A NOVEL DRUG DELIVERY OF LIPOSOMES: A COMPREHENSIVE REVIEW

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ABSTRACT

Liposomes are little spherical artificial vesicles, which is made up of naturally derived phospholipids or pure surfactants. Liposomes can aid with active targeting as it has flexibility in coupling with site-specific ligands. The components of liposomes are Phospholipids, Cholesterol and other additional excipients. Liposomes are vesicular structures consisting of hydrated bilayers. The hydrophilic part is mainly phosphoric acid bound to a water-soluble molecule whereas the hydrophobic part consists of two fatty acid chains with 10-24 carbon atoms and 0-6 double bonds in each chain. Liposomes are classified into different types like., Based on their structural components, based on method of liposome preparation, based on composition and applications etc. There are some advantages and disadvantages of liposomes formulation. The main goal of an ideal method of liposome formulation is to obtain efficient drug entrapment, narrow particles size distribution and long-term stability of liposome products. Method of preparation is based on two techniques i.e., active loading technique and passive loading technique. Physio-chemical and biological characteristics of the liposomal formulations, as well as their stability and drug entrapment efficiency are controlled by different analytical methods. The therapeutic efficacy of the drug molecule is governed by the stability of the liposomes involving manufacturing steps, storage and delivery. The aspects in stability of liposomes are of two types: Physical stability and Chemical stability. The pharmacokinetics of liposomes focused on the total body fluids and tissue distribution and their metabolism. Liposomal based formulations were implemented successfully in the clinical fields. Doxil is the first approved anticancer liposome drug in USA. The use of liposomes as systemic and topical drug delivery systems has attracted increasing attention.

KEYWORDS: liposomes, characteristics, method of preparation, stability, pharmacokinetics, clinical applications.

1.INTRODUCTION

Rational research in drug delivery began in 1950s with the advent of polyclonal antitumour antibodies developed for tumour targeting of cytotoxic drugs to experimental tumours. This had triggered a series of concerted efforts evolved with the emergence of a plethora of delivery systems. Liposomes were first described by Dr Alec D Bangham FRS (British haematologist) at the Babraham Institute, in Cambridge (early 1960s) and R. W. Horne, was the person who tested the institute's new electron microscope by adding negative stain to dry phospholipids. According to legend, he was experimenting with new laboratory equipment, and he made a noted observation about phospholipids forming closed multilamellar vesicle spontaneously in aqueous solution which took two years to be proved. The name liposome is derived from two Greek words: 'Lipos' meaning fat and 'Soma' meaning body. Liposomes are little spherical shaped artificial vesicles, which is made up of naturally-derived phospholipids with mixed lipid chains (like egg phosphatidyl-ethanolamine), or of pure surfactant components like dioleoyl phosphatidyl-ethanolamine. It consists of an internal aqueous compartment entrapped by one or multiple concentric lipidic bilayers.

The phospholipid bilayer envelope is a cell-like boundary appropriate for cellular investigations and affords liposomes a functional scaffold suitable for fundamental cellular functions such as motility and shape change not to mention the ability to mimic the biophysical properties of living cells. Liposomes nanoemulsions are widely used nanoparticles in nanomedicine mainly due to their biocompatibility, stability, ease to synthesize and high drug loading efficiency high bioavailability and their safe excipients used in these formulations. Depending upon the composition and the structure, liposomes can separate hydrophobic or hydrophilic molecules from the solution. When the structural layer of phospholipid is disrupted, they are able to realign themselves into smaller structures. These reassembled bilayer structures are known as liposomes while a monolayer is called micelle. A liposome can be formed at a variety of sizes. Liposomes can aid with active targeting as it has flexibility in coupling with site-specific ligands. They are biocompatible, completely biodegradable, non-toxic, flexible and nonimmunogenic for systemic and no systemic administrations.

Liposomes could provide increased efficacy and therapeutic index. Liposomes have many advantages when compared with other methods of drug delivery but they also have some limitations. The main limitation of the standard liposome drug delivery system is its fast clearance from circulation due to uptake by the reticuloendothelial system, initially in the liver. The cost of liposome formulation is high than other formulations. Liposome-cell interactions

are influenced by a variety of factors, including composition, the diameters of liposomes, surface charge, targeting ligand on the liposome surface, and biological environment.

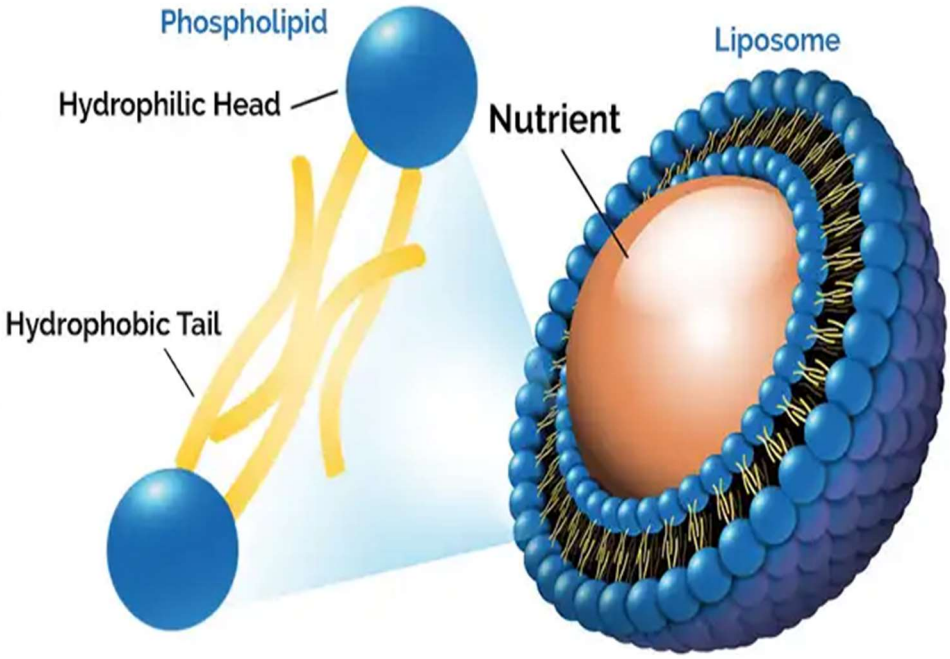


Fig.1 Structure of liposome

2.COMPOSITION OF LIPOSOME

The components of liposomes are:

