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# LIPOSOMES AS TARGETED DRUG DELIVERY SYSTEM: A REVIEW

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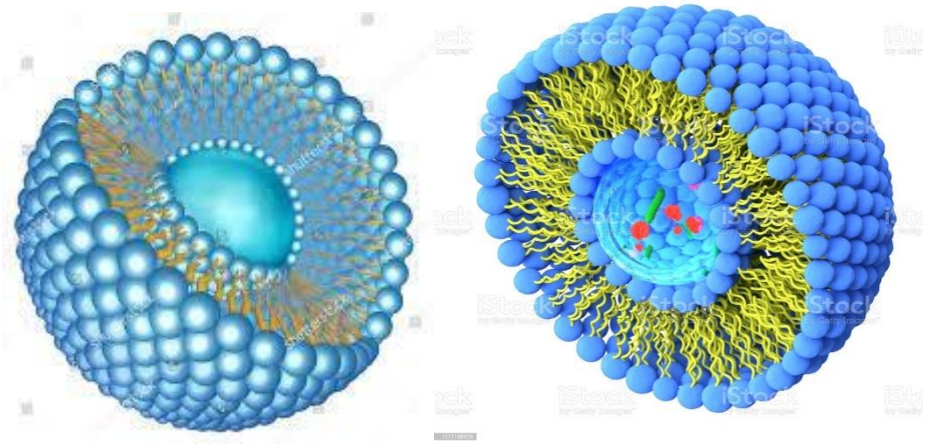
## INTRODUCTION

**"Liposome"** was first discovered in the year 1965 by Bangham and the word was derived from Greek word **"lipo"** means **"fatty constitution"** and **"soma"** means **"structure".**[1]Liposome are relatively small in size and it ranges from 50nm to several micrometers in diameter. These are spherical vesicles in which aqueous core is entirely enclosed by one or more phospholipid bilayers. It is having the unique ability to entrap both lipophilic and hydrophilic compounds. These molecules are inserted into the bilayer membrane, whereas hydrophilic molecules can be entrapped in the aqueous center. Because of their biocompatibility, biodegradability, low toxicity, and aptitude to trap both hydrophilic and lipophilic drugs and simplify site-specific drug delivery to tumor tissues. Many studies have been performed on liposomes inorder to decrease the drug toxicity and cell specific targeting. Various nanocarriers like nanoparticles, microparticles, polysaccharides etc…, can be used to a targeted drug delivery system. Liposomes are very useful because they act as carriers for a variety of drugs, having a potential therapeutic action. These vesicles are formed when phospholipids are hydrated in aqueous medium or aqueous solution.



## ADVANTAGES OF LIPOSOMES:

- Stability is increased if the liposomes are prepared via encapsulation.
- Liposome can increases the toxicity of the encapsulated agent (amphotericin B).
- These helps in decreasing the toxicity towards the sensitive tissues.

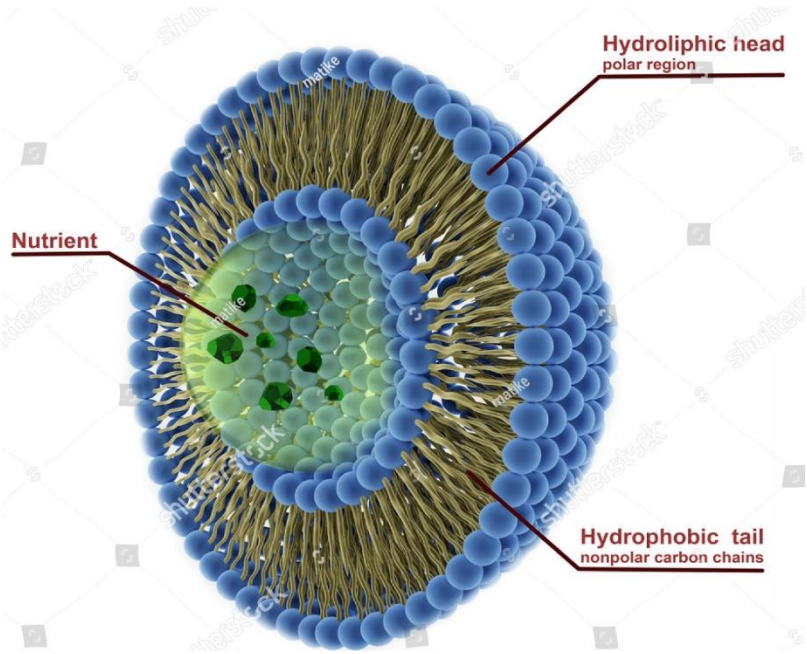
- These liposomes are flexible, non-toxic, biocompatible, completely biodegradable, and non-immunogenic for systemic and non-systemic administrations.
- Flexibility to couple with site-specific ligands to achieve active targeting.

**DISADVANTAGES OF LIPOSOMES:**

- These have short half-life.
- They have low solubility.
- Leakage of encapsulated drug molecules.
- The production cost is high.
- Phospholipids undergoes oxidation and hydrolysis like reaction.

**STRUCTURE OF LIPOSOMES:**

Liposomes are circular soft-matter vesicles formed by one or more bilayer membranes that separate aqueous media from each other. Phospholipid molecules are mainly composed of different polar head groups and two hydrophobic hydrocarbon chains. The polar group may be zwitterionic or negatively charged. Hydrocarbon chain molecules have different lengths and unsaturation.




**COMPONENTS OF LIPOSOMES:**

The major components of liposomes include:

- Phospholipids
- Cholesterol

- **Phospholipids:**
  - Phospholipids are the major constituents of liposomes.
  - Phospholipids are of 3 types:
    - Phosphotidylcholine

- Phosphotidylethanolamines
- Phosphotidyl serine

- **Cholesterol:**
  - Cholesterol itself does not form a bilayer.
  - It acts as a fluid buffer.
  - It provides rigidity to the bilayer.
  - Due to cholesterol bilayer become more stable towards temperature change.
  - Cholesterol generally incorporated in 1:1 or 1:2 molar ratio of PL.

**PROPERTIES OF LIPOSOMES:**

- **Solubilization:**

It solubilizes lipophilic drugs that would otherwise be difficult to administer intravenously.

- **Protection:**

Liposome encapsulated drugs are inaccessibleto metabolizing enzymes.

- **Amplification:**

Liposome can be used as an adjuvant in vaccine formulations.

- **Internalisation:**

Liposomes are endocytosed or phagocytosed by cells,opening up opportunities to use liposome-dependent drugs.

- **Duration of action:**

Liposomes can prolong the drug action by slowly releasing the drug in the body.

**CLASSIFICATION OF LIPOSOMES**

**Classification based on structural features:**

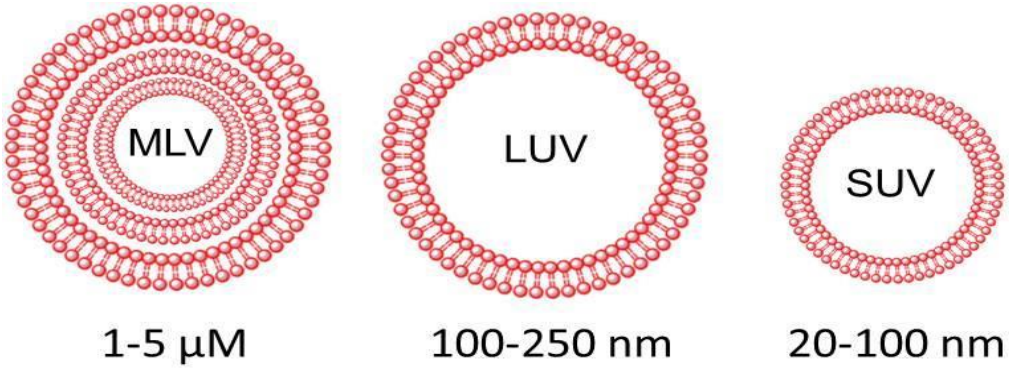
- Small Unilamellar Vesicles: 20-100nm.
- Large Unilamellar Vesicles: >100nm.
- Giant Unilamellar Vesicles: >1000nm.
- Oligo lamellar Vesicles: 100-1000nm.
- Multilamellar Large Vesicles: >500nm.

**Classification based on Preparation Method:**

- Reverse phase evaporation method.
- Stable plurilamellar vesicles.
- Vesicles prepared by fusion.
- Vesicles by extrusion technique.
- Frozen-thawed MLV.

**Classification based on targeting concepts of liposomes:**

- PEG ethylated liposomes.
- Immuno liposomes.
- Cationic liposomes.



