LIPOSOMES: A NOVEL DRUG DELIVERY APPROACH

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ABSTRACT:

Bangham and his colleagues invented liposomes, which are concentric bilayer vesicles, in 1961. They have high drug entrapment ability and are extremely effective. They are the most commonly used vehicles for drug delivery due to their size, hydrophobic and lipophilic properties. The primary goal of this drug delivery system is to deliver the drug directly to the site of action, thus extending and enhancing the drug's impact. Liposomes are biocompatible and stable, and they can hold both hydrophilic and lipophilic drugs in their compartments. The diameter ranges from 0.05 to 5.0 millimeters. Mechanical dispersion methods, solvent dispersion methods, and detergent removal methods are some of the convectional techniques used for liposomal preparation and size reduction. Liposomes can be classified based on their size, charge, lamellarity, and other characteristics due to differences in preparation methods and lipid composition. This article covers the basics of liposomes, including their benefits, drawbacks, mechanism of action, classification, structural structure, planning, assessment criteria, applications, and future prospects.

Keywords- Liposomes**,** Phospholipids, Bilayer, Dispersion.

INTRODUCTION:

The word *liposome* derives from two Greek words: *lipo* ("fat") and *soma* ("body"); it is so named because its composition is primarily of phospholipid.

Liposomes were first described by British haematologist Alec D Bangham in 1961 (published 1964), at the Babraham Institute, in Cambridge.[1]

Liposomes Microscopic phospholipid bubbles with a bilayer membrane structure have received a lot of attention during the past 30 years as pharmaceutical carriers of great potential.[2]More recently many new developments have been seen in the area of liposomal drugs from clinically approved products to new experimental applications with gene delivery and cancer therapy still being the principal area of interest.[3]

Liposomes are microscopic vesicles composed of a bilayer of phospholipids or any similar amphipathic lipids. They can encapsulate and effectively deliver both hydrophilic and lipophilic substances and may use as a non-toxic vehicle for insoluble drugs.[4] Liposomes are composed of small vesicles of phospholipids encapsulating an aqueous space ranging from above 0.03 to 10um in diameter. The membrane of liposomes is made up of phospholipids which have phosphoric acid sides to form liposome bilayer.[5]

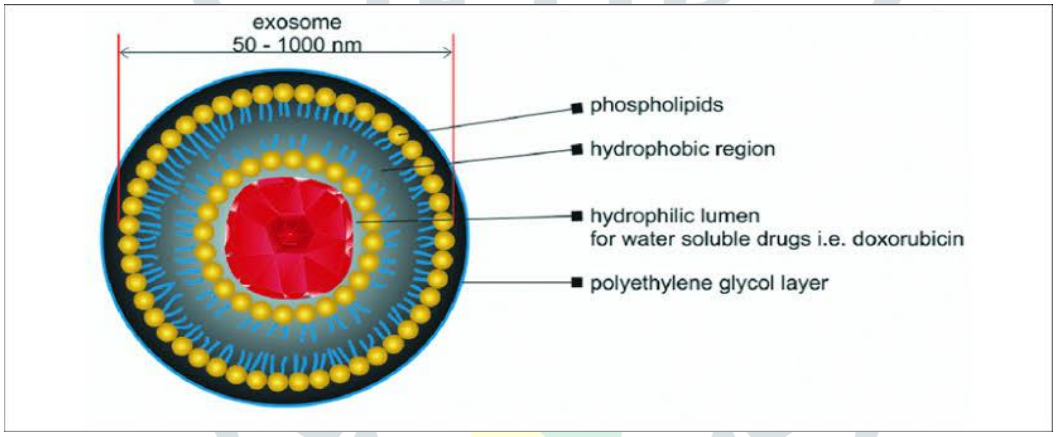

Fig.1 Basic structure of liposome

Structural components of liposomes

The main components are: -A) Phospholipids
B) Cholesterol

A) Phospholipids:Phospholipids are the major structural components of biological membranes. The most common phospholipid used in liposomal preparation is phosphatidylcholine (PC). Phosphatidylcholine is an amphipathic molecule contains

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

The molecules of phosphatidylcholine are not soluble in water. In aqueous media, they align themselves closely in planar bilayer sheets in order to minimizes the unfavorable action between the bulk aqueous phase and the long hydrocarbon fatty chain. Then the sheets fold on themselves to form closed sealed vesicles. There are several phospholipids that can be used for the liposome preparation such asDilaurylphosphatidylcholine (DLPC), Dimyristoylphosphatidylcholine (DMPC),Dipalmitoyl phosphatidylcholine (DPPC), Distearoylphosphatidylcholine (DSPC), Dioleolyphosphatidylcholine (DOPC), Diaurylphosphatidyl ethanolamine (DLPE).

