IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

REVIEW ON: RECENT ADVANCES IN LIPOSOMAL DRUG DELIVERY SYSTEM

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Abstract

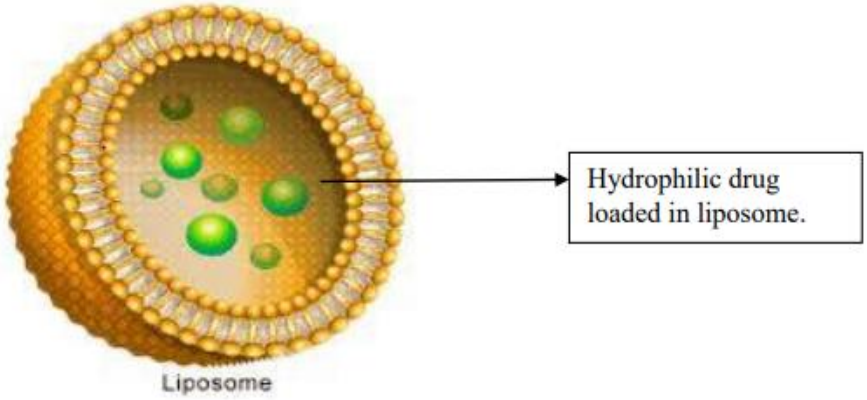
When a lipid bilayer was hydrated, a self-forming enclosed lipid vesicle or liposome appeared. Liposome drug delivery methods have been crucial in the development of powerful drugs that have improved treatments. Because they share lipids with the epidermis, liposomes can also improve cutaneous medicine delivery while reducing systemic absorption. The hydration of the lipid film by hand shaking, sonication, macro-emulsification, and french-pressure cell are some of the numerous ways that liposomes can be made. Liposomes are made up of the sterols, phospholipids, sphingolipids, and polymeric compounds. Vesicle shape and lamellarity, size, and size distribution of the vesicles were used to assess the prepared liposomes. Microscopy Techniques techniques for cryo-transmission electron microscopy and optical microscopy (cryo-tem).

Keywords : Liposome, topical drug delivery, Phospholipid, Vesicle, Macro-emulsification.

INTRODUCTION

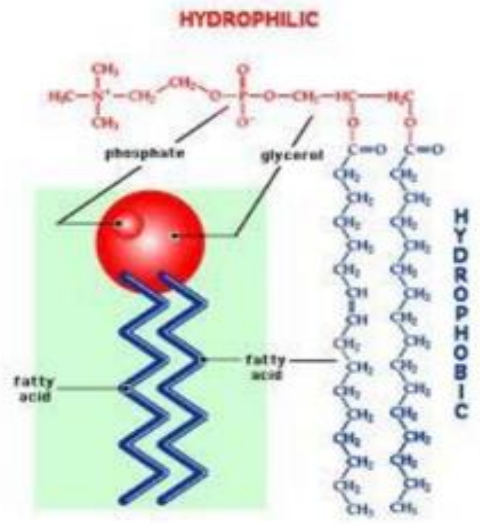
The word "liposome" comes from the Greek, where "lipo" refers to their fatty makeup and "soma" to their structure. A spherical vesicle called a liposome has a membrane made of a bilayer of phospholipid and cholesterol. Liposomes are straightforward tiny vesicles that completely surround an aqueous volume with a lipid-based membrane [1]. It is possible to introduce hydrophilic drugs into the aqueous compartment and lipophilic drugs into lipid bilayers due to the type of structure [2]. The lipid bilayer can combine with the cell membrane to transport the liposome's contents to the active components [1]. Liposomes can also enhance cutaneous medication delivery while lowering systemic absorption because they share lipids with the epidermis [3]. This includes the capacity of lipid vesicles to modify cell membrane fluidity and merge with cells based on lipid content. To improve skin penetration, liposomes containing stratum corneum lipids have been explored in preliminary investigations[4]. According to their size and lamellarity (number of bilayers), liposomes are typically divided into three categories: small unilamellar vesicles (SUVs) or oligolamellar (OLVs), large unilamellar vesicles (LUVs), and multilamellar vesicles (MLVs) [5]. According to their size and lamellarity (number of bilayers), liposomes are typically divided into three categories: small unilamellar vesicles (SUVs) or oligolamellar (OLVs), large unilamellar vesicles (LUVs), and multilamellar vesicles (MLVs) [5]. Due to their distinct characteristics, liposomes are among the most researched drug delivery methods. Mezei and Gulasekharam (1980) [6] carried out the first study utilising liposomes as a drug delivery device for the local treatment of skin disorders. and first entered the cosmetics industry in 1986 [7].

According to Mezei's research, applying liposomal formulations topically to the skin increased drug skin deposition while lowering its systemic biodisposition[8]. The goal of subsequent research was to have a local effect as opposed to a systemic effect [9]. The stratum corneum can be penetrated by a number of different channels, including the transepidermal pathway, which mostly involves intercellular penetration, and the transappendageal routes via sweat glands and hair follicles [10]. Drug solubility, liposome size, surface charge, and lipid composition all have an impact on the stability, pharmacokinetic characteristics, and drug release profile of liposomes[2]. Compared to nonliposomal formulations, many different pharmacological formulations exhibit a significant improvement in therapeutic activity. As a result, the fields of dermatology and cosmetics have recently shown a lot of interest in the usage of liposomes in skin gels or skin creams. As needed for cosmetic goods, liposomes assist in fixing active substances to the epidermis[11]. The first formulations were composed solely of natural lipids; at present they can include natural and/or synthetic lipids and surfactants. They have the capability of entrapping both lipophilic and hydrophilic agents, in the lipid membrane and in the aqueous core, respectively. The size of these nearly spherical lipid vesicles can range from a few nanometers to several micrometers. However, liposomes applied to medical use range between 50 and 450 nm. Liposomes seem to be an almost ideal drug-carrier system, since their morphology is similar to that of cellular membranes and because of their ability to incorporate various substances. Therefore, for the last 50 years liposomes have been widely investigated and they continue to be the subject of intense research. They are valued for their biological and technological advantages as optimal delivery systems for biologically active substances, both in vitro and in vivo, and are considered to be the most successful drug-carrier system known to date.[12]



Liposome structure formed by phospholipids

Fig No.1- Structure of liposome[13]



Shape of phospholipids molecule

Fig No.2- Shape of phospholipids molecule.[13]

Liposome classification [13]

Liposomes are categorised according to

1. Structure.
2. The preparing process.
3. Application and composition.
4. Standard liposome.
5. Unique liposomes.

1. **Structure-Based Classification (Table 1)**

Table No.1 Vesicle types with their dimensions and number of lipid layers

1. Vesicle Type	Abbreviation	Diameter Size	No of Lipid Bilayer
Unilamellar vesicle	UV	All size range	One
Small Unilamellar vesicle	SUV	20-100 nm	One
Medium Unilamellar vesicle	MUV	More than 100nm	One
Large Unilamellar vesicle	LUV	More than 100nm	One
Giant Unilamellar vesicle	GUV	More than 1 micro meter	One
Oligolamellar vesicle	OLV	0.1-1 micro meter	Approx. 5
Multilamellar vesicle	MLV	more than 0.5	5-25

2. **Based on Preparation Method (Table 2)**

Table 2. Various Methods of Preparation and the Vesicles Produced by Each

Preparation Method	Vesicle Type
Single or oligo lamellar vesicle made by reverse phase evaporation method	REV
Multi lamellar vesicle made by reverse phase evaporation method	MLV-REV
Stable pluri lamellar vesicle	SPLV
Frozen and thawed multi lamellar vesicle	FATMLV
Vesicle prepared by extrusion technique	VET
Dehydration- Rehydration method	DR V

3. Considering Composition (Table 3) [14]

Table 3 shows various liposomes and their chemical compositions

Type of Liposome	Abbreviation	Composition
Conventional liposome	CL	Neutral or negatively charge phospholipids and cholesterol
Fusogenic liposome	RSVE	Reconstituted sendai virus envelops
PH sensitive liposomes	-	Phospholipids such as PER or DOPE with either CHEMS or OA
Cationic liposome	-	Cationic lipid with DOPE
Long circulatory liposome	LCL	Neutral high temp, cholesterol, and 5- 10% PEG, DSP

4. Using Traditional Liposomes

1. Make natural lecithin (PC) combinations stable

Two-chain, synthetic phospholipids that are identical.

