-

The unentrapped drug from the vesicles is removed by three methods-

a) Dialysis-The aqueous liposomal/niosomal dispersion is dialyzed in a dialysis tube using phosphate buffer.

b) Gel filtration-The un-entrapped drug is removed by gel filtration of dispersion through Sephadex- G-50 column and elution with phosphate buffer.

c) Centrifugation-Dispersion is centrifuged, and supernatant is separated. Pellets are washed and re suspended to obtain dispersion free from unentrapped drug.

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