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A COMPREHENSIVE REVIEW ON LIPOSOMES: A NOVEL DRUG DELIVERY SYSTEM

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ABSTRACT: Liposome was derived from two Greek words "Lipos" meaning fat, and "Soma" meaning body. Liposome, where spherical-shaped vesicles consist of phospholipids and cholesterol vesicles which are under extensive investigation as drug carriers for improving the bioavailability and delivery of therapeutic agents. Due to innovative developments in liposome technology, numerous liposome-based drug delivery systems are currently in a clinical trial, and recently some of them have been approved for clinical use. Due to their size, hydrophobic and lipophilic character, they are up-and-coming systems for drug delivery. This novel drug delivery system aims to target the drug directly to the site of action. Liposomes are biocompatible and stable They have the unique property to entrap both hydrophilic drug and lipophilic drug (amphipathic nature) to its compartment and lead to a controlled-release effect. They are 0.05to 5micrometertre in diameter. Liposomes are used for the treatment of various diseases like tumours or cancer. This review describes the current state-of-the-art of these new liposomal delivery systems classification, formulation, characterization, and potential applications of liposomes in drug delivery.

INTRODUCTION: Paul Ehrlich in 1906 initiated the era of development for targeted delivery when he envisaged a drug delivery mechanism that would target drug directly to diseased cells, what he called as magic bullets, liposomes are spherical shaped small vesicles that can be produced from cholesterols, non-toxic surfactants, sphingolipids, glycolipids, long-chain fatty acids and even membrane proteins. Phospholipids spontaneously form a closed structure when dissolved in water with internal aqueous environment bounded by phospholipids bilayer membranes; this makes the transport of drug to be easy.

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Because the nature phospholipid that is amphipathic contain hydrophobic and hydrophilic nature in aqueous solution. The phospholipid bilayers membranes of liposomes are able to generate a spherical structure with a hydrophilic compartment internally when the liposomes are introduced in hydrophilic solution.

The hydrophilic drug has stored the center of the liposome; the hydrophobic drug is stored in the hydrophobic region in the liposome [1]. this vesicular system is called liposome [2]. Liposomes are the drug carrier loaded with different variety of molecules such as small drug molecules, proteins, nucleotides, and even plasmids. Liposome very useful because act as a carrier for a variety of drugs, having a potential therapeutic action or other properties. A liposome is colloidal carriers, having a size range of 0.01–5.0 μm in diameter **Fig. 1** Drug encapsulated by liposome achieve therapeutic level for long duration as drug must first be release from

liposome before metabolism and excretion, they are small artificial vesicles of spherical shape that can be created from cholesterol and natural non-toxic phospholipids. Due to their size and hydrophobic and hydrophilic character (besides bio-compatibility), liposomes are promising systems for drug delivery. Liposomes' unique ability to entrap drugs of both aqueous and the lipid phase makes them attractive drug delivery systems for hydrophilic and hydrophobic drugs [3]. Liposomes are a novel drug delivery system that aims to deliver the drug directly to the place of action. They have the potential to accommodate both hydrophilic and lipophilic compounds to protect the drug from degradation and release the active ingredients in a controlled manner [4]. It has been found that glycerol is the backbone of a molecule that's why phospholipid containing glycerol was found to be an essential component of liposomal formulation, and it represents 505 of lipid weight [5].

Furthermore, liposomes have the potential to be engineered to tailor their properties, such as PEGylation, which enables the nanocarrier to be in a "stealth" mode, avoiding being engulfed by phagocytes of the *in-vivo* immune system [6]. Additionally, modification of liposome has successfully enhanced encapsulation, targeted delivery, and release abilities.

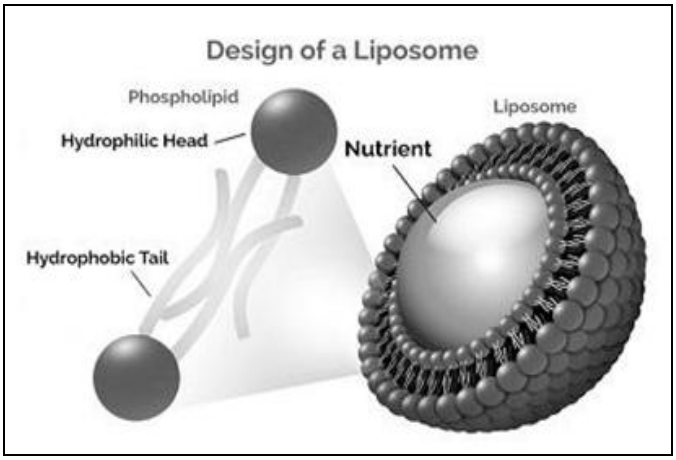


FIG. 1: DESIGN OF LIPOSOME

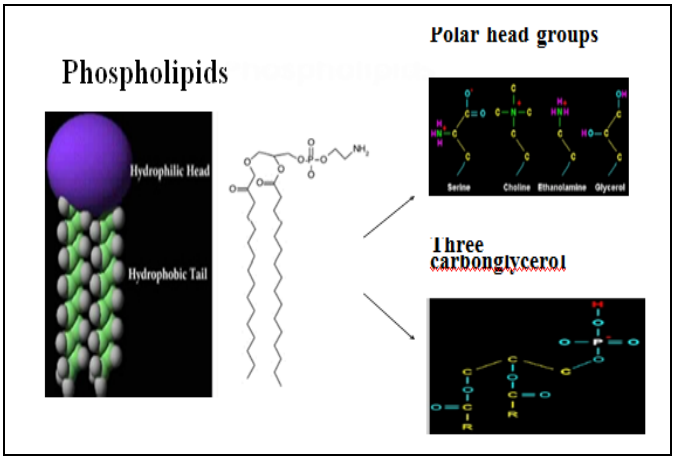


FIG. 2: PHOSPHOLIPIDS

The structural components are:

1) Phospholipids: Phospholipids are the major structural components of liposome. The most common phospholipids used in liposomal preparation are Phosphatidylcholine **Fig. 2**. Phosphotidyl-choline is an amphipathic molecule consist of-

- A hydrophilic polar head group, phosphocholine
- A glycerol bridges
- A pair of hydrophobic acyl hydrocarbon chains

The chemical structure of naturally occurring Phosphatidylcholine has a glycerol moiety attached to two acyl chains which may be saturated or unsaturated. The stability of liposome membrane depends on the packing of hydrocarbon chains of the lipid molecules [7]. The nature of the fatty acid in lipid molecule, such as a number of double bonds in the chain, is responsible for bilayer properties such as elasticity and phase behavior8. Phospholipids are very abundant in nature, and which contains choline is used for the preparation of liposomes. Examples of phospholipids are-

- Phosphotidyl choline (Lecithin) PC
- Phosphotidyl serine (PS)
- Phosphotidyl Glycerol (PG)
- Phosphotidyl ethanolamine (Cephalin)-PE

Lipids all have a temperature at which their fluidity changes. This temperature is also known as transition temperature (TC). The TC is directly proportional to the length of the acyl chain; the longer the chain, the higher the TC and the more rigid the membrane. More rigid membranes keep entrapped drugs inside, or in other words, prevent

leakage. The TC is very important, as it can affect the way the membrane reacts to fusing with other liposomes, stability, aggregation, permeability as well as contributing to the way the liposomes react in the presence of biological systems [9].

2) Cholesterol: Cholesterol is another important structural component of liposome. It is a commonly used sterol. The addition of sterols modulates the function of stability and rigidity, increases the time of circulation in the bloodstream [9]. It does not by itself form a bilayer structure.

It gets incorporated into phospholipids in a very high concentration up to 1:1 or 2:1 molar ratio of cholesterol to phosphotidyl choline. The presence of cholesterol in the lipid bilayer enhances the stability and form a highly ordered and rigid membrane structure [10].

Cholesterol reduces the permeability of water-soluble molecules and improves the fluidity and stability of the biological membrane. The interaction and destabilization of liposomes were prevented by cholesterol [11].