3- Liposomes with glycolipids

5. Utilizing Specialty Liposome

1. Bipolar fatty acid, first

2. Liposomes guided by antibodies.

3. X-Linked Methyl/Methylene Liposome.

4. Liposomes with lipoprotein coating.

5. Liposome with a carbohydrate coating.

6. Liposomes with many encapsulations.

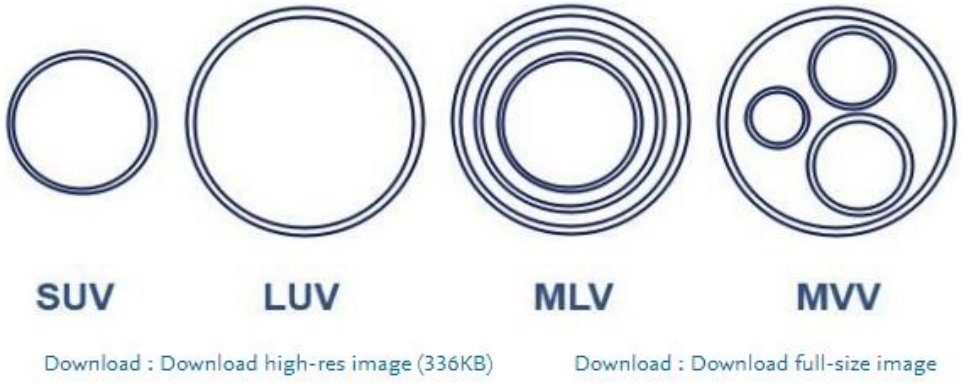


Fig No.3 - Types of vesicles depending on size and lamellarity. SUV, small unilamellar vesicle (20–100 nm); LUV, large unilamellar vesicle (>100 nm); MLV, multilamellar vesicle (>500 nm); MVV multivesicular vesicle (>1000 nm)[15]

MERITS OF LIPOSOMES [13]

The following provides the medicinal and pharmacological basis for using liposomes as medication carriers:

1. Liposomal delivery systems offer both an aqueous milleu interne and a lipophilic environment, making them appropriate for the delivery of hydrophobic, amphipatic, and hydrophilic medicines and agents.

2. Liposomes are well-characterized chemical and physical phenomena.

3. The composition and physical characteristics of liposomes influence their biological destiny after delivery.

4. Due to their biodegradability, low toxicity, and absence of immunogenicity, liosomes are biocompatible.

5. Liposomes can be used as a method for the controlled release of medications inside of cells and in bodily fluids (micro reservoir concept) (after endocytic uptake).

6. Liposomes assist in lowering the amount of hazardous medications that reach delicate tissues.

7. The majority of delivery methods, including intramuscular, subcutaneous, topical, nasal, oral, and intravenous, can be used to administer liposomes.

8. The port of entry, lipid content, and size of liposomes can all affect their pharmacokinetics and in vivo distribution.

DEMERITS OF LIPOSOMES [13]

1. Drug leakage, fusion, and accumulation while being stored.

2. Chemically unstable, meaning it can be broken down by oxidation and hydrolysis

3. High density lipoproteins destabilise them in the physiological environment (HDL)

4. The cost of manufacture and the natural phospholipids' level of purity

5. Complete mediated phagocytosis and lipid exchange processes take place.

Structural elements include:

1. **The phospholipids** [17][16]

Phosphatidic acid is the source of these. Glycerol moieties serve as the molecules' skeleton. The most often utilised component of liposome formulations, phospholipids that contain glycerol,

account for more than 50% of the weight of lipid in biological membranes. A phosphoric acid ester is formed at the C3 OH group.

Phospholipids include, for instance, phospholipids such as phospholipid choline (Lecithin), phospholipid ethanolamine (Cephalin), phospholipid serine (PS), phospholipid inositol (PI), and phospholipid glycerol (PG) Use of saturated fatty acids results in stable liposomes. In general, unsaturated fatty acids are not used.

2. **Sphingolipids-**[17][16]

It is the sphingosine backbone or a similar base. These are the crucial elements found in both plant and animal cells. Three defining components make up this.

• Fatty acids in a mole

• A sphingosine mole

• A head group can range from incredibly complex carbohydrates to basic alcohols like choline.

Sphingomyelin is the most popular sphingolipid.

• Lipid glycosphingoid.

• Gangliosides—Located in the grey matter and employed as a liposome miner component.

This molecule has one or more negative charges at pH neutral because it contains complex saccharides with Sialic acid residues and their polar head groups.

A layer group with a charged surface was added by the liposome.

1. **The sterols**[17][16]

Liposomes frequently comprise cholesterol and its derivatives for-

decreasing the bilayer's fluidity or microviscosity, decreasing the membrane's permeability to water-soluble compounds, and stabilising the membrane when exposed to biological fluids like plasma (This effect used in the formulation of i.v. liposomes) Albumin, transferrin, and macroglobuline are three plasma proteins with which liposomes without cholesterol interact. These proteins have a propensity to remove large amounts of phospholipids from liposomes, depleting the monolayer and causing the vesicles' physical instability.

2. **Polymeric substances** [17]

When exposed to ultraviolet light, synthetic phospholipids with a diactylenic group in the hydrocarbon chain polymerize, creating polymerized liposomes with noticeably higher permeability barriers to entrapped aqueous pharmaceuticals. Examples of additional lipids that can be polymerized include lipids that contain conjugated dienes, methacrylate, etc. Additionally, a number of polymerisable surfactants are created.

3. **Lipid-bearing polymers**[16]

Electrostatic forces that repel one another are largely responsible for the stability of repellent interactions with macromolecules. By covering the liposome surfaces with charge polymers, this repellency can be produced. Higher solubility is conferred by non-ionic, water-compatible polymers such polyethylene oxide, polyvinyl alcohol, and polyoxazolidine. The best outcomes can be obtained by covalently bonding polymers to phospholipids because adsorption of such copolymers, which contain hydrophilic segments with hydrophobic parts, results in liposome leakage.

As an illustration, consider diacyl phosphatidyl ethanolamine and PEG polymer joined by a succinate or carbon at bond.

4. **Lipids with a cation**[16]

For instance, DODAB/C (dioctadecyl dimethyl ammonium bromide or chloride)Dioleoyl propyl trimethyl ammonium chloride, also known as DOTAP, is an analogue of DOTAP and several other compounds, such as other DOTMA analogues and cationic derivatives of cholesterol.

Other ingredients: Different lipids and surfactants are employed.

• Numerous single-chain surfactants can combine with cholesterol to produce liposomes.

5. **Non-ionic lipid**[17]

• A variety of polyglycerol and polyethoxylated mono and dialkyl amphiphiles, which are frequently utilised in the creation of cosmetics.

• Lipids with fluorocarbon chains in both their single and double chains can create exceptionally stable liposomes.

• Dicetyl phosphate and sterylamine.

• Added to liposomes in order to give the structures a negative or positive surface charge.

GENERAL PREPARATION AND DRUGS LOADING METHOD [18]

Aqueous solutions of these materials are used as hydrating fluids in the bulk of the processes used to create liposomes, and at various points along the process, drugs or drug solutions are added to trap water-soluble (hydrophilic) components. Materials that are lipid soluble (also known as "lipophilic") are dissolved in an organic solution that contains the constitutive lipid, which is subsequently evaporated from the dry drug film before being hydrated. These techniques entail loading the agents that have been trapped either before or during the production process (passive loading). After the production of complete vesicles, some types of chemicals can be injected into liposomes.

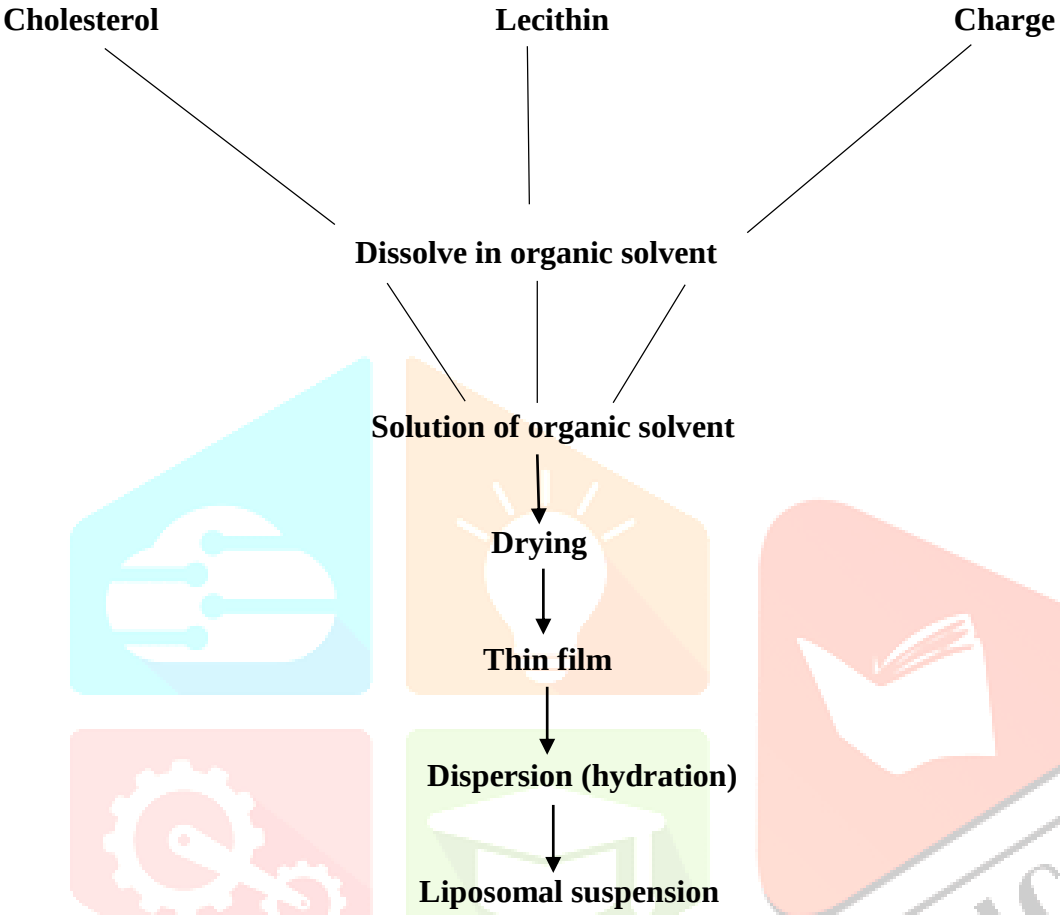


Fig No.4- general method of liposome preparation

Liposome classification:[19]

There are numerous ways to create liposomes. Their nomenclature also depends on the preparation technique, structural factors, or given special purpose.

