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IX—LIPOSOMES

INTRODUCTION

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Learning Objective

- Concept of liposomal drug delivery

Liposomes are simple microscopic vesicles composed of lipid bilayer structures enclosing an aqueous compartment. Their size ranges from 25 nm to 5000 nm.

Target-specific Liposomes

1. The drug encapsulated in liposomes does not come into contact with blood. This prevents harmful effects and the drug undergoes less biodegradation.
2. The encapsulated drug gets deposited in the tissue or organ and remains there for a longer period of time than the free drug.
3. Free liposomes are not site- or target-specific, but when they are attached to affinity ligands, they achieve directional specificity toward the given cell or tissue.
4. By attaching monoclonal antibodies to the outer surface of the liposomes, the drugs entrapped become more target specific.
5. Drugs that have problems with solubility, membrane permeation, or toxicity may be delivered with the help of liposomes.

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STRUCTURE OF LIPOSOMES

Learning Objective

- Composition of liposomes

The liposomes are mainly composed of bilayers of phospholipids that are separated by an aqueous phase within which drug can be incorporated. These phospholipids are amphiphilic in nature with a hydrophilic head (polar portion) and lipophilic tail (nonpolar portion). In aqueous phase, they are arranged as bilayers, which form closed vesicles.

The materials used in the preparation of liposomes include the following:

1. **Phospholipids:** The commonly used phospholipids are phosphatidylcholine (PC) and phosophatidylglycerol. Phospholipids are the major structural components of biological membranes. These are amphipathic molecules in which a pair of hydrophobic acyl hydrocarbon chains and hydrophilic polar head group phosphocholine are linked together with a glycerol bridge.

   Phosphotidylcholine is also known as "lecithin," which can be derived from natural (egg yolk and soya bean) and synthetic sources.
2. **Sterols:** Cholesterol is a major component of natural membrane and its incorporation into liposome bilayer can alter the properties of vesicles. Cholesterol by itself does not form a bilayered structure but can do so if it is incorporated into phospholipid membrane at very high concentrations along with PC (1:1 or 2:1 molar ratios).

   Addition of cholesterol to liposomes alters the permeability and fluidity character. Cholesterol increases the rigidity of bilayers and reduces the permeability, thus reducing the leakage of the drug.

The stability of the lipid membrane is also increased. The use of cholesterol also increases the biological half-life of the drug in the blood and hence enhances therapeutic activity.

3. **Sphingolipids:** Sphingolipids are lipids containing long-chain amino alcohol sphingosine and its derivatives. The most abundant sphingolipid is sphingomylin, which is similar to phospholipids.
4. **Charge-inducing substances:** The charge-inducing substances are incorporated into liposomes to induce a surface charge and prevent aggregation. Examples are stearylamine and dipalmitoyl phosphotidylglycerol.

CLASSIFICATION OF LIPOSOMES

The nomenclature depends upon the following:

1. **Structural parameters:** Figure 6.9.1 shows the classification based on the structural parameters.

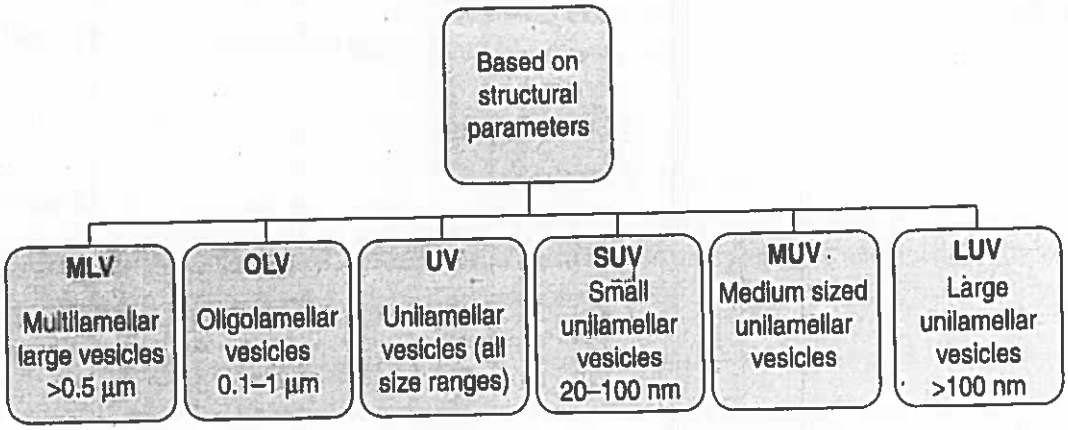

Figure 6.9.1 Classification Based on Structural Parameters

2. **Method of preparation:** Figure 6.9.2 shows the classification based on the method of preparation of the liposomes.

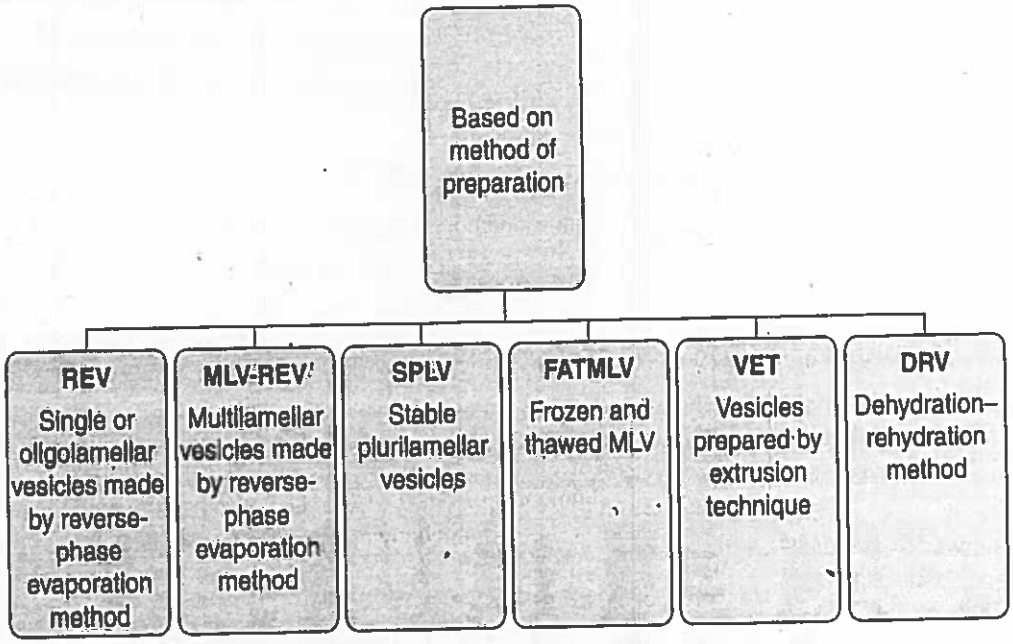

Figure 6.9.2 Classification Based on Method of Preparation

3. **Applications:** Figure 6.9.3 shows the classification based on the composition and applications of the liposomes.