Advantages of Liposomes: Amphipathic in nature so entrap both kind of drugs, either water-soluble or insoluble [12, 13]

- It offers targeted drug delivery
- Increased efficacy and therapeutic index of drug
- Non-ionic
- Liposome helps to reduce exposure of sensitive tissues to toxic drugs
- Provides selective passive targeting to tumour tissue
- Prevent oxidation of drugs
- Liposomes are biodegradable
- Biocompatible
- Liposome increases the stability of drug
- Site avoidance effect
- Improve protein stabilization
- Provide sustained release
- Direct interaction of drug with cell
- Site avoidance effect

Disadvantages of Liposomes:

- Low solubility
- Short half-life
- Production cost is high
- Leakage and fusion of encapsulated drug may occur
- Oxidation of phospholipids may occur
- Allergic reactions may occur to liposomal constituents [14]
- Less stable

Classification of Liposomes: The liposomes may be classified based on various parameters,

1. Structure **Table 1**
2. Method of preparation **Table 2**
3. Composition **Fig. 3** and **Table 3**
4. Conventional liposome
5. Specialty liposome

1. Classification Based on Structure:

TABLE 1: LIPOSOMES BASED ON VESICLE TYPE

Vesicle type	Diameter Size	No. of Lipid Layer
Multi lamellar large vesicles (MLV)	More than 0.5 μm	5-25
Oligo lamellar vesicles (OLV)	0.1-1.0 μm	Approx. 0.5
Uni lamellar vesicles (UV)	All size ranges	1
Small Uni lamellar vesicles (SUV)	20-100 nm	1
Medium sized Uni lamellar vesicles (MUV)	More than 100nm	1
Large Uni lamellar vesicles (LUV)	More than 100nm	1
Giant Uni lamellar vesicles (GUV)	More than 1.0 μm	1
Multi Vesicular vesicles (MVV)	More than 1.0 μm	Multicompartmental structure

2. Based on Method of Preparation:

TABLE 2: DIFFERENT PREPARATION METHODS AND THE VESICLES FORMED BY THESE METHODS

Preparation Method	Vesicle Type
Single or oligo lamellar vesicle made by reverse-phase evaporation	REV (Reverse- Phase Evaporation Method.)
Multilamellar vesicle made by a reverse-phase evaporation method	MLV-REV (Multilamellar vesicles made by Reverse-Phase Evaporation method)
Stable pluri lamellar vesicle	SPLV (Stable PlurilamellarVesicles)
Frozen and thawed multilamellar vesicle	FATMLV (Frozen and Thawed MLV)
Vesicle prepared by extrusion technique	VET (Vesicles prepared by extrusion technique)
Dehydration- Rehydration method	DRV (Dehydration-rehydration method)

3. Based on Composition:

TABLE 3: DIFFERENT LIPOSOME WITH THEIR COMPOSITIONS

Type	Abbreviation	Composition
Conventional	CL	Neutral or negatively charge phospholipids and cholesterol
Fusogenic	RSVE	Reconstituted sendai virus envelops
pH-sensitive	-	Phospholipids such as PER or DOPE with either CHEMS or OA
Cationic	-	Cationic lipid with DOPE
Long circulatory	LCL	Neutral high temp, cholesterol and 5-10% PEG, DSP
Immuno	IL	CL or LCL with attached monoclonal antibody or recognition sequences

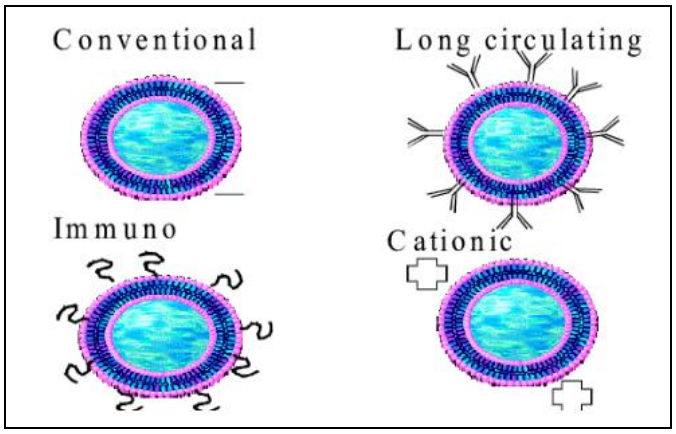


FIG. 3: CLASSES AND COMPOSITION OF LIPOSOMES

4. Based Upon Conventional Liposome

- Natural lecithin mixtures
- Synthetic identical, chain phospholipids
- Liposome with Glycolipids

5. Based Upon Speciality Liposome

- Bipolar fatty acid
- Antibody directed
- Methyl/ Methylene x- linked
- Lipoprotein coated
- Carbohydrate coated
- Multiple encapsulated

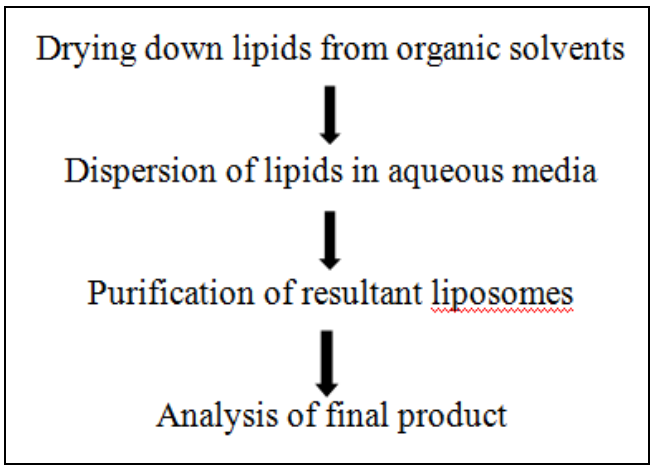
Mechanism of Vesicle Formation: Lipid vesicles are formed when thin lipid films are hydrated and swelled. The hydrated lipid sheets detach during agitation and form large MLV, which prevents water interaction with the bilayer's hydrocarbon core at the edges. Once the particles formed, it is size reduced by sonication or by extrusion

Liposome performs their action by four different mechanisms. They are as follows:

- **Endocytosis:** This takes place by phagocytic cells of the reticuloendothelial system, such as neutrophills
- **Adsorption:** It occurs to the cell surface by nonspecific electrostatic forces or by interaction with cell surface components [15].
- **Fusion:** It occurs by the insertion of liposomal bilayer into plasma membrane with continuous release of liposomal content into the cytoplasm [16].
- **Lipid Exchange:** In this transfer of liposomal lipids to the cellular membrane without association of liposomal contents [17].

Methods of Preparation of Liposomes: There are different methods involved in the preparation of liposomes [18].

1) General Method of Preparation: It involves four steps for the preparation of liposomes



2) Passive Loading Techniques: [19]

A) Mechanical Dispersion: This is the common and most widely used method for the preparation of MLV. A round-bottomed flask can be used for the preparation. The method involves the formation of a thin film by drying the lipid solution and then hydrating the film by adding an aqueous buffer and vortexing the dispersion. The hydration step is done at a temperature above the gel liquid crystalline transition temperature of the lipid or above the transition temperature of the highest melting component in the lipid mixture. Depending upon their solubilities the compounds to be encapsulated are added either to an aqueous buffer or to the organic solvent containing lipids. The disadvantages of the method include low internal volume, less encapsulation efficiency and varying size. The less encapsulation efficiency can be overcome by hydrating the lipids in the presence of immiscible organic solvents like petroleum ether, diethyl ether. Then it is emulsified by sonication. MLVs are formed by removing the organic layer bypassing nitrogen.

Hand Shaken Method: This is the simplest and widely used method. The lipid mixture and charged components are dissolved in chloroform and methanol mixture (2:1 ratio), and then this mixture is introduced in to a 250 ml round-bottomed flask. The flask is attached to a rotary evaporator connected with a vacuum pump and rotated at 60 rpm. The organic solvents are evaporated at about 30 degrees. A dry residue is formed at the walls of the flask and rotation is continued for 15 min after dry residue appeared.

