Liposome: An overview

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Abstract: *The self-forming encapsulated lipid bilayer that results from hydration resulted to the term "liposome" or "lipid vesicle."Nanosized drug delivery techniques known as liposomes are significant. Concentric phospholipid bilayer molecules with a range in molecular weight from low to high have been combined into liposomes. Water-soluble substances and drugs are found in aqueous compartments, whereas lipid-soluble substances and amphiphilic substances insert themselves in phospholipid bilayers. The spherical sac vesicle has at least one lipid bilayer and is composed of lipids. Drug delivery size and size distribution are the primary purposes for liposome development. The key important factor in developing potent drugs is enhancing the therapeutic result. The primary goal of liposome formulation is to increase accumulation at the target site; the subsequent effect is then intended to diminish toxicity. Numerous techniques, including hydration, ethanol injection, ether injection, sonication, and micro-emulsion, French extrusion technique for pressure cells are used to create liposomes.The main drawback of this dosage form is very expensive and time-consuming the process. However, it has important uses such as homogenous size extrusion, long circulating liposomes, triggered release liposomes, remote drug loading, ligand targeted liposomes, and containing combination of drug.*

Keyword :

liposomes, drug delivery system, Liposomes, Preparation Methods, Phospholipids.

Introduction:

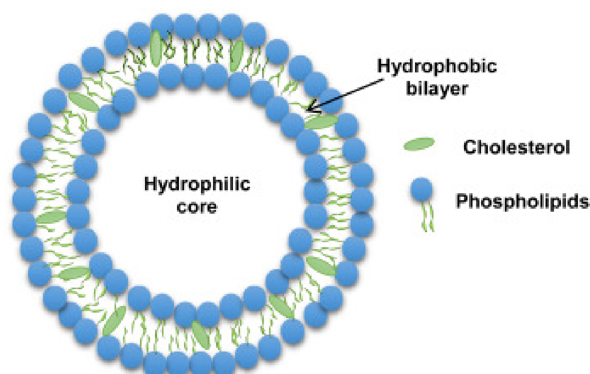

Figure no. 1 Structure of Liposome

In the 1960s, Liposomes were discovered by Alec D Bangham in the 1960s at the Babraham Institute, University of Cambridge, which are composed of one or more concentric lipid bilayers enclosing an aqueous compartment. The first formulations consisted just of natural lipids; today, they can also contain surfactants and/or synthetic lipids. Both lipophilic and hydrophilic substances can be entrapped by them, in the lipid membrane and the aqueous core, respectively. These virtually spherical lipid vesicles can be as small as a few nanometers or as large as several micrometres.(1,2,3) From the Greek terms lipo, which means "fat," and soma, which means "body," comes the name liposome (4,5). A liposome is a type of drug delivery system that resembles a colloidal, vesicular structure and is composed of one or more lipid bilayers (the outer layer), which are surrounded by an equal number of aqueous layers (the inner layer). which is composed of peptides, proteins, hormones, enzymes, antibiotics, antifungal agents, and anticancer agents. In this method of drug delivery, the disease-specific therapeutic effect is achieved without adversely affecting other body parts (6,7) . When phospholipids are mixed with water, a closed structure with an interior aqueous environment enclosed by phospholipid bilayer membranes, known as a liposome, spontaneously arises (8). As spherical vesicles with a phospholipid bilayer membrane, liposomes are a type of drug delivery system (9,10). Liposomes are vesicles having concentric phospholipids bilayer molecules with a range in molecular weight from low to high have been combined into liposomes (11). They provide excellent drug carrier systems for therapeutics since they are biodegradable, biocompatible, and non-immunogenic in nature (25). Water-soluble substances and drugs are found in aqueous compartments, whereas lipid-soluble substances and amphililic substances/drugs insert themselves in phospholipid bilayer vesicle (11, 12, 13). The characterisation of product performance heavily depends on the source of the lipids and the stability of the phospholipids, which are important excipients (8). Because of their poor solubility or because of their physicochemical nature, many medications have a poor pharmacokinetic profile, very low bioavailability, and severe side effects. Nanotechnology has had a substantial impact on improving outcomes and increasing the therapeutic efficacy of medications used in a certain therapeutic class. One of the Nano-components system's with remarkable effects is liposomes (7). The ability to incorporate both hydrophobic and hydrophilic drug molecules, good biocompatibility, increased efficacy and therapeutic index, decreased toxicity, and improved drug stability in the encapsulation system are several benefits of liposomes (9). When nonionic surfactants were used in place of phospholipids, the formulation was described as niosome(4).