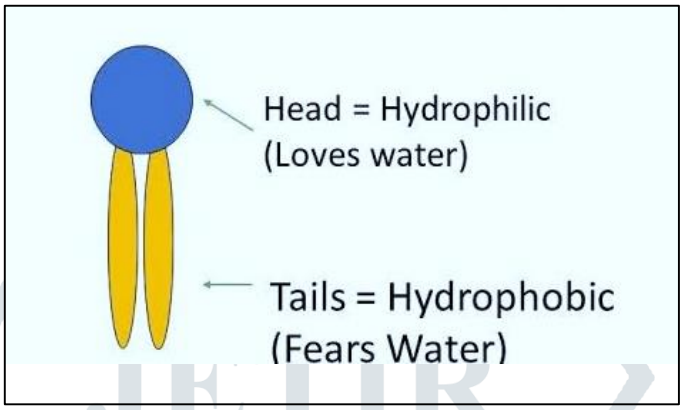

Fig.2 Phospholipid bilayer

B).Cholesterol: The role of cholesterol in formulation of liposomes is

1) Incorporation of sterols in liposomes bilayer produces major changes in the preparation of these membranes.
2) Cholesterol itself does not form a bilayer structure.
3) However, cholesterol acts as a fluidity buffer.
4) So, it makes the membrane less ordered and slightly more permeable below the phase transition and make the membrane more ordered and stable above the phase transition. It can be incorporated into phospholipid membrane in very high concentration up to 1:1 or even 2:1 molar ratios of cholesterol to phospholipids.[6]

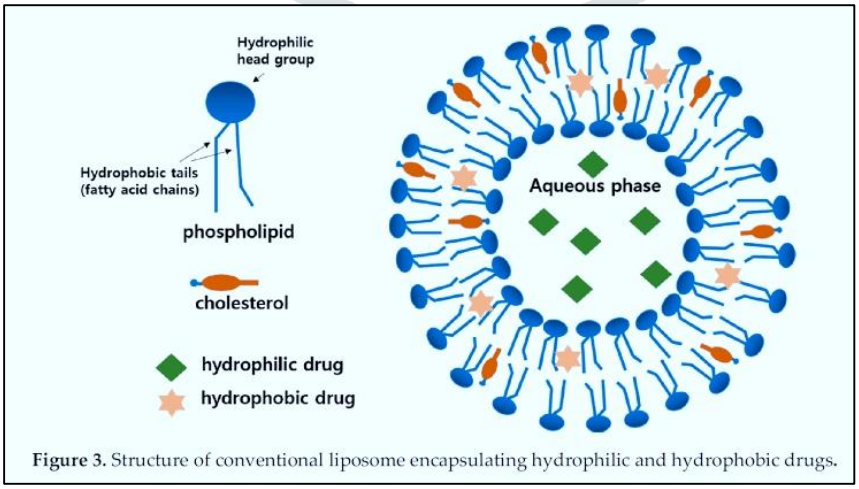

Figure 3. Structure of conventional liposome encapsulating hydrophilic and hydrophobic drugs.

- **Advantages of liposomes**

- They offer targeted drug delivery.
- They are biocompatible, biodegradable and biologically inert.
- They arenon-antigenic, non-pyrogenic and nontoxic.
- They can encapsulate both water soluble and water insoluble drugs.

- Drug's toxicity is removed as other tissues and cells are protected.
- Cellular uptake of drug is enhanced.

Disadvantages of liposomes

- Liposomes are less stable.
- They are rapidly removed by cells of Reticuloendothelial (RES) from blood after iv injection.
- Drug release is slow and influenced by phagocytes.
- Production cost is high
- Short half life
- Sometime liposomes go under oxidation and hydrolysis like reaction.

Classification of liposomes

Liposomes are classified on the basis of:

- 1.Composition
- 2. Structure
- 3. Method of preparation
- 4.Conventional liposomes
- 5.Specialty liposomes

