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Review Article

A COMPLETE REVIEW ON: LIPOSOMES

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ABSTRACT
In this review article, we discussed about liposome, these are one amongst the various drug delivery system used to target the drug to particular tissue. Because of structure similarity between lipid bilayer & cell membrane, liposome can penetrate effectively deliver drug to such that a free drug would not penetrate. The various other drug delivery devices include niosomes, microparticles, resealed erythrocytes, pharmacosomes etc.
The term liposome means lipid body. It has been derived on the basis of name of subcellular particles, ribosome. Liposomes were first made by A.D Bangham in early 1960s. Their size ranges from 25 to 500 nm.
KEYWORDS: Drug delivery system using liposomes, Structural components of liposomes, Methods of preparation of liposomes

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.[1-4]
"Liposomes are colloidal, vesicular structures composed of one or more lipid bilayers surrounding an equal numbers of aqueous compartments". The sphere like shell encapsulated a liquid interior which contain substances such as peptides and protein, hormones, enzymes, antibiotic, antifungal & anticancer agents. A free drug injected in blood stream typically achieves therapeutic level for short duration due to metabolism & excretion. Drug encapsulated by liposomes achieve therapeutic level for long duration as drug must first be release from liposome before metabolism & excretion.[5, 6]

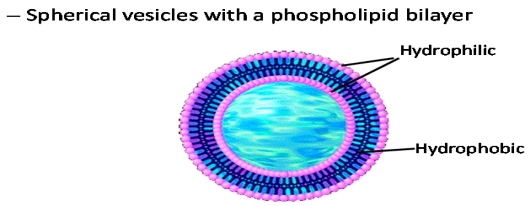

Figure 1: Liposome [6]

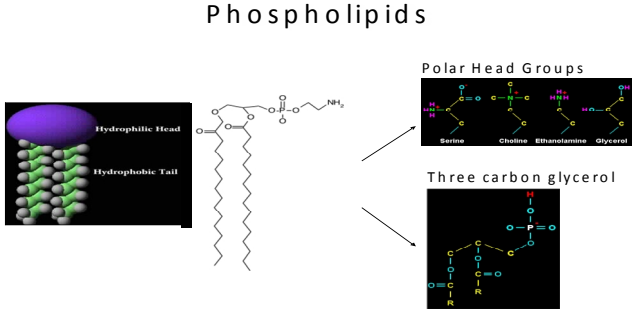

Figure 2: Phospholipids [7]

ADVANTAGES OF LIPOSOMES [1-6]

- Liposomes are biocompatible, completely biodegradable, non-toxic and non immunogenic.
- Suitable for delivery of hydrophobic, amphipathic and hydrophilic drugs.
- Protect the encapsulated drug from the external environment.
- Reduced toxicity and increased stability-As therapeutic activity of chemotherapeutic agents can be improved through liposome encapsulation. This reduces deleterious effects that are observed at conc. similar to or lower than those required for maximum therapeutic activity.
- Reduce exposure of sensitive tissues to toxic drugs.

DISADVANTAGES OF LIPOSOMES [1-6]

- Production cost is high
- Leakage and fusion of encapsulated drug/molecules
- Short half-life

TYPES OF LIPOSOMES [1-6]

Liposomes are classified on the basis of

A) BASED ON STRUCTURAL PARAMETERS:

1. Unilamellar vesicles:

- Small unilamellar vesicles (SUV): size ranges from 20-40 nm
- Medium unilamellar vesicles (MUV}: size ranges from 40-80 nm.
- Large unilamellar vesicles (LUV): size ranges from 100 nm-1,000 nm

2. Oligolamellar vesicles (OLV): These are made up of 2-10 bilayers of lipids surrounding a large internal volume

3. Multilamellar vesicles (MLV): They have several bilayers. They can compartmentalize the aqueous volume in an infinite numbers of ways. They differ according to way by which they are prepared. The arrangements can be onion like arrangements of concentric spherical bilayers of LUV/MLV enclosing a large number of SUV etc.