Hydration of lipid film by hand shacking:

Using a physical dispersion process and various lipid ratios, liposomes were created. In this procedure, chloroform was used to dissolve the lipids. This conical flask has a flat bottom and is covered with the lipid solution in chloroform. The solution was then allowed to evaporate at room temperature without being disturbed. With phosphate buffer (pH 7.4) tilted to one side, aqueous medium containing medication was poured into the side of the flask, and the flask was gently brought back to an upright position. This process was used to hydrate the appearance of the lipid film. The vesicles were harvested by swirling the contents of the flask after the fluid was allowed to pass silently over the lipid layer and the flask was allowed to stand for 2 hours at 37 °C for complete swelling. After that, centrifugation was used to the formulations. A variety of liposome batches were created in an effort to find the best recipe. According to the above-described procedure and lipid composition for liposome manufacture, all batches of liposome were created.

Sonication:

The Sonication process is the one utilised the most frequently to prepare SUVs. MLVs are sonicated in this situation using a passive atmosphere and either a probe-type sonicator or a bath-type sonicator. This method's principal drawbacks include its extremely tiny internal volume, poor encapsulation efficiency, degradation of phospholipids and the elimination of big molecules, metal contamination of the probe tip, and the existence of MLV in addition to SUV. There are two types of sonication:

a) Sonication of the probe: The sonicator is inserted right into the liposome dispersion. This approach has a very large energy input into the lipid dispersion. The vessels must be submerged in water or an ice bath because the coupling of energy at the tip causes localised heat. More than 5% of the lipids can be desterified for up to an hour during the sonication process. Titanium determination slough off and contaminate the solution using the probe sonicator.

b) Bath sonication: Compared to sonication by dispersion directly utilising the tip, placing the cylinder holding the liposomes into a bath sonicator at a controlled temperature is typically a simpler procedure. In contrast to a probes unit, the substance being sonicated can be safeguarded in a sterile vessel or under an inert environment.

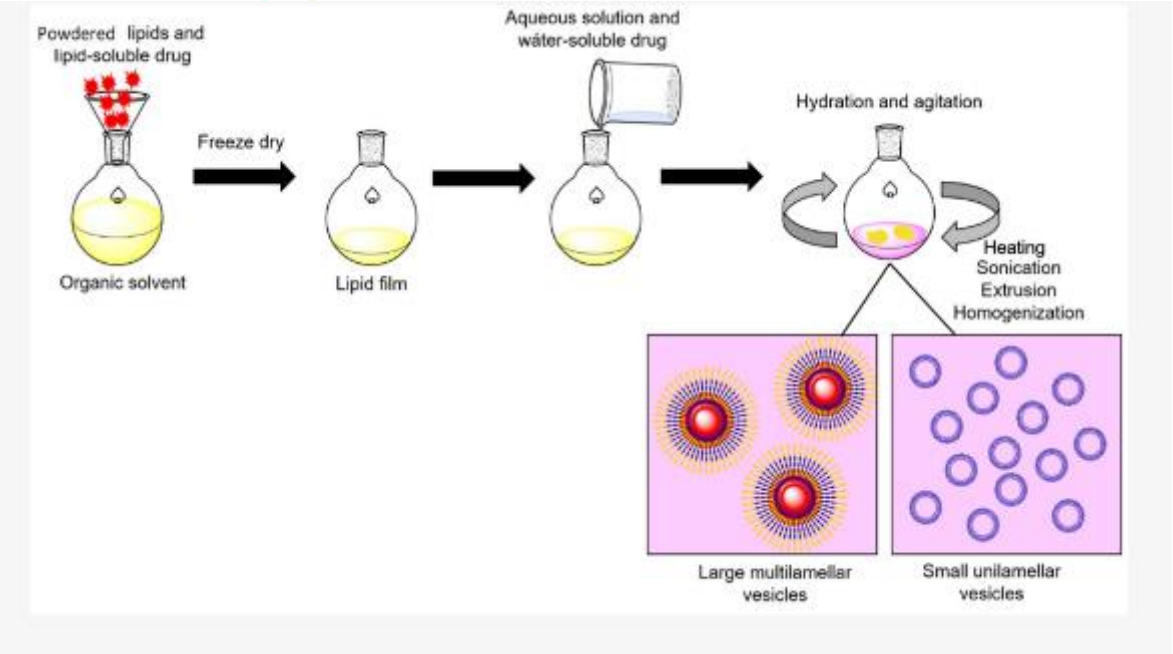


Fig No.5 - Preparation of enzyme-liposomes in general. To create a lipid film, powdered lipids and the medication are dissolved in an organic solvent. The water-soluble medication is then added to a saline buffer, which rehydrates the lipid film. After an overnight incubation, lipid-based vesicles are produced using sonication, heating, extrusion, and homogenization. These vesicles can then be divided by ultracentrifugation into the required sizes.[20]

Liposomes Preparation Techniques

Mechanical dispersion method	**Solvent dispersion method**	**Detergent removal methods**
Lipid film hyadration by hand shaking	Ethanol injection	(1) Detergent (chlorate, Aglycoside Triton X-100) Removal from mixed micelles by
Non-hand shaking or freeze drying	Ether injection	Dialysis
Micro-emulsification	Double emulsion vesicles	Column chromatography
Sonication	Reverse phase evaporation vesicles	Dilution
French-pressure cell	Stable plurilamellar vesicles	Reconstituted Sendai virus enveloped
Membrane extrusion	–	–
Dried reconstituted vesicles	–	–
Freeze- thawed liposome	–	–

Macro-emulsification: Small vesicles are made from concentrated lipid suspension using a microfluidizer. Large MLVs can be used to suspend the lipids before adding them to the fluidizer. The apparatus pushes fluid through a 5 mm screen under very high pressure. Next, it is pushed with a long Two streams of fluid collide at right angles in tiny channels that have dried out over time. Pump and an interaction chamber can be used to recycle the collected fluid until spherical vesicles are produced. A key aspect of the French pressure vesicle approach is that the protein does not appear to change much throughout the process, as they do in sonication.

French-pressure cell: The French pressure cell involves the extrusion of MLV through a tiny opening. The technique calls for delicate manipulation of unstable techniques. The approach provides a number of benefits over sonication. The end product liposomes are bigger than sonicated SUVs. The method's shortcomings include the difficulty in achieving the high temperature and the relatively modest working quantities (about 50 ml as the maximum).

Extrusion of Membranes Liposomes:[21-26] These are reduced in size by passing them through a membrane filter with a defined pore size. Membrane filters come in two varieties. The nucleation track type and the tortuous path type. For sterile filtering, the former is utilised. Between the criss cross strands, a random path emerges. The density of fibres in the matrix determines the average diameter of these fibres. When trying to transmit liposomes through a membrane with a diameter bigger than the channel diameter, they are impacted. The nucleation track is made up of a continuous thin layer of polycarbonate. Because they are made up of straight sided pore holes with precise diameters punched from one side to the other, they will provide less barrier to liposome transit. Both LUVs and MLVs can be processed with this method.

Reverse Phase Evaporation Technique: The lipid mixture is added to a flask with a circular bottom, and the solvent is rotary evaporated under pressure to eliminate it. Vesicles will occur once the system has been nitrogen-purged, when the lipids have been redissolved in the organic phase. The typical solvents of choice are diethyl ether and isopropyl ether. After the lipids have been redissolved, an emulsion is produced. The solvent is then removed from the emulsion by evaporating it to a semisolid gel under reduced pressure, and any non-encapsulated material is then removed. The liposome that results is known as a reverse phase evaporation vesicle (REV). Large macromolecules can be made using this technique quite effectively.