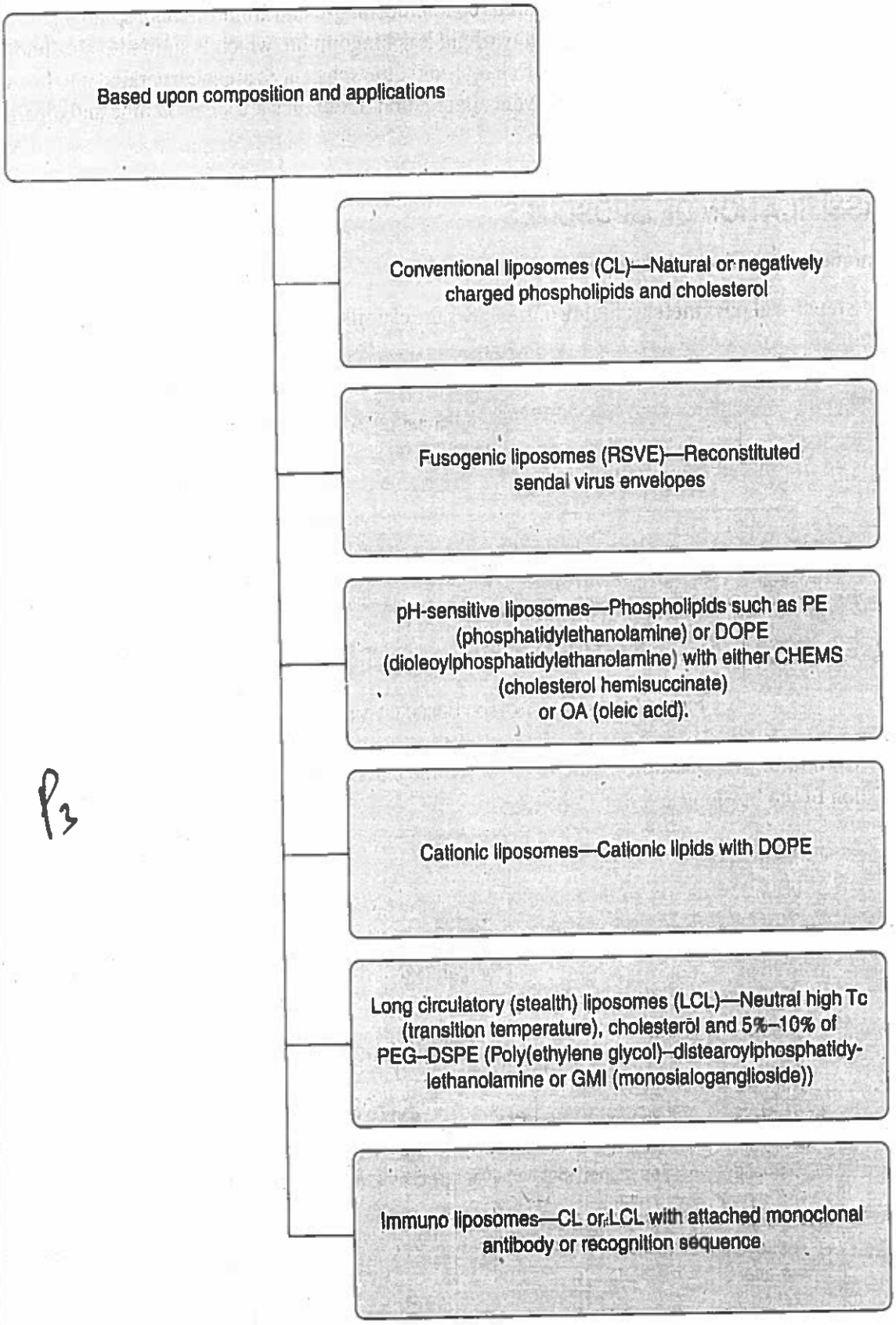

Figure 6.9.3 Classification Based on Composition and Application

METHODS OF LIPOSOMAL PREPARATION

The steps involved in the general method of liposomal preparation are as follows:

1. **Mixing of the components:** The bilayer forming elements PC and cholesterol are mixed in the organic solvent (chloroform and ethanol in the ratio 2:1). Charge-inducing substances are then added.
2. **Drying the lipids from organic solvents:** The organic solvent is then removed under specific conditions of temperature and pressure by using a rotary vacuum dryer at a temperature of 70°C. Drying can also be carried out by spray drying or lyophilization to form a thin film.
3. **Dispersion of lipids in aqueous medium:** The dry lipid mixture is dispersed in an aqueous media containing buffers, chelating agents and antioxidants. If the drug is hydrophilic, it can be incorporated in the aqueous medium at this stage and this process is termed as hydration.

   This step is the rate-limiting step and determines the type of liposomes formed (MLV/ULV/SUV), entrapped volume, surface area and its porosity. After dispersion in the aqueous medium, the liposome suspension is formed, which is further vacuum-dried.
4. **Purification:** It can be carried out by thin layer chromatography or high performance liquid chromatography using Sephadex-25 as the stationary phase or by dialysis process using a semi-permeable membrane.
5. **Analysis of final product:** The final product is characterized using various techniques such as NMR and X-ray diffraction.

Figure 6.9.4 shows the various methods of liposome preparations.

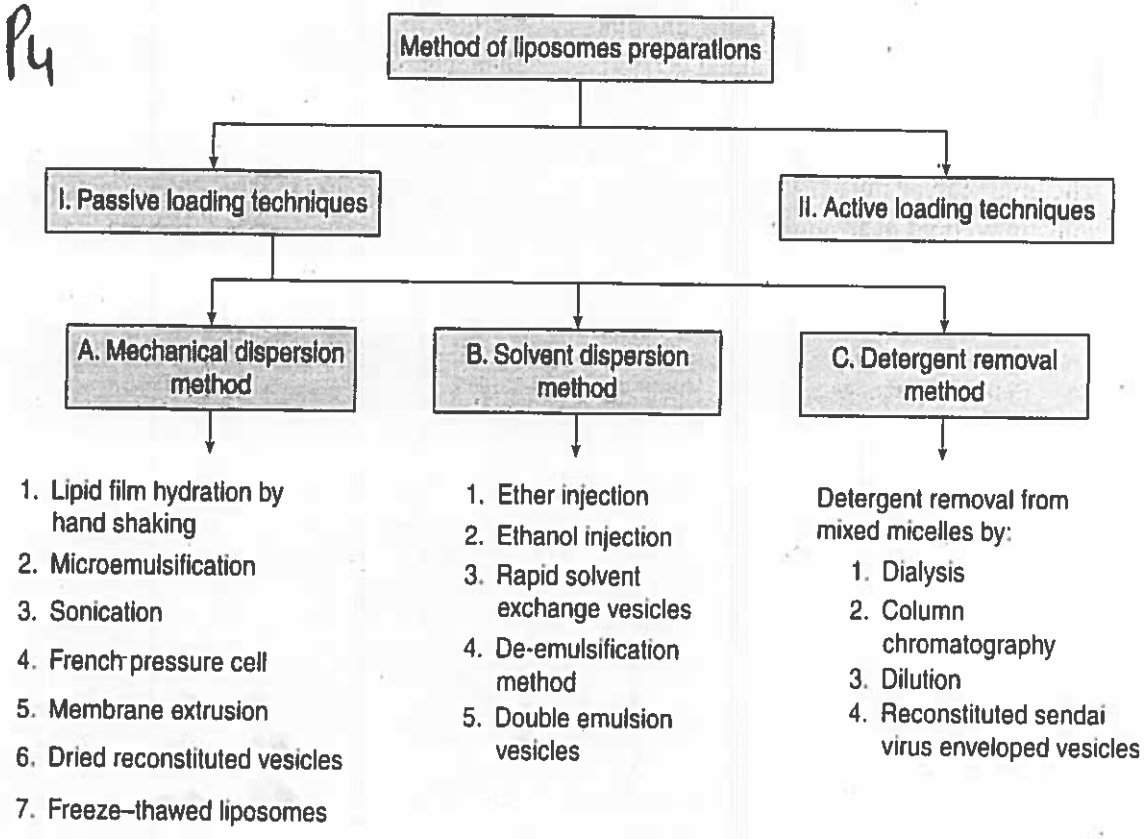

Figure 6.9.4 Various Methods of Liposome Preparations

Passive Loading Techniques

Mechanical Dispersion Methods

1. **Lipid film hydration by hand shaking and non-shaking methods:** In this method, the lipids are obtained as films from their organic solution using a flash rotary evaporator under reduced pressure or by hand shaking. The films are dispersed in an aqueous medium. Upon hydration, the lipids swell and peel off from the wall of the round-bottomed flask and form multilamellar vesicles. The swelling of lipids and dispersion of lipid film is aided by manual agitation (hand shaking technique) or by exposing the film to a stream of water-saturated nitrogen for 15 minutes followed by swelling in aqueous medium without shaking (nonshaken vesicles) (refer Fig. 6.9.5).