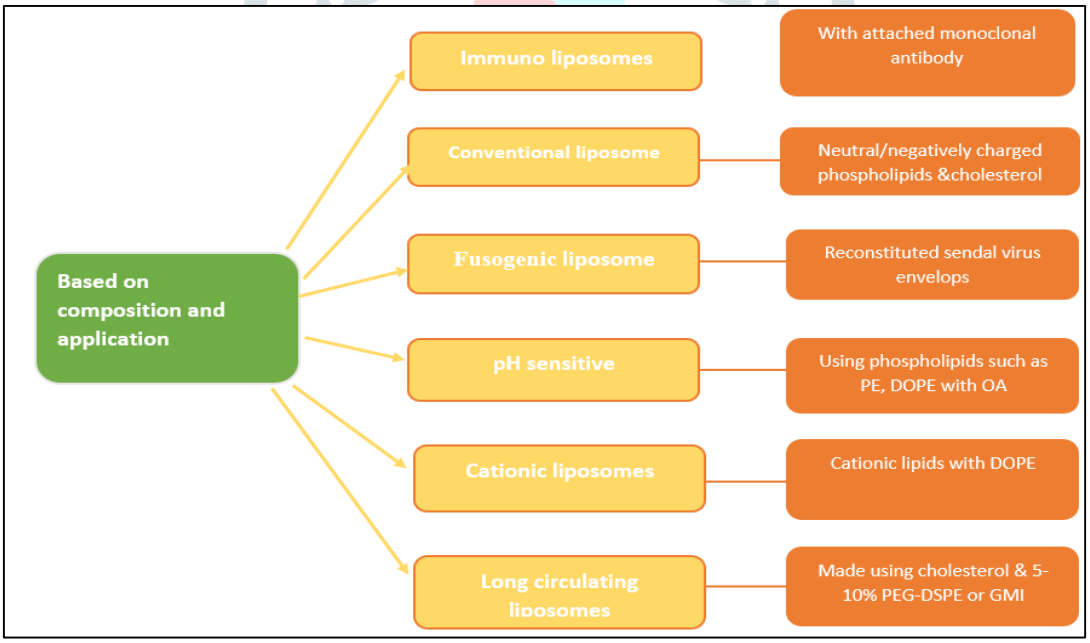

Fig 4. Based on composition and [9]

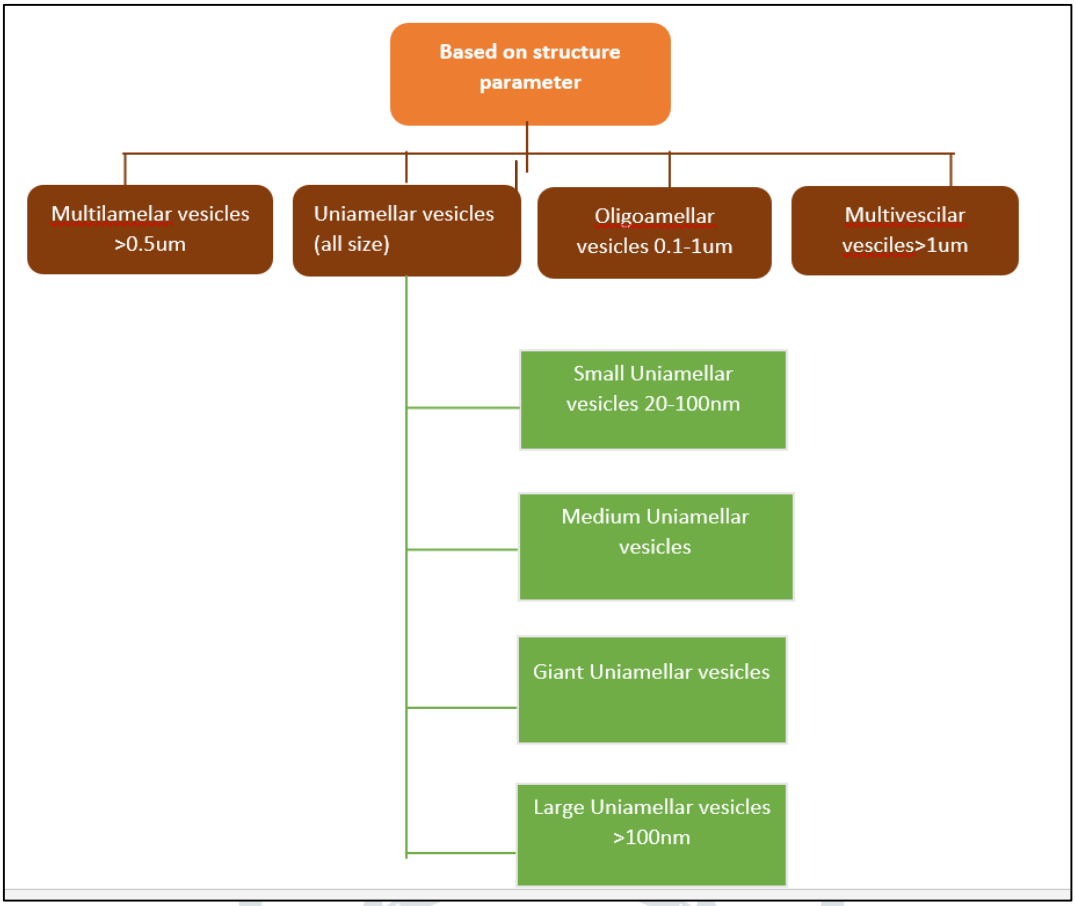

Fig 5. Based on structural parameter [9]