1.**Phospholipids** 2. **Cholesterol** 3. **Additional excipients**

2.1. Phospholipids:

Phospholipid is the major structural components of biological membranes, where two types of phospholipids exist – phosphodiglycerides and sphingolipids, together with their corresponding hydrolysis product. The most common phospholipids used is phosphatidylcholine (PC), an amphipathic molecule, which consists of a hydrophilic polar head group, phosphocholine, a glycerol bridge and a pair of hydrophobic acyl hydrocarbon chains. Glycerophospholipids consists a lipophobic head and a lipophilic tail. Liposomes can be made from both natural and synthetic phospholipids. Molecules of PC are not soluble in water and in aqueous media they align themselves closely in planar bilayer sheets in order to minimize the unfavourable action between the bulk aqueous phase and the long hydrocarbon fatty chain. Such interactions are completely eliminated when the sheets fold on themselves to form closed sealed vesicles. PC molecules contrast markedly with other amphipathic molecules like detergents, lysolecithin in that bilayer sheets are formed in presence to micellar structures. This is thought to be because the double fatty acid chain gives the molecule an overall tubular shape, more suitable for aggregation in planar sheets compared with detergents with the polar head and single chain whose conical shape fits nicely into a spherical micellar structure.

The hydrophilic group in the lipids may be negatively, positively charged, or zwitterionic (both negative and positive charge in the same molecule). The charge of the hydrophilic group provides stability through electrostatic repels. The hydrophobic group of lipids varies in the acyl chain length, symmetry, and saturation. To achieve charged vesicles, charged phospholipids like stearyl amine and diethyl phosphate can been used. Binding of sphingomyelin helps in water permeability reduction and enhancement of proton permeability in some kinds of liposomes. Lipids are capable to manipulate the surface charge, bio distribution, permeability, release and clearance of various formulations of liposomes.

2.2. Cholesterol:

Cholesterol is a waxy, fat-like substance is an essential component of our body. Incorporation of sterols in liposomes bilayer can bring about major changes in the preparation of these membranes. Cholesterol does not by itself form bilayer structure, but can be incorporated into phospholipid membrane in very high concentration upto 1:1 or even 2:1 molar ratios of cholesterols to PC. Cholesterol inserts into membrane with its hydroxy group oriented towards the aqueous surface and aliphatic chain aligned parallel to acyl chains in the centre of the bilayer. Cholesterol incorporation increases the separation between choline head group & eliminates normal electrostatic & hydrogen bonding interactions. The high solubility of cholesterol in phospholipid liposome has been attributed to both hydrophobic and specific head group interaction but there is no equivocal evidence for the arrangement of cholesterol in the bilayer. It helps to produce hormones, vitamin D, and substances that help to digest foods.

2.3. Additional excipient:

Polyethylene glycol [PEG] on the liposome surface offers extended circulation property, protects the captured drug from inactivation or metabolic degradation, further enhances stability and improves intracellular intake. PEG may produce stealth liposomes that are undetectable by the body's reticuloendothelial system. Moreover, PEG assists in decreasing particle's aggregation and improves the stability on storage. Cellular intake of PEGylated liposomes can be enhanced by ligands such as antibodies, vitamins, proteins and nucleic acids, which exists on the receptor surface of target cells. Research on liposomal technologies was continuously refined from conventional vesicles to "second-generation liposomes", i.e., the extended-circulating liposomes with controlled and gradual release of active pharmaceutical ingredient, which can be achieved by modifying the phospholipid composition, dimension and charge of the vesicle. Numerous particles like sialic acid or glycolipids, unmodified dextrans and modified dextrans are used to establish the modified surface liposomes.

2.4. General Ingredients

Generally, liposome composition includes natural and/or synthetic phospholipids (Phosphatidylethanolamine, Phosphatidylglycerol, Phosphatidylcholine, Phosphatidylserine, Phosphatidylinositol) Phosphatidylcholine (also known as lecithin) and phosphatidylethanolamine constitute the two major structural components of most biological membranes. Liposome bilayers may also contain other constituents such as cholesterol, hydrophilic polymer conjugated lipids and water. Cholesterol has been largely used to improve the bilayer characteristics of the liposomes. It improves the membrane fluidity, bilayer stability

and reduces the permeability of water-soluble molecules through the membrane. A clear advantage of liposomes is the fact that the lipid membrane is made from physiological lipids which decreases the danger of acute and chronic toxicity.

3.MECHANISM OF LIPOSOME FORMATION

Liposomes are vesicular structures consisting of hydrated bilayers. Liposomes are formed by phospholipids (amphiphilic molecules having a hydrophilic head and hydrophobic tail). The hydrophilic part is mainly phosphoric acid bound to a water-soluble molecule whereas the hydrophobic part consists of two fatty acid chains with 10-24 carbon atoms and 0-6 double bonds in each chain. They form lamellar sheets when dispersed in aqueous medium by aligning themselves in such a way that the polar head group faces outwards the aqueous region while fattyacid groups face each other forming a spherical, vesicle like structures called as liposomes.