   When compared to hand shaking methods, the vesicles produced by nonshaken methods are large unilamellar vesicles. Large amounts of water-soluble compounds are wasted during swelling as only 10%–15% of the total volume gets entrapped.

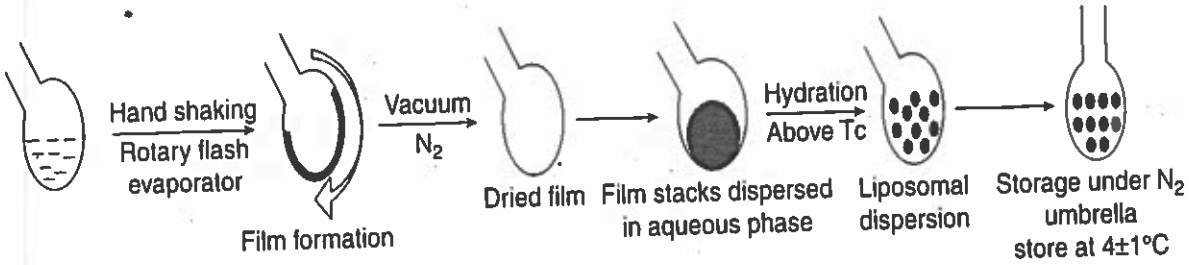

Figure 6.9.5 Liposome Preparation by Lipid Film Hydration Method

2. **Microemulsification technique:** A "microfluidizer" is used to prepare small MLVs from a concentrated lipid dispersion. It pumps the fluid at very high pressure (10,000 psi, 600–700 bar) through an orifice and forces it along defined microchannels, which direct two streams of fluid to collide with each other at right angles at a very high velocity, resulting in an efficient transfer of energy.

   The lipids can be introduced into the fluidizer either as a dispersion of large MLVs or as slurry of unhydrated lipid in an aqueous medium. The fluid collected can be recycled through the pump to the interaction chamber until vesicles of specified dimension are obtained. After a single pass, the size of the vesicles is reduced to a size of 0.1 m and 0.2 m in diameter (refer Fig. 6.9.6).

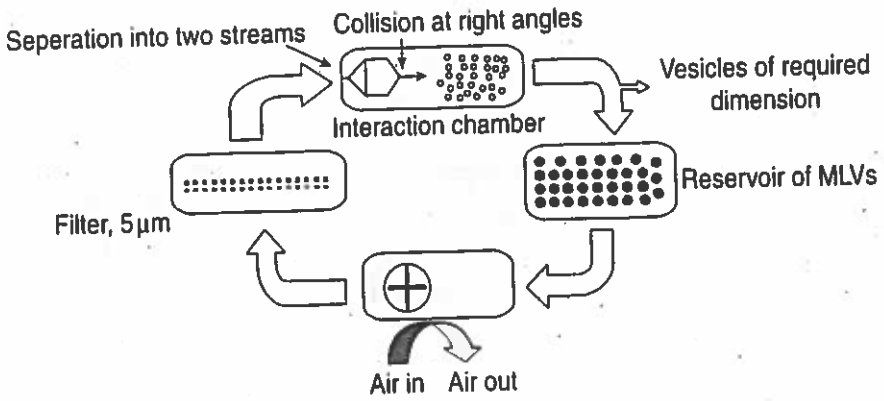

Figure 6.9.6 Liposome Preparation by Microemulsification Method

3. **Sonication method:** There are two methods of sonication based on use, namely probe sonication and bath sonication.

   The probe is employed for small-volume dispersions, which require high energy (e.g., high concentrations of lipids or a viscous aqueous phase), whereas the bath is more suitable for large volumes of diluted lipids.

   Probe tip sonicators supply high energy input to the lipid dispersion and can cause lipid degradation due to overheating of the liposome dispersion. At higher energy levels, the average size of vesicles is further reduced with the aid of ultrasonic irradiation. Sonication tips also tend to release titanium particles into the liposome dispersion, which must then be centrifuged prior to use. For this reason, bath sonicators are most widely used for the preparation of SUVs.

   The MLV dispersion can be sonicated by placing a test tube containing the dispersion in a bath sonicator or placing the tip of the probe sonicator in the test tube and sonicating for 5–10 minutes above the phase transition temperature of the phospholipid used. The lipid dispersion on clarification yields a slightly hazy transparent solution. These particles can then be removed by centrifugation to yield a clear SUV dispersion.

   After centrifugation, the liquid with the top clear layer is decanted leaving a central opalescent layer (containing small multilamellar vesicles) and aggregated lipids at the bottom. The top layer constitutes pure dispersion of SUVs (refer Fig. 6.9.7).

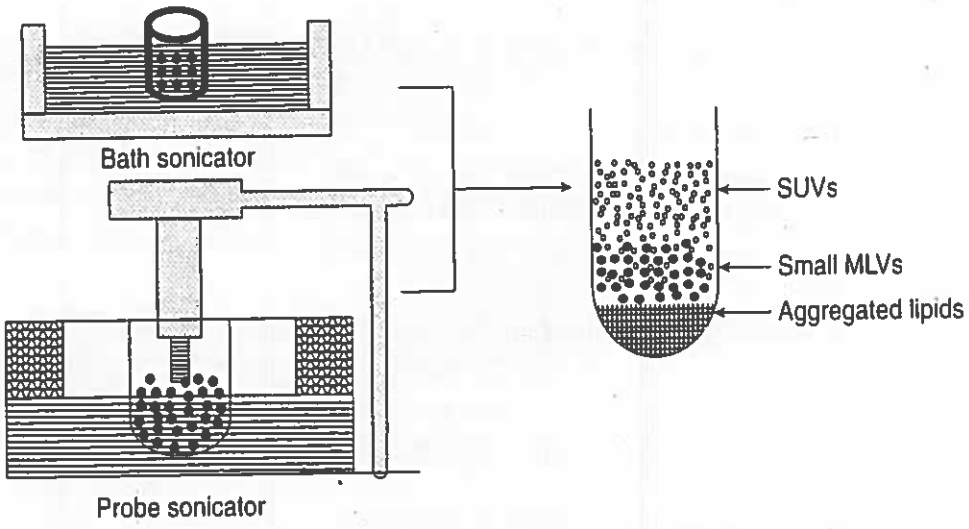

Figure 6.9.7 SUV Preparation by Probe Sonication Method

4. **French press cell liposomes for high pressure exclusion:** French press is an equipment used to reduce the particle size of liposomes by the use of high shear forces. The MLV dispersion is placed in the French press and extruded at a pressure of about 20,000 psi at 4°C. On extrusion, a heterogeneous dispersion consisting of vesicles ranging from several micrometers in diameter to SUV size are produced.