B) BASED ON METHOD OF LIPOSOME PREPARATION

1. REV: Single or oligolamellar vesicles made by Reverse-Phase Evaporation Method.
2. MLV-REV: Multilamellar vesicles made by Reverse-Phase Evaporation Method.
3. SPLV: Stable Plurilamellar Vesicles
4. FATMLV: Frozen and Thawed MLV.
5. VET: Vesicles prepared by extrusion technique
6. DRV: Dehydration-rehydration method.

C) BASED UPON COMPOSITION AND APPLICATION

1. Conventiuonal Liposomes (CL): Neutral or negatively charged phospholipids and Cholesterol.
2. Fusogenic Liposomes (RSVE): Reconstituted Sendai virus envelopes
3. pH sensitive Liposomes: Phospholipids such as PE or DOPE with either CHEMS or OA
4. Cationic Liposomes: Cationic lipids with DOPE
5. Long Circulatory (Stealth) Liposomes (LCL): They have polyethylene glycol (PEG) derivatives attached to their surface to decrease their detection by phagocyte system (reticuloendothelial system; RES). The attachment of PEG to liposomes decreases the clearance from blood stream and extends circulation time of liposomes in the body. The attachment of PEG is also known as pegylation.
6. Immuno-Liposomes: CL or LCL with attached monoclonal antibody or recognition sequence.

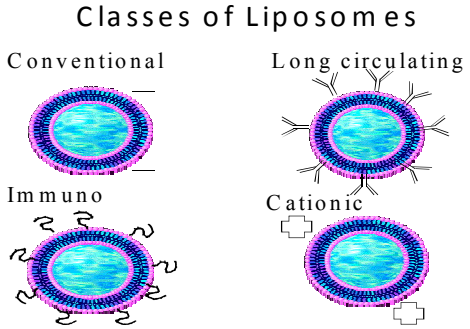

Figure 3: Classes of Liposomes [6]

STRUCTURAL COMPONENTS

1) Phospholipids

Glycerol containing phospholipids are most common used component of liposome formulation and represent greater than 50% of weight of lipid in biological membranes. These are derived from Phosphatidic acid. The back bone of the molecule is glycerol moiety. At C_3 OH group is esterified to phosphoric acid. OH at C_1 & C_2 are esterified with long chain. Fatty acid giving rise to the lipidic nature. One of the remaining OH groups of phosphoric acid may be further esterified to a wide range of organic alcohols including glycerol, choline, ethanolamine, serine and inositol. Thus the parent compound of the series is the phosphoric ester of glycerol.

Examples of phospholipids are

- Phosphatidyl choline (Lecithin) – PC
- Phosphatidyl ethanolamine (cephalin) – PE
- Phosphatidyl serine (PS)
- Phosphatidyl inositol (PI)
- Phosphatidyl Glycerol (PG)

For stable liposomes, saturated fatly acids are used. Unsaturated fatty acids are not used generally.

2) Sphingolipids

Backbone is sphingosine or a related base. These are important constituents of plant and animal cells. This contain 3 characteristic building blocks

- A mol of F.A
- A mol of sphingosine
- A head group that can vary from simple alcohols such as choline to very complex carbohydrates.

Most common Sphingolipids – Sphingomyelin. Glycosphingo lipids.

Gangliosides – found on grey matter, used as a minor component for liposome production.

This molecule contain complex saccharides with one or more Sialicacid residues in their polar head group & thus have one or more negative charge at neutral pH. These are included in liposomes to provide a layer of surface charged group.

3) Sterols: Cholesterol & its derivatives are often included in liposomes for

- decreasing the fluidity or microviscocity of the bilayer
- reducing the permeability of the membrane to water soluble molecules
- Stabilizing the membrane in the presence of biological fluids such as plasma.(this effect used in formulation of i.v. liposomes)

Liposomes without cholesterol are known to interact rapidly with plasma protein such as albumin, transferrin, and macroglobulin. These proteins tend to extract bulk phospholipids from liposomes, there by depleting the outer monolayer of the vesicles leading to physical instability. Cholesterol appears to substantially reduce this type of interaction. Cholesterol has been called the mortar of bilayers, because by virtue of its molecular shape and solubility properties, it fills in empty spaces among the Phospholipid molecules, anchoring them more strongly into the structure. The OH group at 3rd position provides small Polar head group and the hydrocarbon chain at C_{17} becomes non polar end by these molecules, the cholesterol intercalates in the bilayers.