Advantages of liposome:

1. Liposomes are entirely biodegradable, non-toxic, and immune-suppressive.

2. Suitable for hydrophobic, hydrophilic, amphipathic drugs. Suitable for hydrophobic, hydrophilic, and amphipathic medications.

3.Protect the encapsulated drug from external environment.

4. Provide sustained release.

5. Improve protein stabilization.

6. Can be administered through various routes.

7. Reduce exposue of sensitive tissues to toxic drugs.

Disadvantages: (15,16,24)

1. Production cost is high.
2. Leakage and fusion of encapsulated drug/molecule.
3. Short half-life.
4. Stability problems.

- **Liposome raw materials:**

For the formation of lipid bilayers, different lipids and amphiphiles are available as liposome basic materials or additives.

Phospholipids:

Phospholipids are the basic molecular building block of the liposome. Phospholipids is lipid which is amphiphilic which consist of hydrophic polar head and hydrophic tail.

Natural Phospholipids: Sphingolipids and phosphodiglycerides are two kinds of phospholipids that make up the majority of the structural components of living cells. The most prevalent phospholipid is the phosphatidylcholine (PC) molecule. phosphatidylcholine particles cannot dissolve in aqueous solutions like water, they organise themselves into planar bilayer sheets to minimise contact between the long hydrocarbon fatty chain and the bulk watery phase. Phospholipids make up the majority of the lipid weight in biological membranes and are the most common component of liposome composition (14). The most often utilised components of liposome formulation are phospholipids that contain glycerol and account for more than 50% of the weight of lipid in biological membranes. From phosphatdic acid, these are obtained. The glycerol moiety serves as the molecule's backbone.A phosphoric acid ester is formed at the C3 OH group. Along the chain, OH at C1 and C2 are esterified. Lipidic nature is being induced by fatty acids. One of the phosphoric acid's remaining OH groups can be further esterified to a variety of organic alcohols, including glycerol. Serine, choline, inositol and ethanolamine. The phosphoric ester of glycerol is hence the series' parent component.
Phosphotidyl choline,
Phosphotidyl serine,
Phosphotidyl ethanolamine,
Phosphotidyl inositol
Phosphotidyl glycerol
Use of saturated fatty acids results in stable liposomes. Unsaturated fatty acids are not rarely used. (15)
Synthetic Phospholipids:
1, 2-Dilauroyl-sn-Glycero-3-Phosphocholine (DLPC);
1, 2-Dioleoyl-sn-Glycero-3-[Phospho-L-Serine] (Sodium Salt) (DOPS);
Dipalmitoylphosphotidylcholine,
Distearoylphosphotidylcholine;
Dipalmitoylphosphotidylserine,
Dipalmitoylphosphotidylglycerol;
1,2-Dilauroyl-sn-Glycero-3-Phosphocholine (DLPC)
Unsaturated:
1-Stearoyl-2-Linoleoyl-sn-Glycero-3-[Phospho-L-Serine] (Sodium Salt)**;**
Dioleaylphosphotidylcholine
Sphingolipids: Shingomyelin
Glycosphingolipids: Gangliosides

Steroids: Cholesterol:

Cholesterol does not play a role during the formation of bilayers, although it is integrated in high concentrations—equal to or less than the concentration of phosphatidylcholine—into phospholipid membranes. A kind of cholesterol with the hydroxyl group facing the aqueous surface and the aliphatic chain oriented in betwee bilayers. It has excellent solubility in phospholipid due to both its hydrophilic and lipophilic components, but cholesterol is not clearly arranged in the bilayer (14).