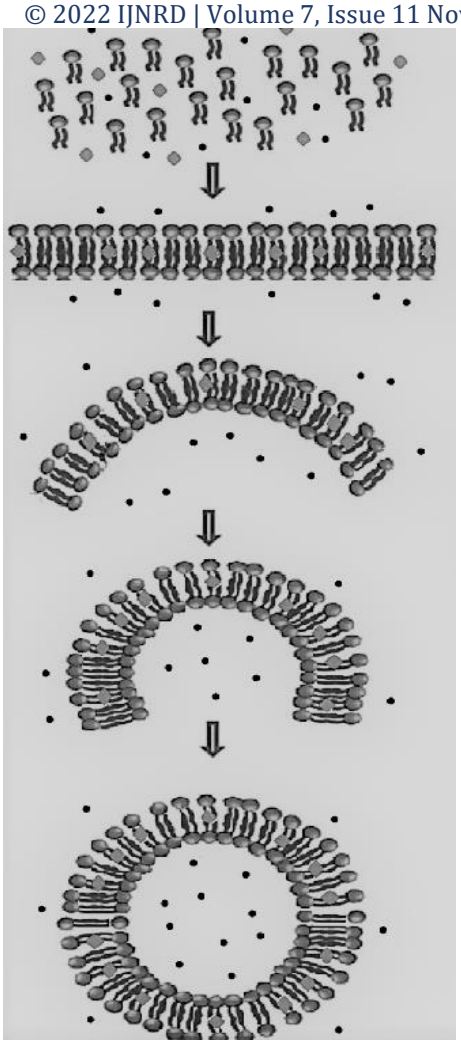

**MECHANISM OF FORMATION OF LIPOSOMES:**

The mechanism involves the following steps:

a) Phospholipids together with hydrophobic compound are placed in an aqueous media containing hydrophilic compounds.

b) Phospholipids form aggregated complexes to shield their hydrophobic sections from the water molecules. Hydrophobic compounds dissolved in lipids are entrapped into the liposomal bilayers.

c) Providing a sufficient amount of energy to the aggregated phospholipids makes the bilayer sheet to arrange in the form of organized, closed bilayer vesicles. During this process, liposomes can entrap hydrophilic compounds present in the hydration media inside their aqueous core.



**Formation of liposomes**

## LIPOSOME ACTION OF DRUG DELIVERY

The steps involved in the liposome action of drug delivery include:

1. Adsorption
2. Endocytosis
3. Fusion
4. Lipid exchange

1. **Adsorption:**

   Liposome adsorption to cell membrane is one of the important mechanism that is involved in the intracellular drug delivery. The adsorbed liposomes, in the presence of cell surface proteins, become leaky and release those contents that are required into the cell membrane. Thus, results in a higher concentration of drug close to cell membrane and facilitates cellular uptake of drug by passive diffusion or transport.[2]

2. **Endocytosis:**

   Adsorption of liposomes on the surface of cell membrane is followed by engulfment and internalization into endosomes. Endosomes transport liposomes to lysosomes. Subsequently, lysosomal enzymes degrade the lipids and release the entrapped drug into the cytoplasm.[3]

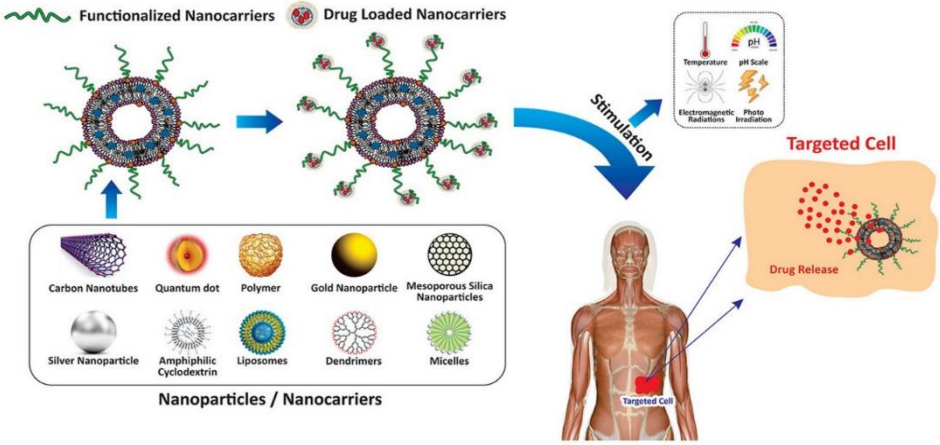
3. **Fusion:**

   Fusion of lipid bilayer of liposomes with lipoidal cell membrane by intermixing and lateral diffusion of lipids results in direct delivery of liposomal contents into the cytoplasm.[4]

4. **Lipid exchange:**

   Due to similarity of liposomal membrane lipids with the cell membrane phospholipids, lipid transfer proteins in the cell membrane recognize liposomes and consequently causes lipid.[5]Thisresults in the destabilization of liposomal membranes and intracellular release of drug molecules.

An understanding of the mechanisms of the intracellular drug delivery by liposomes provides the basis for bringing about manipulations in the characteristics of liposomes to enhance their favourable interaction with cellmembranesandhencethedrugdelivery.




**Action of drug delivery**

## PREPARATIVE METHODS OF LIPOSOMES:

### GENERAL METHOD OF PREPARATION:

**Step1:** Dissolve 10-20mg/lit of lipids in chloroform.

**Step2:**Discard the solvent by using rotary evaporator to produce thin film of lipids.

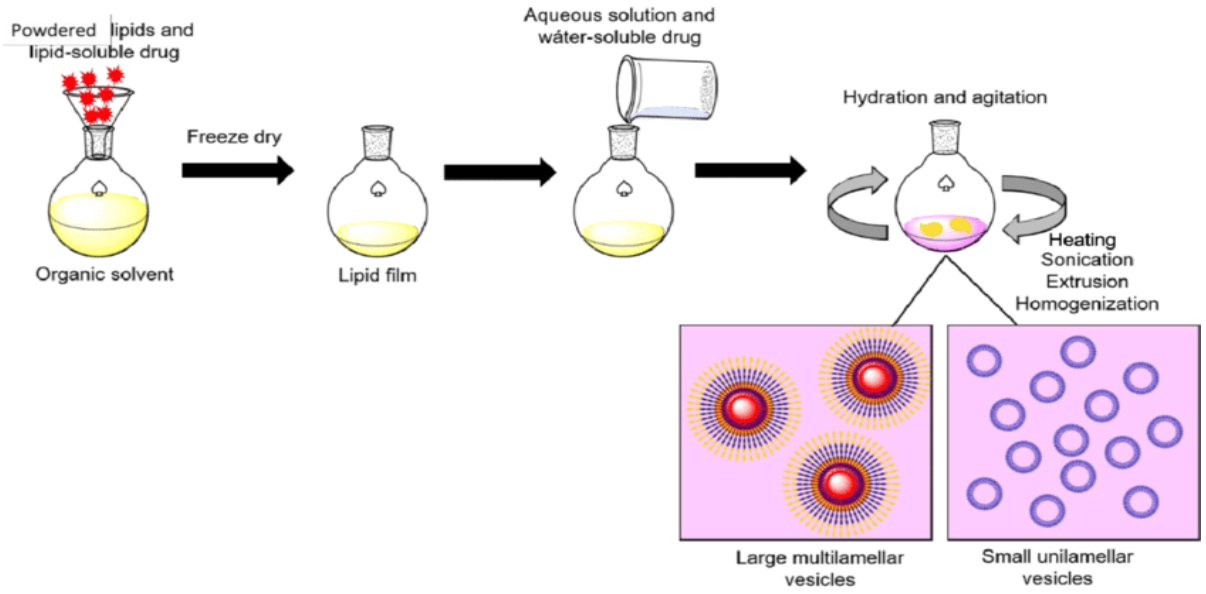
**Step3:** Desiccate the thin film for required time.

**Step4:** Hydrate desiccate product for required time. After complete hydration, the liposomes of multilamellar vesicles are produced in the size range of 200-1000nm.

**Step5:** Reduce the MLVs size by extrusion.

**Step6:** Purify the resultant liposome.

**Step7:** Analyse the final product.




**OTHER METHODS:**

The different methods involved in preparation of liposomes include:

1. **Passive loading techniques:**
    i. Mechanical dispersion method
        a. Sonication
        b. French pressure cell
        c. Membrane extrusion
        d. Microencapsulation
        e. Lipid hydration method
        f. Membrane extrusion
    ii. Solvent dispersion method
        a. Ethanol injection
        b. Ether injection
        c. Double emulsion
        d. Reverse phase evaporation
    iii. Detergent removal method
2. **Active loading techniques:**
    i. Proliposomes
    ii. Lyophilization

1. **Passive loading techniques:**

This technique is used for the encapsulation of drug during the formation of liposomes. The hydrophilic drugs are loaded in the internal core of the liposomes by mixing with the hydrating buffer which is used to hydrate the thin lipid film during the formation of liposomes. The untrapped drug molecules are removed from liposome suspension by dialysis, gel-filtration chromatography.

**i. Mechanical Dispersion Method:**

**a. Sonication:**

Most widely used method. The MLVs are sonicated either with bath sonicator or probe sonicator.