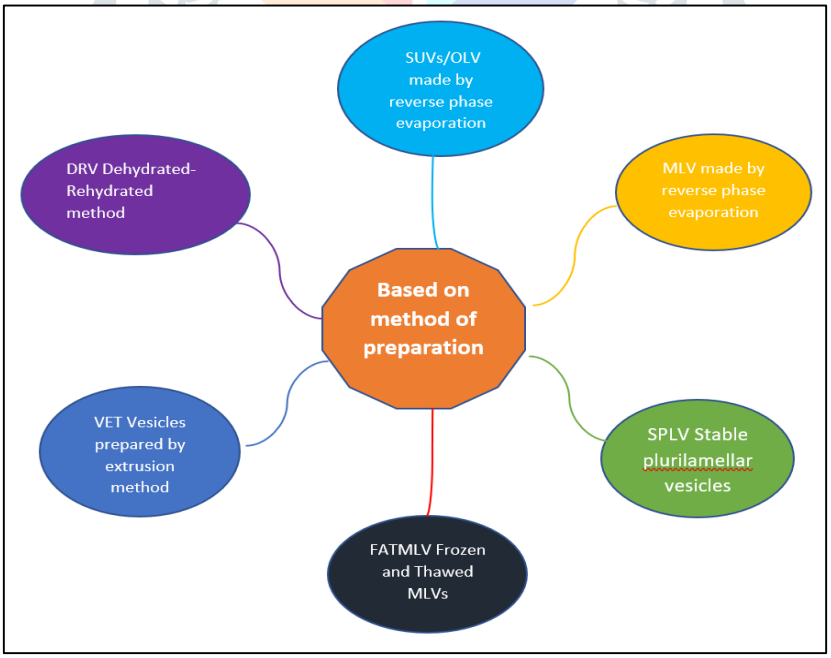

Fig 6. Based on methods of preparations [9]

Methods of preparations of liposomes:

- **Passive Loading Technique:**
  - **I.** Mechanical dispersion
    a. Lipid hydration method
    b. Micro emulsification
    c. Sonication
    d. French pressure cell method
    e. Membrane extrusion
    f. Dried reconstituted vesicles
    g. Freeze Thaw method
    h. pH induced vesiculation
    i. Calcium induced fusion
  - **II.** Solvent Dispersion
    a. Ethanol injection method
    b. Ether infusion method
    c. Double emulsification
    d. Reverse phase evaporation
  - **III.** Detergent Removal

- **Active Loading Technique:**
  a) Proliposomes
  b) Lyophilization

- **Passive Loading technique:**

Lipid hydration method by hand shaking and non-handshaking: -

In this method the lipid is casted as stacks of film from their organic solution using `flash rotatory evaporator` or hand shaking the film formation will be takes place and the film will be dried in presence of reduced nitrogen after the film stacks are dispersed in aqueous phase. Open hydrogen the liquid will swell and peel off from wall of round bottom flask and vesiculate forming multi lamella vesicles (MLVs). liposomes stored under the nitrogen umbrella store.

Process:

- **Step1:**

Lipid mixtureof different phospholipid and charge components in chloroform: methanolsolvent mixture (2:1v/v) is prepared first and then introduced into a round bottom flask with a ground glass neck, this flask is then attached to a rotary evaporator and rotated at 60 rpm.The organic solvent isevaporated at about 30 degree Celsius or above the transition temperature of the lipids used theevaporator is isolated from the vacuum source by closing the tep.The nitrogen is introduced into the evaporator and the pressure at the cylinder head is gradually raised till there isno difference between inside and outside the flask. The flask is then removed from the evaporator and fixed on to the manifold of lyophilizer to remove residue solvent.

- **Step-2:**

  After releasing the vacuum and removal from the lyophilizer, the flask is flushed withnitrogen,5 ml of saline phosphate buffer (containing 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 lack is left rotating for 30 minutes 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 hours at room temperature or at a temperature above the transition temperature of the lipid in order to complete the swelling process to give MLVs.14[8]

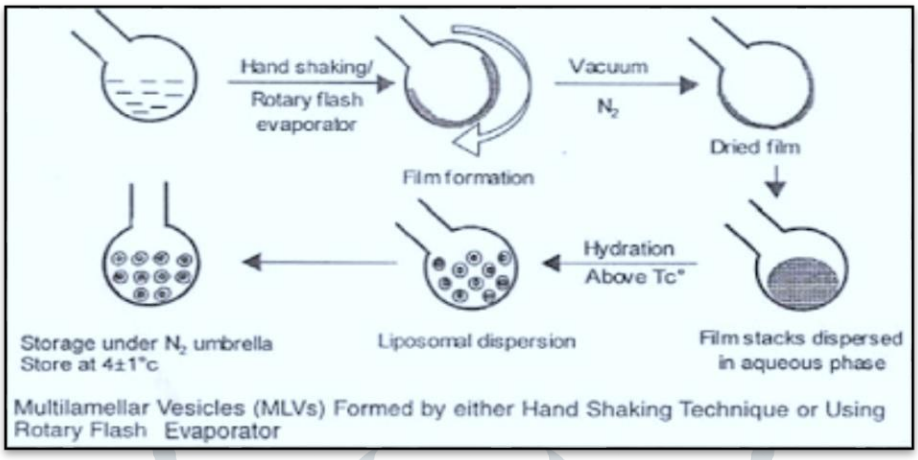

Fig 7. Lipid hydration method by hand shaking

b.MicroEmulsification: -

'Micro fluidizer" is used to prepare smallMLVs from concentrated Lipiddispersion. Micro fluidizer pumps the fluid at very 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 highvelocity, there by affecting an efficient transfer of energy. The lipids can be introduced into the fluidizer, either as large MLVs or as the slurry of un hydrated lipid in organic medium. The fluid collected can be recycled through the pumpand interactionchamber until vesicles of spherical dimensions are obtained.[9]

c. French pressure cell: -

This method is having the mechanism of high pressure. This method will give the either uni- or oligo-lamellar liposomes of intermediate size(30-80nm), these liposomes are more stablecompared to the sonicated liposomes. This method is having some drawbacks are that initial high cost for the press and the pressure cell liposomes prepared by this method having less structural defects unlike sonicated liposome.[7][10]