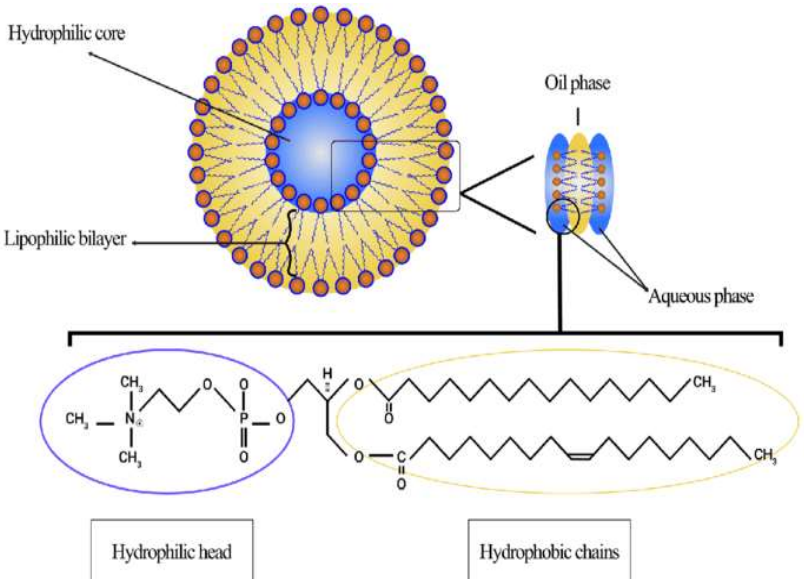


Fig.2 Formation of liposome

A Lamella is a flat plate-like structure that appears during the formation of a liposome. The phospholipids bilayer first exists as a lamella before getting converted into spheres. Several lamellas of phospholipids bilayer are stacked one on top of the other during the formation of liposome to form a multilamellar structure.

The reason for bilayer formation includes:

• The unfavourable interactions created between hydrophilic and hydrophobic phase which can be minimized by folding into closed concentric vesicles.

• The large free energy difference existing between the hydrophilic and hydrophobic environment is reduced by the formation of large vesicle formation. Since spherical structures have minimum surface tension and maximum stability. Hence there is maximum stability of self-assembled structure by forming vesicles.

Liposomes which are targeted to specific tissues are developed by stabilization of phosphatidylethanolamine into bilayer with antibody derivatives of fatty acids like palmitic acid. After binding to the target's cell surface, immunoglobulin molecules concentration at contact point leads to the destabilization of bilayers. Finally, liposomal content is released at this site. Specific sub-cellular targeting is still in novice and is in in vitro research phase.

Israelachvili and co-workers defined critical packing parameter p by

$$P = v/ a0lc$$

were,

v = molecular volume of the hydrophobic part

a0 = optimum surface area per molecule at the hydrocarbon water interface,

lc=critical half thickness for the hydrocarbon region which must be less than the maximum length of the extended lipid chains.

- For p <1/3, spherical micelles are formed. In this category fall single chain lipids with large head groups area. E.g., Lys phosphatidylcholine.
- For 1/3 < ½ globular or cylindrical micelles are formed.
- Double chain "fluid state" lipids with large head area (1/2< p <1) form bilayers and vesicles.
- This occurs also with double chain "gel state" lipids with small head groups and p ~ 1. for p > 1 inverted structure such as the inverted hexagonal phase can be observed.
- An additional condition required for bilayer formation is that the compound can be classified as a non-soluble swelling amphiphile.

4.CLASSIFICATION OF LIPOSOMES

Liposomes are classified into different types:

1. Based on their structural components

- Multilamellar large vesicles (MLV)
- Oligolamellar vesicles (OLV)
- Unilamellar vesicles (UV)
- Medium sized unilamellar vesicles (MUV)
- Large unilamellar (LUV)
- Giant unilamellar vesicles (GUV)
- Multivesicular vesicles (MVV)

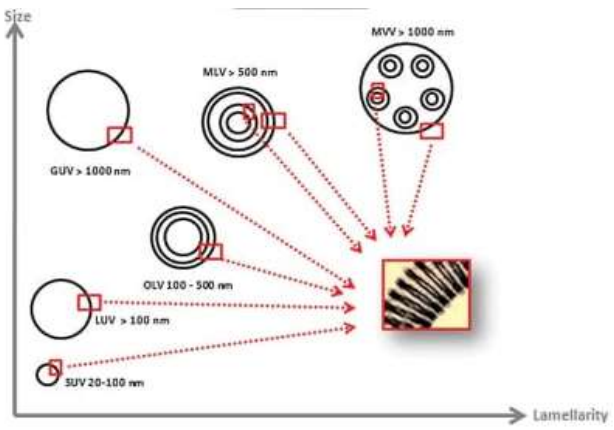


Fig.3 Size and lamellarity of liposomes

2. Based on method of liposome preparation

- Single or oligolamellar vesicle made by reverse phase evaporation method (REV)
- Multilamellar vesicles made by reverse phase evaporation method (MLV / REV)
- Stable plurilamellar vesicles (SPLV)

- Frozen and thawed MLV (FATMLV)
- Vesicles prepared by extrusion method (VET)
- Vesicles prepared by fusion (FUV)
- Vesicles prepared by French press (FPV)
- Dehydration- rehydration vesicles (DRV)
- Bubblesomes (BSV)

3.Based on composition and applications

- Conventional liposomes
- Fusogenic liposomes
- pH sensitive liposomes
- Stealth liposomes
- Immune liposomes

Liposome encapsulation efficiency increases with liposome size and decreases with the number of bilayers for hydrophilic compounds only. The size of the vesicles is an important factor that controls the circulation half-life of liposomes. Both the size and number of bilayers influence the amount of the encapsulated drug. When liposomes are employed for drug delivery, the desired vesicles usually extend from 50 nm to 150 nm.