   Passing the dispersion repeatedly through the press results in a progressive decrease in the mean particle diameter. Approximately 95% of the vesicles can be converted to SUVs (30–50 nm) by this method and liposomes produced by this method are more stable than those produced by sonication (refer Fig. 6.9.8).

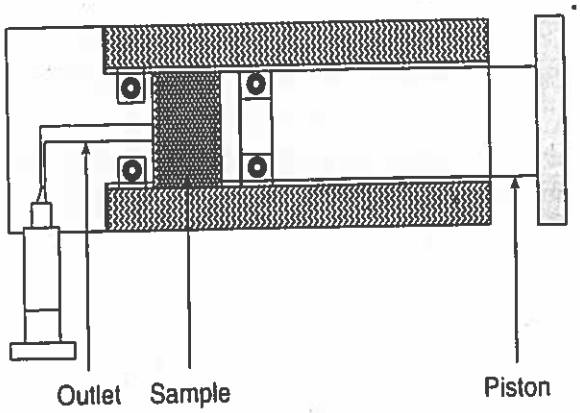

Figure 6.9.8 French Press Cell for the Preparation of Uni- or Oligolamellar Vesicles

5. **Vesicles prepared by membrane extrusion technique:** The vesicles prepared by this technique are termed as LUVETs. In this method, the liposomes are size reduced by gently passing them through a membrane filter of defined pore size. This method utilizes much lower pressure when compared to a French press cell. The membrane extrusion technique can be used to process LUVs as well as MLVs. In this process, during their passage through the membrane, the phospholipid bilayers break and reseal leading to an exchange of contents with the dispersion medium.

   In order to achieve high entrapment of water-soluble drugs, the drug should be dispersed in the suspending medium during the extrusion process. The material that is not entrapped can be removed subsequently (refer Fig. 6.9.9).

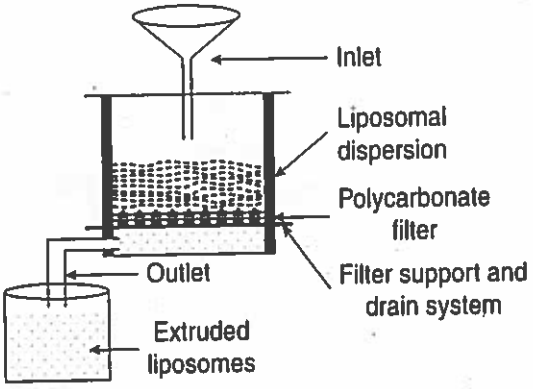

Figure 6.9.9 Liposome Preparation by Membrane Extrusion Technique

6. **Dried reconstituted vesicles method (DRV):** A dispersion of empty SUVs along with the water-soluble materials are freeze-dried and then rehydrated with the aqueous fluid containing the material to be entrapped. A dispersion of solid lipids in finely subdivided form is obtained. The freeze drying method is used to freeze preformed SUV dispersion instead of drying the lipids from an organic solution.

   Organized membrane structure is formed, which on addition of water can rehydrate, fuse and reseal to form vesicles with high encapsulation efficiency. The final product obtained by this method is usually uni- or oligolamellar in nature.

7. **Freeze–Thaw sonication method:** This method is an extension of the classical DRV method and involves freezing of unilamellar (mainly SUV) dispersion, which is then thawed by allowing it to stand at room temperature for 15 minutes and finally sonicated. Sonication reduces the permeability of the liposome membrane (refer Fig. 6.9.10).

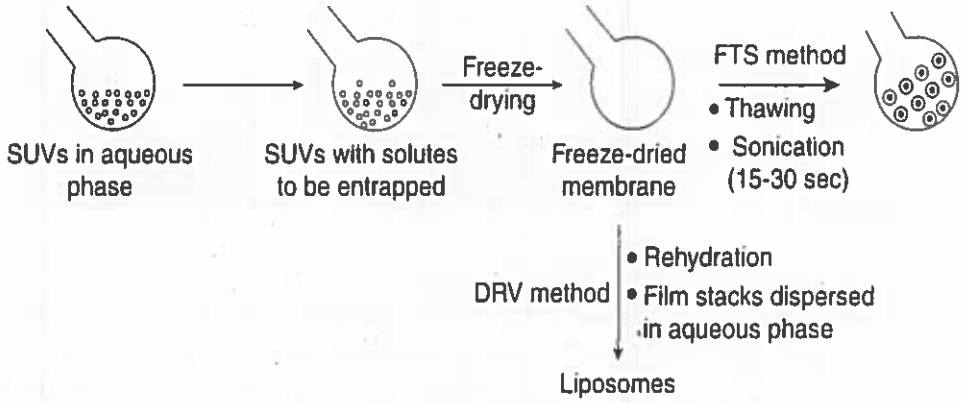

Figure 6.9.10 Liposome Preparation by Freeze–Thaw Sonication Method

Solvent Dispersion Methods

In the solvent dispersion method, the lipids are first dissolved in an organic solution and then the resulting solution is added to the aqueous phase containing materials to be entrapped within the liposomes.

Solvent dispersion methods can be categorized on the basis of miscibility of the organic solvent and aqueous solution.

1. **Ether injection method:** In this method, lipids dissolved in diethyl ether (immiscible organic solvents) are slowly injected into an aqueous solution containing the material to be encapsulated, using a syringe-type infusion by pump at 55°C–56°C or under reduced pressure. The organic solvents are then removed by evaporation at elevated temperature and/or under reduced pressure. A single layer of vesicles is formed depending on the solvent removal method used. The diameter of the resulting vesicles range from 50 nm to 200 nm.
2. **Ethanol injection method:** This method involves the preparation of SUVs without sonication. A solution of lipids in ethanol is injected rapidly through a fine needle into an excess of saline or other aqueous medium. Ethanol mixes with water instantaneously and the phospholipid molecules are dispersed evenly throughout the medium. This procedure yields a high proportion of SUVs, but if the mixing is not adequate, lipid aggregates and larger vesicles may be formed. This method is very simple and has very less chances of degradation of sensitive lipids.

   By varying the concentration of lipid in ethanol or by changing the rate of injection of ethanol solution in preheated aqueous solution, vesicles of 100 nm size may be obtained. The drawback of this method is that it is difficult to remove residual ethanol from the phospholipid membrane.
3. **Rapid solvent exchange vesicles (RSEVs):** In this method, the lipid mixture is quickly transferred between an aqueous pure organic solvent environment and pure aqueous environment. This method is specifically designed to form homogeneous dispersion by sudden precipitation of a lipid mixture in an aqueous buffer.

   This method involves the passage of an organic solution of lipids through the orifice of the blue-tipped syringe (injection needle) under vacuum into a tube containing the aqueous buffer. The tube is mounted on a vortexer. The solvent is vaporized and removed within seconds before

coming into contact with the aqueous environment, while a precipitate of the lipids is formed in the aqueous buffer. This method of preparation requires less time and the liposomes will have high entrapment volumes (refer Fig. 6.9.11).