Polymeric material:

When exposed to ultraviolet light, synthetic phospholipids with a diactylenic group in the hydrogen chain polymerize, forming polymearied iposomes with noticeably higher permeability barriers to entrapped aqueous drugs. Other lipids that can be polymerized include those that contain conjugated dienes, methacrylate, etc. Additionally, a number of polymerisable surfactants are formed (15).

Charge-inducing lipids:

Dioctadecyldimethyl ammonium bromide/chloride (DODAB/C); Dioleoyl trimethylammonium propane (DOTAP)

Other Substances:

Stearylamine & Dicetylphosphates, Polyglycerol & polyethoxylated mono & dialkyl amphiphiles(8).

- **Depending upon size and shape:**

a) Multilamellar vesicle (MLV)-

Multilamellar vesicles typically range in size from 100 to 1000 nm and are made up of two or more bilayers. The process for making multilamellar vesicles is relatively simple and involves hydrating lipids in excess of an organic solvent using the thin-film hydration method. Because they are mechanically stable, they can be stored for a very long time.

b) Large unilamellar vesicle (LUV)-

A single bilayer or single lamella makes up the enormous unilamellar vesicles of a liposome. LUVs have sizes greater than 0.1 micrometres and up to 1000 nm. They can contain huge volumes of solutions in their cavity, which leads to their high encapsulation efficiency. They resemble multilamellar vesicles in structure. Large unilamellar vesicles are produced using a variety of techniques, including detergent dialysis, ether injection and reverse phase evaporation.

c) Small unilamellar vesicle (SUV)-

In comparison to multilamellar vesicles and large unilamellar vesicles, small unilamellar vesicles are typically smaller in size (0.1 micrometre). Single bilayers are present in small unilamellar vesicles. SUVs are created using the solvent injection technique (16).

- **Factors affecting selection lipid:**

1. Phase transition temperature:

Controlling the lipid's transition temperature may be helpful when creating novel products, processes, or methods. The phase transition temperature is defined as the temperature required needed to cause a change in the lipid's physical state from the ordered gel phase, where the hydrocarbon chains are fully extended and tightly packed, to the disordered liquid crystalline phase, where the chains are fluid and randomly oriented. A non-leaky packing method might be achieved by selecting a high transition lipid in which the lipid vesicle would always remain in the gel phase. a lipid having a transition temperature between the system's starting temperature and ending temperature would instead provide a means of releaseing packed material as the lipid passes through its phase transition temperature and the vesicle starts to leak. One should also think about how the processing stages might be affected by

the lipid's transition temperature. When filtration is required, using a high transition lipid could cause some technical issues.

2. **Charge:**

The charge may provide the membrane a unique function. A lipid membrane is necessary for several phases of the blood coagulation cascade. A negatively charged surface is necessary for the assembly of protein aggregates on the surface of platelets. Not only is a negative surface necessary for the conversion of prothrombin to thrombin, but the requirement is also relatively limited to phosphatidylserine (PS) and phosphatidic acid (PA). Although coagulation proteins attach to negatively charged surfaces containing phosphatidylglycerol and phosphatidylinositol almost as strongly as they do to PS or PA membranes, the activity is only a fraction of what is achieved with PS or PA membrane. Therefore, in some systems, Not only must the charge requirement be satisfied in some systems, but also the system specificity for a specific species.

3. **Lipid mixture:**

Many times, a single lipid species is insufficient to create the precise physical characteristics required for a given system, or it is insufficiently able to mimic the natural system for which it is intended to duplicate. For these issues, Consider a complex lipid mixture made up of two or more different lipid species that is made to construct or replicate a specific charge ratio, unsaturation ratio, phase transition temperature, or biological function.

• Stability consideration of liposomes:

Regarding the physical, chemical, and biological stability of liposomes, this is a significant issue in the development of pharmaceutical applications.

Physical stability:

Liposome aggregation could result in fusion. The components of the bilayer, particle size, ionic strength of the medium, medicine that is encapsulated, and temperature all affect this property. It can be observed visually, through light scattering, and using the probe fluorescence technique. In order to maximise stability adjust the bilayer's lipid composition and the aqueous solvent's pH. Physical stability can also be increased through proliposomes and lyopiliation.