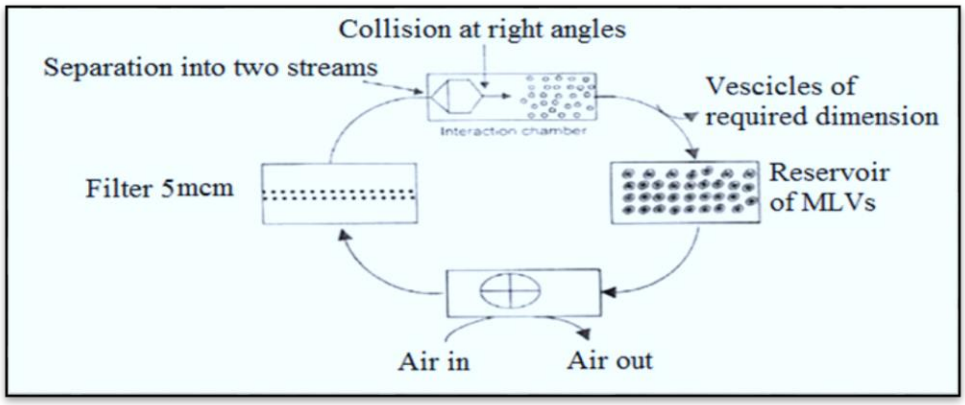

Fig 8.Micro fluidizer method

d. Sonication: -

This is the method in which Multi lamellar vesicles are transferafter 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 varying diameter as size is influenced by composition and concentration, temperature, sonication, volume and sonication tuning. Med to the small uni lamellar vesicles. The ultra-sonic irradiation is provided to the MLVs to get the SUVs.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 over-heating of liposomal dispersion causing lipid degradation. sonication tip also releases titanium into the liposome dispersion which will be removed from the it by centrifugation prior to use.[7][9]

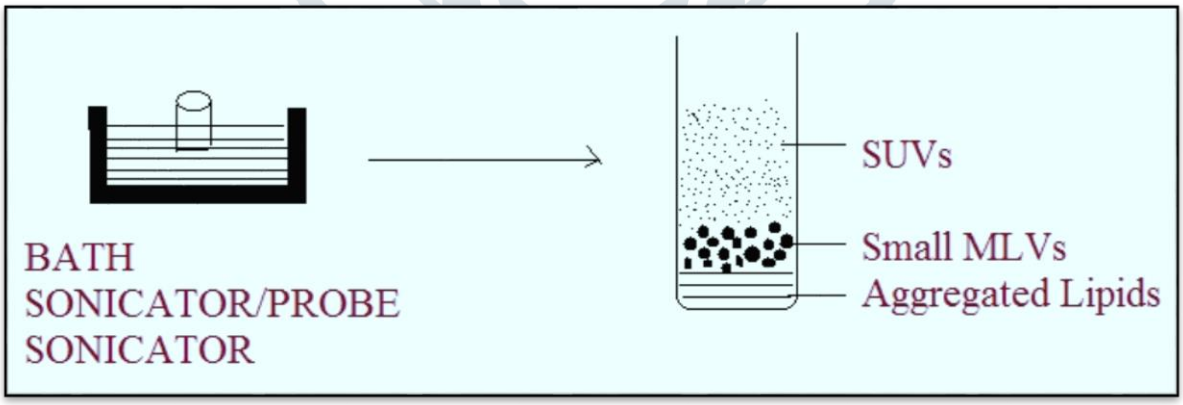

Fig 9. Sonication method

e.Membrane Extrusion technique: -

The technique can be used to process LUVs as well as MLVs.The size of liposomes is reduced by gently passing them through membrane filter of defined pore size achieved at much lower pressure(<100psi). I this process, the vesicles contents areexchanged with the dispersion medium during breaking and resealing of phospholipids bilayers as they pass through the polycarbonate membrane. The liposomes produced by this technique have been termed LUVETs.This technique is most widely used method for SUV and LUV production for in vitro and in vivo studies.[9][11]