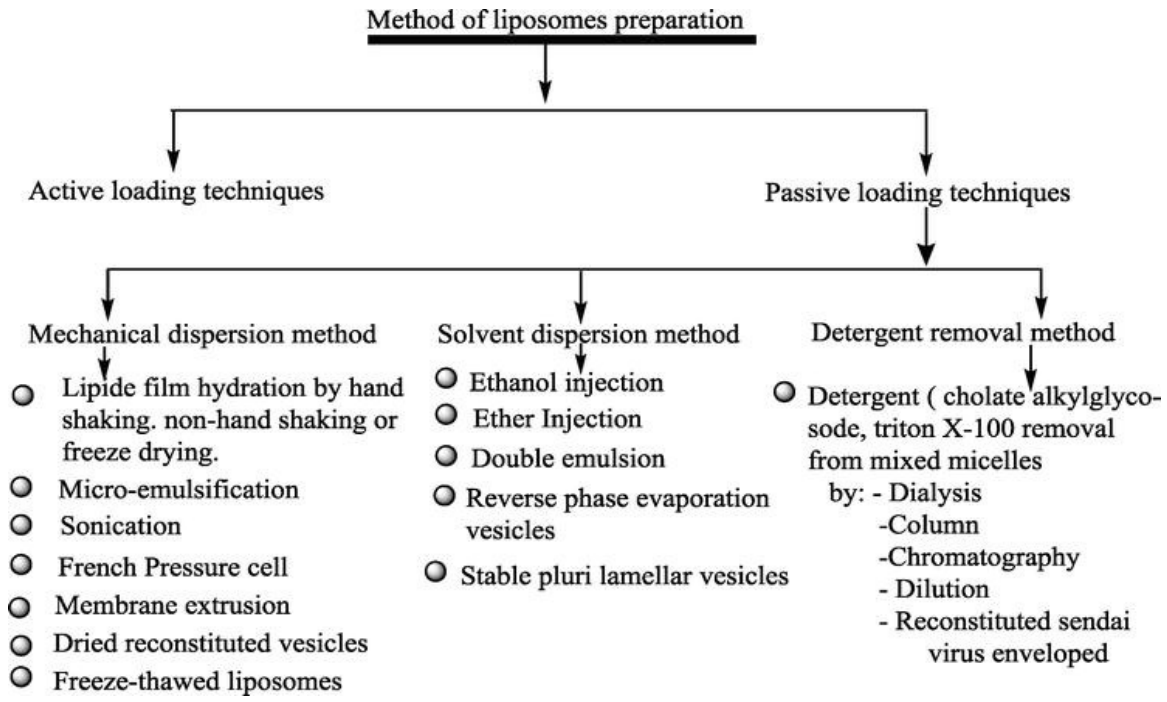
5. ADVANTAGES OF LIPOSOMES

- Liposome increased efficacy and therapeutic index of drug.
- Liposome is non-toxic, flexible, biocompatible, completely biodegradable and non-immunogenic for systemic and non-systemic administration.
- Liposome provide controlled and sustained release.
- Suitable for delivery of hydrophobic, hydrophilic and amphipathic drugs and agents.
- Site avoidance effect.
- Improved pharmacokinetic effects (reduced elimination, increased circulation life times).
- Flexibility to couple with site specific ligands to achieve active targeting
- Liposome increase stability via encapsulated drug.

6. DISADVANTAGES OF LIPOSOMES

- Sometimes phospholipid underdogs oxidation and hydrolysis like reaction.
- Sometimes, they are less stable.
- Short half-life.
- Leakage and fusion of encapsulated drug/molecules.
- Difficult in large scale manufacture and sterilization.
- Very high production cost
- Low solubility and oxidation off bilayer phospholipid.
- Low therapeutic index and dose effectiveness.

7. METHODS OF LIPOSOME PREPARTION



The main goal of an ideal method of liposome formulation is to obtain efficient drug entrapment, narrow particles size distribution and long-term stability of liposome products.

7.1. ACTIVE LOADING TECHNIQUE

a. Prollposome:

Lipid and active substances(drug) are covered onto a solvent transporter to shape free-streaming granular material in supportive of liposomes which structure an isotonic liposomal suspension of hydration. The favorable to pro-liposome approach may give a chance for cost-effective large scale manufacturing liposomes containing particularly lipophilic drugs.

b. Lyophilization:

The expulsion of water from items in a solidified state at incredibly decreased weight is called lyophilization (freeze-drying).

7.2.PASSIVE LOADING TECHNIQUE

I.MECHANICAL DISPERSION METHOD:

a. Lipid film hydration

The Bangham method is the first commonly used method for liposome preparation. On this method firstly prepare the homogeneous mixture of lipids. By dissolving and mixing a lipid component in an organic solvent (chloroform). Once the lipid is thoroughly mixed in the organic solvent, the solvent is removed to yield a lipid film. The lipid film is thoroughly

dried by placing the vial or flask on a vacuum pump overnight by removing the residual organic solvent. Lipid solution was frozen by placing the container on a block of dry ice or swirling the container in dry ice- acetone or alcohol.

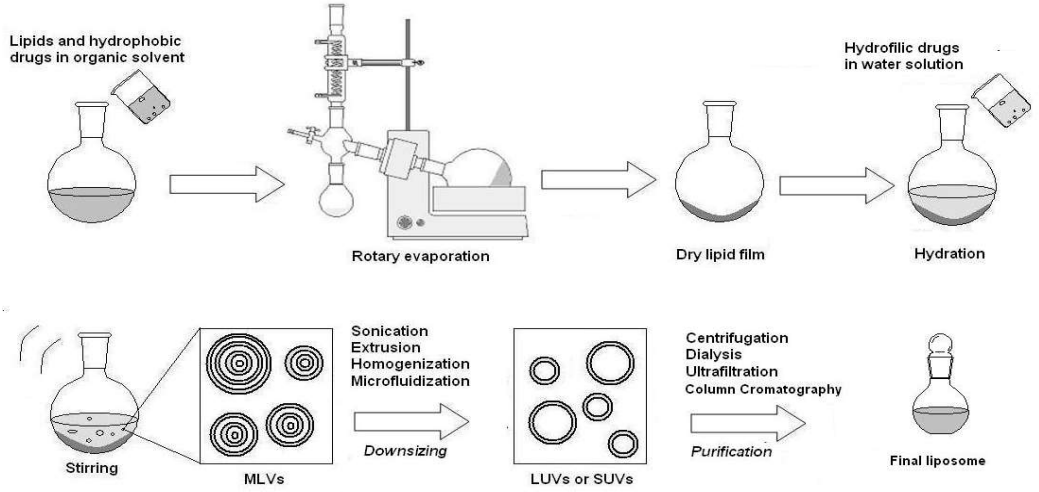


Fig.4 Lipid film hydration method

Advantages:	Disadvantage:
i. Simple process	i. Water soluble drugs exhibit low entrapment efficiency.
ii. Straightforward approach	ii. Difficulty in scaling up.
iii. Used for all kinds of lipid mixtures	iii. Time-consuming method.

b. Micro emulsification:

In this method, small vesicles are prepared by micro emulsifying lipid composition using high shearing stress generated from high-pressure homogenizer. (Speed of rotation 20 to 200 for biological). These methods are used to prepare the small lipids vesicles on a commercial scale.