Chemical stability:

This primarily pertains to phospholipid stability. The stability of phospholipids is primarily influenced by hydrolytic and peroxidation reactions. The hydrolysis kinetics of phospholipids are highly influenced by temperature, bilayer, rigidity, and pH.

Biological stability:

Protein binding and membrane fusion, or simply aggregation and fusion, are significant factors in the instability of liposomes. Plasma proteins like albumin, globulin, and lipoproteins interact with liposomes to cause destabilisation in blood and plasma.

Blood pH has an impact on pH sensitive liposome stability as well. Low pH of the stomach environment, degradation by phospholipases, surfactants, and the presence of bile salts in the intestinal environment are all factors that affect liposome stability in the gastrointestinal tract (GIT) (8).

- **Liposome preparation techniques:**

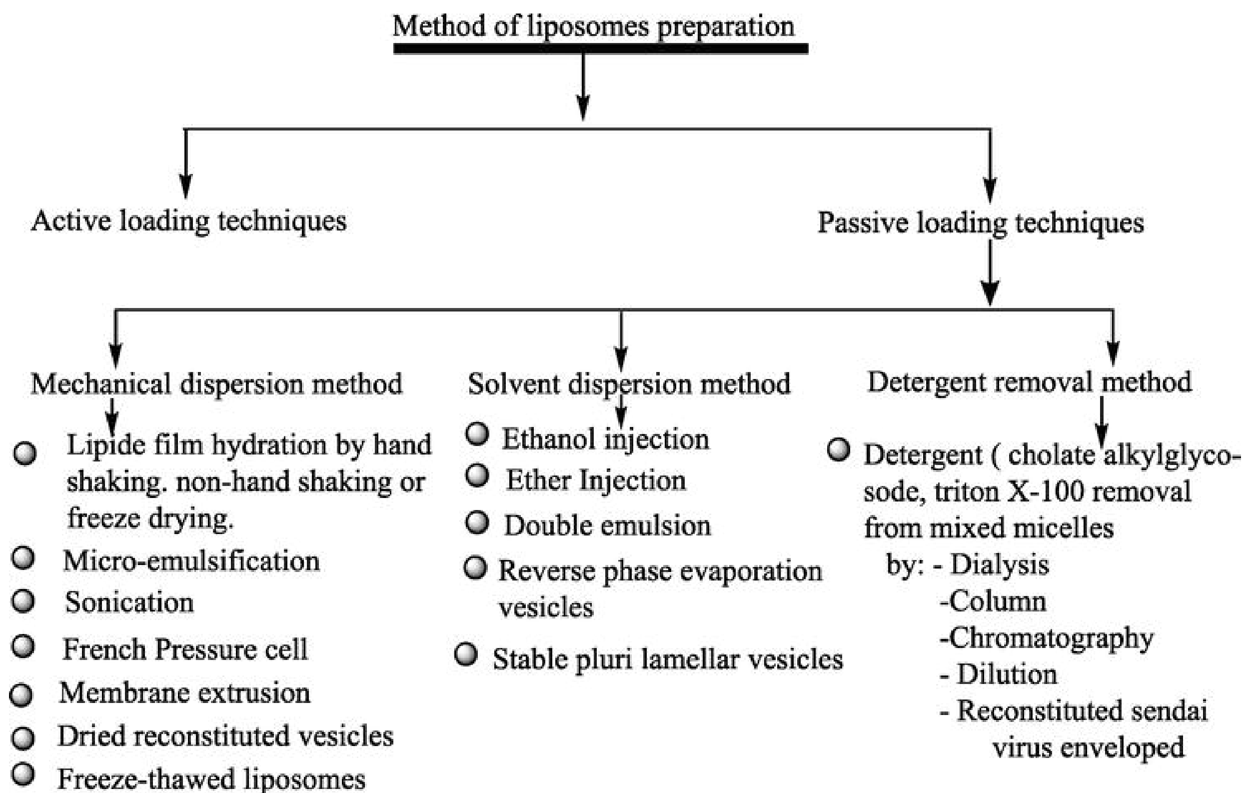

Figure 2. Different methods of liposomes preparations.