**Advantages:**

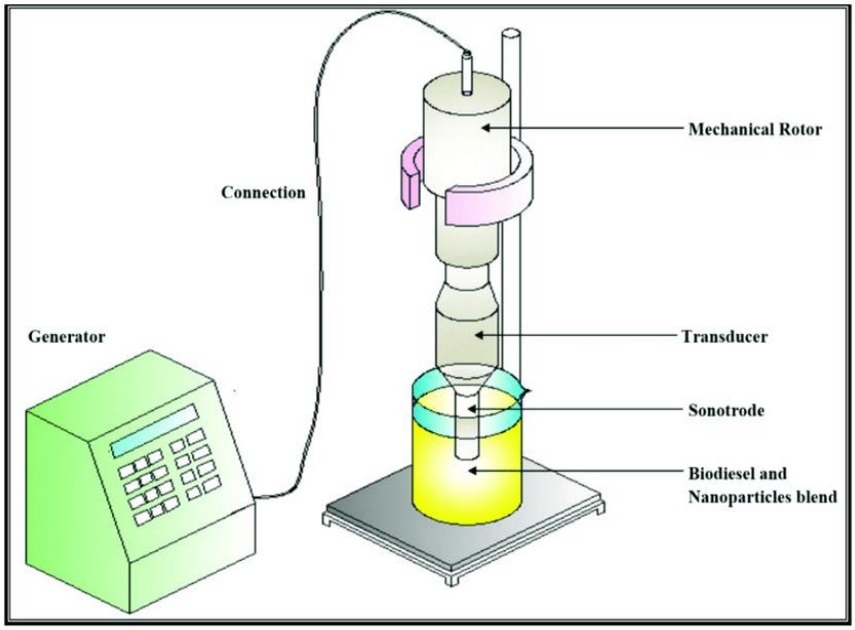
- This helps in speed dissoloution.
- It helps in stirring the sample in NMR tubes.
- It also produce energy for certain chemical reactions to proceed.

**Disadvantages:**

- Chance of metal pollution.
- Very low internal volume.
- Elimination of large molecules.

➢ **Probe Sonication:**

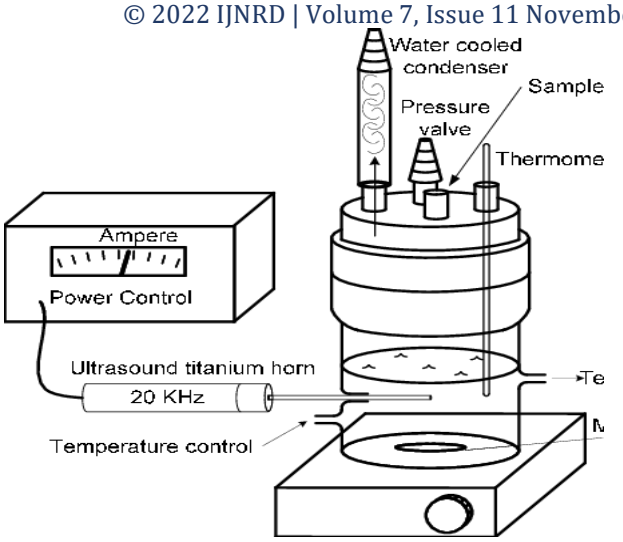
- The tip of a sonicator is directly engrossed into the liposome dispersion.
- In this method the energy input is very high.
- The coupling of energy at the tip results in local hotness, such that the vessel must be engrossed in ice.
- Continue the same process for upto 1hr, more than 5% of the lipids can be desterified.
- With this, titanium will slough off and pollute the solution.




**Probe Sonicator**

➢ **Bath Sonicator:**

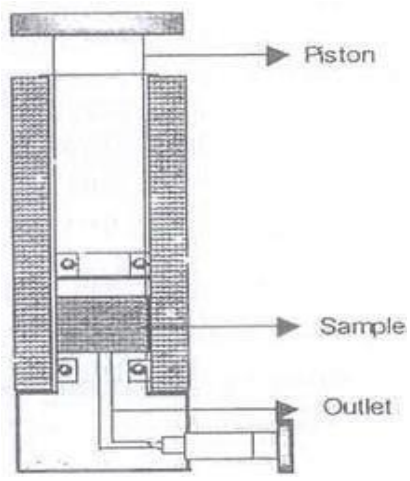
- Bath sonicator is like a big water bath.
- The sample which is to be sonicated is kept in the tub of the bath sonicator.
- The assembly comes with a temperature regulator, thus maintain the temperature.
- The high energy of sound waves breaks the MLVs into Small unilamellar vesicles.
- The material being sonicated can be protected in a sterile vessel, dissimilar the probe units, or under an inert atmosphere.[6]




**Bath Sonicator**

**b. French Pressure Cell:**

- It involves extrusion of MLV through a small orifice at a very high pressure of 20,000psi and temperature of 4°C.
- The important feature is that the proteins do not seem to be significantly pretentious during the procedure as they are in sonication.
- French press vesicles appears to recall entrapped solutes significantly longer than SUVs do, produced by sonication.
- The method involves the gentle handling of the unstable materials. This method has various advantages when compared with sonication.
- The liposomes are larger than sonicated SUVs.




**French Pressure Cell**

**c. Membrane extrusion:**

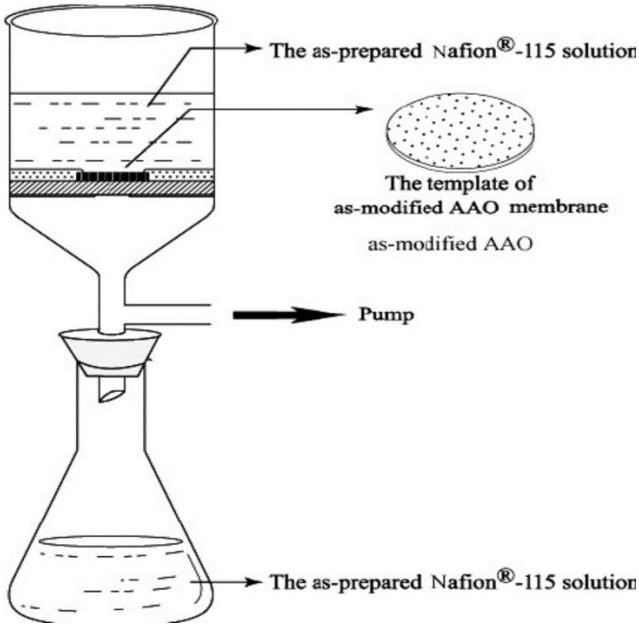
- In this method multi lamellar vesicles are made to pass through a membrane which is madeup of polycarbonate under high pressure of around 250psi.
- The layers of multi lamellar liposomes get peeled off, until only one layered vesicles of small size are left.

**Advantages:**

- Produce homogenous size range and suitable to produce stable liposomes with a variety of lipids.

**Disadvantages:**

- High pressure is required.
- Not effective as sonication and produces relatively larger vesicles.



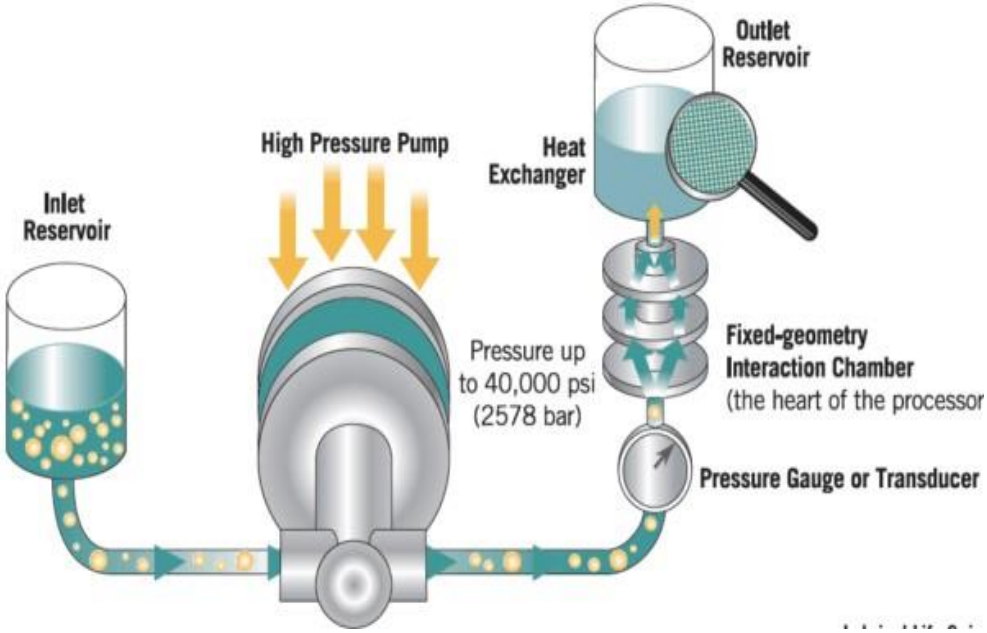

**Membrane extraction**

**d. Microemulsification method:**

- It is used for preparing SLVs.
- It can be achieved by microemulsifying lipid compositions using high shearing stress generated from high pressure homozenizer.