The evaporator is detached from the vacuum pump, and nitrogen is introduced into it. **Fig. 4**. The flask is then removed from the evaporator and fixed onto lyophilizer to remove residual solvent. Then the flask is again flushed with nitrogen, and 5 ml of phosphate buffer is added. The flask is attached to the evaporator again and rotated at about 60 rpm speed for 30 min or until all lipid has been removed from the wall of the flask. A milky white suspension is formed finally. The suspension is allowed to stand for 2 h in order to complete the swelling process to give MLVs.

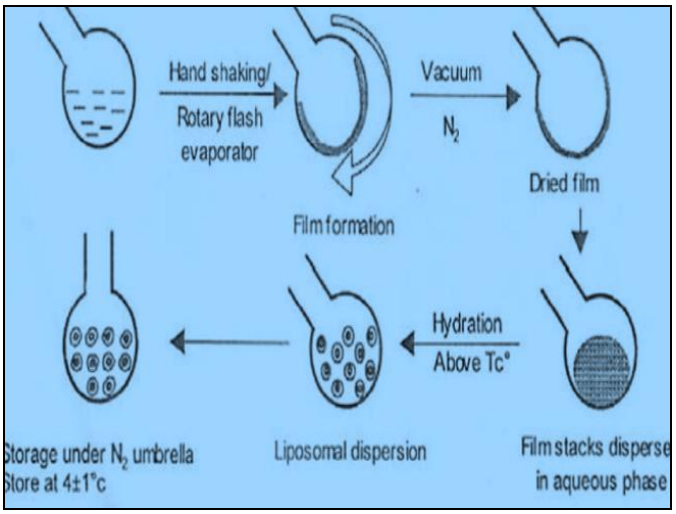


FIG. 4: METHOD OF PREPARATION OF LIPOSOMES BY HANDSHAKING METHOD

Non –Shaking Vesicles: Method described by reeves and down in 1996 by which large unicellular vesicles (LUVv) can be formed with higher entrapment volume. The procedure differs from the hand-shaken method in that it uses a stream of nitrogen to provide agitation rather than rotational movements. Solution of lipid in chloroform: methanol mixture is spread over the flat bottom conical flask.

The solution is evaporated at room temperature by the flow of nitrogen through the flask without disturbing the solution; after drying, water-saturated nitrogen is passed through the flask until the opacity of the dried film disappears (15-20 mins). After hydration, the lipid is swelled by the addition of bulk fluid. The flask is inclined to one side, 10-20ml of 0.2 sucrose in distilled water (degassed) is introduced down the slide of the flask and the flask is slowly returned to upright orientation. The fluid is allowed to run gently over the lipid layer on the bottom of the flask.

The flask is flushed with nitrogen, sealed and allowed to stand for 2 h at 37 degrees Celsius. Take care not to disturb the flask in any way. After swelling the vesicles are harvested by swirling the contents to yield a milky suspension.

Sonication: This is the method in which Multi lamellar vesicles are transformed to the small Unilamellar vesicles. The ultra-sonic irradiation is provided to the MLVs to get the SUVs. **Fig. 5** There are two methods are used. a) Probe sonication method. b) Bath sonication method.

The probe is employed for dispersion, which requires high energy in small volume (*e.g.*, high conc. of lipids or a viscous aqueous phase) while is more suitable for large volumes of diluted liquid Probe tip sonicator provides high energy input to the liquid dispersion but suffer from overheating of liposomal dispersion causing lipid degradation. The Sonication tip also releases titanium into the liposome dispersion, which will be removed from it by centrifugation prior to use.

Due to above reason most widely the bath sonicator are used Sonication of MLVs is accomplished by placing dispersion into the bath sonicator or placing tip of probe sonicator into the test tube of dispersion. (5-10 min.) After sonication applied the resultant dispersion is centrifuged and according to diagram the SUVs will stay on the top and the small MLVs and aggregated lipids will get settled down. The top layer constitutes pure dispersion of SUVs with a varying diameter as size is influenced by composition and concentration, temperature, sonication, volume and sonication tuning.

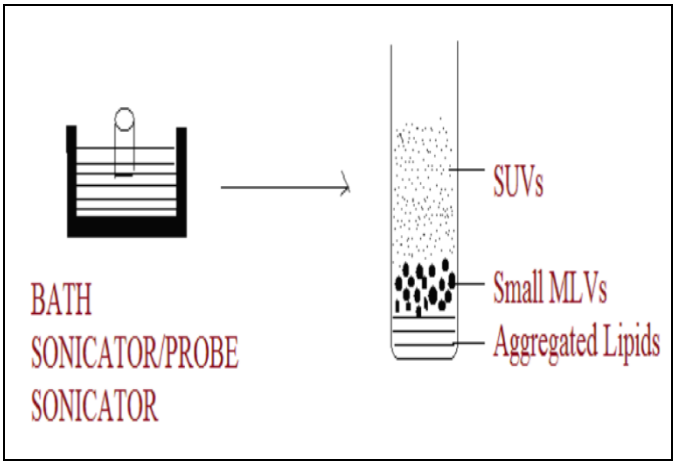


FIG. 5: METHOD OF PREPARATION OF LIPOSOMES BY SONICATION

Hydration of Lipid Layer: After releasing the vacuum and removal from the lyophilizer, the flask is flushed with nitrogen, 5 ml of saline phosphate buffer (containing a solute to be entrapped) is added. The flask is attached to the evaporator again (flushed with N2) and rotated at room temperature and pressure at the same speed or below 60 rpm.

The flask is left rotating for 30 min or until all lipid has been removed from the wall of the flask and has given homogenous milky–white suspension free of visible particles.

The suspension is allowed to stand for a further 2 h at room temperature or at a temperature above the transition temperature of the lipid in order to complete the swelling process to give MLVs

Micro Emulsification Liposomes: A microfluidizer is used to prepare small MLVs from concentrated Lipid dispersion. Micro fluidizer pumps the fluid at very high pressure (10,000 psi), through a 5-micrometer orifice.

Then, it is forced along defined micro-channels which direct two streams of fluid to collide together at the right angles at a very high velocity, thereby affecting an efficient transfer of energy. The lipids can be introduced into the fluidizer, either as large MLVs or as the slurry of unhydrated lipid in an organic medium.

The fluid collected can be recycled through the pump and interaction chamber until vesicles of spherical dimensions are obtained. Diameter After a single pass, the size of vesicles is reduced to a size 0.1 and 0.2 um **Fig. 6.**

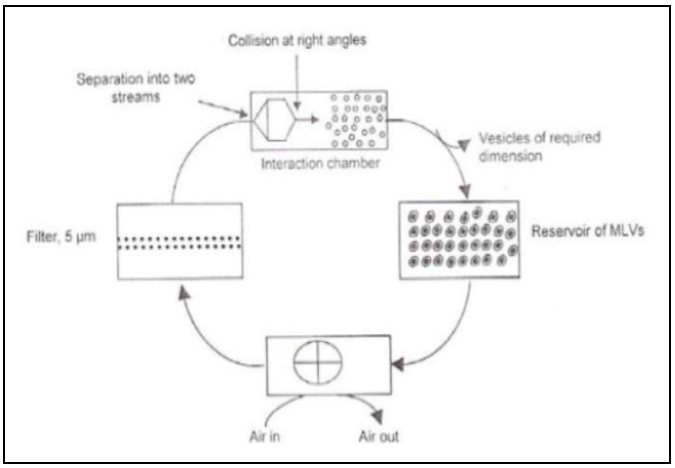


FIG. 6: MICRO EMULSIFICATION LIPOSOMES

Proliposomes: In Proliposomes, lipid and drug are coated onto a soluble carrier to form free-flowing granular material, which on hydration forms an isotonic liposomal suspension.

The Proliposomes approach may provide an opportunity for cost-effective large-scale manufacture of liposomes containing the particularly lipophilic drug.

Freeze-Thaw Sonication: This method is explained based upon freezing of a unilamellar dispersion (SUV) **Fig. 7**.