c. Sonication:

Sonication is perhaps the most extensively used method for the preparation of SUV. Here, MLVs are sonicated either with a bath type sonicator or a probe sonicator under a passive atmosphere. The main disadvantages of this method are very low internal volume/encapsulation efficacy, possible degradation of phospholipids and compounds to be encapsulated, elimination of large molecules, metal pollution from probe tip, and presence of MLV along with SUV.

a. French pressure cell:

Extrusion French pressure cell involves the extrusion of MLV through a small orifice. An important feature of the French press vesicle method is that the proteins do not seem to be significantly pretentious during the procedure as they are in sonication. An interesting comment is that French press vesicle appears to recall entrapped solutes significantly longer than SUVs do, produced by sonication or detergent removal.

b. Membrane extrusion:

In this method the processed liposome has a narrow size distribution and selected average size less than about 0.4 microns.

c. Dried reconstituted vesicle:

This method starts with freeze drying of a dispersion of empty SUVs and then rehydrating it with the aqueous fluid containing the material to be entrapped

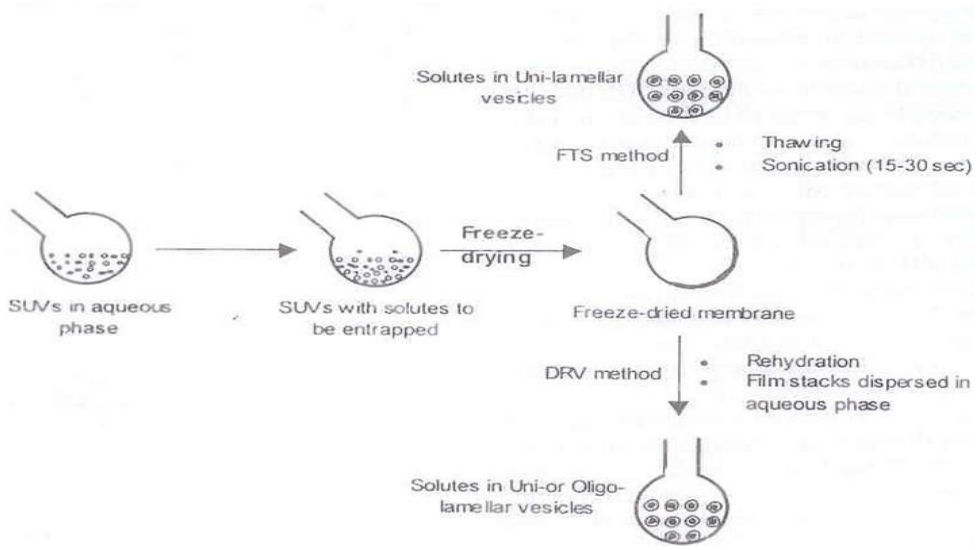


Fig.5 Dried reconstituted vesicle method

However, this leads to dispersion of solid lipids in finely subdivided form. Liposomes obtained from this method are usually uni- or oligo- lamellar of the order of 1.0 μm or less in diameter. Entrapment yield can vary, but 40% is fairly standard compared with 2-10% for MLVs prepared by hand-shaking method.

d. Freeze-thawed liposomes:

SUVs are rapidly frozen and thawed slowly. The short-lived sonication disperses aggregated materials to LUV. The creation of unilamellar vesicles is as a result of the fusion of SUV throughout the processes of freezing and thawing [26-28]. This type of synthesis is strongly inhibited by increasing the phospholipid concentration and by increasing the ionic strength of the medium. The encapsulation efficacies from 20% to 30% were obtained

II.SOLVENT DISPERSION METHOD:

a. Ethanol injection:

An ethanol solution of lipid is injection rapidly into an excess of saline or other aqueous medium, through a fine needle. The force of the injection is unusually sufficient to achieve complete mixing, so that the ethanol is diluted almost instantaneously in water, and phospholipid molecules are dispersed evenly throughout the medium.

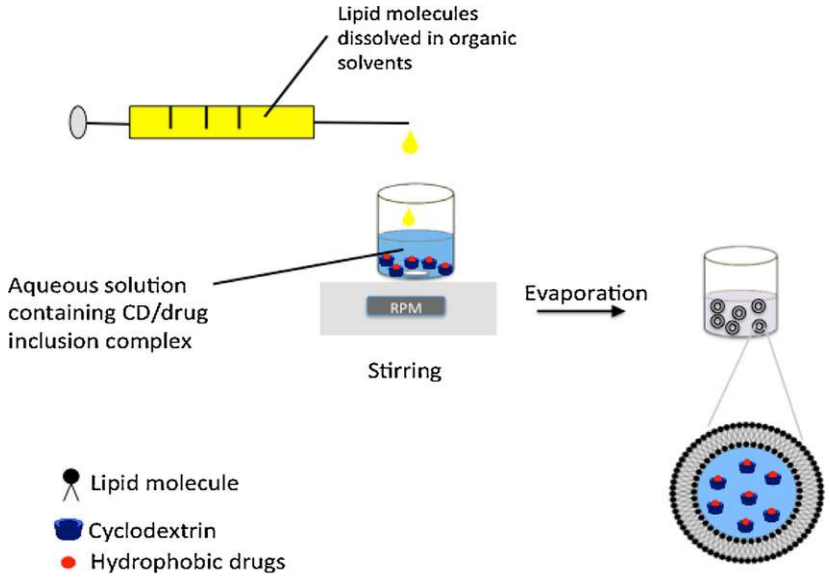


Fig. 6 Ethanol injection method

b. Ether injection:

A solution of lipids dissolved in diethyl ether or ether-methanol mixture is gradually injected to an aqueous solution of the material to be encapsulated at 55°C to 65°C or under reduced pressure. The consequent removal of ether under vacuum leads to the creation of liposomes. The main disadvantages of the technique are that the population is heterogeneous (70 to 200 nm) and the exposure of compounds to be encapsulated to organic solvents at high temperature.

c. Double emulsion:

This method firstly prepared the emulsion by dissolving the drug in the aqueous phase(W1), which is then emulsified in an organic solvent of a polymer is called primary emulsion(W/O). After that this primary emulsion further mixed in an emulsifier-containing aqueous solution(W2) to make a W1/O/W2 double emulsion. And after than microspheres are obtained by removal of the solvent and filtration process.

d. Reserve phase evaporation vesicles:

Reverse phase evaporation method, where lipids are dissolved in an organic solvent and desired drugs are dissolved in an aqueous media; further the mixture is sonicated to produce w/o emulsion or inverted micelles, followed by slow removal of organic solvent using rotary evaporator, leading to conversion of these micelles into viscous state or gel product. In this process, at a critical point, the gel state collapses and some inverted micelles were distributed. The excess phospholipids form the bilayer around the residual micelles which results in the formation of liposomes. Modified reverse phase evaporation method and the main advantage of this method is the liposome which had high encapsulation (about 80%).

e. Stable plurilamellar vesicles:

In this method, water in oil dispersion is prepared earlier with excess lipid, but drying process is accompanied by continued bath sonication with a stream of nitrogen. The redistribution and equilibration of aqueous solvent and solute occurs during this time in between the various bilayer in each plurilamellar vesicle.

III.DETERGENT REMOVAL METHOD:

Detergents are used to solubilize the lipids at their critical micellar concentrations. LUVs are shaped by eliminating the detergent by dialysis and combining the micelles. In this method, the liposomes are formed in homogenous size. And the retention of detergent contaminants is the drawback of this method.

8.CHARACTERISATION OF LIPOSOMES

Physio-chemical and biological characteristics of the liposomal formulations, as well as their stability and drug entrapment efficiency are controlled by different analytical methods. The liposomes produced by different techniques may have different physiochemical characteristics. These differences do have an impact on their behaviour in vivo(disposition) and in vitro (e.g., sterilisation and shelf life). The characteristics parameters of liposomes are based on different categories:

1. Physical characterisation

2. Chemical characterisation
3. Biological characterisation

8.1. Physical characterisation:

The control of physical parameters is based on measuring vesicle shape, surface morphology, mean vesicle size and size distribution, surface charge, lamellarity, phase behaviour, percent of free drug/percent capture and drug release.

a). Size and size distribution

The vesicle size is crucial to determine the in vivo release of drug-loaded liposomes. The average size of liposomes depends on the method of preparation and phospholipid composition. The particle size and distribution are critical parameters especially when liposomes are intended for therapeutic use by inhalation or parenteral route. Liposomes have sizes of 30nm to several micrometres. The size of deformable liposomes in the size range of 300-350nm has been reported to maintain the drug in the skin layer for topical use and avoid its absorption into the systemic circulation. The most precise method to determine size of the liposomes is electron microscopy since it permits one to view each individual liposomes and to obtain exact information about the profile of liposome population over the whole ranges of sizes. Unfortunately, it consumes more time and requires equipment that may not always be immediately to hand. So, other methods used d to evaluate the size and size distribution such as:

i). Microscopic techniques:

Microscopic techniques such as optical microscopy, scanning electron microscopy (SEM), negative stain TEM and freeze-fracture TEM. SEM and TEM techniques are used for imaging of liposomes and also provide information about bilayer thickness and inter-bilayer distance of liposome. One of the newly established microscopic methods is atomic force microscopy (AFM), which is a very high-resolution scanning probe microscopy that produce 3D micrographs through resolution of nano-meter and A0 scale to evaluate the liposome morphology, stability, size and dynamic process of lipid nano-capsule.

ii). Hydrodynamic techniques:

Hydrodynamic techniques such as ultracentrifugation, field flow fractionation and gel exclusion chromatography and analytical centrifugation procedures are used to estimate molecular mass of compound and also used for comparison of size distribution, elution characteristics and uniformity of the liposomes. Large vesicles of 1-3 μm diameter usually fail to enter the gel and are retained on the top of the column. A thin layer chromatography system using agarose beads has been introduced as a convenient, fast technique for obtaining a rough estimation of the size distribution of a liposome preparation. However, it was not reported if this procedure was sensitive to a physical blockage of the pores of the agarose gel as is the more conventional column chromatography.

iii). Diffraction light scattering techniques:

Diffraction light scattering techniques such as laser light scattering, quasi-elastic light scattering and photon correlation spectroscopy give information about the size of the lipid vesicles. Proton correlation spectroscopy (PCS) is the analysis of the time dependence of intensity fluctuation in scattered laser light due to the Brownian motion of particles in solution/suspension. Since small particles diffuse more rapidly than large particles, the rate of fluctuation of scattered light intensity varies accordingly. Thus, the transitional diffusion coefficient (D) can be measured, which in tur can be used to determine the mean hydrodynamic radius (Rh) of the particles using Stokes-Einstein equation.

b). Surface charge

A technique has been developed that separates extruded vesicles on the basis of their surface charge by electrophoresis on a cellulose acetate plate in a sodium borate buffer pH 8.8. The lipid samples (5nmoles) are applied to the plate and electrophoresis is carried out at 4°C on a flatbed apparatus for 30 min at 18 V/cm. The plate is dried and the phospholipids are visualized by the molybdenum blue reagent. Liposomes upto 0.2 μm diameter can migrate on this support and with this technique as little as 2 moles % of charged lipids can be detected in a liposome bilayer. This sensitive assay should prove valuable for examining the charge heterogeneity in liposome preparation for following fusion between two populations of vesicles with different charge and for determining the presence of charge impurities e.g., fatty acids in liposome. Liposome characterization is the surface charge of the liposome measured by the zeta potential. The zeta potential is the key factor that affects the cellular uptake and targeted drug delivery. The laser Doppler electrophoresis and Zetasizer are used to measure the zeta potential of the liposomal dispersion by applying an electric field based on the scattering of incident laser on the moving particles. The particles move in an electrical of known strength in the interference pattern of two laser beams and produce scattered light which depends on the speed of the particles. However, if the zeta potential value of a particle is too small, there will be a force of attraction greater than the repulsion force so that it causes coagulation and flocculation which indicate colloidal instability.