4) Synthetic phospholipids

E.g.: for saturated phospholipids are

- Dipalmitoyl phosphatidyl choline (DPPC)
- Distearoyl phosphatidyl choline (DSPC)
- Dipalmitoyl phosphatidyl ethanolamine (DPPE)
- Dipalmitoyl phosphatidyl serine (DPPS)
- Dipalmitoyl phosphatidic acid (DPPA)
- Dipalmitoyl phosphatidyl glycerol (DPPG)

E.g.: for unsaturated phospholipids

- Dioleoyl phosphatidyl choline (DOPC)
- Dioleoyl phosphatidyl glycerol (DOPG)

5) Polymeric materials

Synthetic phospholipids with diactylenic group in the hydrocarbon chain polymerizes when exposed to U.V, leading to formation of polymerized liposomes having significantly higher permeability barriers to entrapped aqueous drugs. E.g.: for other Polymerisable lipids are – lipids containing conjugated diene, Methacrylate etc. Also several Polymerisable surfactants are also synthesized.

6) Polymer bearing lipids

Stability of repulsive interactions with macromolecules is governed mostly by repulsive electrostatic forces. This repulsion can be induced by coating liposome surfaces with charged polymers.

Non ionic and water compatible polymers like polyethylene oxide, polyvinyl alcohol, and Polyoxazolidines confers higher solubility. But adsorption of such copolymers containing hydrophilic segments with hydrophobic part leads to liposome leakage, so best results can be achieved by covalently attaching polymers to phospholipids. E.g.: Diacyl Phosphatidyl ethanolamine with PEG polymer linked via a carbon at or succinate bond.

7) Cationic lipids

E.g.: DODAB/C – Dioctadecyl dimethyl ammonium bromide or chloride

DOTAP – Dioleoyl propyl trimethyl ammonium chloride – this is an analogue of DOTAP and various others including various analogues of DOTMA and cationic derivatives of cholesterol

8) Other Substances

- Variety of other lipids of surfactants are used to form liposomes
- Many single chain surfactants can form liposomes on mixing with cholesterol
- Non ionic lipids
- A variety of Polyglycerol and Polyethoxylated mono and dialkyl amphiphiles used mainly in cosmetic preparations
- Single and double chain lipids having fluoro carbon chains can form very stable liposomes
- Sterylamine and Dicetyl phosphate
- Incorporated into liposomes so as to impart either a negative or positive surface charge to these structures

PREPARATION OF LIPOSOMES [7-20]

- General method of preparation
- Specific methods of preparation

A) GENERAL METHOD OF PREPARATION: The lipid is dissolved in organic solvent. The solvent is evaporated leaving a small film of lipids on the wall of the container. An aqueous solution of drug is added. In first procedure the mixture is agitated to produce multi lamellar vesicle and then sonicated to get SUVs. In the second procedure the mixture is sonicated and the solvent is evaporated to get LUVs. After extrusion SUVs are formed. Drug can be incorporated into the aqueous solution or buffer if it is water soluble or included in organic solvent if it is hydrophobic. Free drug and liposomes can be separated by gel chromatography.

B) SPECIFIC METHODS: These are classified as 3 types based on the modes of dispersion. They are

1. Physical Dispersion methods
2. Solvent Dispersion methods
3. Detergent Solubilization methods

1) PHYSICAL DISPERSION METHODS: In these methods the aqueous volumes enclosed within lipid membranes is about 5- 10%, which is very small proportion of total volume used for preparation. So large amount of water soluble drug is wasted during preparation. But lipid soluble drug can be encapsulated to high percentage. In these methods, MLVs are formed and further treatment is required for preparation of Unilamellar vesicles

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 rotary evaporator connected with 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 minutes after dry residue appeared. The evaporator is detached from vacuum pump and nitrogen is introduced into it. The flask is then removed from evaporator and fixed onto lypholizer 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 evaporator again and rotated at about 60 rpm speed for 30 minutes 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 hours in order to complete swelling process to give MLVs.