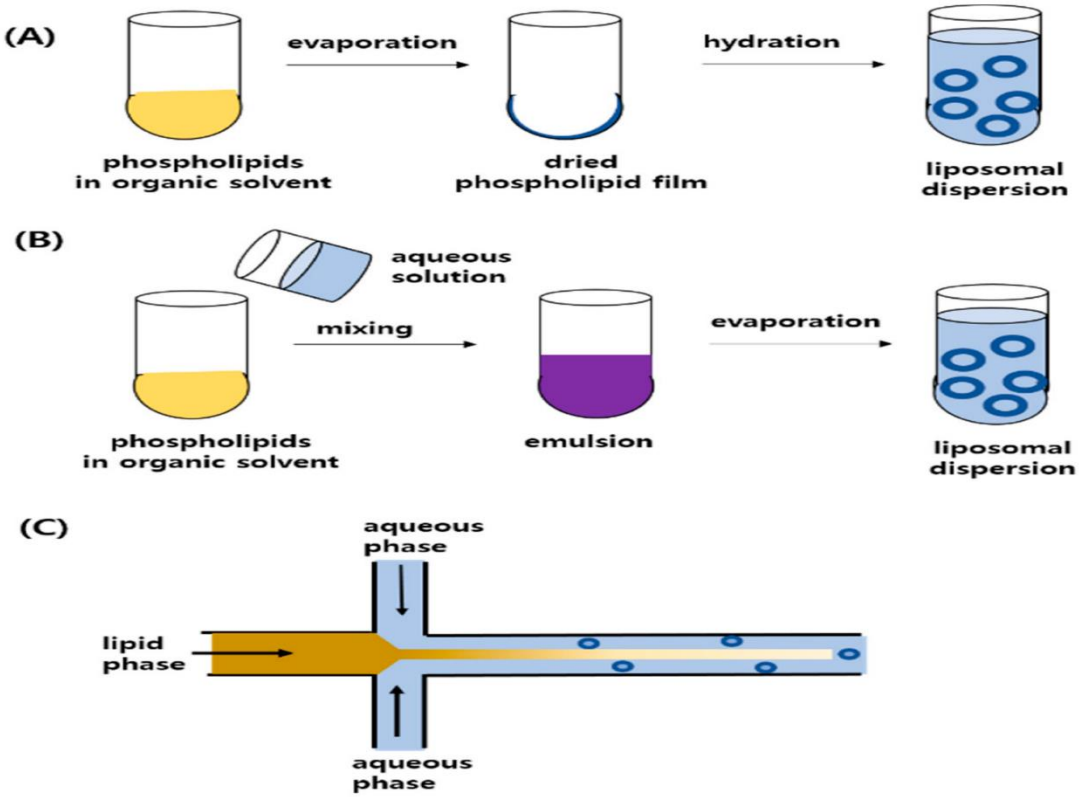


Fig No.6 -The reverse phase evaporation method (B), the microfluidic method (A), and the film hydration method (C) are examples of liposome preparation techniques.[27]

Ether injection: A solution of lipids is dissolved in ether, diethyl ether, or methanol, and then gently injected into an aqueous solution of the substance to be encapsulated. Liposomes are created by successively removing the organic solvent under decreasing pressure. Heterogeneous population and exposing the substance that will be encapsulated to organic solvents or high temperatures are the method's principal drawbacks.

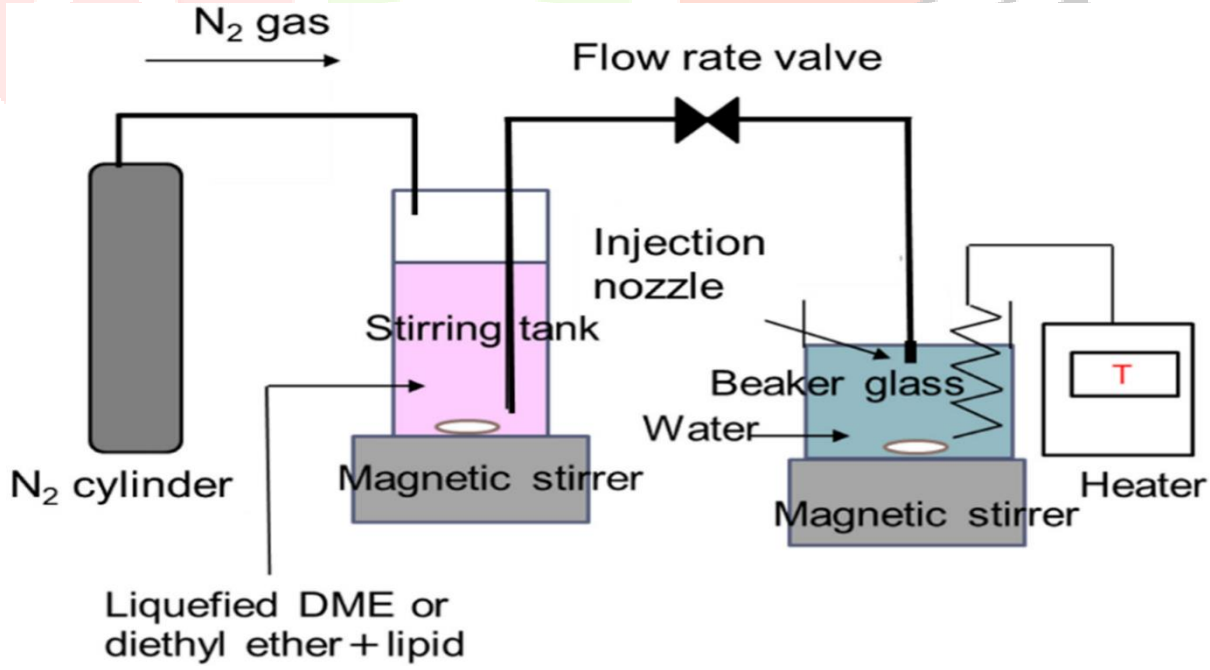


Fig No.7 - Diagrammatic representation of the mechanism for making liposomes from liquefied DME. [28]

Double Emulsification Technique: This technology, also known as DepoFoamplatformTM, has been used to create MVLs in three commercial products: DepoCyte, DepoDur, and Expel. The four operations that are frequently included in the complete production are as follows:

(1) the creation of a water-in-oil-in-water emulsion

(2) solvent extraction utilising stripping gas or vacuum pressure

(3) microfiltration for free drug removal, concentration, and external solution exchange.

Hydrocarbon injection: The ethanol injection method involves rapidly injecting an enormous amount of warmed distilled water or TRIS-HCl buffer with an ethanolic lipid solution. The hydrophilic/hydrophobic nature of the substance determines whether it will be incorporated into the liposomal vesicle. Comparatively to 5-fluorouracil, which migrates to the exterior aqueous phase, nimesulide is a lipid soluble component that integrates well in liposomes. The inclusion of a non-harmful solvent in the ethanol injection method is its principal benefit. Its usefulness is limited by the potential for azeotrope production in water.

Detergent Removal Method[19]: A variety of vesicle forms and very homogeneous liposomes can be produced using the detergent depletion approach, which is a gentle process. The procedure is based on the creation of detergent-lipid micelles, which are then removed to create liposomes. This approach has the drawbacks of a low final concentration of liposomes in the solution and a poor level of hydrophobic chemical entrapment. The composition still contains the detergent. The rate of detergent removal and the initial detergent to phospholipid ratio determine the size and homogeneity of liposomes made by detergent depletion. The procedure takes a long time, and while removing the detergent, other tiny hydrophilic compounds might also be removed.

A microfluidic technique for managed liposome production was created by Microfluidic Channel Method. A stream of lipid dissolved in alcohol is passed between two aqueous streams in a microfluidic channel during the procedure. Mixing happens at the liquid interfaces, which leads to the formation of liposomes. The channels' laminar flow makes it possible to regulate the liposomes' size and size distribution. This microfluidic technique for liposome self assembly can be employed for drug encapsulation right before usage.

Heating Technique: The phospholipid components are hydrated in an aqueous solution that contains 3% (vol) glycerol as part of Mozafari's heating technique, which then raises the temperature to 60°C or 120°C depending on whether cholesterol is present or not. Since glycerol is a medically acceptable chemical that is water soluble and capable of acting as an isotonizing agent, it is used to strengthen the stability of lipid vesicles by inhibiting coagulation and sedimentation. There have been no reports of the lipid components in liposomes made using the heating technique degrading. Additionally, once high temperature (i.e. 120°C) is employed in this procedure, sterilisation is not required. The encapsulation and targeted administration of the food-grade antibacterial nisin have recently been accomplished using a further enhanced variation of the heating technique known as the Mozafari method. The Mozafari method enables the production of carrier systems on a wide scale in a single step without the necessity for prehydrating the constituent material or using hazardous solvents or detergents.

Dense Gas Methods: The phrase "dense gas" is a catch-all phrase used to describe a substance in the vicinity of the critical point. Dense gases have mass transport characteristics similar to those of gases and solvent characteristics comparable to those of liquids. The special characteristics of dense gases have been used to improve processing methods, particularly separation, purification, and size reduction procedures, and to replace numerous organic solvents. Since carbon dioxide is nonflammable, nontoxic, noncorrosive, affordable, ecologically friendly, and possesses crucial values that are simple to get (31.1°C and 73.8 bar), it is the most commonly utilised dense gas. After processing, the solvent is easily recovered by simply restoring to atmospheric pressure. Dense gas processing can offer clean working environments and one-step production, which can solve the liposome sterilisation problems that are now present.