General methods of preparation

general preparation techniques

There are four basic stages to all liposome preparation techniques:

1. Drying down lipids from organic solvent.
2. Dispersing the lipid in aqueous media.
3. Purifying the resultant liposome.
4. Analyzing the final product.

Method of liposome preparation and drug loading

1. Passive loading techniques
2. Active loading technique.

Passive loading techniques include three different methods:
1. Mechanical dispersion method.
2. Solvent dispersion method.
3. Detergent removal method (removal of none capsulated material)

Mechanical dispersion method: (17,18)

1) Thin Film Hydration Method.
2) French pressure cell: extrusion.
3) Membrane Extrusion Method.
4) Dried Reconstituted Vesicles:
5) Freeze-Thaw Method.
6) Sonication.

Thin Film Hydration Method:

The most popular and simple technique for making MLV is through the thin-film hydration process, which involves dissolving phospholipids in organic solvents such chloroform, dichloromethane, ethanol, and chloroform-methanol combination (19,20). In a suitable organic solvent, all lipids and the hydrophobic drug are dissolved in a flask with a round bottom. When solvent evaporates under vacuum at a temperature between 45 and 60 C, a thin, homogenous lipid layer forms. The formed thin film is subsequently hydrated with an aqueous buffer solution at the lipid's transition temperature (Tm). A hydrophilic drug or drugs may be present in the hydration solution that will be added to the aqueous core of the liposomes(21). Depending on how soluble they are, the compounds to be encapsulated are either added to an aqueous buffer or an organic solvent containing lipids (22). The efficiency of drug encapsulation is determined by the rate of hydration; the higher the encapsulation efficiency, the slower the rate of hydration (21). In order to totally eliminate the remaining solvent, nitrogen gas is used. In the hydration process, a mixture of distilled water, phosphate buffer, phosphate saline buffer with a pH of 7.4, and regular saline buffer is utilised. The hydration procedure took between one and two hours at a temperature of 60 and 70 oC. The liposomal suspension is kept overnight at 4 ^{0}C to achieve complete lipid hydration (20).

French pressure cell: extrusion:

The process includes extrusion of MLV through a small orifice at 20,000 pressure and 4°C (22). The proteins do not appear to be as pompous during the French press vesicle approach as they do during sonication, which is an important feature. It's interesting to note that French press vessels seem to retain entrapped solutes for a lot longer than SUVs do, whether they were formed by sonication or detergent removal. The technique involves gentle handling unstable materials. The process is quick, easy to repeat, and it handles unstable materials with care. The resulting liposomes are a little bigger than SUVs that have been sonicated (23). The method's disadvantages include the difficult temperature to obtain and the working volumes are relatively small (about 50 mL maximum) (22 ,19).

Membrane Extrusion Method:

This technique is frequently used to transform MLVs into SUVs and LUVs. By putting them through a polycarbonate membrane filter with a specific pore size at low pressure (less than 100 psi), the size of the liposomes should be reduced. LMV are affected by freeze-thaw cycles and prefiltering through large pore size 0.2-1 m before extrusion. In this procedure, the phospholipid layers are broken and then resealed as they pass through the polycarbonate membrane, causing the vesicle contents to be extruded numerous times with the dispersion media. With a rise in transmembrane pressure and the quantity of extrusion cycles, the mean size of the vesicles produced by extrusion decreases (19).

Dried Reconstituted Vesicles:

Preformed liposomes are added to an aqueous solution containing an active ingredient in the dried reconstituted vesicles method, or they are combined with a lyophilized protein, then the mixture is dehydrated.

Freeze-Thaw Method:

SUVs freeze up quickly, then followed by slowl thawing. Aggregated materials are dispersed to LUV by a brief sonication. Due to the SUV's fusion during the freezing and/or thawing procedures, unilamellar vesicles are formed. By boosting the medium's ionic strength of medium and increase phospholipid concentration, this form of fusion is severely prevented. The obtained encapsulation efficiencies ranged from 20 to 30% (22).