Non-Shaking Method: This is similar to shaking method except that care is taken in swelling procedure. The solution of lipid in chloroform and methanol mixture is spread over the flat bottom of the conical flask. The solution is evaporated at room temperature by 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. After hydration, lipid is swelled by addition of bulk liquid. The flask is inclined to one side, 10 to 20 ml of 0.2M sucrose in distilled water is introduced down the side of the flask and then flask is slowly returned to up right position. The solution 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 hours at 37 degrees for swelling. After that the vesicles are mixed to yield a milky suspension. The suspension is centrifuged at 1200 rpm for 10 minutes. The layer of MLVs floating on the surface is removed. From the remaining fluid, LUVs are produced.

Freeze Drying: Another method of dispersing the lipid in a finally divided form prior to 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. In order to modify the size the prepared MLVs are further processed using the following procedures.

PROCESSING OF LIPIDS HYDRATED BY PHYSICAL MEANS

Micro-emulsification of liposomes: An equipment called micro fluidizer is used to prepare small vesicles from concentrated lipid suspension. The lipids can be introduced in to the fluidizer as a suspension of large MLVs. This equipment pumps the fluid at very high pressure through 5 micrometer screen. Then it is forced long micro channels, which direct two streams of fluids collide together at right angles at very high velocity. The fluid collected can be recycled through the pump and interaction chamber until vesicles of spherical dimensions are obtain

Sonication: This method reduces the size of the vesicles and imparts energy to lipid suspension. This can be achieved by exposing the MLV to ultrasonic irradiation. There are two methods of sonication A) using bath sonicator B) using probe sonicator. The probe sonicator is used for suspensions which require high energy in small volume. (eg: high concentration of lipids or viscous aqueous phase) The bath sonicator is used for large volume of dilute lipids. The disadvantage of probe sonicator is contamination of preparation with metal from tip of probe. By this method small unilamellar vesicles are formed and they are purified by ultra centrifugation.

Membrane Extrusion Liposome: In this method the size is reduced by passing them through a membrane filter of defind pore size. There are two types of membrane filter. The tortuous path type and the nucleation track type. The former is used for sterile filtration. In this random path arise between the criss cross fibres. The average diameter of these fibers is controlled by the density of fibres in the matrix. Liposomes that are larger than the channel diameter get struck when one tries to pass them through such membrane. The nucleation track type is composed of thin continuous sheet of polycarbonate. They will offer less resistance to passage of liposomes as these consist of straight sided pore holes of

exact diameter bored from one side to another. This method can be used to process both LUVs and MLVs.

Freeze and Thaw Sonication: This is a method in which rupture and refusing of SUVs are done during which the solute equilibrates between the inside and outside. This process increases the entrapment volume and entrapment efficiency. This method will result in the formation of vesicles with in vesicled and vesicle between lamellae. This method can increase the entrapment volume upto 30%

2) SOLVENT DISPERSION METHODS: In these methods lipids are first dissolved in an organic solution and then brought into contact with aqueous phase containing materials to be entrapped within liposome. At the interface between the organic and the aqueous phases the phospholipids align themselves to form a monolayer, which is important step to form the bilayer of liposome.

Ethanol injection method: This is simple method. In this method an ethanol solution of the lipids is directly injected rapidly to an excess of saline or other aqueous medium through a fine needle. The ethanol is diluted in water and phospholipids molecules are dispersed evenly through the medium. This procedure yields a high proportion of SUVs (about 25nm diameter).

Ether injection: This method is similar to above one. It involves injecting the immiscible organic solution very slowly into an aqueous phase through a narrow needle at temperature of vaporizing of organic solvent. In this method the lipids are carefully treated and there is very less risk of oxidative degradation. The disadvantage is that long time is required for the process and careful control is needed for introduction of lipid solution.