**Lipid hydration method is modified to:**

- Dried reconstituted method
- Freeze thawed liposomes




**Micro Encapsulation Method**

**e. Lipid hydration method:**

- This method involves 3 main steps:
    - Preparing a dry film of lipid.
    - Hydration of lipid film.
    - Size reduction of the prepared large unilamellar or multi lamellar liposomes.

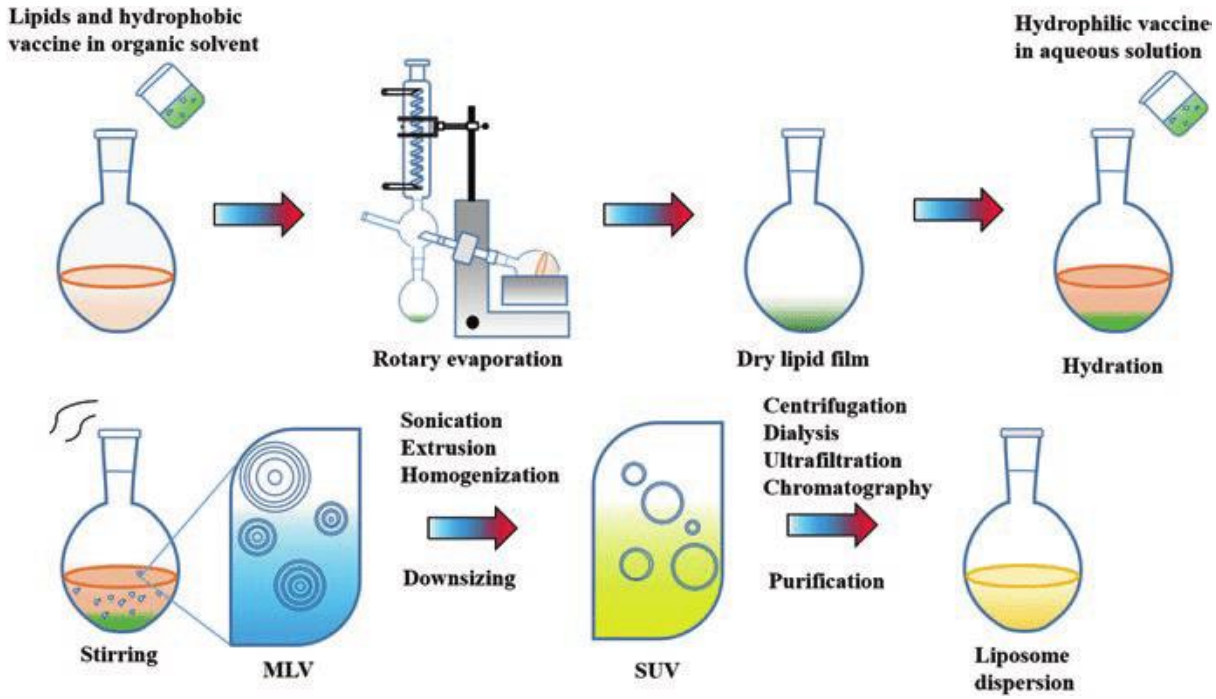
- **Step1:** Dissolve the lipids in a suitable organic solvent like chloroform.
- **Step2:** Evaporate solvent by using rotary evaporator in round bottomed flask so as to get thin film of lipid.
- **Step3:** Ensure complete removal of organic solvent by using vacuum pump for suitable period of time.
- **Step4:** Add aqueous solvents like distilled water, saline solution, buffer solution and non-electrolyte into RBF and agitate solution for 1hr. The hydration step is done at a temperature above the gel liquid crystalline transition temperature.
- **Step5:** After complete hydration, large and multi lamellar vesicles formed in RBF. Size of the liposome is reduced by using suitable method.

**Advantages:**

- It is the simplest method for preparation of liposomes.
- Less expensive.
- It does not require high pressure or temperature.

**Disadvantages:**

- The liposomes formed are generally large unilamellar or multi lamellar.
- Encapsulation efficiency is low.
- Liposomes formed are heterogenous in size.

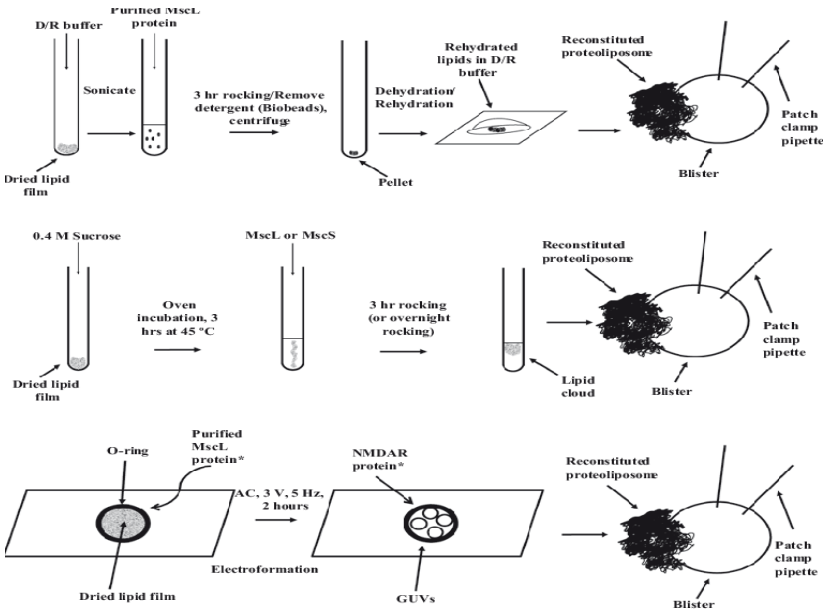



**Lipid Hydration Method**

**Lipid hydration method is modified to:**

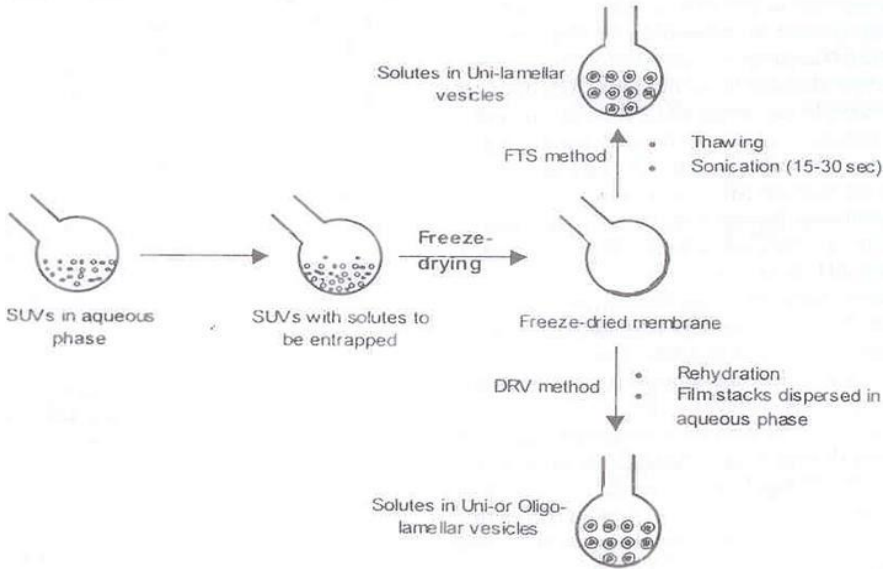
- Dried reconstituted method
- Freeze thawed liposomes

- **Dried reconstituted method:**
  - In this technique the liposomes are first freeze dried, and are then reconstituted by adding aqueous phase.
  - The hydrophilic materials that are to be encapsulated are added to the dispersion of small unilamellar vesicles and then the mixture is freeze dried.
  - Then the freeze dried membrane is subjected to rehydration by dispersing the film stacks in an aueous phase.
  - This results in the formation of uni or oligo lamellar vesicles entrapping the hydrophilic materials in their core.




**Dried reconstituted Method**

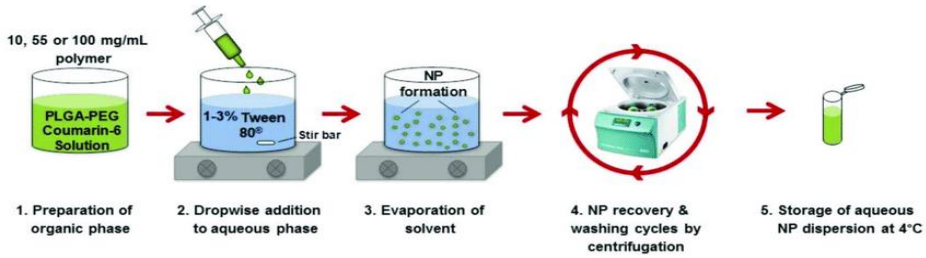
- **Freeze thaw method:**
  - In this we freeze te SLVs and thawed slowly.
  - The aqueous dispersion of small unilamellar liposomes is first freeze dried with solutes to be entrapped, and then mass is slowly thawed.
  - Thawing means to make a frozen mass soft by warming it up.
  - The repeated cycles of freezing and thawing increase the space between the consequetive layers.
  - The obatained mass is now subjected to sonication for 15-30 seconds.
  - The short cycle of sonication causes the dispersion of the aggregated material into large unilamellar vesicles.
  - The large umilamellar vesicles are formed due to the fusion of small lamellar vesicles during the entire process of freezing and thawing.