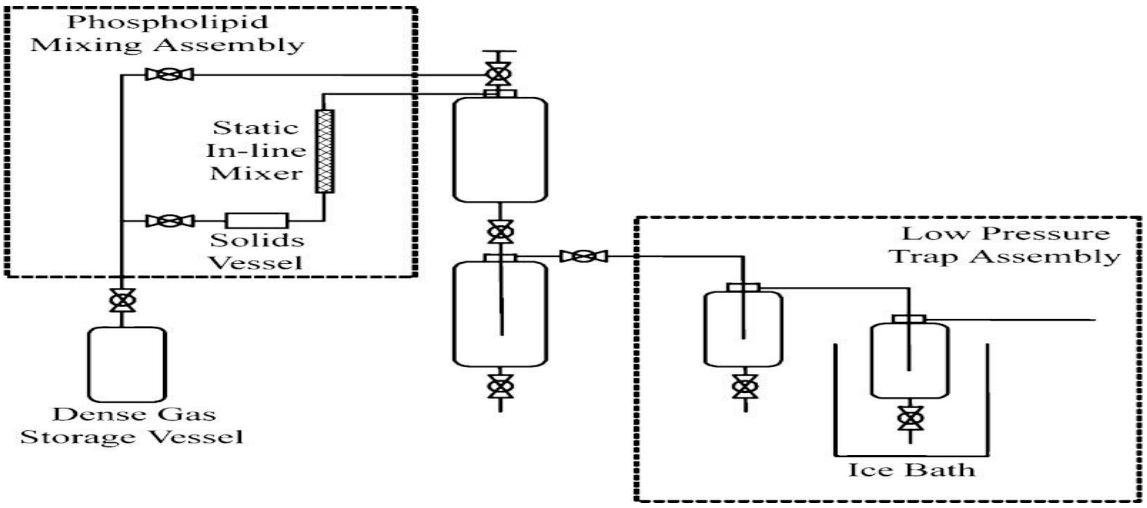


Fig No.8 -Traditional and Dense Gas Methods for Liposome Production[29]

Method for Supercritical Fluid Injection and Decompression: Castor and Chu first disclosed the injection and decompression procedures, the first dense gas approaches for the production of liposomes, in 1998. The injection method involves injecting compressed gas, lipid, and organic solvent through a nozzle into an aqueous solution. Alternately, the decompression approach entails the formation of liposomes through the decompression of a mixture of lipid, organic cosolvent, compressed gas, and aqueous solution. While the aqueous phase is included into the compressed phase that is sprayed into air in the decompression technique, the compressed phase is sprayed into water in the injection method. The injection and decompression procedures can create sterile, pharmaceutical-grade liposomes with narrow particle size distributions that are a predetermined size.

Supercritical Fluid Method: The injection method created by Castor and Chu is similar to the supercritical liposome approach disclosed in 1994 by Supercritical Fluid Method, which created small unilamellar vesicles (SUVs) with particle sizes between 20 and 50 nm. Lipid and cholesterol are dissolved into supercritical carbon dioxide during the procedure. The hydrophilic molecule to be trapped is then contained in an aqueous phase created by quickly expanding the solution. The encapsulation efficiency was less effective than what was obtained utilising standard liposome production methods.

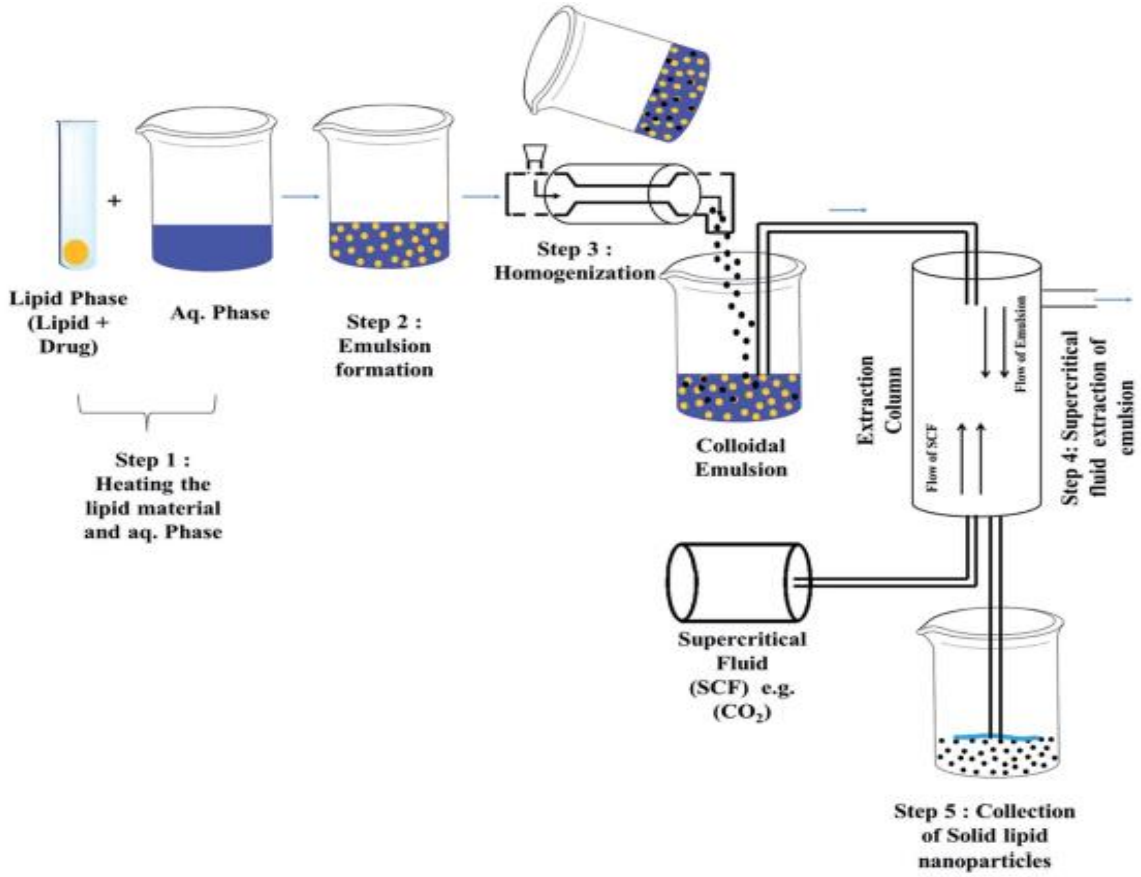


Fig No.9- Incredibly important fluid approach for making solid lipid nanoparticles.[30]

Process of Supercritical Reverse Phase Evaporation: Otake et al. created the Supercritical Reverse Phase Evaporation (SCRPE) technology. In a stirred variable volume cell operating at a temperature above the lipid phase transition temperature, the lipid, organic co-solvent, and compressed gas are mixed. The cell is then progressively injected with an aqueous solution. When the compressed gas is released, the pressure is lowered and liposomes are created. The SCRPE method works on the same principles as the decompression method. The achieved trapping efficiencies were incredibly poor. The improved supercritical reverse phase evaporation (ISCRPE) approach was recently created by Otake et al. to replace the usage of organic solvents in liposome synthesis and improve the stability and drug loading effectiveness of the vesicles.

Method of High-Pressure Homogenization (HPH): Due to their propensity to disrupt vesicles, high-pressure homogenizers are employed to create liposomes and lipid dispersions. The sample is injected at a high, steady pressure into the homogenizer's specially constructed area, where turbulence, cavitations, and/or shear processes cause the liposome structure to reorganise. The amount of pressure and the number of times the material is processed affect the properties of the liposomes created by high-pressure homogenization (number of cycles). Because they are best used intravenously, HPH is extremely helpful in the manufacture of very small liposomes.