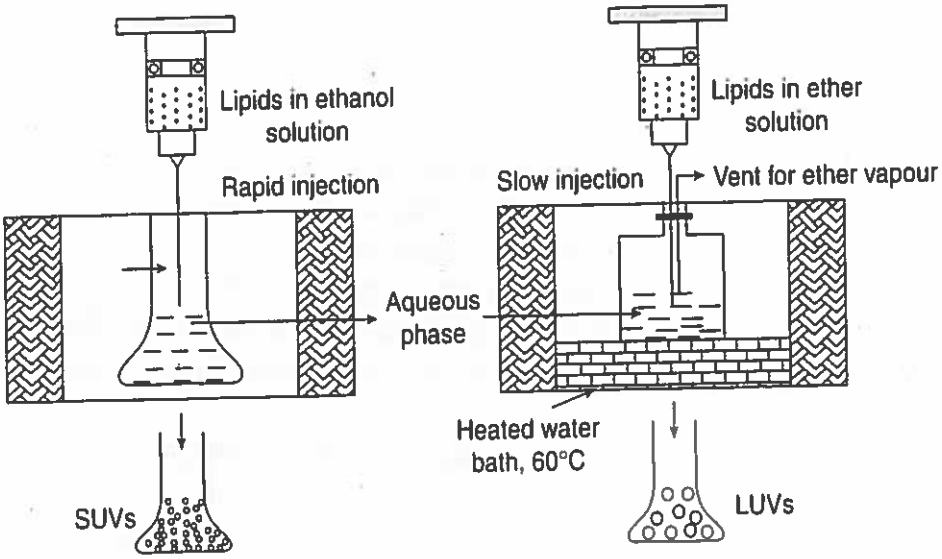

Figure 6.9.11 Liposome Preparation by Rapid Solvent Exchange Method

4. **De-emulsification method:** This method is a two step process: first the inner leaflet of the bilayer is formed and then the outer half is formed.

   The method involves the introduction of a small quantity of aqueous medium containing material to be entrapped into large volumes of immiscible organic solution of lipid to form "water-in-oil" (w/o) emulsion. The emulsion is then homogenized to convert the aqueous phase into microscopic droplets. These droplets are stabilized by the presence of phospholipid monolayer at the interface. The size of droplets is determined by the intensity of homogenization required to form the emulsion and the ratio of lipid to volume of aqueous phase. The aqueous solution surrounded by the monolayer of the phospholipid forms the central core of the final liposome.
5. **Double emulsion vesicles:** In this method, water is emulsified in an organic solution (w/o emulsion) to obtain the outer half of the liposome membrane. This dispersion is then introduced into excess of aqueous medium followed by mechanical dispersion to obtain multicompartment vesicles. The dispersion so obtained is w/o/w system (i.e., double emulsion). The two aqueous compartments will be separated from each other by a thin film of organic solvent. Removal of the solvent results in an intermediate-sized unilamellar vesicle. This method ensures drug entrapment up to 90%..

Detergent Solubilization Method

In this method, the phospholipids are brought in contact with detergents, which helps to bring the phospholipids in close contact with the aqueous phase but still protects the hydrophilic part of the phospholipid. These detergents are often soluble in both aqueous and organic solutions. The structures formed are known as micelles. The chemical nature of detergents, the content and the other lipids involved determines the shape and size of the liposomes. The concentration of detergent in water at which the micelles just start to form is known as the critical micellar concentration (CMC). Below the CMC, detergent molecules exist entirely in free solution, and as the concentration of detergent is increased, micelles are formed.

Membrane-solubilizing detergents have a higher affinity for phospholipid membranes than the pure detergent micelles. Thus, as the detergent is added in increasing amounts to the membrane preparation, more and more detergents get incorporated into the bilayer, until a point is reached where there is a transition from lamellar to spherical micellar phase.

Active (Remote) Loading Techniques

The lipid bilayer membrane is generally impermeable to ions and polar molecules. Some weak acids or bases, however, can be transported through the membrane due to various transmembrane gradients, such as electrical, electrochemical, pH, or specific salt gradients.

Active (remote) loading methods involve the loading of drug molecules into performed liposomes using pH gradients and potential difference across liposomal membranes. A concentration difference in proton concentration across the membrane or liposomes can drive the loading of amphipathic molecules (refer Fig. 6.9.12).

Active loading methods have the following advantages over passive encapsulation:

1. High encapsulation efficiency and capacity
2. Reduced leakage of the encapsulated compounds
3. "Bed side" loading (when the components are mixed at the bed-side and used immediately) of the drugs into liposomes, thus limiting loss of drugs by diffusion or chemical degradation during storage
4. Flexibility for the use of constitutive lipids since drug is loaded after the formation of carrier units

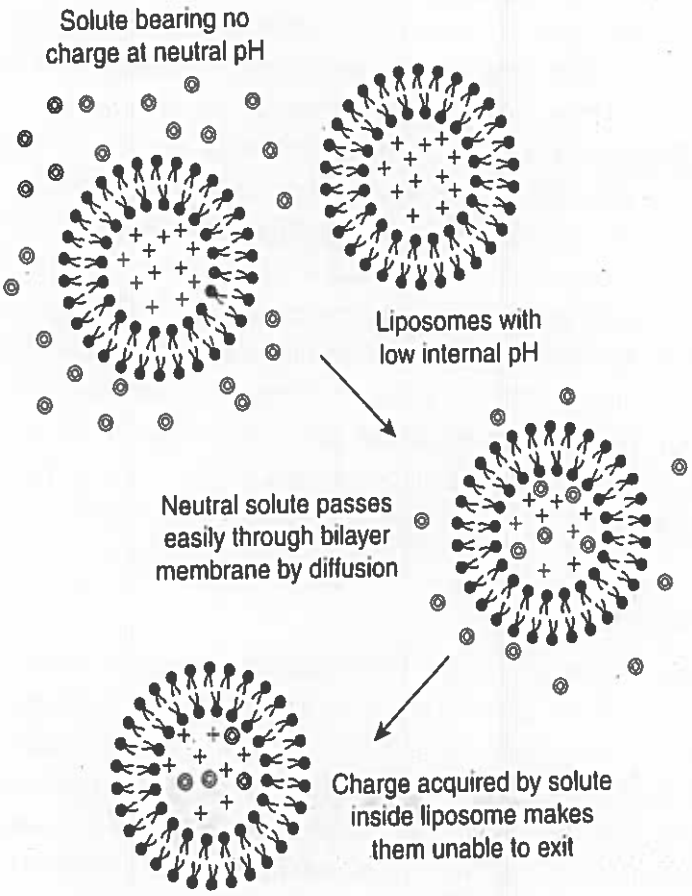

Figure 6.9.12 Preparation of Liposomes by Active Loading Technique

INCORPORATION OF DRUGS INTO LIPOSOMES

Drug loading can be carried out by three methods depending on the characteristics of liposomes and the physical and chemical properties of the drug.

1. **Encapsulation:** This method is useful for water-soluble drugs. Encapsulation involves hydration of a lipid with an aqueous drug solution. A small volume of dissolved drug is entrapped in the interlamellar spaces, leading to the formation of liposomes.
2. **Partitioning:** The drug is dissolved along with phospholipids in a suitable organic solvent. It is either dried first or added directly to the aqueous phase and the residual solvent is removed under vacuum. The acyl chains of the phospholipids help in solubilizing the drug molecule.
3. **Reverse loading:** This method is used for certain drugs (such as weak acids) that may exist in both ionized and unionized forms depending on the pH of their environment. Such drug molecules can be added to an aqueous phase in the unionized state to permeate into liposomes through the lipid bilayers. Then, the internal pH of the liposomes is adjusted to create a charge on the drug molecule. Once ionized, the drug substance loses its lipophilicity and returns to the external medium.