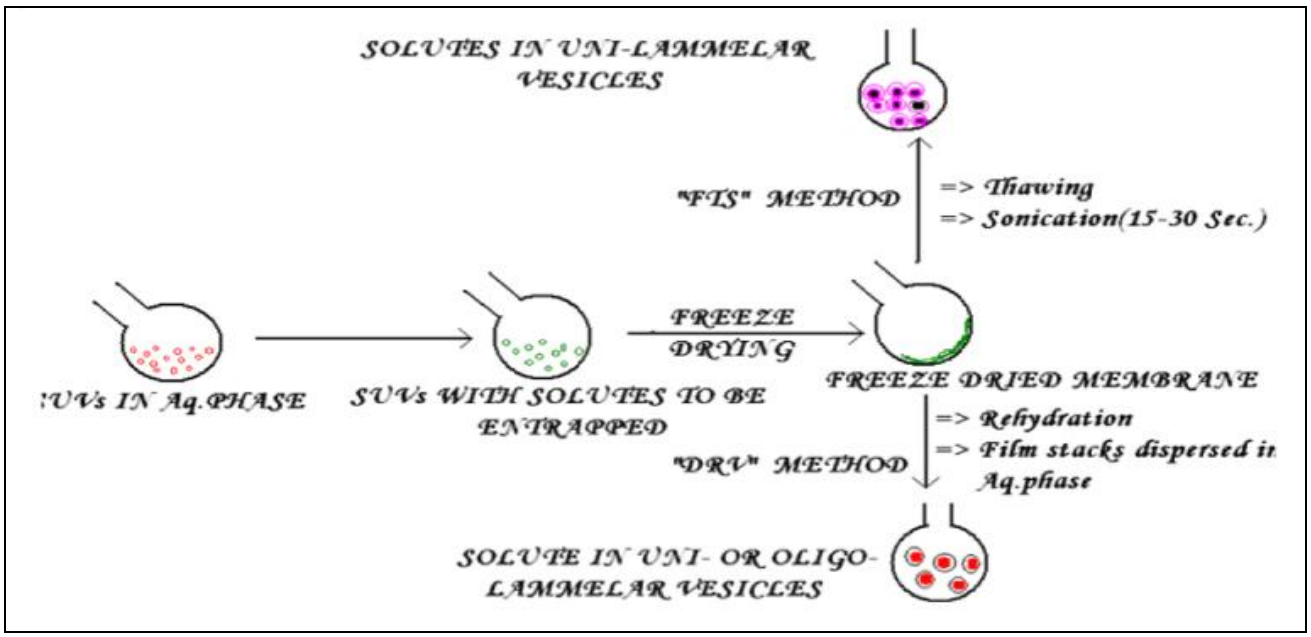


FIG. 7: METHOD OF PREPARATION OF LIPOSOMES BY FREEZE-THAW SONICATION

Freeze Drying: Another method of dispersing the lipid in a finely divided form prior to the addition of aqueous media is to freeze-dry the lipid dissolved in a suitable organic solvent. The solvent usually used is tertiary butanol. All the above methods produce MLVs. These are too large or too heterogeneous. To modify the size, the prepared MLVs are further processed using the following procedures.

B) Solvent Dispersion:

Ether and Ethanol Injection:

Ethanol Injection: The method involves the extrusion of MLV at 20,000 psi at 4 °C through a small orifice **Fig. 8.** The method has several advantages over the sonication method. The method is simple rapid, reproducible, and involves gentle handling of unstable materials [20]. A lipid solution of ethanol is rapidly injected to a vast excess of buffer. The MLVs are immediately formed. The drawbacks of the method are that the population is heterogeneous (30-110 nm), liposomes are very dilute, it is difficult to remove all ethanol because it forms azeotrope with water and the possibility of various biologically active macromolecules to inactivation in the presence of even low amounts of ethanol [21].

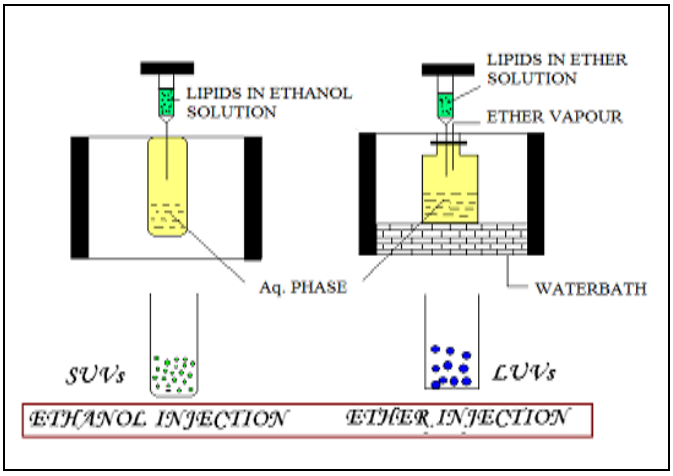


FIG. 8: METHOD OF PREPARATION OF LIPOSOMES BY SOLVENT DISPERSION

Reverse Phase Evaporation Method: This method provided progress in liposome technology. Reverse-¬-phase evaporation is based on the creation of inverted micelles these are shaped upon sonication of a mixture of a buffered aqueous phase, which contains the water-soluble molecules

to be encapsulated into the liposomes and an organic phase in which the amphiphilic molecules are solubilized. Elimination of the organic solvent leads to the conversion of these inverted micelles into viscous state and gel form. At a critical point in this process, the gel state collapses, and some of the inverted micelles were disturbed, and excess of phospholipids in the environment donates to the formation of a complete bilayer around the residual micelles, which results in the creation of liposomes **Fig. 9.** The method's main disadvantage is the contact of the materials to be encapsulated to organic solvents and brief periods of sonication, which may result in the breakage of DNA strands or the denaturation of some proteins.

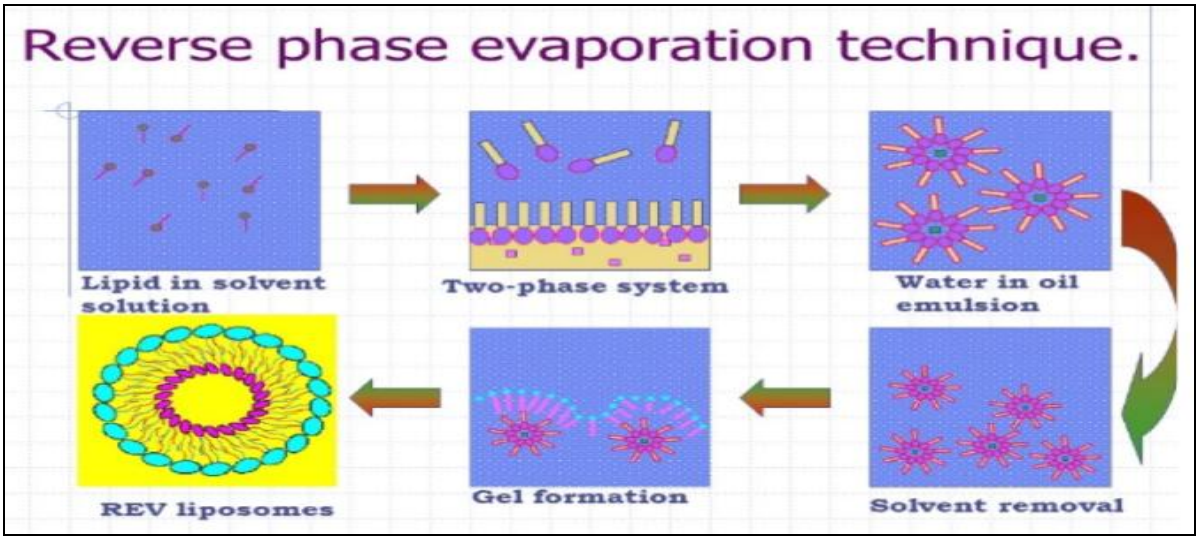


FIG. 9: METHOD OF PREPARATION OF REVERSE-PHASE EVAPORATION METHOD

Double Emulsification: In this method, a primary emulsion is prepared by dissolving the drug in an aqueous phase (w1) which is then emulsified in an organic solvent of a polymer to make a primary w1/o emulsion. This primary emulsion is further mixed in an emulsifier containing an aqueous solution (w2) to make a w1/o/ w2 double emulsion. The removal of the solvent leaves' microspheres in the aqueous continuous phase, which are collected by filtering/centrifuging **Fig. 10**.