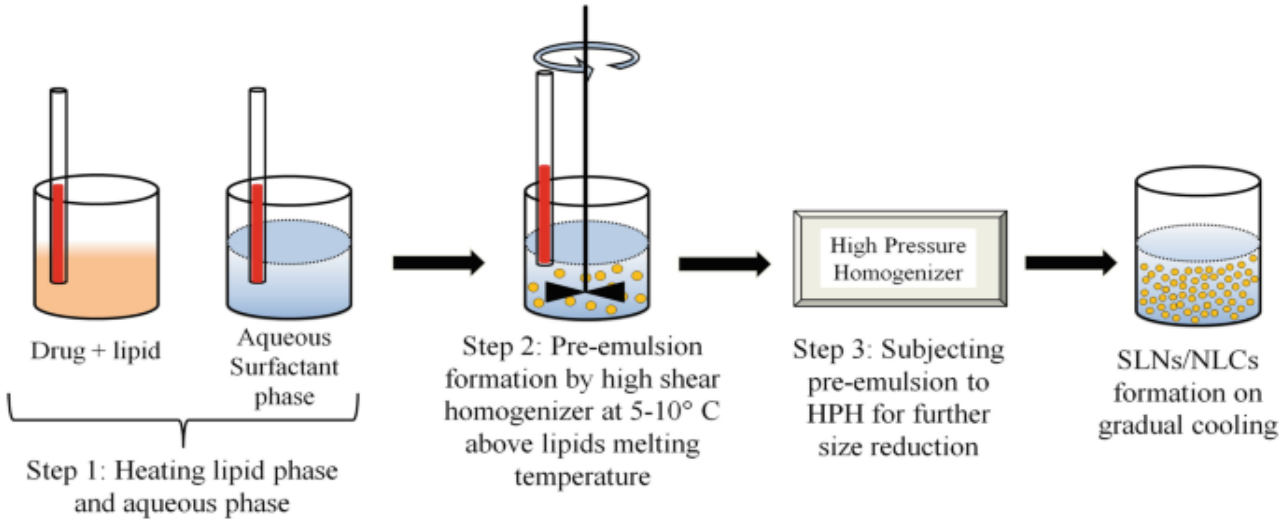


Fig No.10 -Technique for High-Pressure Homogenization [31]

Asymmetric Dual Centrifugation (ADC): Dual asymmetric centrifugation (DAC) is a unique form of centrifugation in which, as is customary, a vial is rotated around the primary rotation axis at a specific speed and distance. The fundamental distinction between DAC and conventional centrifugation is that during conventional centrifugation, the vial is rotated about its own centre (vertical axis). Nano liposomes with a uniform size distribution and a diameter of 60 nm are created when mechanical turbulence and cavitations transmit energy to the material. DAC has a high trapping effectiveness, although this approach works best for batches that are a gramme or less in size.

Encapsulating bioactive agents: techniques: The choice of an encapsulation protocol is heavily influenced by factors such encapsulation efficiency, drug/lipid ratio, drug retention, sterility, ease of preparation and scaling up, compliance with regulatory authorities, cost effectiveness, and liposome and drug stability.

Bioactive substances can be encapsulated in liposomes in two different methods. Distinguish between I loading the bioactive into intact vesicles and (ii) passive encapsulation of the bioactive during vesicle production (active loading).

Techniques for Passive Trapping: The passive entrapment methods rely on liposomes' capacity to collect a specific amount of aqueous fluid during vesicle formation, including any dissolved solutes. The encapsulation effectiveness after passive encapsulation is proportional to the aqueous volume encompassed by the vesicles for water soluble chemicals that do not interact with the bilayer, -which in turn is reliant on the vesicle shape, lamellarity, and concentration of phospholipids in the dispersion. Both encapsulation factors will depend more on phospholipid content and selection than on morphological features, similar to the less water-soluble medicines that interact with the bilayer. Water-soluble (hydrophilic) molecules will be contained inside the aqueous phase of the liposome during this process, whereas lipid-soluble (hydrophobic) agents will be found in the liposome's bilayer (lipidic phase). Amphiphilic molecules will be positioned so that their water-soluble portion is in the liposomal aqueous phase and their lipid-soluble portion is embedded between the liposomal lipids.

Techniques for Active Trapping: Active trapping approach, in theory, involves mixing "empty" liposomes with a concentrated drug solution and then incubating the mixture until the drug is evenly dispersed through diffusion. This approach has certain benefits since the vesicle bilayers are porous enough to allow medications to diffuse into the liposomes within an acceptable amount of time. Following the concentration gradient, the drug permeates through the lipid bilayers into the vesicles until equilibrium is reached between the interior of the vesicles and the surrounding medium. As a result, liposome formulations for this class of medications vary dramatically amongst agents since the amount of hydrophobic drug that can enter a liposome really depends on packing limitations in the lipid bilayer. Liposomes interact with the polar head groups of phospholipids to sequester water-soluble medicines, however amphiphilic medications are frequently challenging to keep inside liposomes due to their rapid lipid bilayer permeability. . However, the active loading method is only effective with a limited number of medicines that behave as weak amphipathic bases or acids and can permeate bilayers in the uncharged state but not in the charged state. Because the active component is not yet present during the liposome synthesis, there are some benefits to active loading that may reduce the need for handling-related safety precautions.

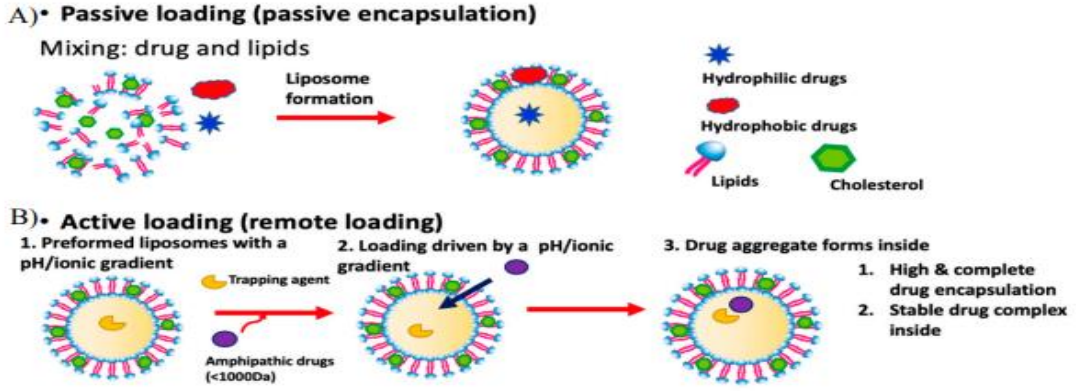


Fig No.11 -The two main techniques for putting drugs into liposomes. Co-current loading and liposomal production are both aspects of (A) passive loading. (B) Active loading creates liposomes with a gradient that load medicines.[26]

Liposome Characterization

The features of the liposomal formulations can be used to estimate the fate of the liposomes under storage conditions and the various therapeutic applications. There are numerous ways to describe the liposomes, some of which are provided in the following section, according to a review by Crommelin and Storm[27].

Size and polydispersity Because liposome size is crucial for parenteral administrations, size and polydispersity determination offer a crucial technique for characterising liposomes. They also offer a rapid estimation of manufacturing variances and batch quality. Numerous methods, such as dynamic light scattering (DLS), size exclusion chromatography (SEC), nuclear magnetic resonance (NMR), and various microscope technologies, including as transmission electron microscopy (TEM), cryogenic-TEM (Cryo-TEM), and atomic force microscopy, can be used to determine size (AFM).

DLS is the fastest and most popular method for measuring the liposome size and size distribution. When particles collide, DLS examines their Brownian motion, resulting in light that was incidentally scattered. The difference in refractive indices between the solvent and suspended particles affects how light scatters. The mean particle size of the suspended liposomes is determined by calculating and analysing the amount of light scattered. This method has the benefits of being simple to use, having a large measurement range (20–1000 nm), and being able to evaluate the liposomes in their natural habitats. There are certain drawbacks, though, such as the system's inability to distinguish between a single liposome and an aggregate. False readings could be acquired because it treats an aggregate as a single particle. Additionally, this method is sensitive to even small levels of contaminants.[28-29]

HPLC-SEC size analysis is connected to high resolution with modest sample requirements. The samples are run through columns with the proper porosity packing while being pumped under pressure by HPLC pumps. Low to intermediate size particles can be separated to varying degrees. It is a method that may be used in conjunction with DLS and is dependable and repeatable. The assay can be affected by the chemistry of the liposomes as well as the column packing, even if the separation is dependent on size. Deformable lipids in the liposomes allow them to squeeze through pores and produce erroneous results. On the other hand, there is a chance that the liposomes will cling to the lipid packing in the columns, which could cause problems with recovery.[30-31]

The liposomes can be seen and their sizes can be determined using microscopy. Cryo-TEM and TEM techniques have both been widely used to produce liposomal pictures. The liposomes are positioned on a tiny dry copper grid for negative staining TEM, where the aqueous buffer is allowed to dry and a negative stain (uranyl acetate, phosphotungstic acid) is used to label the backdrop so that the liposomes show as brilliant vesicles.[32] The lipid chemistry may be altered by the removal of liposomes from their natural environment, which could result in artefacts in the image that is produced. Negative staining of liposomes made for aerosols was avoided in a recent study during the TEM imaging to prevent caused artefacts.[33] Alternatively, The liposomes can be kept together using cryo-TEM. to their original state to avoid chemical shrinkage or shape distortion harm.[34] Liquid nitrogen flash freezing of the liposomes and subsequent transfer to the controlled environment in the cryoTEM apparatus are the preparative processes. However, samples with a lower nanometer range do better in the study. During the blotting process, larger liposomes may be eliminated from the sample film. A description of the cryo-TEM method is given along with a discussion of the sample and sample preparation parameters. Without changing the sample,[35] AFM provides a rapid, dependable, and high resolution method of determining liposome size.[36]

Nanoparticle tracking analysis (NTA), a recently discovered method, uses light scattering caused by the presence of nanoparticles to identify them. The liposomes are injected into a view cell irradiated by a laser beam while still in their natural buffer environment. Digital photography is used to record and trace the motions of the individual liposomes as they move through the medium. The sphere equivalent influences the particle movement speed. Calculated using the Stokes-Einstein equation is the hydrodynamic radius.[37][38] It has an edge over competing methods because of the instrument software's ability to calculate liposome size on a per-particle basis. The NTA-based Nanosight LM10, NS300, and NS500 systems from Nanosight, Amesbury, UK, can quickly evaluate liposomes and other nanoparticles ranging in size from 10 to 2000 nm.[39-40]

Zeta Potential. The charge of the liposomes in a dispersion is calculated as part of the zeta potential measuring process. Each liposome contains a charge, which can be neutral, positive, or negative depending on the ligands it is attached to. The zeta potential can be used to determine the liposomes' stability in the medium. While liposomes with a higher negative or positive charge will have repulsive forces in the medium that prevent agglomeration, uncharged or low-charged liposomes have a tendency to cluster over time.