MECHANISM OF DRUG RELEASE FROM LIPOSOMES

When liposomes are injected into the animal or human body, various hydrolytic or lipolytic enzymes act on the amide or ester bonds formed by the natural lipids. The enzymes cut the acyl chains to form lysolipids, which destabilize the lipid layer and release the entrapped bioactive components.

The release of the drug from liposome depends on the composition of liposome, type of drug encapsulated and nature of the cell. Drug is released from liposomes by the following mechanisms:

1. Stable adsorption to the cell surface
2. Endocytosis
3. Fusion with the plasma cell membrane
4. Transfer of lipid into the cellular or subcellular membrane

CHARACTERIZATION OF LIPOSOMES

Learning Objective

- Evaluation of liposomes

Factors such as physical size, membrane permeability, percentage of entrapped solutes, chemical composition, quantity and purity of the starting materials govern the behavior of liposomes in both physical and biological systems.

Liposomes can be characterized by the following methods.

Physical Characterization

Table 6.9.1 shows the physical characterization of liposomes.

Table 6.9.1 Physical Characterization of Liposomes

Parameter	*Method*
Vesicle shape and surface morphology	Determined by transmission electron microscopy and freeze-fracture electron microscopy
Vesicle size and size distribution	Determined by dynamic light scattering, transmission electron microscopy and zetasizer
Submicron range Micron range	Determined by transmission electron microscopy, freeze-fracture electron microscopy, gel permeation and gel exclusion
Surface charge	Determined by free-flow electrophoresis
Electrical surface potential and surface pH	Determined by zeta potential measurements and pH sensitive probes
Lamellarity	Determined by small angle X-ray scattering freeze-fracture electron microscopy
Face behavior	Determined by freeze-fracture electron microscopy and DSC
Percentage capture or percentage of free drug	Determined by ion exchange chromatography, gel exclusion chromatography and minicolumn centrifugation
Drug release	Determined by using a diffusion cell or dialysis method

Chemical Characterization

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Table 6.9.2 shows the chemical characterization of liposomes.

Table 6.9.2 Chemical Characterization of Liposomes

Characterization Parameter	*Analytical Method*
Phospholipid concentration	Lipid phosphorus content can be determined using Barlett assay or Stewart assay and HPLC
Cholesterol concentration	Assayed by cholesterol oxidase assay and HPLC
Drug concentration	Appropriate methods given in the monograph for the individual drug
Phospholipid peroxidation	Determined by UV absorbance, TBA for endoperoxidase, iodometric for hydroperoxidase and GLC
Phospholipid hydrolysis	Determined by HPLC, TLC and fatty acid concentration
Cholesterol auto-oxidation	Determined by HPLC and TLC
Antioxidation degradation	Determined by HPLC and TLC
pH	Determined using a pH meter
Osmolarity	Determined using a osmometer

Biological Characterization

Table 6.9.3 shows the biological characterization of liposomes.

Table 6.9.3 Biological Characterization of Liposomes

Parameter	*Method*
Sterility	Determined using aerobic or anaerobic cultures
Pyrogenicity	Determined by rabbit fever response test or LAL test (Limulus Amebocyte Lysate test)
Animal toxicity	Determined by monitoring survival rates, histology and pathology

STABILITY OF LIPOSOMES

Industrially produced liposomes will reach the patient only after a prolonged time. Thus, during storage or transport, the liposome dispersion should not change its characterization or lose the associated drug or antigen. In general, a shelf life of at least one year is a minimum prerequisite for liposomes.

Chemical Stability

Phospholipids form the backbone of the liposomes and hence their chemical stability is important. The performance of phospholipids bilayer can be affected by hydrolysis of the ester bonds or peroxidation of unsaturated acyl chains.

Chemical degradation can be prevented by the following:

1. Using freshly prepared and purified solvents
2. Manufacturing the liposomes in an oxygen-free environment
3. Avoiding procedures that require high temperatures
4. Using complexing agents such as EDTA to remove traces of metals that can potentiate oxidation
5. Including antioxidants as components of lipid membranes (e.g., tocopherols and BHT)
6. Storing prepared liposomes in an inert atmosphere

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Physical Stability

Physical processes that affect shelf life include loss of liposome associated drug and change in size and aggregation or fusion of liposomes. The liposomes are considered to be physically stable if the size distribution and the ratio of lipid to active agent of liposomes remains constant. The stability can be improved by the use of an aqueous dispersion, by proper selection medium and bilayer components, by freeze drying of liposomes, or by the use of proliposomes approach.

ADVANTAGES OF LIPOSOMES

1. Liposomes are completely biodegradable and nontoxic in nature.
2. They are biologically inert and nonantigenic in nature.
3. They are biocompatible and can also be made bioadhesive.

4. They can be used for delivering hydrophilic substances.
5. The encapsulated drug is protected from degradation.
6. The frequency of drug administration can be decreased with the use of liposomes.
7. Liposomes can alter the tissue distribution of certain drugs by targeting the elements of reticulo-endotheleial system.

DISADVANTAGES OF LIPOSOMES

1. Liposomes above a certain size range can block the capillaries causing embolism.
2. The positively charged surface may "blind out" circulating alpha-2-macroglobulin resulting in higher coagulation time.
3. The lipid components of liposomes may induce metabolic changes in the body resulting in toxicity.
4. Since the liposomal products are administered by the parenteral route, sterility should be maintained at each step during production and the ingredients used in the formulation should be of highest purity.

APPLICATIONS OF LIPOSOMES

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Learning Objective

- Therapeutic uses of liposomes

1. **Treatment of cancer:** Antitumor drugs such as actinomycin-D, vinblastine, methotrexate and bleomycin can be encapsulated into liposomes for drug delivery to the target tissue.
2. **Diseases caused by intracellular parasites:** Drug-loaded liposomes can be used in the treatment of rickettsial infections, malaria, amebiasis, lieshmaniasis and viral diseases.
3. **Metal toxicity:** Liposomal EDTA can be used in heavy metal poisoning. Chelating agents cannot pass through the biological membranes, so these are incorporated in liposomes so that they can easily pass through the biological membranes.
4. **Diabetes:** Researchers have studied the potential of liposomes as carriers for oral administration of insulin. Studies have shown that liposomes have protective effects against the proteolytic digestive enzymes, pepsin and pancreatin. They can increase the intestinal uptake of macromolecules and are thus capable of enhancing insulin uptake.
5. **Liposomes as radiopharmaceutical carrier:** For diagnostic purposes, liposomes may act as carriers of radio pharmaceuticals.
6. **Cell biological application:** Liposomes can be used to incorporate functional DNA and RNA molecules into the cells during the preparation of polio vaccine.
7. **Liposomes in cosmetics and dermatology:** The potential of liposomes as topical drug delivery systems has been explored. These are highly effective in the treatment of skin disorders. Drugs from different categories of antitumors, local anesthetics, antimicrobials, NSAIDs and antiseptics can be formulated as liposomes.
8. **Liposomes in immunology:** It is used as an immunoadjuvant. Examples are antigen-influenza subunit antigens, immunodiagnostics and immunomodulators.

9. **Liposomes in gene delivery:** Liposomes are used in genetic vaccination and gene and antisense therapy.
10. **Liposomes in antimicrobial, antifungal and antiviral therapies:** Amphotericin B is used in the treatment of candidiasis and leishmaniasis, gentamycin is used in the treatment of staphylococcal pneumonia and rifampicin is used in the treatment of tuberculosis.
11. **Liposomes in ophthalmic therapy:** Liposomes have the ability to remain in intimate contact with the corneal and conjuctival surfaces, thereby increasing the ocular absorption.
12. **Vaccine adjuvant:** Vaccines can be prepared by entrapping microbes, soluble antigens, DNA, or cytokines inside liposomes.