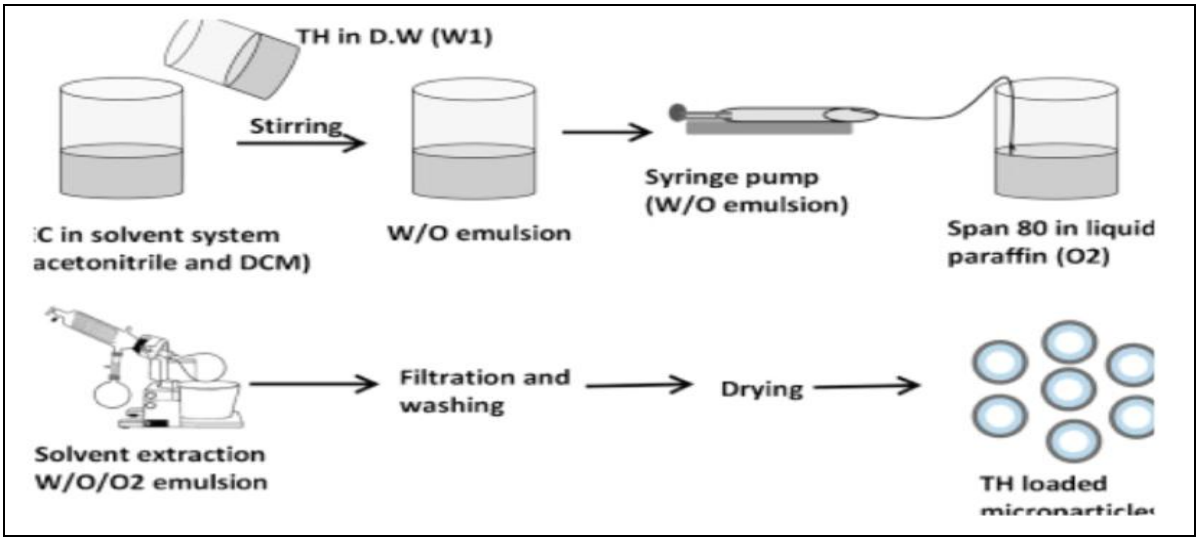


FIG. 10: METHOD OF PREPARATION OF DOUBLE EMULSIFICATION

C) Detergent Removal Method:

Solubilization and Detergent Removal Method: This method is used in the preparation of LUVs and it involves the use of detergent (surfactant) for the solubilization of the lipids. Detergents used include the nonionic surfactants, e.g., n-octyl beta, D-glucopyranose (octyl gluside), anionic surfactants (*e.g.*, dodecyl sulphate), and cationic

surfactants (*e.g.,* hexadecyl trimethyl ammonium bromide). The procedure involves the solubilization of the lipids in an aqueous solution of the detergent and the protein(s) to be encapsulated. The detergent should have a high critical micelle concentration (CMC) to be easily removed. The detergent is subsequently removed by dialysis or column chromatography. During detergent removal, LUVs of diameter 0.08–0.2 μm are produced [37]. Detergent/Phospholipid's mixtures can form large unilamellar vesicles upon removal of non-ionic detergent using appropriate adsorbents for the detergent. in this method, the phospholipid is brought into intimate contact with the aqueous phase via the intermediary of detergent, which is associated with phospholipid molecule from water, in structural formed as a result of this associated are known as micelles and can be composed of several hundred component molecules. Their shape and size depend on the chemical nature of the detergent, the concentration, and other lipid involved. The concentration of detergent in water at which micelles from is known as critical micelle concentration (CMC) [22].

To Remove the Detergent and all the Transition of Mixed Micelles to Concentric Bilayered from 3 Methods:

Dialysis: Dialysis In contrast to phospholipids, detergents are highly soluble in both aqueous and organic media, and there is equilibrium between the detergent molecules in the water phase and in the lipid environment of the micelles. Upon lowering the concentration of the detergent in the bulk aqueous phase, the molecules of the detergents can be removed by dialysis. *E.g.,* of detergent: bile salts sodium cholate and sodium deoxycholate and synthetic detergents such as octyl glucoside. Dialysis: Egg PC + sodium cholate (2:1) vesicles (100 nm) Trade name-LIPOREP dialysis **Fig. 11**.

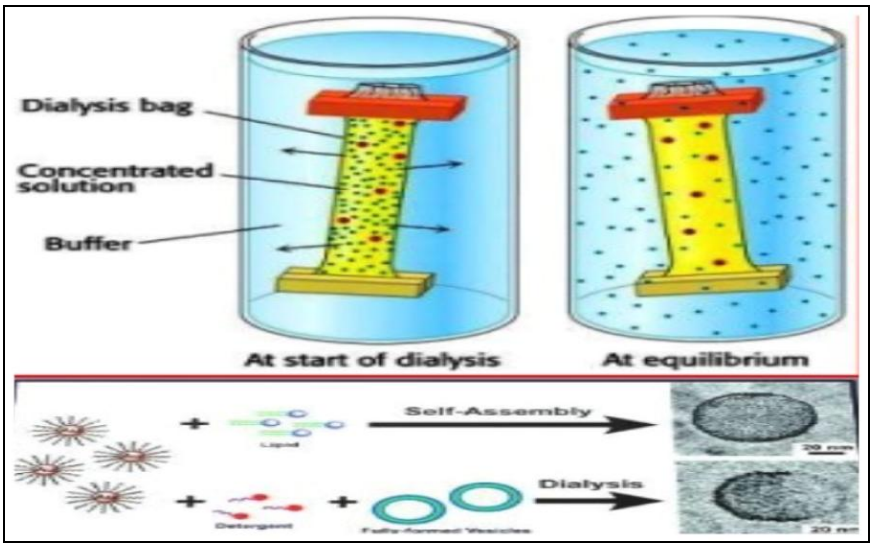


FIG. 11: DIALYSIS METHOD

Column Chromatography: Column chromatography Phospholipids + deoxycholate (sonicated vesicles 2:1 or as a dry film) removal of deoxycholate by Column chromatography (Sephadex G-25) Unilamellar vesicles (100 nm).

Detergent Adsorption using Bio-Beads: Detergent adsorption using bio-beads detergent (non-ionic)/phospholipids mixtures can form LUVs by removal of non-ionic detergent (Triton X-100) using appropriate adsorbents for the detergent. *E.g.,* Casted lipid film + 0.5-1.0% Triton X-100 + washed bio-beads (0.3g/ml of dispersion) and rocked for about hrs at 4 ± 1 ºC gives LUVs.

3) Active Loading Technique: The utilization of liposomes as a drug delivery system is stimulated with the advancement of efficient encapsulation procedures. The membrane from the lipid bilayer is in general impermeable to ions and larger hydrophilic molecules. The ionospheres can regulate ion's transport while concentration gradients can control the permeation of neutral and weakly hydrophobic molecules. However, some weak acids or bases can be transported through the membrane due to various transmembrane gradients, such as electric, ionic (pH) or specific salt (chemical potential) gradient. Several methods

exist for improved loading of drugs, including remote (active) loading method, which loads drug molecules into preformed liposome using pH gradient and potential difference across liposomal membrane. A concentration difference in proton concentration across the membrane of liposomes can drive the loading of amphipathic molecule.