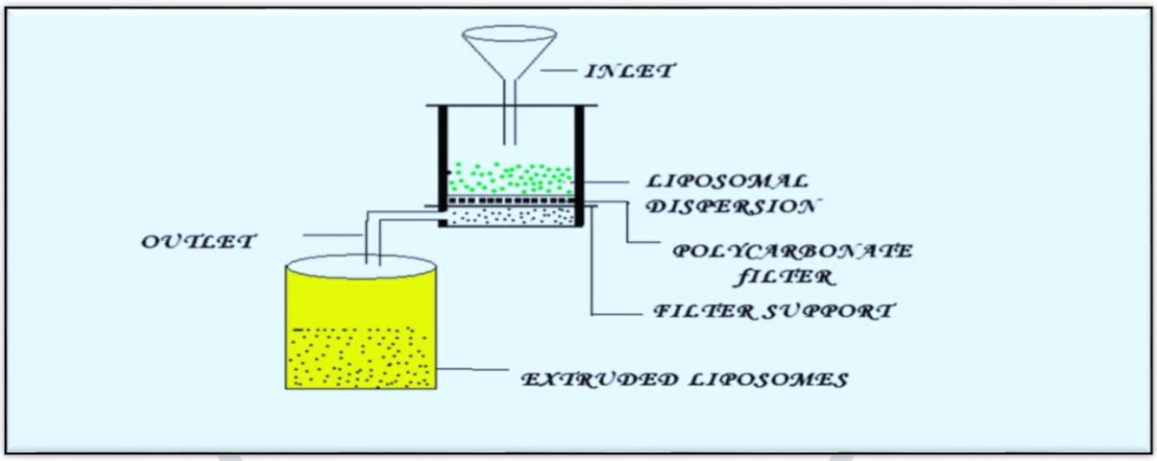

Fig 10. Membrane extrusion technique

f.Dried Reconstituted Vesicles (DRVs): -

This method starts with freeze drying of a dispersion of empty SUVs. After freeze drying the freeze-dried membrane is obtained. Then these freeze-dried SUVs are rehydrated with the use aqueous fluid containing the material to be entrapped. This leads to formation of the solutes in uni- or oligo- lamellar vesicles.[11]

g. Calcium Induced fusion: -

It uses concept of aggregation and fusion of acid phospholipid vesicles in the presence of calcium .In this method ,lipid is dried down and suspended in sonication buffer (NaCl 0.385g,histidin 31.0 mg ,this – base 24.2 mg ,water 100ml ,PH 7.4)The large liposomes and lipid particles are removed by centrifugation at 100,000g.Equimolar proportion of calcium solution precipitate is formed It is incubated for 60 mins at 37 degree Celsius and the precipitate is separated by spinning the container at 3000g for 20 min at room temperature .the supplementing is discarded pellet is resuspended is buffered saline containing the material to be entrapped and incubated at 37 degrees Celsius for 10 min The EDTA (170mm) is added in buffer with mixing The cloudy precipitate will clear rapidly ,then incubate for 15 min at 37 degree Celsius and further 15 min mix at room temperature.Finally, the ca/EDTA complex is removed by dialyzing again a liter of buffer.[9][12]

h.pH induced vesiculation: -

This method prepares ULVs from MLVs withoutsonication or high-pressure application, they are reassembled by simply charging the ph. it is an electrostatic phenomenon, the transferringchange in PH bring about an increase in the surface charge density of lipid bilayer, provided this exceeds a certain threshold valve of around 1-2uc/cm2, spontaneous vesiculation will occur. the period of exposure of the

phospholipid to high pH. is less than 2min s and not long enough to cause detectable degradation of phospholipid this method ,dry film of lipid is obtained is the round bottom flask using the rotatory evaporator and last traces of the solvent are removed using freeze dryer.Then,the film is hydrated with minimum quantity of water by hand shaking at room temperature. At this stage material to be entrapped inside the vesicles may be added in the water beforeadditionto thelipid. The dispersionis completedby subjectingthe suspension to six freeze thawing cycle between 15 degree Celsius and 5 degrees Celsius. [10][12]

I. Freeze Thaw Method: -

This method is based upon freezing of a Uniamellardispersion (SUV). Then thawing by standing at room temperature for 15min.Finally subjecting to a briefSonication cycle which considerably reduces thepermeability of the liposome's membrane. In order to prepare GIANT VESICLES of diameter between 10 and 50um, the freeze thaw technique has been modified to incorporate a dialysis step against hypo- osmolar buffer in the place of sonication.[9] The method is simple, rapid and mild for entrapped solutes, and results in a high proportion of large Uniamellar vesicles formation which are useful for study of membranetransport phenomenon. Thismethod is based upon freezing of a Uniamellardispersion (SUV). Then thawing by standing at room temperature for 15min. Finally subjecting to a brief Sonication cycle which considerably reduces the permeability of the liposome's membrane. In order to prepare GIANT VESICLES of diameter between 10 and 50um, the freezethaw techniquehas been modified to incorporate a dialysis step against hypo- osmolar buffer in the place of sonication.[7][10]