REVIEW QUESTIONS

Answer in Detail

1. Explain the principle, advantages, disadvantages and methods of manufacture of liposomal drug delivery systems.
2. Write in detail about the different methods used for the manufacture of liposomes?
3. Discuss drug targeting with liposomes.
4. Write a note on passive loading of drug into liposomes.
5. Write in detail about characterization of liposmes.

Answer in Brief

1. State the applications of liposomes.
2. Write a note on the structural components of liposomes.
3. Classify liposomes with examples.
4. Write a note on dried reconstituted vesicles.
5. State the therapeutic applications of liposomes.
6. Discuss freeze–thawed liposomes.
7. Define liposomes.
8. Write a note on rapid solvent exchange vesicles.
9. Discuss the merits and demerits of liposomal preparations.

Answer in One or Two Sentences

1. Differentiate between liposomes and niosomes.
2. Name the basic components of liposomes.
3. How do you improve the shelf life of liposomes?
4. What are the advantages of active loading in the manufacture of liposomes?

Session: 2013—2018

X—NIOSOMES

INTRODUCTION

Learning Objective

- Concept of niosomes

Niosomes are novel drug delivery systems in which the drug is encapsulated in a vesicle formed by the self-assembly of hydrated surfactant monomers. They are also referred to as nonionic surfactant vesicles (NSVs).

Niosomes are essentially nonionic surfactant-based unilamellar or multilamellar vesicles in which an aqueous solution is enclosed in a highly ordered bilayer made of nonionic surfactant, with or without cholesterol and diacetyl phosphate. The niosomes are very small and microscopic in size and exhibit an *in vivo* behavior, which is similar to that of liposomes.

STRUCTURE OF NIOSOMES

Niosomes are microscopic lamellar structures. They are formed by the combination of nonionic surfactant (alkyl or dialkyl polyglycerol ether class) and cholesterol followed by hydration in aqueous media. In niosomes, an aqueous solution of solute is enclosed by a bilayered membrane, which is made up of nonionic surfactants as compared to phospholipids in liposomes. The bilayered membrane is arranged in such a way that the hydrophobic tail of the surfactant faces away from the central aqueous core.

Niosomal vesicle can be formed by a nonionic surfactant such as Span-60. Addition of cholesterol results in an ordered liquid phase, which gives a more rigid and less leaky membrane. Addition of diacetyl phosphate increases the size of the vesicle, provides charge and also increases the drug-loading efficiency of the niosomes. Figure 6.10.1 shows a diagrammatic representation of the niosome structure.

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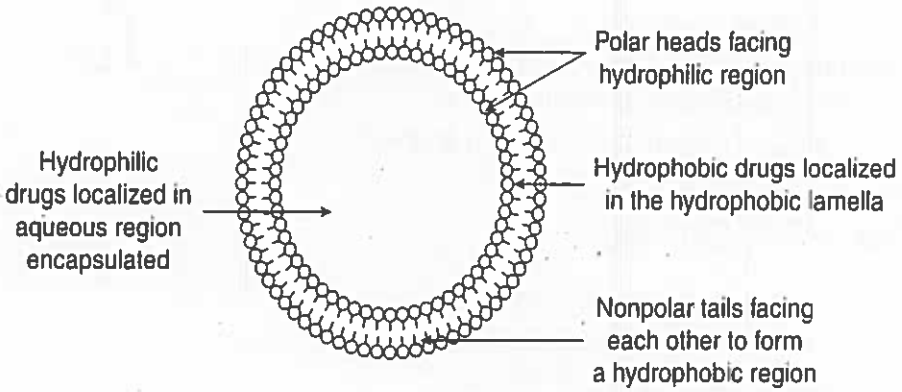

Figure 6.10.1 Diagramatic Representation of Niosome Structure

METHODS OF PREPARATION OF NIOSOMES

Learning Objective

- Techniques involved in the preparation of niosomes

Figure 6.10.2 depicts the various methods of preparation of niosomes, which are discussed in this section.

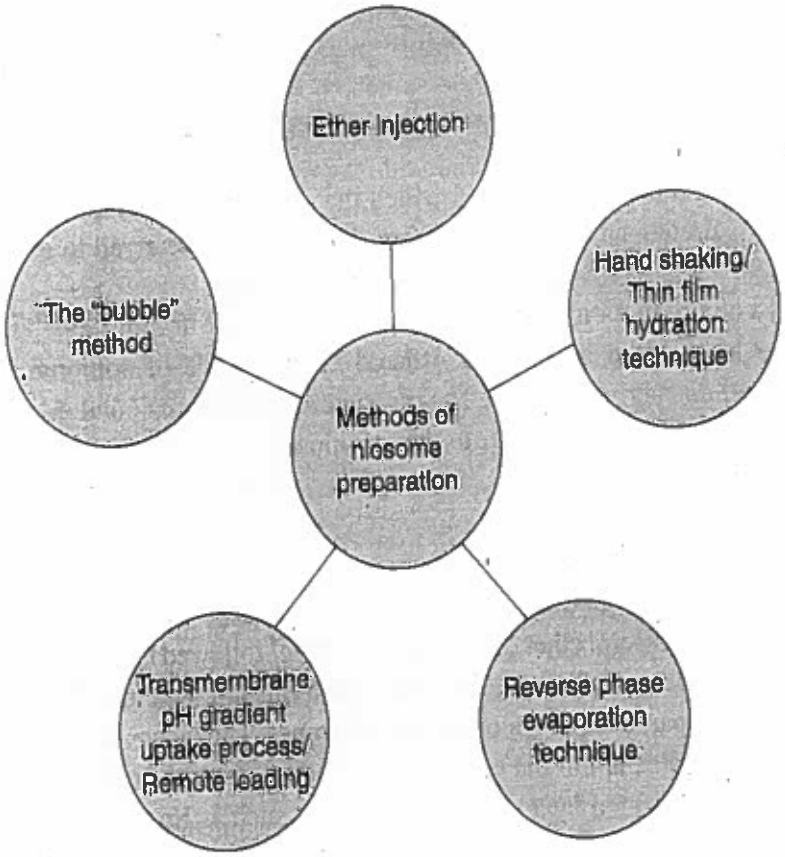

Figure 6.10.2 Methods of Preparation of Niosomes

Ether Injection Method

In this method, a solution of niosomal ingredients in ether is made. This solution is slowly injected into an aqueous phase (e.g., buffer) using a 14 gauge needle at the rate of approximately 0.25 ml/min. The aqueous phase is preheated to 60°C during the injection of the ether solution. This causes evaporation of the ether leading to the formation of single-layered vesicles. The particle size of the niosomes formed can range between 50 µm and 1000 µm. This method has the disadvantage that a small amount of residual ether frequently remains in the final product and is difficult to remove.

Hand Shaking Method (Thin Film Hydration Technique)

In this method, the vesicle-forming agents such as the surfactant and cholesterol are dissolved in a volatile organic solvent such as diethyl ether, chloroform or methanol in a round-bottomed flask. The organic solvent is evaporated under reduced pressure using a rotary evaporator. A thin film of solid mixture remains deposited on the walls of the flask. This dried surfactant layer is rehydrated with the aqueous phase (containing the drug) at normal temperature with gentle agitation to yield multilamellar niosomes. The multilamellar vesicles can be further processed to yield unilamellar niosomes or smaller niosomes using sonication, microfluidization or membrane extrusion techniques.