c). Lamellarity

Lamellarity is defined as the number of lipid bilayers present around the lipid vesicles. Liposomal lamellarity can be measured by using cryo-electron microscopy, 31P-nuclear magnetic resonance (NMR) and small angle X-ray scattering (SAXS) technique that provide information about size, homogeneity and lamellarity of liposomes. Liposome lamellarity is often accomplished by methods that are based on the visible or fluorescence signal change of lipids marker upon reagents addition. This approach is reviewed in more detail, since it is a relatively simple procedure that can be easily carried out in a standard lab. Several lipids can be used and results rely on the comparison of the total signal to the signal achieved from the reaction between the lipids marker and the specified reagents. To confirm the lamellarity results by an imaging method, freeze fracture technique with subsequent transmission electron microscopy was used. For this purpose, carbon film grids were used for specimen preparation. To confirm the lamellarity results by an imaging method, freeze fracture

technique with subsequent transmission electron microscopy was used. For this purpose, carbon film grids were used for specimen preparation.

d). Phase behaviour of liposomes

An important feature of lipid membrane is the existence of a temperature dependent reversible phase transition, where the hydrocarbon chains of the phospholipid undergo a transformation from an ordered(gel) state to more disordered fluid (liquid crystalline) state. These changes have been documented by freeze fracturing electron microscopy but most easily demonstrated by differential scanning calorimetry.

e). Percent capture (Entrapment)

The efficiency of entrapment is a key parameter in developing liposome-based delivery. High absorption efficiency can reduce cost and increase efficacy. After removal of unincorporated material by the separation techniques, one may assume that the quantity of material remaining is 100% entrapped, but the preparation may change upon storage. For longer term stability test and for developing new liposome formulation or method of preparation, a technique is need ed for separating free from entrapped material. In general, two methods may be used i.e., mini column centrifugation and protamine aggregation.

f). In vitro Drug release studies

The in vitro drug release studies are assessed at 370 C through in vitro diffusion cell or by using dialysis bag. The cell or bag must be wet through receptor medium containing pH 7.4 buffer with constant stirring under sink conditions, which mimics the in vivo conditions. At regular time intervals, the required volume of the medium was collected, and the concentration was determined by using HPLC and UV–Visible spectrophotometry, and at the same time equal volume of fresh medium was added to receptor media. Some millilitres aliquot of liposome suspension is placed in the dialysis bag, hermetically tied and dropped in the receptor compartment containing the dissolution medium. The entire system is kept at 37° C under continuous magnetic stirring and the receptor medium is closed to avoid evaporation of the dissolution medium.

g). In vivo drug release studies

The in vivo behaviour of drug-loaded liposomes might be influenced by several pharmacokinetic properties of the vesicle. To study the in vivo performance of liposomal drug delivery systems, the liposomes are administered intravenously to reveal rapid clearance from spleen and liver. The large liposomes having particle size greater than 0.5 μm diameter taken by phagocytosis and for liposomes having particle size less than 0.1 μm were taken up by liver parenchymal cells. Cholesterol incorporated in liposomes will increase stability by evading the leakage of drug.

8.2. Chemical characterisation

Chemical analysis of liposomes is important to determine the purity and potency of various liposomal constituents. The most common chemical parameters which are analysed are: phospholipid concentration, cholesterol concentration, phospholipid peroxidation, phospholipid hydrolysis, cholesterol auto-oxidation and osmolarity. The quantification of phospholipids is important due to control the efficiency of the preparation method. Phospholipids content in liposomes was determined by Stewart's method based on the formation of a coloured complex between phospholipids and ammonium ferro thiocyanate reagent. Phospholipid peroxidation is quantitatively determined using UV absorbance, iodometry (for hydroperoxidase) and GLC techniques. Phospholipid hydrolysis as well as cholesterol autooxidation can be determined using HPLC and TLC. pH of the liposomal dispersion can be determined using pH meter.

8.3. Biological characterisation

The importance of determining biological parameters is helpful in determining the safety of formulation for therapeutic application. Sterility, pyrogenicity and animal toxicity are determined during the biological characterization of the liposomes.

9. STABILITY OF LIPOSOMES

Liposomes face a number of chemical and physical destabilisation processes. Liposomes can be degraded chemically through oxidation and hydrolysis. So, liposomes stability is an important consideration while studying liposomes. The therapeutic efficacy of the drug molecule is governed by the stability of the liposomes involving manufacturing steps, storage and delivery. The aspects in stability of liposomes are of two types:

- **Physical stability**
- **Chemical stability**

9.1. Physical stability:

The vesicles obtained during the liposomal formation processes are of different sizes, aggregation and fusion. During its storage, vesicles tend to aggregate and increase in size to attain a thermodynamically favourable state. While storage, the drug may leak from the vesicles that may cause its fusion and breaking. The leakage rate strongly depends on the bilayer composition and the physiochemical nature of the drug. Aggregation is the formation of larger units of liposome material, these units are still composed of individual liposomes. This process can also undergo reversibly, e.g., by applying mild shear forces, or by changing the temperature or by binding metal ions that initially induced aggregation. Bilayer permeability is not necessarily a constant parameter. Changing the bilayer permeability can occur as a result of chemical degradation processes, such as the formation of lypo-

PC and FA. Physical stability can be maintained by avoiding the excess unsaturation in the phospholipids as they are subjected to mere peroxidation and by maintaining the pH conditions.

9.2.Chemical stability:

The two acyl ester bonds are most liable to hydrolysis. The glycerophosphate and phosphocholine ester bonds are more stable. pH, ionic strength, solvent system and buffered species also play a major role in maintaining a liposomal formulation. The polyunsaturated acyl chains of phospholipids are sensitive to oxidation via free radical reactions. Phospholipids are chemically unsaturated fatty acids, prone to oxidation and hydrolysis, which may alter the stability of the drug products. Cyclic peroxides, hydroperoxides, malondialdehyde, alkanes are the major degradation products.

9.3.Stability protocols:

The liposomal stability can be determined by storing it under some conditions. The conditions are:

1. Visual or microscopic examination.
2. Highest and lowest temperatures likely to be encountered (1 month)
3. Room temperature (12-24 months)
4. 2-3 freeze-thaw cycles. (20-25°C)
5. 6-8 heat-cool cycle (5-45°C).