- Active loading methods have the following advantages over passive encapsulation Technique
- A high encapsulation efficiency and capacity.
- A reduced leakage of the encapsulated compounds.
- "Bed side" loading of drugs thus limiting loss of retention of drugs by diffusion, or chemical degradation during storage.
- Flexibility of constitutive lipid, as the drug is loaded after the formation of carrier unit.
- Avoidance of biologically active compounds during preparation step in the dispersion thus reducing safety hazards.
- The transmembrane pH gradient can be developed using various method. Depending upon the nature of the drug to be encapsulated.

Drug Loading in Liposomes: Drug loading can be attained either passively (drug is encapsulated during liposome formation) or actively (after liposome formation). Hydrophobic drugs can be directly combined into liposomes during vesicle formation, and the amount of uptake and retention is governed by drug lipid interactions. Trapping effectiveness is dependent on the solubility of the drug in the liposome membrane. Passive encapsulation of hydrophilic drugs depends on the ability of liposomes to trap aqueous buffer containing a dissolved drug during vesicle formation [23].

Purification of Liposomes: Liposomes are commonly purified by either gel filtration column chromatography or by dialysis or centrifugation. In column chromatographic separation Sephadex G-50 is the most widely used material. In this column, chromatographic separation liposome membrane may bind or interact with the surface of the polydextran beads. There may be a small amount of lipid lost, resulting into the destabilization of the membrane leading to permeability changes and subsequent leakage of entrapped solute. This problem can be overcome either by avoiding forming too small size liposomes of the same lipid composition as the test sample either before or after the column's packing. In the dialysis method, hollow fiber dialysis cartridge may be used. In the centrifugation method, SUVs in normal saline may be separated by centrifuging at 200000 g, for 10-20 hours. MLVs are separated by centrifuging at 100000 g for less than one hour [24, 25].

Sizing of Liposomes: Size characteristics of liposome have a major effect on the application they can be used. Physical integrity and stability of lipid bilayer's structure influence the therapeutic applications of liposome. Therefore, the particle size of the liposome must be considered for the liposome Production procedure, and it must be predictable and reproducible with particle size distribution within a certain size range. Sequential extrusion, gel chromatography, and sonication are the common methods of sizing liposomes,26but ultimately, these methods have the following

Disadvantages:

- Exclusion of oxygen is difficult, which results in a peroxidation reaction.
- Titanium probes shed metal particles resulting in contamination.
- They can generate aerosols, which exclude them from.

Use with Certain Agents: These above problems are mainly related to the probe sonication, but these problems can be removed using bath sonication.

Pharmacokinetics of Liposomes:

- Liposomal drugs can be applied through various routes, but mainly i.v and topical administration is preferred. After reaching in the systemic circulation or in the local area, a liposome can interact with the cell by any of the following methods.

- Endocytosis by phagocytotic cells of the R.E.S such as macrophages and neutrophils.
- Adsorption to the cell surface either by nonspecific week hydrophobic or electrostatic forces or by specific interaction with cell surface components.
- Fusion with the plasma cell membrane by insertion of the lipid bilayer of liposome into plasma membrane with the simultaneous release of liposomal contents into the cytoplasm.
- Transfer of liposomal lipids to cellular or sub-cellular membrane or vice versa without any association of the liposome contents.
- It is often difficult to determine what mechanism is operative and more than. One may operate at the same time **Fig. 12**.

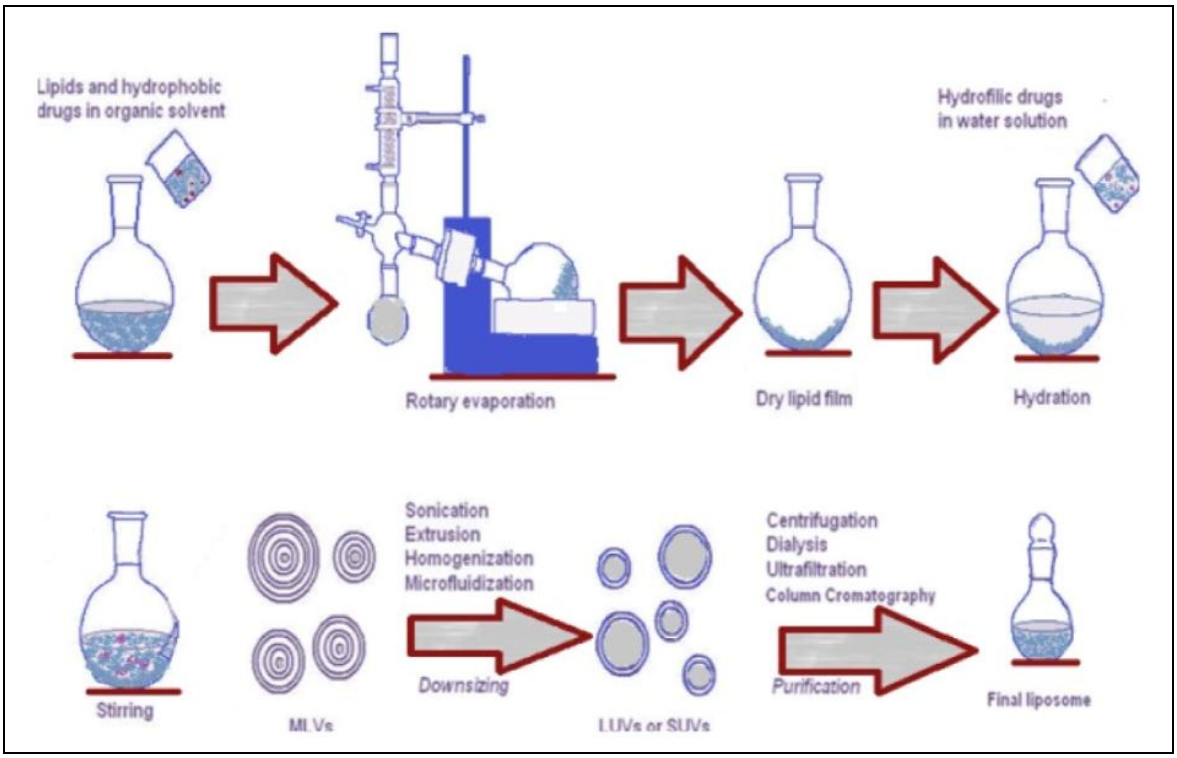


FIG. 12: REPRESENTATION OF LIPOSOME PRODUCTION BY LIPID HYDRATION FOLLOWED BY VORTEX

Pharmacodynamics of Liposome Encapsulated Drugs: To get the action of drugs to a particular site in the body, the general approach is to deposit drug bearing liposome directly into the site where therapy is desired. The liposomes slowly release drug into the target site, and otherwise, the drug-loaded liposomes might interact directly with cells in the target site, without producing release. The goal of this approach is to maximize the amount of effective drug at the target site and to decrease systemic toxicity.

Stability of Liposomes: Therapeutic efficacy of drug molecules is governed by the stability of liposomes. A stable dosage form maintains the physical stability and chemical integrity of the active molecule during its developmental procedure and storage [27]. There are two types of stability-

Physical Stability: There are various physical processes that affect liposomes' shelf life like fusion, aggregation, and shape and size. The general problem that occurs is leakage of drug material.

The morphology and size distribution are important parameters for accessing stability. Physical stability can be maintained by avoiding excess unsaturation in the phospholipids. They must be stored at 4 ºC with no freezing and light exposure [28].

Chemical Stability: Phospholipids are unsaturated fatty acids prone to hydrolysis alter the stability of drug products. Liposomes can be prevented from oxidative degradation by adding antioxidants such as butylated hydroxyanisole. Usually, liposomes may create problems in stability during the storage

periods. In general, certain parameters should be considered to achieve the successful formulation of the stable liposomal drug product.

- Processing with fresh, purified lipids and solvents.
- Avoidance of high temperature and excessive
- Shearing stress.
- Maintenance of low oxygen potential
- Use of antioxidant or metal chelators.
- Formulating at neutral pH.
- Use of lyo-protectant when freeze-drying.

Entrapment of Drugs into Liposome Bilayers: Liposomes, because of their biphasic character, can act as a carrier for both lipophilic and hydrophilic drugs.