**Freeze thaw Method**

**ii. Solvent Dispersion Method:**

- Lipids are dissolved in organic solvents to make homogenous lipid solution.
- The lipid solution is added to an excess of aqueous phase which contains the hydrophilic materials which are to be encapsulated in the liposomes.
- As a result of interaction of organic phase and aqueous phase, the lipid molecules align themselves at the interface of the organic and queous phase and form a single layer.
- This monilayer of the phospho lipids contributes to the formation of the bilayer of liposomes.




**Solvent Dispersion Method**

Solvent dispersion method is further classified into 4 different methods:

a. Ether injectioon
b. Ethanol injection
c. Double emulsion
d. Reverse phase evaporation

**a. Ether injection:**

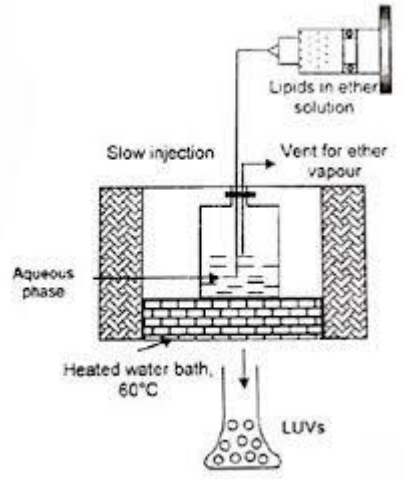
- This method is also called as **"Solvent Vaporization".**
- A solution of lipids dissolved in diethyl ether mixture and is gradually injected to an aqueous solution of the material to be encapsulated at 55°C-65°C.
- The addition is done using a mechanical drive at an extremely low rate, i.e., around 0.25ml per minute.
- The consequent removal of ether under vacuum leads to creation of liposome.

**Advantages:**

- Does not cause oxidative degradation of the lipids.

**Disadvantages:**

- Liposomes formed are heterogenous.
- Compounds which are encapsulated are exposed to organic solvents at high temperature.[7]




**Ether injection**

**b. Ethanol injection:**

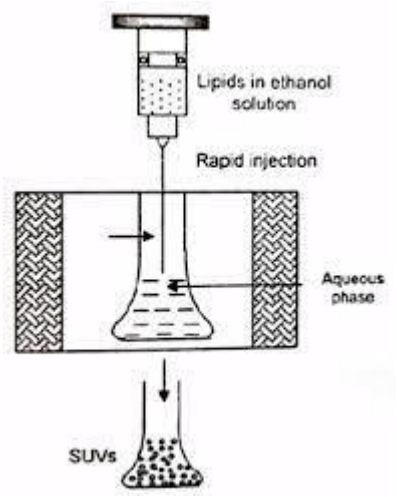
- A lipid solution of ethanol is rapidly injected to a huge excess of buffer. The MLVs are formed at once.

**Advantages:**

- This method is very simple and convenient.
- The sensitive lipids are at very low risk of degradation.

**Disadvantages:**

- The population is heterogenous.
- The liposomes are very dilute.
- Removal of ethanol is difficult.




**Ethanol Injection**

**c. Double emulsion evaporation:**

- An aqueous solution containing drug is dispersed in an organic solvent in which the lipids were in it and creates the water-in-oil emulsion then both phases are homogenized by proper agitation to form a primary emulsion.
- Then, the primary emulsion is emulsified with the outer aqueous phase containing appropriate stabilizer to form double emulsion.

- Formation of double emulsion is followed by evaporation of the organic solvent from the dispersed phase leading to a point of insolubility and consequently, hardening of the polymer encapsulating the active material.
- Usually, chloroform-ether mixture is used as a solvent which may be evaporated under reduced pressure through rotary evaporator at ambient temperature depending on the boiling point of organic solvent.

**Advantages:**

- Vesicles formed are of intermediate size.
- 100% theoretical yield can be obtained.

**d. Reverse phase evaporation method:**

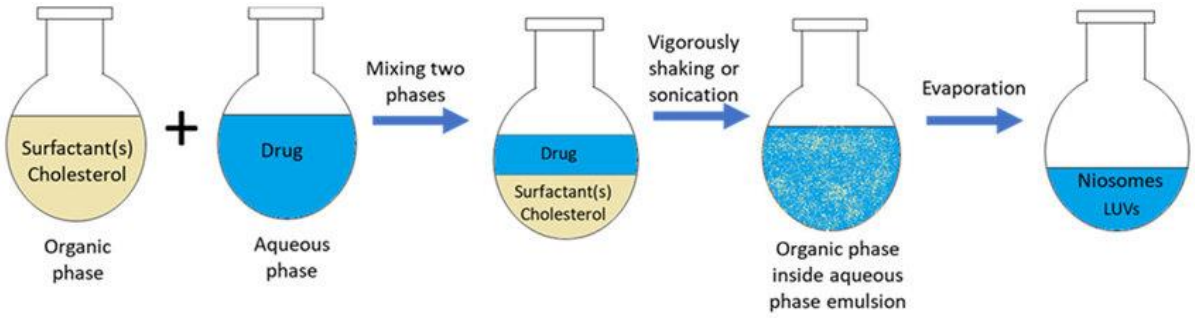
- This method is based on the creation of inverted micelles.
- The technique is carried out by dissolving the lipids in an organic solvent, adding a small volume of aqueous phase, then sonicating the solution to produce inverted micelles.
- Usually diethyl ether or isopropyl ether, a mixture of isopropyl ether and chloroform is used as organic solvent.
- The organic solvent is removed using a rotary evaporator and a viscous gel is mixed with a mechanical stirrer and thus forms liposomes.

**Advantages:**

- High encapsulation rate upto 50%.

**Disadvantages:**

- Materials to be encapsulated are made to come in contact with the organic solvents and sonication cycles.




**Reverse phase Evaporation Method**

**iii. Detergent Removal Method:**

- In this method, the detergents are first used to solubilize the molecules of phospholipids in an aqueous phase, and then are removed to form liposome suspension.
- When detergents are added to an aqueous solvent at a concentration above their CMC value, along with the lipids, the detergents and lipids form mixed micelles, where the hydrophilic part of the detergent molecules face towards aqueous phase, and their hydrophobic part covers the lipid molecules, thus shielding lipid molecules from aqueous solvent.
- Removal of detergent is critical.
- When the detergents leave the solution, the micelles progressively become devoid of detergent molecules.

- Finally, the micelles which are now made up entirely of phospholipids combine together to form a large unilamellar vesicles.

The detergents can be removed by any of the following methods:

- Dialysis
- Detergent absorption
- Gel permeation chromatography

- **Dialysis:**
  - The detergents at their CMC is used to solubilize lipids.
  - As the detergent is detached, the micelle becomes increasingly better-off in phospholipid and lastly combine to form LUVs.
  - The detergents are removed by dialysis.
  - A commercial device called LipoPrep, which is a version of dialysis system, is obtained for the elimination of detergents.
  - The dialysis can be performed in dialysis bag engrossed in large detergent free buffers.

  **Advantages:**
  - Offers good reproducibility.
  - Liposome population is homogenous.

  **Disadvantages:**
  - The main limitation of this method is the inefficient removal of the detergents which may lead to retention of their traces in the liposomes.

- **Detergent absorption:**
  - It is attained by shaking mixed micelle solution with beaded organic polystyrene adsorbers such as XAD-2 beads.
  - The great benefit is that they can eliminate detergents with a low CMC, which are not entirely depleted.