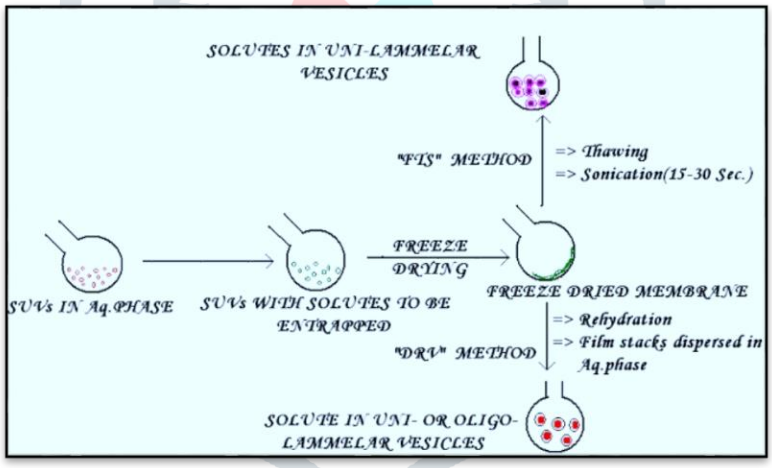

Fig 11. Freeze thaw method

B. Solvent Dispersion method:

a.Ether and Ethanol Injection: -

The method involves the extrusion of MLV at 20,000 psi at 4°C through a small orifice. The method has several advantages over sonication method. The method is simple rapid, reproducible and involves gentle handling of unstable materials (Hamilton and Guo, 1984).[10][12] Ethanol Injection Method A lipid solution of ethanol is rapidly injected to a vast excess of buffer. The MLVsare 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.[5]

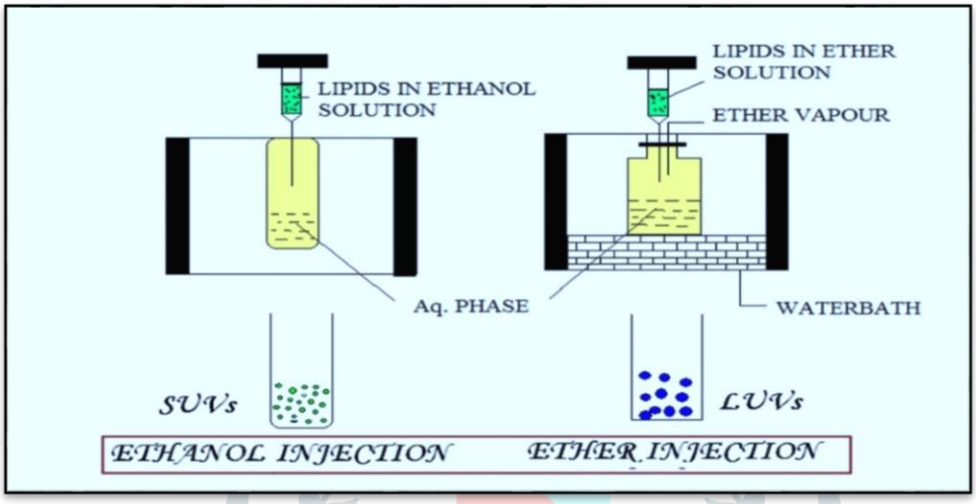

Fig 12. Ethanol and Ether injection method

b.Double Emulsion Vesicles: -

In this method, the outer half of the liposome membrane is created at a second interphase between two phases by emulsification of an organic solution in water. If the organic solution, which already contains water droplet, is introduced into excess aqueous medium followed by mechanical dispersion,multi compartment vesicles are obtained. The ordered dispersion so obtained is described as W/O/W system. If the organic solution, which already contains water droplet, is introduced into excess aqueous medium followed by mechanical dispersion, multicompartment vesicles are obtained. The ordered dispersion so obtained is described as W/O/W system. At this step monolayers of phospholipids surrounding each water compartment are closely opposed by each other. The next step is to bring about the collapse of a certain proportion of the water droplets by vigorous shaking by using mechanical vortex mixer. [10][12]

c.Reverse phase evaporation:

Sonicated methods (stable plurilamellar vesicles –SPVs) in this method, w/o dispersion is prepared as described earlier with excess lipid, butdrying process is accompanied by continuedbath sonicationwith a stream of nitrogen. The redistribution and equilibration of aqueous solvent and solute occur this time in between the various bilayer in each plurilamellar vesicles the internal structure of SPVs is different from that of MLV-REV, in that they lack a large aqueous medium being located in compartment in betweenadjacent lamellae. Thepercent entrapment is normally 30.[12]

C.Detergent Removal Method

Detergent/Phospholipids mixtures can form large Uniamellar 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 associated with phospholipid molecule from water, in structural formed as a resultof this associated are known as micelles, and can be composed of several hundred component molecules. Their shape and size depend on chemical nature of the detergent, theconcentration and other lipid involved. The concentrated of detergent in water at which micelles from is known as critical micelle concentration (CMC).[9]

Active Loading techniques

Proliposomes Lipid and drug are coated onto a soluble carrier to form free flowing granular material in pro liposomes which forms an isotonic liposomal suspension on hydration. The pro liposome approach may provide an opportunity for cost affection large scale manufacture of liposomes containing particularly lipophilic drugs.[6]

Lyophilizationthe removal of water from products in the frozen state at extremely reduced pressure is called Lyophilization (freeze drying). The process is generally used to dry products that are thermolabile which may be destroyed by heat drying. This technique has a great potential to solve long term stability problems with respect to liposomal stability.[6]

Therapeutic applications of liposomes:

- Drug targeting: - An ideal targeted drug deliverydeliversfor only to its site of action. Drug targeting leads to increased efficacy at low dose with decreased toxicity. Methods to achieve active targeting via liposomes involves use of ligand e.g.,cell specific antibodies, sugar residues, apoproteins or hormones etc. which are tagged on lipid vehicle, these ligandsrecognizespecific sites so cause targeting of liposomal drug at those target sites. Ligands is selected on the basis on its recognition and specifically to target site. In cancer treatment, drug targeted to tumor cells via receptor specific ligands which may be specific antibodies for antigens produced by tumor cells. E.g., MT_1-MMP (Membrane type -1 matrix metalloproteins).[14]

- **Topical drug deliver** :-Liposomes have shown great potential in dermatology and cosmetogy ,when applied topically ,liposomes exhibited an increased penetration of , thus enhanced permeability through skin but offered less side effects ,because of reduced dose and limited systemic absorption .in an experiment in guinea pigs, liposomes lipocaine was found to have higher concentration than its cream formulation (o/w) which proves enhanced penetration by liposome carrier system causing drug release in epidermis.[27]Liposomes applied to skin in the form of solution and hydrogen where hydrolic polymers are used thinners' study shows enhanced penetration in to skin by hydrogels prepared from xanthngum. Liposomal encapsulated drug of ketoconazole showed sustained release, increased antifungal efficacy and less adverse effect.[16]

- **Antimicrobial therapy: - Incorporation of antibiotics in liposomes offer two benefits:**
  - Protection of drug e.g., penicillin's,cephalosporins, etc. against enzymatic degradation (e.g., by beta lactamase).
  - Enhanced cellular uptake of antibiotic in microorganism, thus reducing effective dose and toxicity ae in liposomal amphotericin. Meglumineantimonite incorporated liposomes may

provide better treatment against visceral leishmaniasis, allowing lower number of injections compared to conventional treatment.[17]

- **Antiviral therapy:** -A study showed effectiveness of liposomes as earlier of anti-retroviral agent dideoxycytidine-5-triphosphate. Encapsulation of this antiretroviral agent into liposomes reduces the effective dose which prevents the dose related toxicities associated with agents.[14]

- **Local therapy:** - Antioxidants like calabash SOD delivered via anionic liposomes may provide better targeted treatment in chronic inflammation of colonic epithelium like ulcerative colitis.[18]

- **Immunotherapy:-** Liposomes are used as carrier of vaccine agents. They are now employed as oral vaccines in immunization procedure. They can elicit both humoral cells mediated immunity and exhibits potential base for oral vaccine against hepatitis A. Multiple antigen peptides (MAP) enclosed liposome triggered strong immune response suggesting in role for design of therapeutic vaccines.[19]

- **Protection against enzymatic degradation:** - Lipids in liposomes formulation not prone to enzymatic degradation, so entrapped drug is protected when lipid vesicle in circulation in extracellular fluid. Inside the cell, entrapped drugs get released either by diffusion or dissolution of shell or degradation by lysosomal enzymes. Liposomes protect drug in GI environment and facilitates GI transport of different types of compounds. Thus, liposomes have great potential for delivery of insulin and proteins which are orally biodegradable.[21]

- **Prophylaxis:** -Butyl cholinesterase encapsulating bio adhesive liposomes may prophylaxis against organophosphate poisoning by preventing loss of intracellular enzyme activity.[22]

- **Liposome potentialities for intravitreal administration:** - Marketedliposome-based medicines are all given parenterally (Ambisome*, Doxil*, DaunoXome*, Novasome* and Nyotran*) and only one named Visudyne* is employed for the treatment of age-related macular degeneration. However, by the intravenous route. Liposomes are able to control drug release and improve significantly theirvitreoushalf-life. Moreover, they might reduce the amount of drug that needs to be administered and consequently, the volume to be injected since large volume could result in an intraocularpressure increase.[23][24]

CONCLUSION:

Liposomes over the years have been investigated as the major drug delivery systems due to their flexibility to be tailored for varied desirable purposes. The flexibility in their behavior can be exploited for the drug delivery through any route of administration and for any drug or material irrespective of its physicochemical properties. The uses of Liposomes in the delivery of drugs and genes to tumor sites are promising and may serve as a handle for focus of future research. Liposomes are best tool for targeting brain; hence it is made up of lipid bilayer. And also, it's having a site-specific delivery and it produces long term therapy. BBB is made up of lipid bilayer so when compared to other dosage form Liposomes are easily crosses the B.B.B. Hence, we can achieve very good site-specific action via Liposomes.

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ABBREVIATIONS:

1.PC - Phosphatidylcholine
2.DLPC –Dilaurylphosphatidylcholine
3.DMPC – Dimyristoylphosphatidylcholine
4.DPPC – Dipalmitoyl phosphatidylcholine
5.DSPC – Distearoylphosphotidylcholine
6.DOPC – Dioleolyphosphatidylcholine
7.DLPE – Diaurylphosphatidyl ethanolamine
8.SUVs -Small Uniamellar vesicles
9.MLVs –Multilamellar vesicles
10.LUVs – Large Uniamellar vesicles
11.EDTA – Ethylene diamine tetra acetic acid
12.SPVs – Stable Plurilamellar vesicles

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