Depending upon their solubility and partitioning characteristics, the drug molecules are located differently in the liposomal environment and exhibit different entrapment and release properties **Table 4**.

TABLE 4: ENTRAPMENT OF DRUGS INTO LIPOSOME BILAYERS

Lipophilic drugs	Hydrophilic drugs	Amphiphilic drugs	Biphasic insoluble drugs
High Entrapment	Low Entrapment	High Entrapment	Poor Entrapment
Low Leakage	Bilayer Composition Dependent Leakage	Rapid Leakage	
Chemical Stability	Hydrolytic Degradation		

Characterization of Liposomes: Liposome should be characterized for visual appearance, turbidity, size distribution, lamellarity, concentration, composition, presence of degradation products, and stability. These factors govern the behavior of liposomes in both physical and biological system; therefore, liposomes are characterized for physical attributes and chemical compositions [29, 30].

A. Biological Characterization Table 5:

TABLE 5: BIOLOGICAL CHARACTERIZATION

Characterization parameters	Analytical method
Sterility	Aerobic/anaerobic culture
Pyrogen city	Temperature (Rabbit) response
Animal toxicity	Monitoring survival of animals (rats)

B. Chemical Characterization Table 6:

TABLE 6: CHEMICAL CHARACTERIZATION

Characterization parameters	Analytical method/Instrument
Phospholipid's concentration	HPLC/Barrlet assay
Cholesterol concentration	HPLC / Cholesterol Oxide assay
Drug concentration	Assay method
Phospholipid's peroxidation	UV observance
Phospholipid's hydrolysis	HPLC/ TLC
Cholesterol auto-oxidation	HPLC/ TLC
Anti-oxidant degradation	HPLC/ TLC
PH	PH meter
Osmolality	Osmometer

C. Physical Characterization Table 7:

TABLE 7: PHYSICAL CHARACTERIZATION

Characterization parameters	Analytical method /Instrument
Vesicle shape and surface morphology	Transmission electron microscopy, Freeze-fracture electron microscopy
Mean vesicle size and size distribution (submicron and micron range)	Dynamic light scattering, zetasizer, Photon correlation spectroscopy, laser light scattering, gel permeation and gel exclusion
Surface charge	Free-flow electrophoresis
Electrical surface potential and surface pH	Zeta potential measurements & pH-sensitive probes
Lamellarity	Small angle X-ray scattering, 31P-NMR, Freeze-fracture electron microscopy
Phase behaviour	Freeze-fracture electron microscopy, Differential scanning colorimetry
Percent of free drug/ percent capture	Minicolumn centrifugation, ion-exchange chromatography, radiolabelling
Drug release	Diffuse cell / dialysis

1. Visual Appearance: Liposome suspension can range from translucent to milky, depending on the composition and particle size. If the turbidity has a bluish shade, this means that particles in the sample are homogeneous; a flat, gray color indicates that presence of a nonliposomal dispersion and is most likely a disperse inverse hexagonal phase or dispersed microcrystallites. An optical microscope (phase contrast) can detect liposome> 0.3 μm and contamination with larger particles.

2. Determination of Liposomal Size: It is usually measured by dynamic light scattering. Liposomes with relatively homogeneous size distribution are reliable for this method. Gel exclusion chromatography is a simple method in which a truly hydrodynamic radius can be detected. Sephacryl-S100 can separate liposome in size range of 30-300 nm. Sepharose -4B and -2B columns can separate SUV from micelles.

3. Determination of Lamellarity: The lamellarity of liposomes is measured by electron microscopy or by spectroscopic techniques. The nuclear magnetic resonance spectrum of liposome is frequently recorded with and without the addition of a paramagnetic agent that shifts or bleaches the signal of the observed nuclei on the outer surface of liposome. Encapsulation efficiency is measured by encapsulating a hydrophilic marker.

4. Liposome Stability: Liposome should be physically, chemically, and biologically stable. Physical stability indicates the ratio of lipid to therapeutic agent and the steadiness of the size. The chemical stability may be affected by two degradation pathways, oxidative and hydrolytic. Oxidation of phospholipids in liposomes mainly takes place in unsaturated fatty acyl chain-carrying phospholipids. These chains are oxidized in the absence of particular oxidants. Reduction of oxidation can be achieved by storage at low temperatures and protection from light and oxygen Physical stability indicates mostly the constancy of the size and the ratio of lipid to active agent. The cationic liposomes can be stable at 4 °C for a long period of time, if properly sterilized.

5. Entrapped Volume: The entrapped volume of a population of liposome (in µL/ mg phospholipid) can often be deduced from measurements of the total quantity of solute entrapped inside liposome assuring that the concentration of solute in the aqueous medium inside liposomes is the same after separation from unentrapped material.

For example, in two-phase preparation methods, water can be lost from the internal compartment during the drying down a step to remove organic solvent [31].

% Entrapment efficiency = Entrappeed drug (mg) / Total Drug Added (mg) × 100

6. Surface Charge: Liposome is usually prepared using charge imparting constituting lipids, and hence it is imparting to study the charge on the vesicle surface. In general, two methods are used to assess the charge, namely free-flow electrophoresis and zeta potential measurement. From the mobility of the liposomal dispersion in a suitable buffer, the surface charge on the vesicles.

Applications of Liposomes: Liposomes have great pharmaceutical applications in oral and transdermal drug delivery systems. This drug delivery system achieves a reduction in the toxic effect and enhancement of the effectiveness of drugs. The targeting of liposome to the site of the action takes place by the attachment of amino acid fragments that target specific receptors cells. Several modes of drug delivery application have been proposed for the liposomal drug delivery system, a few of them are as follows:

1. Enhancement of Solubilisation (Amphotericin-B, Paclitaxel)

2. Protection of sensitive drug molecules (Cytosine arabinose, DNA, RNA, Ribozymes)

3. Enhancement of intracellular uptake (Anticancer, antiviral and antimicrobial drugs)

4. Alteration in pharmacokinetics and bio-distribution (prolonged or SR drugs with short circulatory half-life)

Several recent applications of liposomal drug delivery system are as follows

A. Liposome for Respiratory Drug Delivery System: Liposome is widely used in several types of respiratory disorders. Liposomal aerosols can be formulated to achieve sustained release, prevent local irritation, reduced toxicity, and improved stability. Whilst preparing liposomes for lung delivery, composition, size, charge, Drug/lipid ratio and drug delivery method should be considered. The liquid or dry form is taken for inhalation during nebulization. Drug powder liposome is produced by milling or by spray drying [32].

B. Liposomes in Ophthalmic Disorders: Dry eyes, keratitis, corneal transplant rejection, uveitis, endophthalmitis, and proliferative vitro retinopathy

are the examples of eye disorders against which liposomes have been found to possess beneficial effects. The drug verteporfin that is found to be effective against eye disorders has been recently approved as liposomal formulation.

C. Liposome as Vaccine Adjuvant: Liposome has been established firmly as an immune adjuvant that is potentiating both cells mediated and noncell mediated immunity. Liposomal immuno-adjuvant acts by slow release of encapsulated antigen on intramuscular injection and also by passive accumulation within the regional lymph node.

Depending on the lipophilicity of antigens, the liposome can accommodate antigens in the aqueous cavity or incorporate them within the bilayers [33]. The targeting of liposome does the accumulation of liposome to lymphoid with the help of phosphotidyl serine. The liposomal vaccine can be prepared by inoculating microbes, soluble antigens and cytokinesis of deoxyribonucleic acid with the liposomes [34, 35].

D. Liposomes for Brain Targeting: The biocompatible and biodegradable character of liposomes makes it's used in brain drug delivery system. Liposomes with a small diameter (100 nm) and large diameter undergo free diffusion through the BBB. However small unilamellar vesicles (SUVs) coupled to brain drug transport vectors may be transported by Receptor-mediated or absorptive mediated transcytosis through the BBB.