  **Advantages:**
  - Elimination of detergents even with a very low CMC.

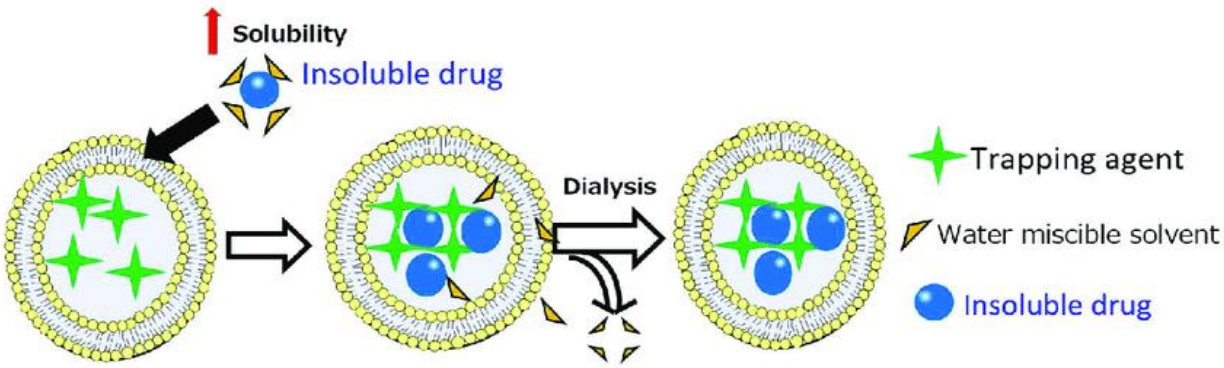
  **Disadvantages:**
  - Additional detergents are required to carry out this process.
- **Gel Permeation Chromatography:**
  - Detergents can also be removed by using gel permeation chromatography, where the detergent is depleted by size special chromatography.
  - The liposomes percolate through the spaces between the beads.
  - Pre-treatment is essential as the swollen polysaccharide beads would absorb significant quantities of the ampiphilic lipids.
  - The pre-treatment can be done by saturating the gel filtration column prior to use with lipids using blank liposomes suspensions.

- Columns that used for gel filtration include Sephadex G-50, Sephadex G-100, Sepharose 2B-6B, Sephacryl S200-S1000.

- **Active loading technique:**
  - Active loading techniques involve the loading of drugs into the pre-formed empty liposomes using various techniques such as pH gradient, electric gradient and potential difference across the lipid membrane.
  - In active loading, several drugs such as lipophilic amines are taken up and accumulated in the liposomes which exhibit a membrane potential.
  - Active loading of drugs through pH gradient involves the preparation of the liposome in a buffer having low pH and then adjusting the external pH to 7 or higher.
  - Subsequently, the drug is added and the dispersion is incubated for short period of time.
  - The lipid bilayer is generally impermeable to ionic molecules and large hydrophilic moieties.
  - Ions can be transported across the lipid bilayer by the use of ionophores, whereas that of the neutral and weakly hydrophobic molecules can be regulated by the concentration gradients.
  - Amphipathic compounds can be drawn inside the liposomes due to a difference in the proton concentration across the liposomal membrane.

    **Advantages:**
    - Encapsulation efficiency and capacity is higher.
    - Leakage of encapsulated drug is less.
    - It gives freedom of using any type of lipid.

    **Disadvantages:**
    - This technique is not suitable for hydrophobic drugs.
    - It also requires presence of weakly basic functional groups.
    - This process requires certain conditions, such as pH or electrostatic gradient which will help the drug to cross the lipid membrane.




**Active loading Technique**

## STABILITY AND EVALUATION OF LIPOSOMES:

The stability of liposomes is of major concern in their development for pharmaceutical applications. A drug containing liposomes can be unstable because of physical or chemical stability. The stability studies can be physical or chemical stability. The stability studies could be broadly covers under two main sections.

Stability in-vitro, which covers the stability aspects once the formulation is, administered via, various routes of biological fluids.

**Stability of in-vitro mainly covers:**

i. **Chemical degradation:**

a. **Oxidation:**[8]

The oxidative degradation of liposome can be prevented by following:

- Start with freshly purified lipids and freshly distilled solvent.
- Avoid procedures which involves high temperature.
- De-oxygenate aqueous solution in an inert atmosphere.

b. **Hydrolysis:** [9]

- Using lipid containing ether linkage instead of ester linkage.
- Sphingomyelin prevents in-vivo hydrolyses.

ii. **Physical degradation:**

- Sedimentation, leaching of drugs aggregation or fusion. High manufacturing temperature and many other factors can include this

**Physical stability can be achieved by**

Manufacturing and storing at temperature below its transition temperature.

- Manufacturing and storing at temperature below its transition temperature
- Adding 10% of P.A [Phosphatidic acid] or P.O to neutral liposome for providing negative pH.
- By cross linking membrane components covalently using glutaraldehyde fixation or polymerization of alkyne containing phospholipid.

1) Buffers at neutral pH can decrease hydrolysis

2) Addition of antioxidants such as sodium ascorbate, can decrease oxidation

3) Freeze-dried liposome formulations should incorporate a lipoprotectant like non-reducing disaccharides such as trehaloes and sucrose.

iii. **Particle size:**

The particle size and particle size distribution of liposomes influence their physical stability. These can be determined by the following methods:

a. **Laser Light Scattering:**

It is simple method which requires expensive instruments. In this method, a light of suitable wavelength is passes through a lipoidal suspension. The intensity of light scattered by the liposomes is proportional to their

diameter. Therefore, measuring the fluctuations in scattered light helps to indicate the particle size of liposomes. It is given by Stokes-Einstein equation,

$$D = kT/6\pi\eta R_h$$

Where,

D = Translation diffusion coefficient.

K = Boltzmann constant.

T = Absolute temperature.

$\eta$ = Viscosity of solvent.

$R_h$ = Mean hydrodynamic radius.

**b. Transmission Electron Microscopy:**

The best method for determining the particle size of individual liposomes is electron microscopy. Freeze fracture electron microscopy is found to be effective for evaluating large-sized vesicles. This technique is also helpful in examining the morphological changes that occur in liposomes when they undergo phase transition.

**Note:** The size of liposome vesicles decreases with increasing sonication time and extraction cycle.[10]

**iv. Surface Charge:**

The positive, negative or neutral charge on the surface of liposomes is due to the composition of the head group. The surface charge of liposomes governs the kinetics and extent of distribution in vivo, as well as interaction with and uptake by the target cells.

- It utilizes a cellulose acetate plate sipped in sodium borate buffer of pH 8.8.
- About 5 moles of lipid samples are applied on to the plate, which is then subjected to electrophoresis at 4°C for 30mins at 18V/cm
- This technique can be used for determining the hetero genecity of charges in the liposome suspension as well as to detect any impurities such as fatty acids.

**Note:** If the zeta potential value of a particle is too small, there will be a force of attraction greater than the repulsion force so that it causes coagulation and flocculation which indicate colloidal stability.[11]

**v. Percent Drug Encapsulation:**

Two methods are used to separate the free drug from the sample.

**a. Mini-Column Centrifugation Method:**

- Mini-Column is constructed by using a syringe without its plunger. The syringe is filled with a hydrated gel and its end is closed with a Whatmann filter pad.
- The barrel of the syringe is placed in a centrifuge tube rotated at 2000rpm for 3min. This helps to remove any excessive saline solution from the hydrated gel through the collection tube.
- Liposome sample is then added drop-wise to the top portion of the gel bed.

- The column is again rotated at 2000rpm for 3mins to fill any void spaces between the liposomes in the centrifuge tube.
- The elute so obtained is then assayed.

**vi. Phase Behaviour:**

Liposomes at transition temperature undergo reversible phase transition i.e . , the polar head group in gel state become disordered to form the liquid crystalline state. The phase behaviour of liposomes can be determined by differential scanning calorimetry. The transition temperature is indicative of stability, permeability of liposomes and also indicates the region of drug entrapment.

**vii. Drug Release Rate:**

The rate of drug release from the liposomes can be determined by in vitro assays which help to predict the pharmacokinetics and bioavailability of drug. However, quantitative in vivo studies are found to provide more information. Liposomes encapsulating the tracer inulin are employed for the study. This inulin is preferred as a marker, as it is injected only in the extra cellular fluid and undergoes rapid renal excretion. The rate of release of inulin from the liposomes is estimated by the time course of renal excretion of the free tracer in urine coupled with the first-order degradation rate constant of the tracer release from the liposomes.