Liposomes are illuminated by incident light in a sample cell, and the zeta potential is determined by changes in the scattered light when the liposomes move as a result of an applied electric field. The relationship between the related charge and the liposomes' movement is linear. [41] The mobilisation of the liposomes causes a Doppler shift in the frequency of the laser light that is being monitored. [42-43]

Encapsulation efficiency (EE). Entrapped medication must first be taken out of the liposomes in order to examine the EE. This can be accomplished by adding an organic phase in place of the aqueous media (acetonitrile, ethanol, methanol, Triton X-100). Several methods, such as UV and/or fluorescence spectroscopy, enzyme- or protein-based assays, and gel electrophoresis, can be used to determine the concentration, depending on the substance being studied. The unencapsulated (free) medication must be taken out of the formulation before the EE estimate. Ultracentrifugation, dialysis, or column separation can all be used to accomplish this. The utilisation of tools like HPLC, UPLC, and LC-MS can also help with EE measurements.[44]

Assay for lamellarity. The number of bilayers has a significant impact on the in vivo fate and uses of liposomes, as previously mentioned. While a number of chemically based methods[45-47] employ labelled reagents or radiolabeled ions to gauge the number of layers or the quantity of lipids in the surface, the predictions frequently presuppose that the reagents were evenly distributed on the surface of the outer layer. This could produce inaccurate readings.[48] Cryo-TEM analysis is a frequently employed technique for studying the liposomal lamellarity and shape. One-dimensional The lamallerity, more particularly the ratio of the number of phospholipids in the outer to inner layers, has also been determined by 31P NMR[49]. 14 On the NMR, the MLV and SUV produce, respectively, broad and narrow line spectra. Due to interactions between the paramagnetic ions and the bilayers, the measured resonance shifts when paramagnetic ions like Mn2+, Co2+, and Pr3+ are introduced to the preparation of the NMR sample. Lamellarity can be calculated by comparing the spectra before and after the addition of Mn2+. 14 The use of methods like trapped volume measurement[53] and small-angle X-ray scattering (SAXS)[50-52] has also been made.

In-vitro drug release. This straightforward analysis is done under dialysis settings. With the proper molecular weight cut-offs, liposome samples are placed in dialysis bags and constantly swirled in a dissolving media. In order to replicate an in vivo environment, the setup is often maintained in an enclosed space at 37 °C with buffered saline at pH 7.4. Samples are collected and examined using procedures such the drug-specific HPLC and UV/fluorescence spectroscopy at predetermined time intervals. The intensity of the By switching out the dissolving medium with fresh ones, samples are kept consistent. An estimation of the drug release by the liposomal carrier is provided by a release profile plot.

Proliposomes formulations, liposomal dry powder for inhalation (DPI), etc. need additional analysis to completely understand their properties in addition to the ones listed above. Angle of repose, flow-ability by bulk/tapped density, moisture content, and proliposomal granule size by are important considerations when using proliposomes for oral and topical distribution via tablets or gels. Measurements are made of the proliposomes' conversion rate to liposomes upon rehydration, sieve analysis, and rheological behaviour.[54-56] Formulations for skin delivery are also examined for rheological and viscosity characteristics, deformability, and even ex vivo permeability/diffusion characteristics.[57-59]

APPLICATIONS[60]

The liposomal drug delivery method has been used for a variety of drug delivery applications, some of which are as follows:

increase medication emulsion (Amphotericin-B, Minoxidil, Paclitaxel, and Cyclosporine). safeguarding delicate medication molecules (Cytosine arabinosa, DNA, RNA, Anti-sense oligo-nucleotides, Ribozymes)

increasing intracellular uptake (Anticancer, anti-viral and antimicrobial drugs)

altered biodistribution and pharmacokinetics (prolonged or sustained released drugs with short circulatory half-life)

The following are a few recent uses for liposomal drug delivery systems:

In Respiratory Drug Delivery System:

-Many different forms of respiratory illnesses are treated with liposomes. The following are some benefits of liposomal aerosol over regular aerosol:

1. Persistent release

2. Reducing local irritability

3. Lessening of toxicity and

4. Greater stability in the substantial watery core.

By avoiding the loss of intracellular enzyme function, butylcholinesterase-encapsulating bioadhesives liposomes may offer prophylaxis against organophosphate poisoning.

In Cosmetics:

The usage of liposomes in skin gels or skin creams has recently sparked a lot of attention in the cosmetics industry. Due to their high quantity of esterified essential fatty acids, particularly linoleic acid, which is thought to improve the skin's barrier function and reduce water loss shortly after application, vegetable phospholipids are frequently utilised for topical applications in cosmetics and dermatology.[20] The properties of liposomes can be utilized also in the delivery of ingredients in cosmetics. Liposomes offer advantages because lipids are well hydrated and can reduce the dryness of the skin which is a primary cause for ageing. Also, liposomes can supply replenish lipids and importantly linolenic acid to the skin. The first liposomal cosmetic product to appear on the market was the anti-ageing cream “Capture” launched by Christian Dior in 1986. Liposomes have been also used in the treatment of hair loss; minoxidil, a vasodilator, is in the active ingredient in products like “Regaine” that claim to prevent or slow hair loss. The skin care preparations with empty or moisture loaded liposome reduce the transdermal water loss and are suitable for the treatment of dry skin. They also enhance the supply of lipids and water to the stratum corneum. Various liposome formulations were compared in vivo for cosmetics application; liposome formulations prepared from egg phospholipids exhibited a 1.5-fold increase in skin water content, whereas liposome formulations prepared from soya phospholipids showed no advantage compared to the references. Skin water content was measured daily and the results showed that skin humidity was increased significantly for the formulation containing 20% egg phospholipids during 6 days.

Since 1987, several cosmetic products have been commercially available; they range from simple liposome pastes which are used as a replacement for creams, gels and ointments to formulations containing various extracts, moisturizers, antibiotics, etc. Unrinsable sunscreens, long lasting perfumes, hair conditioners, aftershaves, lipsticks, make-up and similar products are also gaining large fractions of the market. Some of the liposomal cosmetic.[61]

Systemic liposomal drugs

After systemic (usually intravenous) administration, liposomes are typically recognized as foreign particles and consequently endocytosed by the mononuclear phagocytic system cells (MPS), mostly fixed Kuppfer cells, in the liver and spleen. Liposomes can serve as an excellent drug-delivery vehicle to these cells. Thus, sterically stabilized liposome, which are not avidly taken up by MPS cells, have different biodistributions properties and have shown enhanced accumulation in sites of trauma, such as tumours, infections and

inflammation. This accumulation is simply due to their prolonged circulation and small size; enabling them to extravasate.

Based on the liposome properties introduced above, several techniques of drug delivery can be envisaged: —Liposomes can be applied to protect the entrapped drug against enzymatic degradation whilst in circulation. The lipids used in their formulation are not susceptible to enzymatic degradation; the entrapped drug is thus protected while the lipid vesicles circulate within the extracellular fluid. As an example, - lactamase sensitive antibiotics such as the penicillins and cephalosporins have been encapsulated in order to be protected against the - lactamase enzyme. Rowland et al. reported that liposomes offer protection in the gastrointestinal tract environment of encapsulated drug and facilitate the gastrointestinal transport of a variety of compounds. As clearly evidenced by Dapergolas, liposomes are candidates to be explored for oral delivery of peptides (insulin) and proteins (vaccines), which are orally degradable.

Liposomes can be used for drug targeting. It has been proved that restricting the distribution of the drug to the specific target site should allow efficacy increase at low dose with attendant decrease of toxicity. Indeed, pumping a drug through the whole body is not only wasteful but, more fundamentally, increase undesirable side effects. Hence, the benefits of drug targeting include reducing drug waste, and it is possible to deliver a drug to a tissue or cell region not normally accessible to the free or untargeted drug. Liposomes have been widely applied in drug targeting especially in cancer treatment. Effective chemotherapy is severely limited by the toxic side effects of the drugs. Liposome encapsulation can alter the spatial and temporal distribution of the encapsulated drug molecules in the body, which may significantly reduce unwanted toxic side effects and increase the efficacy of the treatment. The first step, therefore, is to determine the antigens that are produced by the tumour cells. Then to target the drug via specific receptor ligands, which may be specific antibodies for antigens produced by tumour cells. Two liposomal formulations have been approved by the US food and drug administration (FDA) and are commercially available in the USA, Europe and Japan for the treatment of Kaposi's sarcoma. Doxil® is a formulation of doxorubicin precipitated in sterically stabilized liposomes (on the market since 1995) and DaunoXome® is daunorubicin encapsulated in small liposomes (on the market since 1990). Doxil® has been shown to have a 4.5-times-lower medium-pathology score for doxorubicin induced cardiotoxicity than the free drug. In squamous cell lung carcinoma, the same drug is capable of reducing tumor burden to a significant extent.