3) DETERGNT SOLUBILIZATION TECHNIQUE: In this method the phospholipids are brought into close contact with the aqueous phase via detergents, which associate with phospholipids molecules. The structures formed as a result of this association are known as micelles. They are composed of several hundereds of component molecules. The concentration of detergent in water at which micelles start to form is called CMC. Below CMC the detergent molecule exist in free solution. As the detergent molecule is dissolved in water at concentrations higher than the CMC, micelle form in large amounts. As the concentration of detergent added is increased more amount of detergent is incorporated into the bilayer, until a point is reached where conversion from lamellar form to spherical micellar form take place. As detergent concentration is further increased, the micelles are reduced in size.

MECHANISM OF FORMATION OF LIPOSOMES [8-10]

Lipids capable of forming liposomes exhibit a dual chemical nature. Their head groups are hydrophilic and their fatty acyl chains are hydrophobic.

It has been estimated that each Zwitter ionic head group of Phosphatidyl choline has on the order of 15 molecules of water weakly bound to it, which explain it's over whelming preference for the water phase. The hydrocarbon fatty acid chains on the other hand vastly prefer each others company to that of H_2O. This can be understood by taking the CMC of P.C into account. The CMC of Dipalmitoyl P.C found to be 4.6 $^{-10}$ M in water, which is a small number indicating the over whelming preference of this molecule for a hydrophobic environment such as that found in the core of micelle or bilayer.

The free energy of transfer from water to micelle is 15.3K cal/mol for Dipalmitoyl PC and 13.0K cal/mol for Dimyristoyl P.C. These results clearly point out the thermodynamic basis for bilayer assembly that has been termed the hydrophobic effect. The large free energy change between a water and a hydrophobic environment explains the over whelming preference of typical lipids to assemble in bilayer structures, including water as much is possible from the hydrophobic core in order to achieve the lowest energy level, hence the highest stability for the aggregate structure.

PURIFICATION OF LIPOSOMES [10, 11]

Liposomes are generally purified by gel filtration chromatography, Dialysis and centrifugation. In chromatographic separation, Sephadex-50 is most widely used. In dialysis method hollow fibre dialysis cartridge may be used. In 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.

EVALUATION OF LIPOSOMES [11-15]

Liposomal formulation and processing for specified purpose are characterized to ensure their predictable in vitro and in vivo performance. The characterization parameters for purpose of evaluation could be classified into three broad categories which include physical, chemical and biological parameters.

- Physical characterization evaluates various parameters including size, shape, surface features, lamellarty, phase behaviour and drug release profile.
- Chemical characterization includes those studies which establish the purity and potency of various lipophillic constituents
- Biological characterization parameters are helpful in establishing the safety and suitability of formulation for therapeutic application.

Some of parameters are:

1. Vesicle shape and lamellarity: Vesicle shape can be assessed using Electron Microscopic Techniques. Lamellarity of vesicles i.e. number of bilayers presents in liposomes is determined using Freeze-Fracture Electron Microscopy and P-31 Nuclear Magnetic Resosance Analysis.

2. Vesicle size and size distribution: Various techniques are described in literature for determination of size and size distribution. These include Light Microscopy, Fluorescent Microscopy, Electron Microscopy (specially Transmission Electron Microscopy), Laser light scaterring Photon correlation Spectroscopy, Field Flow fractionation, Gel permeation and Gel Exclusion. The most precise method of determine size of liposome is Electron Microscopy since it permit one to view each individual liposome and to obtain exact information about profile of liposome population over the whole range of sizes. Unfortunately, it is very time consuming and require equipments that may not always be immediately to hand. In contrast, laser light scattering method is very simple and rapid to perform but having disadvantage of measuring an average property of bulk of liposomes. All these methods require costly equipments. If only approximate idea of size range is required then gel exclusion chromatography techniques are recommended, since only expense incurred is that of buffers and gel material. Another more recently developed microscopic technique known as atomic force microscopy has been utilized to study liposome morphology, size, and stability.