## APPLICATIONS OF LIPOSOMES:

**1. Cationic liposomes for Delivery of Nucleic acid:**

Cationic lipids, which are the core components of nanoparticles, have a structure of positively charged head group and one or two hydrophobic tail region made of hydrocarbon chains or steroid structures. Lipoplexes of cationic liposomes and nucleic acids still suffer from several limitations. One is their low stability in the bloodstream, which is caused by characteristics of cationic lipids. Until they reach their target cells, cationic lipid components can interact with serum proteins, potentially disrupting the integrity of liposomal structure or forming aggregates that are too large to be taken up by cells. Ti increase the in-vivo stability of lipoplexes in the blood, researchers often include PEG-conjugated lipids and cholesterol as components of the cationic liposomes. Another limitation is the relatively weak delivery of nucleic acids into target cells. For anti-cancer therapy, enhanced retention and permeability may serve as an initial driving force for delivery of lipoplexes to tumour tissues. Once in the tumour tissues, the effective recognition off tumour cells and intracellular delivery should proceed.[13]Cisplatin is another widely used anticancer drug, which induces DNA lesions and mitochondrial apoptosis. This sometimes, may lead to toxicities including nephrotoxicity, neurotoxicity, and ototoxicity which demand new formulations to be developed to reduce toxicity and potentiate efficacy such as Cisplatin liposomes.[14]

**2. Liposomes used in Cancer:**

A significant treatment in the treatment of cancer involving chemotherapy is the efficient delivery of cytotoxic agents to tumour tissues while at the same time minimizes the undesired negative side effects with these drugs. The use of DDS such as liposomes can modify drug pharmacokinetic and distribution in a manner that improves the overall pharmacological properties of generally used chemotherapeutics. Liposomes are particularly

attractive to DDS in part due to the ease with which they can be generated and modified such that they can be used to treat a wide variety of cancers. Breast cancer, in particular, has been the focus of many studies concerning liposome based chemotherapeutics in part due to the clinical success of various drugs such as Doxil, which is a liposomal formulation currently employed to treat recurrent breast cancer.[15]

**3. Sustained release liposomes:**

Anticancer drugs can be delivered to systemic circulation in a sustained release mode by encapsulating in the liposomes. DepoFoam, a sustained release injectable technology of Skye Pharma, is applied in DepoCyt that is used in the treatment of lymphoma, i.e., lymphomatous meningitis. In comparison to unentrappedCytarabine, DepoCyt administered through intrathecal route maximized the therapeutic potential of cytotoxic agents that are specific to the S-phase of cell cycle. Furthermore, the dosing frequency reduced due to the prolonged CSF t1/2 of Cytaribine.[15]

**4. Liposome Vaccine:**

Typically, either a purified antigen or an attenuated pathogen is used as an immunogen in a known vaccine. However, a long-term immune response may not be induced by purified antigens and even sometimes does not induce a response at all. On the other hand, attenuated vaccines can produce a reaction in the patient under immunization.[15]

**5. Liposomes used for Fungal Treatment:**

A polyene antibiotic, AmpicillineB, is used for the treatment of fungal infections. It binds to sterols in fungal membrane as well as mammalian membranes which reslt in the formation of transmembrane, pores where leakage of vital intracellular components takesplace leads to cel death. Binding to the cholesterol-containing mammalian membranes results in toxicity. Nephrotoxicity was reported in the systemic use of AmpB and often resulted in central nervous system side effects on chronic use. Studies revealed the effectiveness of liposomal AmpB in experimental fungi and parasitic diseases.[15]

**6. Immuno liposomes:**

Among the various types of liposomes, immune liposomes have gained wide attention due to their targeting capabilities. Due to the presence of antibodies attached on to their surface, these liposomes exhibit immunologic response. The preparation of immune liposomes, i.e., conjugation of antibodies to liposomes, is not that straight forward and even can pose a challenge during their formulation. Protein molecules and monoclonal antibodies can be conjugated directly in to the liposome, PEGylated liposome, or PEG chain of the PEGylated liposomes. Similar to other liposomes, the RES can scavenge and clear the immunoliposome from systemic circulation. Increase circulation half-life, targeting specificity, and minimized drug loss and degradation are the major advantages of immune liposomes. Apart from the promising applications, immunoliposomes suffer from a major drawback, i.e., immunogenicity and increase rate of clearance from circulation can be observes due to repeated injections.

### 7. Liposomes used for gene delivery:

Liposomes have been extensively studied in areas such as gene therapy and drug delivery due to their observed stability and favourable toxicity profile over traditional treatments. Liposomes can encapsulate biomolecules or drugs that are hydrophilic and increase their internalization and solubility through the lipid bilayer of the cell.[16]Various discoveries related to human genomes and their use in disease treatment have become more approachable with the advances in science and technology. In spite of these developments, choosing a right carrier for the delivery of the gene to the target is of paramount importance. One such important carrier is liposomes, which can deliver DNA, antisense oligonucleotides, RNA, and other potential agents into the nucleus. Especially, engineered liposomes such as cationic liposomes, pH-sensitive liposomes, fusogenic liposomes, and genomes are explored for gene delivery.

### 8. Liposomes used for Protein and Peptide Delivery:

Proteins and peptides are potent therapeutic agents used in the treatment of various diseases. However, due to their unstable nature and degradation at physiological conditions, the delivery of these drugs at the targets site is extremely complicated. Most of the protein and peptide drugs produce their mechanism of action extra cellular by interacting with the receptors, Encapsulation of proteins and peptides into lipid vesicles for improving the therapeutic properties has been extensively investigated.[16]

### 9. Liposomes used in Inhales:

The pulmonary route is a promising drug delivery route. The carriers used for this purpose are target selective and can control drug release. The advantages of the carrier system include decrease drug toxicity, increased the stability of drug, and the local irritation which is prevented. Nebulizers are used in the actual liposome formulation for inhalation. The nebulizers used include ultrasonic, air-jet, and passively vibrating mesh nebulizers.

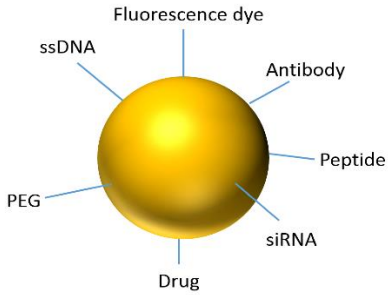
## ADVANCES OF LIPOSOMES IN DRUG DELIVERY

### 1) INORGANIC NANOPARTICLES:

Inorganic nanoparticles have gained significant attention in preclinical development as potential diagnostic and therapeutic systems in oncology for a variety of applications, including tumour imaging, tumour drug delivery or enhancement of radiotherapy. The ability of these inorganic nanoparticles to expose multiple surface binding sites are widely explored.[17]The inorganic nanoparticles include:

#### i. Gold nanoparticles:

Gold nanoparticles have been widely employed in bio nanotechnology based on their unique properties and multiple surface functionalities. A relevant attributes is the ease of functionalization using the strong bond between gold and thiol ligands.[18]




**Gold nanoparticle**

**ii. Silver nanoparticles:**

Silver nanoparticles are increasingly used in various fields, including medical, food, health care, consumer and industrial purposes, due to their physical and chemical properties.[19]

The biological activity of silver nanoparticles depends on the factors including surface chemistry, size, size distribution, shape, particle morphology, particle composition, are crucial for the determination of cytotoxixity.[20]

**Silver nanoparticles**

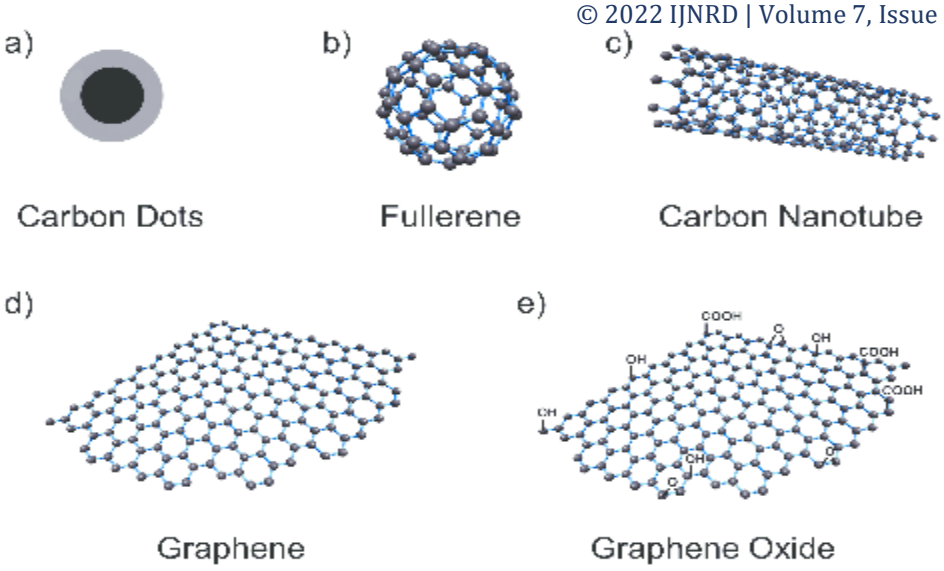
**iii. Iron Oxide Nanoparticles:**

There are 16 iron oxides, including oxides, hydroxides, and oxide-hrdoxides.[21]The magnetic characteristics of iron oxides are related to the super paramagnetic behaviour of small nanoparticles that appear in response to an external magnetic field.[22]

**2) ORGANIC NANOPARTICLES:**

**i. Carbon Nanotubes and Graphene Nanoparticles:**

These are extensively used as electrochemical sensing composites due to their interesting chemical, electronic and mechanical properties giving rise to increased performance. The functionalization of carbon nano tubes with hyaluronic acid increases the interaction of nano systems with bronchial cells and prevents the inflammatory process in the pulmonary tissues since they are not captured by alveolar macrophages.[23]

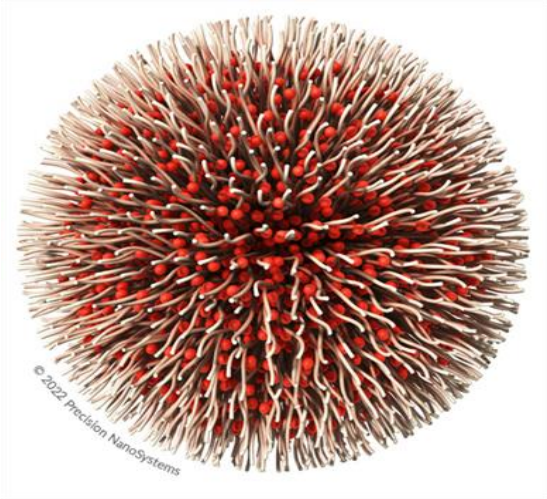



**Carbon nanotubes and Graphene Nanoparticles**

**ii. Polymeric Nanoparticles:**

Polymeric nanoparticles are particles within size range from 1-1000nm and can be loaded with the active compounds entrapped within or surface-absorbance on to the polymeric core.