Cationic liposomes undergo absorptive mediated endocytosis into cells whereas the same undergoing absorptive mediated transcytosis through the BBB has not yet been determined. Liposomes coated with the mannose reach brain and assist the transport of loaded drug through BBB. The neuropeptides, leuenkephaline and mefenkephalin kyoforphin normally do not cross BBB when given systemically. The anti-depressant amitriptyline normally penetrates the BBB, due to the versatility of this method.

E. Liposome as Anti-Infective Agents: The diseases like leishmaniosis, candidiasis, aspergillosis, histoplasmosis, erythrocytosis, gerardiasis, malaria and tuberculosis can be treated by incorporating and targeting the drug-using liposomal carrier [2].

F. Liposome in Cancer Therapy: All cancer drugs on long-term usage produce stern toxic effects. The liposomal approach causes targeting of drug to tumor with lesser toxic effects. The small and stable liposome is passively targeted to the different tumor because they can circulate for longer time. Nowadays, many anti-cancer herbal drugs are also formulated into liposomes to provide better targeting with enhanced bioavailability.

G. Liposomes as Protein Drug Delivery: They are used to enhance drug solubilization.

H. Liposomes in Cosmetics: They are used in cosmetics because their physiology is similar to the cell membrane, and they release materials to the cells [36].

I. Liposomes in Intracellular Drug Delivery: Drugs with intracellular receptors are required to cross the plasma membrane to show pharmacological activity. Liposomal delivery of drugs that normally enter the cells by pinocytosis can be very effective because liposomes can contain greater drug concentrations than the extracellular fluid.

The endocytosis process by which negatively charged liposomes are predominantly taken up by the cells is more efficient than pinocytosis. Liposomes can be used to increase cytosolic delivery of certain drugs which is normally poorly taken up into cells [37].

J. Liposomes in Sustained Release Drug Delivery: Sustained release systems are required to achieve and then to maintain the concentration of drug administered within the therapeutically effective range needed for medication, it is often necessary to take this type of drug delivery systems several times a day. This results in a fluctuated drug level and consequently undesirable toxicity and poor efficiency to minimize this fluctuation, novel drug delivery systems have been developed, which include niosomes, liposomes [38].

K. Liposomes in Gene Therapy: Liposomes have been used widely in the analytical sciences as well as for drug and gene delivery. Several systemic diseases are caused by a lack of enzymes or factors which are due to missing or defective genes. In recent years, several attempts have been made to

restore gene expression by delivery of the relevant exogenous DNA or genes to cells.

Because of the polyanionic nature of DNA, cationic (and neutral) lipids are typically used for gene delivery, while the use of anionic liposomes has been fairly restricted to the delivery of other therapeutic macromolecules.

Some of the widely used cationic liposome formulations are lipofectin, cytofectin, lipofectamine, transfectace.

Other Applications of Liposomes Table 8:

TABLE 8: APPLICATIONS OF LIPOSOMES IN MULTI DISCIPLINES

S. no.	Discipline	Application
1	Mathematics	Topology of 2-Dimensional surfaces in 3-Dimensional space governed only by bilayer elasticity
2	Physics	Aggregation behaviour, fractals, soft and high-strength material
3	Biophysics	Permeability, Phase transition in 2-Dimension, Photophysics
4	Physical chemistry	Colloid behaviour in a system of well-defined physical characteristics, inter-and intra-aggregate forces, DLVO
5	Chemistry	Photochemistry, artificial photosynthesis, catalysis, micro compartmentalization
6	Biochemistry	Reconstitution of membranes, cell function, fusion, recognition studies of drug action
7	Biology	Model biological membranes, Cell function, fusion, recognition
8	Pharmaceutics	Studies of drug action
9	Medicine	Drug delivery and medical diagnostics, gene therapy

Advancements in Liposomes:

- **Ethosomes:** They are efficient at delivering to the skin composed of soya phosphati dylcholine and 30% ethanol.
- **Immuno Liposomes:** They were modified with antibodies.
- **Niosomes:** They is small unilamellar vesicles made from nonionic surfactants
- **Stealth Liposomes:** They are a new type of liposomes which were prepared to improve stability and lengthen their half-life in circulation.

  Coating of liposomes should be done by polyethylene glycol (PEG) for preparing these liposomes.

TABLE 9: LIST OF CLINICALLY APPROVED LIPOSOMAL DRUGS

Name	Trade name	Company	Indication
Liposomal Amphotericin B	Abelcet	Enzon	Fungal infections
Liposomal Amphotericin B	Ambisome	Gilead Sciences	Fungal and protozoal Infections
Liposomal cytarabine	Depocyt	Pacira (Formerly Skye Pharma)	Malignant lymphomatous Meningitis
Liposomal Daunorubicin	DaunoXome	Gilead Sciences	HIV-related Kaposi's Sarcoma
Liposomal Doxorubicin	Myocet	Zeneus	Combination therapy with cyclophosphamide in metastatic breast cancer
Micellular estradiol	Estrasorb	Novavax	Menopausal therapy
Vincristine	Onco TCS		Non-Hodgkin's lymphoma
Lurtotecan	NX211		Ovarian cancer
Nystatin	Nyotran		Topical antifungal agent
Liposomal Vaccine	Epaxal	Berna Biotech	Hepatitis A
Liposomal Vaccine	Inflexal V	Berna Biotech	Influenza
Liposomal morphine	DepoDur	Skye Pharma, Endo	Postsurgical analgesia
Platinum compounds	Platar		Solid tumours
DNA plasmid encoding HLA-B7andα2 microglobulin	Allovectin-7		Metastatic melanoma

TABLE 10: MARKETED LIPOSOMAL COSMETICS FORMULATIONS

S. no.	Product	Manufacturer	Liposomes and key ingredients
1	Capture	Cristian Dior	Liposomes in gel with ingredients
2	Efect du Soleil	L'Oréal	Tanning agents in liposomes
3	Niosomes	Lancôme (L'Oréal)	Glyceropolyether with moisturizes
4	Nactosomes	Lancôme (L'Oréal)	Vitamins
5	Formule liposome gel	Payot (Fedinand Mushlens	Thymoxins, hyaluronic acid
6	Future Perfect Skin gel	Estee Launder	TMF, Vitamin-E, A Palmitate, cerebroside ceramide, phospholipid
7	Symphatic 2000	Biopharma GmbH	Thymus extract, vitamin A palmitate
8	Natipide II	Nattermann PL	Liposomal gel for do-it-yourself cosmetics
9	Flawless finish	Elizabeth Arden	Liquid make-up
10	Inovita	Pharm/Apotheke	Thymus extract, hyaluronic acid, vitamin E
11	Eye perfector	Avon	Soothing cream to reduce eye irritation
12	Aquasome LA	Nikko Chemical Co.	Liposome with humectants

CONCLUSION: Liposomes are one of the classical and extremely useful carrier systems for controlled and targeted drug delivery systems. Liposomes are acceptable and superior carriers having the ability to encapsulate hydrophilic and lipophilic drugs and protect them from degradation. It also has an affinity to keratin of horny layer of skin and can penetrate deeper into the skin and hence give better absorption. Applied on the skin, liposomes may act as a solubilizing matrix for poorly soluble drugs, penetration enhancers, and local depot at the same time diminishing the side effects of these drugs. These systems can be administered through oral, parenteral as well as topical routes.

This wide range of selection of route of administration makes it flexible in designing the drug delivery system. Also, these systems provide as an effective carrier for cosmetic formulations also. The major problem in the formulation of liposome is their stability problem. These problems can be overcome by employing modification in the preparation method and using some specialized carriers.

Nowadays, liposomes are used as a carrier for a wide variety of drugs. In spite of its few disadvantages, liposomes serve as versatile carriers for a wide range of drugs. The success of liposomes as drug carriers has been reflected in a number of liposome-based formulations, which are commercially available or are currently undergoing clinical trials. The mechanisms giving rise to the therapeutic advantages of liposomes, such as the ability of long-circulating liposomes to preferentially accumulate at disease sites such as tumours, sites of infection, and sites of inflammation, are increasingly well understood. The use of liposomes in the delivery of drugs and genes is promising and is sure to undergo further developments in the future.

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