Most of methods used in size, shape and distribution analysis can be grouped into various categories namely microscopic, diffraction, scaterring, and hydrodynamic techniques.

a) Microscopic Techniques

i. Optical Microscopy: The microscopic method includes use of Bright-Field, Phase Contrast Microscope and Fluorescent Microscope and is useful in evaluating vesicle size of large vesiscle.

ii. Negative Stain TEM: Electron Microscopic Techniques used to assess liposome shape and size are mainly negative-stain TEM and Scanning Electron Microscopy. The latter technique is less preferred. Negative Stain Electron Microscopy visualizes bright areas against dark background (hence termed as negative stain)

The negative stains used in TEM analysis are ammonium molybdate or Phosphotungstic acid (PTA) or uranyl acetate. Both PTA and ammonium molybdate are anionic in nature while uranyl acetate are cationic in nature.

iii.Cryo-Transmission Electron Microscopy Techniques (cryo-TEM): This technique has been used to elucidate the surface morphology and size of vesicles.

b) Diffraction and Scaterring Techniques

i. Laser Light Scattering: Photon correlation spectroscopy (PCS) is analysis of time dependence of intensity fluctuation in scattered laser light due to Brownian motion of particles in solution/suspension. Since small particles diffuse more rapidly than large particles, the rate of fluctuation of scattered light intensity varies accordingly. Thus, the translational diffusion coefficient (D) can be measured, which in turn can be used to determine the mean hydrodynamic radius (Rh) of particles using the Stoke-Einstein equation. Using this technique one can measure particles in range of about 3nm.

c) Hydrodynamic Techniques: This technique includes Gel Permeation and Ultracentrifuge. Exclusion chromatography on large pure gels was introduced to separate SUVs from radial MLVs. However, large vesicles of 1-3μm diameter usually fail to enter the gel and are retained on top of column. A thin layer chromatography system using agarose beads has been introduced as a convient, fast technique for obtaining a rough estimation of size distribution of liposome preparation. However, it was not reported if this procedure was sensitive to a physical blockage of pores of the agarose gel as is the more conventional column chromatography.

3) Encapsulation Efficiency and Trapped Volume: These determine amount and rate of entrapment of water soluble agents in aqueous compartment of liposomes.

a) Encapsulation Efficiency: it describes the percent of the aqueous phase and hence percent of water soluble drug that become ultimately entrapped during preparation of liposomes and is usually expressed as % entrapment/mg lipid. Encapsulation efficiency is assessed using 2 techniques including minicolumn centrifugation method and Protamine aggregation method. Minicolumn centrifugation is generally used both as a mean of purification and separation of liposomes on small scale. In mini column centrifugation method, the hydrated gel is filled in a barrel of 1ml syringe without plunger which is plugged with whatman GF/B filter pad. This barrel is rested in a centrifuge tube. This tube is spun at 2000 rpm for 3 min. to remove excess saline solution from gel. After centrifugation the gel column should be dried and have come away from side of barrel. Then eluted saline is removed from collection tube. Liposome suspension (0.2ml) is applied dropwise to top of gel bed, and the column is spun at 2000 rpm for 3 min. to expel the void volume containing the liposomes into centrifugation tube. The elute is then removed and set aside for assay.

Protamine aggregation method may be used for neutral and negatively charged liposomes.

b) Trapped volume: It is an important parameter that governs morphology of vesicles. The trapped or internal volume is aqueous entrapped volume per unit quantity of lipids. This can vary from 0.5 to 30 microlitre/micro mol. various materials including spectroscopically inert fluid, radioactive markers and fluorescent markers are used to determine trapped/internal volume.

The best way to measure internal volume is to measure quantity of water directly, by replacing external medium (water) with spectroscopically inert fluid (deuterium oxide) and then measuring water signal using NMR.

Trapped volume is also determined experimentally by dispersing lipid in an aqueous medium containing non-permeable radioactive solute. The proportion of solute trapped is determined by removing external radioactivity by centrifugation and subsequently residual activity per lipid is determined.