Reverse Phase Evaporation Technique

In this method, cholesterol and surfactant (1:1 ratio) are dissolved in a mixture of ether and chloroform. An aqueous phase containing the drug to be loaded is added to this and the mixture is sonicated at 4°C–5°C until a clear gel is formed. Phosphate buffered saline (PBS) is added to it and further sonicated. The temperature is raised to 40°C and the organic phase is removed under reduced pressure. A viscous niosome suspension is obtained, which can be diluted with PBS and heated on a water bath at 60°C for 10 minutes to yield niosomes.

Transmembrane pH Gradient (Inside Acidic) Drug Uptake Process (Remote Loading)

In this method, the surfactant and cholesterol are solubilized in chloroform. Chloroform is then evaporated under reduced pressure to get a thin film on the wall of the round-bottomed flask. The formed film is then hydrated using 300 mM citric acid solution (pH 4.0) by vortex mixing. The resulting multilamellar vesicles are frozen and thawed three times. The mixture is sonicated to obtain a niosomal suspension. Aqueous solution of drug (10mg/ml) is added and vortexed. The pH of the sample is then raised to 7.0–7.2 using 1M disodium phosphate. This causes the drug, which is outside the vesicle, to become nonionized, which then enters the niosomal membrane. Once inside, it is again ionized since the interior of the vesicle is acidic. Thus, it is prevented from exiting the vesicle. The mixture is later heated at 60°C for 10 minutes to give the desired vesicles.

The "Bubble" Method

This method allows the preparation of niosomes without the use of organic solvents. The niosomes are prepared in a bubbling unit, which consists of a round-bottomed flask with three necks. The flask is positioned in a water bath to control the temperature. Water-cooled reflux is positioned in the first neck and thermometer in the second. The third neck is used to bubble nitrogen gas into the mixture. A dispersion of cholesterol and surfactant in a buffer (pH 7.4) is taken in the flask and maintained at 70°C. The dispersion is then mixed with high shear homogenizer. Nitrogen gas is immediately bubbled into it at 70°C to yield niosomes.

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ADVANTAGES OF NIOSOMES

1. They do not require special conditions of storage such as low temperature or inert atmosphere for protection and storage.
2. They are chemically stable.
3. They possess both hydrophilic and lipophilic regions within their structure. Hence, they are suitable for entrapment of both hydrophilic and lipophilic drugs.
4. They enhance absorption of drugs and hence improve their oral bioavailability.
5. When used in transdermal preparations, they enhance the percutaneous permeation of the drug.
6. They can be used for oral, parenteral and topical use.
7. The nonionic surfactants used in niosome preparations are biodegradable, biocompatible and nonimmunogenic.
8. The niosomal vesicles can act as depot preparations, thereby providing a sustained effect of the drugs in the body.

9. The therapeutic performance of the drug is also improved, since the drug is protected from the surrounding environment. The drug effects are restricted only to the target cells, thus reducing the overall dose of the drug.
10. Further control over the drug release can be obtained by dispersing an aqueous dispersion of the niosomes in a nonaqueous phase.
11. The relatively low cost of materials makes it suitable for industrial manufacture.
12. They are osmotically active and they increase the stability of the entrapped drug.

CHARACTERIZATION TECHNIQUES

Learning Objective

- Evaluation studies of niosomes

1. **Size, shape and morphology:** Electron microscopy is useful in studying the morphology of the vesicles. Photon correlation microscopy can be used to determine the mean diameter of the vesicles. The vesicular structure of the vesicles can be visualized using freeze-fracture electron microscopy.
2. **Entrapment efficiency:** The entrapment efficiency determines how much of the drug is entrapped in the niosomes. The drug entrapment is estimated after separating the untrapped drug by dialysis. The vesicles can be disrupted using solvents such as 50% n-propanol or 0.1% Triton X-100 to release the entrapped drug. The resultant solution can then be analyzed for the drug content. The entrapment efficiency of niosomes varies depending on the type of method used in their preparation. Nonionic surfactants prepared by ether injection method demonstrated higher entrapment efficiency as compared to those prepared by hand shaking method.
3. ***In vitro* drug release rate:** The drug release from the niosomes can be determined by the use of an *in vitro* diffusion cell. For example, a Keshary–Chein cell can be used for transdermal niosomal preparations.

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APPLICATIONS OF NIOSOMES

Learning Objective

- Therapeutic uses of niosomes

The niosomal technology finds a wide range of therapeutic applications. The following text summarizes some of the uses and applications of niosomes, which are either proven or under research.

1. **Drug targeting:** Niosomes are preferentially taken up by the reticulo-endothelial system (RES). The uptake of niosomes by RES is controlled by certain serum factors called "opsonins." The niosomes are coated with opsonins and are taken up by the RES-for clearance. Such localization of drugs is utilized to treat tumors that are known to metastasize to the liver and spleen and also parasitic infections of the liver.

   By conjugating the niosomes with a targeting carrier such as a monoclonal antibody, the niosomes can be made to target organs other than the RES. The antibodies act as carriers and target the drugs to the specific organ.

2. **Anti-neoplastic treatment:** Most of the side effects associated with anticancer drugs are due to their nonspecific distribution in the body. Niosomes can alter the metabolism, can prolong circulation and half life of the drug and can also target the drug to the specific tumor site, thus decreasing the side effects of the drugs. Niosomal entrapment of doxorubicin and methotrexate showed decreased rate of proliferation of the tumor because of higher drug plasma levels accompanied by slower elimination as compared to the free drug.
3. **Treatment of leishmaniasis:** The targeting activity of niosomes to the RES can be exploited to treat diseases such as leishmaniasis. It is a parasitic disease affecting the liver and spleen. Commonly prescribed drugs for the treatment are derivatives of antimony (antimonials), which in higher concentrations can cause cardiac, liver and kidney damage. Experiments with niosomes showed that it was possible to administer higher levels of the drug without causing side effects.
4. **Delivery of peptide drugs:** Oral peptide drug delivery is a challenge, since the peptide drug is broken down by enzymes. Entrapment within niosomes can protect the peptide from enzymatic degradation.
5. **Studying immune response:** Niosomes can be used to study the nature of the immune response provoked by antigens. Formulation of antigens as a niosome further increases the immunogenic activity of the antigen.
6. **Niosomes as carriers for hemoglobin:** The niosomal vesicle is permeable to oxygen and hence can act as a carrier for hemoglobin in anemic patients.
7. **Transdermal drug delivery systems utilizing niosomes:** Niosomes enhance the percutaneous absorption of drugs. Transdermal drug delivery utilizing niosomal technology is widely used in cosmetics. Antibiotics in niosomal preparations can be used in acne treatment.
8. **Other applications:** The drug-sustaining activity of niosomes can be used in controlled and localized delivery of drugs. Toxic drugs that need higher doses can possibly be delivered safely using niosomes.

REVIEW QUESTIONS

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Answer in Detail

1. How are niosomes prepared?
2. Discuss drug targeting with liposomes and niosomes.

Answer in Brief

1. State the application of niosomes.
2. What is the bubble method?
3. State the application of niosomes.
4. Write a note on the applications of niosomes.
5. Discuss the physicochemical aspects of nonionic surfactant vesicles.

Answer in One or Two Sentences

1. Define niosomes.
2. Differentiate between liposomes and niosomes.
3. Write a note on the ether injection method.