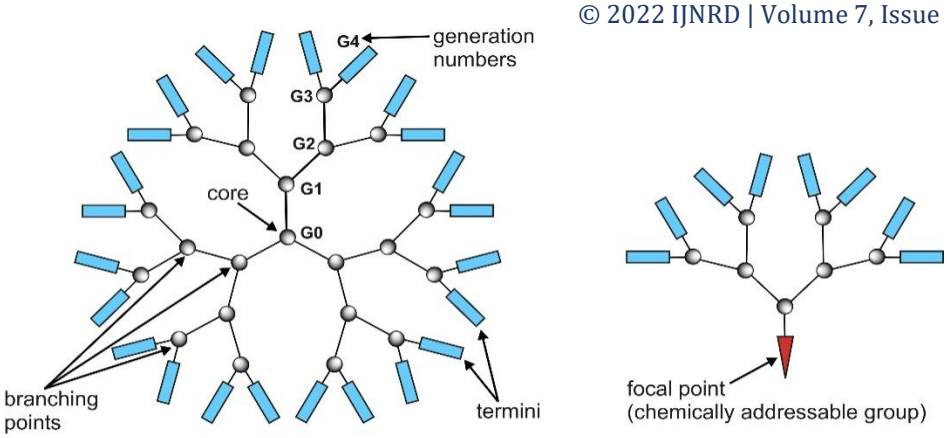
The delivery of active molecuules using nanoparticles has been proven efficient in inhibiting virus replication.[24]




**Polymeric nanoparticle**

**iii. Dendrimers:**

Dendrimers are nano-sized, radially symmetric molecules with well-defined, homogenous, and monodispersed structure that has a typically symmetric core, an inner shell, and an outer shell.Dendrimers have been widely studied for applications against HIV infections. The ability of dendrimers to establish strong interactions with viruses may increase antiviral activity, making them promising systems against viral infections.[25]




**DENDRIMER** **DENDRON**

**Dendrimer**

## CONCLUSION:

Liposomes are very widely used control drug delivery system in the present scenario. They play a crucial role for controlled or targeting of drug to the specific site. The size of liposomes at nanosize is very important for target delivery and have proven to be a safe medication over a period of time. Liposome have a flexibility of formulating into a dosage form that can be delivered by oral route or parenteral route or any other specific targeted drug delivery formulations. They have wide acceptance in cosmetic industry also.

Inspite of some stability related problems liposomes are being used extensively in pharmaceuticals and cosmetic industry. New methods are being developed for addressing the stability issues and increase the entrapment efficiency. Inspite of certain drawbacks liposomes are widely found in the therapeutic and cosmetic products.

## REFERENCES:

1. Dua, J., Rana, A., and Bhandari, A. (2012).
2. Allen TM, McAllister L, Mausolf S, Gyorffy E. (1981). Liposomecell interactions. A study of the interactions of liposomes containing entrapped anti-cancer drugs with the EMT6, S49 and AE1 (transport-deficient) cell lines. BiochimicaetBiophysicaActa 643: 346–62.
3. Lipowsky R. (1995). Generic interactions of flexible membranes. In: Lipowsky R, Sackmann E, eds. Handbook of biological physics. Chapter 11. Vol 1. The Netherlands: B.V. Elsevier Science, 521–602.
4. Knoll G, Burger KNJ, Bron R, et al. (1988). Fusion of liposomes with the plasma membrane of epithelial cells: fate of incorporated lipids as followed by freeze fracture and autoradiography of plastic sections. J Cell Biol 107:2511–21.
5. Wojewodzka J, Pazdzior G, Langne M (2005). A method to evaluate the effect of liposome lipid composition on its interaction with the erythrocyte plasma membrane. ChemPhys Lipids 135:181.
6. Kataria S, Sandhu P, Bilandi A, Akanksha M, Kapoor B, Seth GL, Bihani SD. Stealth liposomes: a review. *IJRAP*. 2011;**2**(5):1534–1538.
7. Deamer D. and Bangham A.D. Biochim. Biophys. Acta.; 1976; 443: 629.
8. Hunt C and Tsang S, Int J Pharma, 1981; 8: 101.

9. Frfkjaer S, Hjorth E and Wfatis O, In., Gregoriadis, G.Eds., Liposome Technology, Vol.1, CRC Press, Boca Raton, FL, 1984, 235.
10. Nava G, Piñón E, Mendoza L, Mendoza N, Quintanar D, Ganem A. Formulation and in vitro, ex vivo and in vivo evaluation of elastic liposomes for transdermal delivery of ketorolac tromethamine. Pharmaceutics. 2011:3 (4):954 970.
11. Channarong s, Chaicumpa W, Sinchaipanid N, Mitrevej A. Development and evaluation of chitosan-coated lipoosmes for oral DNA
12. MaheswaranA,Brindha P, MullaicharamAR,Masilamani K. Liposomal drug delivery systems-a review.Int J Pharm Sci Rev Res 2013;23:295-301.
13. Fan Y, Zhang Q. Development of liposomal formulations: From concept of clinical investigations. Asian J Pharm Sci 2013;8:81-7.
14. Bonde S, Nair S. Advances in liposomal drug delivery system-fascinating types and potential applications. Int J App Pharm 2017;9:1-7**.**
15. Nekkanti V, Kalepu S. Recent advances in liposomal drug delivery: A review. Pharm Nanotechnology 2015;3:35-55.
16. Lamichhane N, Udayakumar TS, D'Souza WD, Simone CB, Raghavan SR, Polf J, *et al*. Liposomes: Clinical applications and potential for image-guided drug delivery. Molecules 2018;23:1-17.
17. Kerry, R, G., Malik, S., Redda, Y.T., Sahoo, S., Patra, J.K., and Majhi, S.(2019). Nano-based approach to combact emerging viral (NIPAH virus) infection. Nanomedicine. Nanotechnology. Biological. Medicine. 18, 196-220. doi:10.1016/j.nano.2019.03.004.
18. Yang, X., Yang, M., Pang, B., Vara, M., and Xia, Y. (2015). Gold nanomaterials at work in biomedicine. *Chem. Rev.* 115, 10410–10488. doi: 10.1021/acs.chemrev.5b00193.
19. Gurunathan S., Park J.H., Han J.W., Kim J.H. Comparative assessment of the apoptotic potential of silver nanoparticles synthesized by *Bacillus tequilensis* and *Calocybeindica* in MDA-MB-231 human breast cancer cells: Targeting p53 for anticancer therapy. *Int. J. Nanomed.* 2015;10:4203–4222. doi: 10.2147/IJN.S83953.
20. Carlson C., Hussain S.M., Schrand A.M., Braydich-Stolle L.K., Hess K.L., Jones R.L., Schlager J.J. Unique cellular interaction of silver nanoparticles: Size-dependent generation of reactive oxygen species. *J. Phys. Chem. B.* 2008;112:13608–13619. doi: 10.1021/jp712087m.
21. chwertmann U, Cornell M. 2018. *Iron Oxides in the Laboratory: Preparation and Characterization.* 2. John Wiley & Sons.
22. Williams, M. J., and Corr, S. A. (2013). Magnetic nanoparticles for targeted cancer diagnosis and therapy. *Front. Nanosci.* 5, 29–63. doi: 10.1016/B978-0-08-098338-7.00002-9.
23. Hussain, S., Ji, Z., Taylor, A. J., DeGraff, L. M., George, M., Tucker, C. J., et al. (2016). Multiwalled carbon nanotube functionalization with high molecular weight hyaluronan significantly reduces pulmonary injury. *ACS Nano* 10, 7675–7688. doi: 10.1021/acsnano.6b03013.
24. Lembo, D., Donalisio, M., Civra, A., Argenziano, M., and Cavalli, R. (2018). Nanomedicine formulations for the delivery of antiviral drugs: a promising solution for the treatment of viral infections. *Expert Opin. Drug Deliv.* 15, 93–114. doi: 10.1080/17425247.2017.1360863.
25. Kim, Y., Park, E. J., and Na, D. H. (2018). Recent progress in dendrimer-based nanomedicine development. *Arch. Pharm. Res.* 41, 571–582. doi: 10.1007/s12272-018-1008-4.