In order to enhance solubilisation, the amphotericin B, which is the drug of choice in the treatment of systemic fungal infections, has been widely studied for liposome encapsulation. Owing to its aqueous insolubility, amphotericin B is typically formulated into detergent micelles. But, micelles are unstable upon systemic administration, and several neuro- and nephrotoxicity limit the dose that can be administered. However in a stable colloid particle, such as liposomes, encapsulated drug is delivered much more efficiently to macrophages and, additionally, toxicity can be significantly reduced. Following this rationale, a lipid-based amphotericin B formulation is actually commercially available in the Europe and US market (respectively since 1990 and 1997): AmBisome® including amphotericin B into small liposomes.

In Food Applications

The majority of microencapsulation techniques currently used in the food industry are based on biopolymer matrices composed of sugars, starches, gums, proteins, synthetics, dextrin and alginates. Nevertheless, liposomes have recently begun to gain in importance in food products. Indeed, the ability of liposomes to solubilise compounds with demanding solubility properties, sequester compounds from potentially harmful medium, and release incorporated molecules in a sustained and predictable way can be used in food processing industry. Based on studies on liposomes for pharmaceutical and medical uses, food scientists have begun to utilize liposomes for controlled delivery of functional components such as proteins, enzymes, vitamins, antioxidants, and flavours. The applications are for example dairy products preparation, stabilization of food components against degradation, and delivery and enhanced efficiency of antimicrobial peptides.

The sustained release system concept can be used in various fermentation processes in which the encapsulated enzymes can greatly shorten fermentation times and improve the quality of the product. A classical example is cheese-making; after preliminary studies in which liposome systems were optimized the cheese ripening times were shorten by 30 to 50%. This means a substantial economic profit knowing that ripening times of some cheeses, such as Cheddar, are about one year during which they require well controlled conditions.

In addition to improved fermentation, liposomes were tried in the preservation of cheeses. Addition of nitrates to cheese milk to suppress the growth of spore-forming bacteria is questioned due to health concerns and natural alternatives are under study. Lysozyme is effective but quickly inactivated due to binding to casein. Liposome encapsulation can both preserve potency and increase effectiveness because liposomes become localized in the water spaces between the casein matrix and fat globules of curd and cheese. These applications of enhancing natural preservatives, including antioxidants such as vitamin E and C, will undoubtedly become very important due to recent dietary trends which tend to reduce the addition of artificial preservatives and increase portion of unsaturated fats in the diet.[27]

Targeted for the Brain

Liposomes are currently being investigated as a drug delivery mechanism for the brain due to their biocompatible and biodegradable behaviour [61]. Both large and small liposomes (more than 100 nm) freely diffuse through the blood-brain barrier (BBB). A small unilamellar vesicles (SUVS) connected to brain drug transport vectors, however, is a possibility. may be transcytosed by a receptor- or an absorptive-mediated mechanism through the BBB. Similar to this, newly created cationic liposomes demonstrated that these particles might enter cells by absorptive driven endocytosis. It is yet unknown if cationic liposomes can successfully cross the BBB via absorptive induced transcytosis. It has been extensively researched how liposomes can transport drugs over the BBB. The key discovery from their investigations is that the capacity of various current applications to cross BBB is increased when sulphatide (a sulphur ester of galactocerebroside) is added to the liposome composition [62]. According to Wang et al., mannose-coated liposomes can penetrate the blood-brain barrier (BBB) and reach brain tissue[63]. When administered systemically, neutropeptides, leu-enkephaline, and mefenkephalin kyoforphin typically do not cross BBB. Amitriptyline, an antidepressant, typically penetrates the BBB thanks to this method's adaptability. In order to create nanoparticles (NP), various stabilisers were used. When the drug was adsorbed onto the NP and coated with polysorbate 85 or particle stabilised, it was discovered that the amount of amitriptyline was dramatically increased in the brain. [64].

As Anti-Infective Agents

The liver and spleen are home to intracellular pathogens such protozoa, bacteria, and fungi; therefore, to eliminate these pathogens, the therapeutic drug may be targeted to these organs employing a liposome as a transport system. [65] Amphotericin B, a polyene antibiotic, is linked to severe kidney damage when used to treat systemic fungal infection. Amphotericin B works by attaching to sterol in the membrane of susceptible fungus and causing an increase in membrane permeability. This substance's toxicity is caused by its non-specificity and binding to the cholesterol in mammalian cells. The first liposomal formulation of amphotericin B (ambisome), which just cleared all clinical testing, is now used to treat fungus infections. Liposomal Amphotericin B lowers renal and general toxicity by passively targeting the liver and spleen, but renal toxicity develops when the drug is administered at an increased dose because liver and spleen macrophages become saturated. By covering the vesicle with ostearoyl amylopectin, liposomes can also be directed toward the lungs. either mono-sialogangliocyte or polyoxylethylene. Antitubercular medicines like isoniazid and rifampicin can be encapsulated in lung-targeted liposomes to reduce their toxicity and increase their effectiveness [66,67]. Several clinical trials had authorised different Amphotericin formulations, and they are now available in several European nations [68].

In cancer therapy

Anticancer medication long-term therapy has a number of hazardous adverse effects. The development of liposomal therapy, which targets tumour cells with the fewest possible side effects, has transformed the field of cancer treatment. Small, stable liposomes are allegedly passively targeted to certain tumours because they can circulate for a longer period of time and extravasate in tissue with increased vascular permeability. [67,68]

Doxil is the intravenous chemotherapy drug doxorubicin's liposomal formulation. The innovative technology known as stealth technology, or stealth liposome, is used to prepare Doxil. These lengthy circulatory liposomes are made in a variety of ways. The liposomal versions of doxorubicin are Caelyx and Myocet. Advanced breast cancer is being treated with Caelyx, which was previously only used to treat metastatic ovarian cancer. For metastatic breast cancer, Myocet has been approved [69-71].

Marketed Preparations [72]

Molecule	Treated disease	Product	Company	Status
Doxorubicin	Kaposi's sarcoma and AIDS-related cancers. Ovarian cancer and multiple myeloma.	DOXIL	Ben Venue Laboratories for Johnson and Johnson, USA	On the market since 1995 (USA) and 1996 (Europe)
Amphotericin B	Systemic fungal infections	AMBISOME	First, NeXstar Pharmaceuticals which was acquired by Gilead Sciences in 1999. Thus, the drug is marketed by Gilead in Europe and licensed to Astellas Pharma (formerly Fujisawa Pharmaceuticals) for marketing in the USA, and Sumitomo Pharmaceuticals in Japan	On the market since 1990 (Europe) and 1997 (USA)
		FUNGISOME AMPHOTEC ABELCET AMPHOLIP AMPHOCIL	Lifecare Innovations, India Intermune, USA Enzon Pharmaceuticals, USA AMPHOLIP Bharat Serums & Vaccines, India Samaritan pharmaceuticals, USA	
Daunorubicin	Specific types of leukaemia (acute myeloid leukemia and acute lymphocytic leukemia).	DAUNOXOME	First, NeXstar Pharmaceuticals. Then the drug was sold to Diatos in 2006	On the market since 1996 (USA and Europe)
Inactivated hepatitis A virus	Hepatitis A	EPAXAL	Crucell Company who merged with the Swiss Serum and Vaccine Institute in 2006	On the Swiss market since 1994 and now in more than 40 country
Lidocaine	Anaesthesia for skin itching, burning or pain.	LMX4 LMX5	Ferndale Laboratories, USA	On the US market since 1998

Conclusion

The current work has been a successful attempt to create liposomes and put them into cream before evaluating how well they provide sustained drug delivery. The produced liposomes may prove to be a possible option for safe and effective sustained drug administration over an extended length of time, which can minimise dosing frequency, according to results of a skin permeation study and an in vivo investigation. Advantages of liposomes include the fact that they are suitable for delivering hydrophobic, amphipatic, and hydrophilic drugs and agents since they provide both an aqueous microenvironment and a lipophilic environment. The market offers a variety of liposome products, including DOXIL, AMBISOME, FUNGISOME, ABELCET, AMPHOLIP, AMPHOTEC Liposomes are a useful tool for improving the stability of active chemicals in cosmetic products, hydrating the skin by adhering to the skin's surface, improving dermal bioavailability and skin targeting, and shielding skin cells from external stressors like sweat and sunlight.

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