Sonication:

This process reduces the size of vesicles and gives the lipid suspension energy (11). Perhaps the most popular technique for preparing SUV is sonication. Here, MLVs are sonicated using a probe sonicator or a bath type sonicator in a passive atmosphere (17). When high energy in a small volume is needed for suspension, the probe Sonication is used. When processing a large amount of diluted lipids, a bath sonicator is used (11). The key drawbacks of this approach are its extremely low internal volume/encapsulation efficacy, the potential for phospholipid and chemical degradation, the exclusion of large molecules, and metal pollution probetip, and the presence of an MLV and an SUV (17).

a) Probe sonication: The tip of a sonicator is directly engrossed into the liposome dispersion. The energy input into lipid dispersion is very high in this method. The vessel needs to be submerged in a water/ice bath because the coupling of energy at the tip causes local hotness. More than 5% of the lipids can be deesterified for up to an hour during the sonication process. Titanium will also slough off and contaminate the fluid while using the probe sonicator.

b) Bath sonication: The bath sonicator is filled with the liposome dispersion in a cylinder. In contrast to sonication by direct dispersal using the tip, it is typically simpler to regulate the temperature of the lipid dispersion using this method. It is possible to safeguard the substance being sonicated in a sterile vessel, unlike the probe units, or under an inert environment (17,19).

Solvent dispersion methods:

Ether Injection Method:

At 55-65°C or under reduced pressure, a lipid solution mixed in diethyl ether or an ether/methanol mixture is gently injected into an aqueous solution of the substance to be encapsulated. Liposomes are formed when the ether is subsequently removed under vacuum. The method's major drawbacks are the population's heterogeneity (70-190 nm) and the compounds that must be encapsulated's exposure to organic solvents or high temperatures.

Ethanol Injection Method:

A lipid solution of ethanol is rapidly injected to a vast excess of buffer. The MLVs are created immediately. The method's disadvantages include the population's heterogeneity (30–110 nm), the dilute nature of liposomes, the challenge of completely eliminating ethanol because it forms an azeotrope with water, and the possibility for a number of biologically active macromolecules to become inactive in the presence of even small amounts of ethanol (18,11,23).

Reverse Phase Evaporation Method:

The first water-in-oil emulsion is created by briefly sonicating a two-phase system made up of phospholipids in an organic solvent (diethylether, isopropylether, or a combination of isopropyl ether and chloroform), as well as an aqueous buffer. Under reduced pressure, the organic solvents are eliminated, which causes the development of a thick gel. When leftover solvent is eliminated through continuous rotating evaporation at a low pressure, liposomes are formed. With the help of this technique, it is possible to achieve a high encapsulation efficiency of up to 65% in a medium with low ionic strength, such as 0.01M NaCl. Both small and large macromolecules have been encapsulated using this technique. The main drawback of the technique is that the materials that will be encapsulated will be exposed to organic solvents and brief durations of sonication (18).

3. Detergent removal:

Lipids have been solubilized by the detergents when they are present in crucial micelles concentrations. The micelles get richer in phospholipid when the detergent is eliminated, and they eventually gather together to form LUVs. Dialysis was used to eliminate the detergents. The detergent dialysis approach has the benefits of great repeatability and homogeneous-sized liposome populations. The method's primary drawback is the persistence of detergent (or detergents) traces inside the liposomes (11).

- **Application of liposome: (23,16)**

1) Gene therapy
2) Liposomes as carriers for vaccines
3) Liposomes as a drug delivery system for oral therapy
4) Liposomes for topical applications
5) Liposomes for pulmonary delivery
6) Against Leishmaniasis
7) Lysosomal storage disease
8) Cell biological application
9) Metal storage disease
10) Ophthalmic delivery of drugs.

Conclusion: The liposomal formulation's effectiveness depends on its capacity to transport the therapeutic molecule to the targeted site over an extended period of time. The phospholipid bilayer contains the drugs, which are intended to diffuse from the bilayer slowly. Many biologically active compounds have been delivered using liposomes to increase cellular absorption. Thus, the liposomal technique can be used to successfully increase the pharmacokinetics and therapeutic efficacy of several extremely potent drugs while concurrently lowering their toxicity.

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