Majority of therapeutic liposome formulations are parenteral products and therefore must be sterilized to remove the microbial contamination from the product. Thus, it is important to control microbial stability of liposomal preparations.

10.LIPOSOMAL PHARMACOKINETICS

The pharmacokinetics of liposomes focused on the total body fluids and tissue distribution and their metabolism. It mainly includes liposome chemical degradation and excretion, which is achieved through uptake and RES clearance. The pharmacokinetics of liposomal-based drugs depends on the physicochemical characteristics of the lipid vehicle, such as lipid composition, size, membrane lipid packing, steric stabilization, surface charge, dose, and route of administration. If the therapeutic agent is loaded into the liposomes, it changes as carrier's pharmacokinetics until it is delivered to the targeted site. As the result, it modifies both the tissue distribution as well as the rate of clearance of loaded drug in liposomes.

11.CLINICAL APPLICATION OF LIPOSOMES:

Liposomal based formulations were implemented successfully in the clinical fields. Doxil is the first approved anticancer liposome drug in USA. The use of liposomes as systemic and topical drug delivery systems has attracted increasing attention.

Liposomes can be formulated in liquid (suspension), solid (dry powder) or semi-solid (gel, cream) forms. In vivo, they can be administered topically or via parenteral route.

11.1. Cancer treatment:

- Liposomal anthracyclines have achieved highly effective drug encapsulation, resulting in substantial anticancer activity with reduced cardio toxicity.
- liposomal daunorubicin and pegylated liposomal doxorubicin versions has greatly prolonged circulation.
- Doxil was designed as a polyethylene glycol coated doxorubicin (DOX) liposome intended for the treatment of Kaposi's sarcoma.
- LipoDox is FDA approved PEGylated liposomal formulation encapsulating DOX manufactured by Sun Pharma.
- Daunorubicin was the second anthracycline antineoplastic drug loaded in liposomes to treat acute myeloid leukaemia (AML) under the generic name DaunoXome.
- Pegylated liposomal doxorubicin has shown substantial efficacy in breast cancer treatment both as monotherapy and in combination with another chemotherapeutics.
- The anticancer drug release follows two mechanisms into the tumour site viz pH responsive (pH difference between the blood (7.4) and tumour cell (acidic pH)) and the other is polymer degradation by lysozyme enzyme.
- Lipoplatin is developed recently by using cisplatin as a carrier for treating cancer.
- The thermo-sensitive liposomal formulation ThermoDox, which contains Lys phosphatidylcholine and is employed in the treatment of various cancers like liver cancer, breast cancer, pancreatic cancer.

11.2. Liposomes in vaccinations:

- Liposome formulations could protect DNA/RNA and proteins payload from biodegradation.
- Liposomes can be used for enhancing the immune response by encapsulating the adjuvants. Depending on the lipophilicity of antigens, the liposome can accommodate antigens in the aqueous cavity or incorporate within the bilayers.
- The liposomes are used as adjuvants in vaccine delivery by modifying the surface with different molecules such as peptide antigens/virus antigens, to boost the immunity and immunological response.
- To enhance the immune response of diphtheria toxoid, liposomes were first used as immunological adjuvants.
- Two commercial vaccines based on virosome technology are currently on the market, Epaxal and Inflexal are hepatitis A vaccine.

11.3. Ophthalmic treatment

- Various drugs are used to treat eye disorders like dry eyes, keratitis etc.
- The pharmaceutical preparations may be suspension form or ointment for topical application as well as in solution forms but these preparations exhibit poor ocular bioavailability. To avoid this barrier, liposomal formulation is used.
- The ciprofloxacin/ciprocin is the most widely used eye drops which is effective against gram +ve and gram −ve bacteria.

11.4. Fungal treatment

- A two major approved anti-fungal liposomes formulation were Ambisome and Fungisome.
- They encapsulate Amphotericin B anti-fungal drug with many advantages compared free drug because they are stabilized in saline and have longer bioavailability and less toxicity and side effects.

11.5. Pain management

- DepoDur is a morphine formulation which is formulated in sustained release formula using DepoFoam Technology with prolonging the clinical effect time.
- Exparel to release Bupivacaine, uses the DepoFoam technology for sustained pain relief.

12.CONCLUSION

In conclusion, liposomes have a diverse range of uses ever since it was first noted that it was able to self-assemble into vesicles. Vital progress has been made in the development of long circulating liposomes that are not immediately recognized and removed by the cells of mononuclear phagocyte system. Liposomes with enhanced drug delivery to disease locations, by ability of long circulation residence times, are now achieving clinical acceptance. Also, liposomes promote targeting of particular diseased cells within the disease site. The fact that all issues associated with scale-up, stability, and satisfying regulatory demands have also been successfully addressed points to a plethora of new and increasingly sophisticated lipid-based therapeutics in the future.

REFERENCE

1. http://www.jpsr.pharmainfo.in/Documents/Volumes/vol5issue09/jpsr05091304.pdf
2. https://doi.org/10.31069/japsr.v3i3.2
3. https://innovareacademics.in/journal/ijcpr/Issues/Vol3Issue2/292.pdf
4. https://doi.org/10.18231/j.joapr.2019.v.8.i.1.003
5. https://doi.org/10.1155/2011/939851
6. https://doi.org/10.1007/s12668-022-00941-x
7. https://eijppr.com/n4DZcRj
8. https://eijppr.com/LOdV6nk
9. https://portal.issn.org/resource/ISSN/0976-2779
10. https://doi.org/10.1166/jcsb.2012.1020
11. https://doi.org/10.4172/2155-952X.1000276
12. https://doi.org/10.1080/10717544.2016.1177136
13. https://doi.org/10.1016/j.heliyon.2022.e09394
14. http://dx.doi.org/10.2139/ssrn.2960975
15. Controlled and novel drug delivery – N.K. Jain (pg.no., 321,322,324)
16. Theory and practice in novel drug delivery system – S.P. Vyas (pg.no., 161,162,163)