4) Phase Response and Transitional Behaviour: liposome and lipid bilayers exhibit various phase transitions that are studied for their role in triggered drug release or stimulus mediated fusion of liposomal constituents with target cell. An understanding of phase transitions and fluidity of phase transistions and fluidity of phospholipids membranes is important both in manufacture and exploitation of liposomes since phase behaviour of liposomal membrane determine such properties such as permeability, fusion, aggregation, and protein binding.

The phase transistion have been evaluated using freeze fracture electron microscopy. They are more comprehensively verified by differential scanning colorimeter (DSC) analysis.

5) Drug Release: The mechanism of drug release from liposomes can be assessed by use of well calibrated in vitro diffusion cell. The liposome based formulation can be assisted by employing in vitro assays to predict pharmacokinetics and bioavailability of drug before employing costly and time-consuming in vivo studies. The dilution-induced drug release in buffer and plasma was employed as predictor for pharmacokinetic performance of liposomal formulations and another assay which determined intracellular drug release induced by liposome degradation in presence of mouse-liver lysosome lysate was used to assess the bioavalability of drug.

TARGETING OF LIPOSOMES [15-20]

Two types of targeting

1. Passive targeting

As a mean of passive targeting, such usually administered liposomes have been shown to be rapidly cleared from the blood stream and taken up by the RES in liver spleen. Thus capacity of the macrophages can be exploited when liposomes are to be targeted to the macrophages. This has been demonstrated by successful delivery of liposomal antimicrobial agents to macrophages.

Liposomes have now been used for targeting of antigens to macrophages as a first step in the index of immunity. For e.g. In rats the i.v administration of liposomal antigen elicited spleen phagocyte mediated antibody response where as the non liposome associated antigen failed to elicit antibody response.

2. Active targeting

A pre-requisite for targeting is the targeting agents are positioned on the liposomal surface such that the interaction with the target i.e., the receptor is tabulated such as a plug and socket device. The liposome physically prepared such that the lipophilic part of the connector is anchored into the

membrane during the formation of the membrane. The hydrophilic part on the surface of the liposome, to which the targeting agent should be held in a stericaly correct position to bond to the receptor on the cell surface. The active targeting can be brought about the using

i. Immuno liposomes: These are conventional or stealth liposomes with attached Antibodies or other recognition sequence [e.g. Carbohydrate determinants like glycoprotein]
The antibody bound, direct the liposome to specific antigenic receptors located on a particular cell. Glycoprotein or Glycolipid cell surface component that play a role in cell-cell recognition and adhesion

ii. Magnetic liposomes: Contain magnetic iron oxide. These liposomes can be directed by an external vibrating magnetic field in their delivery sites.

iii. Temperature or heat sensitive liposomes: Made in such a way that their transition temperature is just above body temperature. After reaching the site, externally heated the site to release the drug.

APPLICATIONS [20]

- Cancer chemotherapy
- Gene therapy
- Liposomes as carriers for vaccines
- Liposomes as carrier of drug in oral treatment
- Liposomes for topical applications
- Liposomes for pulmonary delivery
- Against Leishmaniasis
- Lysosomal storage disease
- Cell biological application
- Metal storage disease
- Ophthalmic delivery of drugs

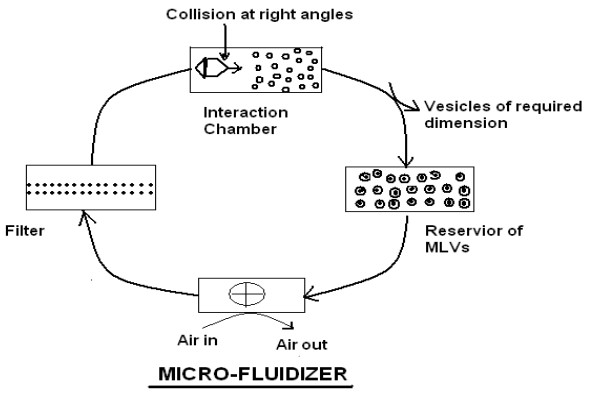

Figure 4: Micro- fluidizer [20]

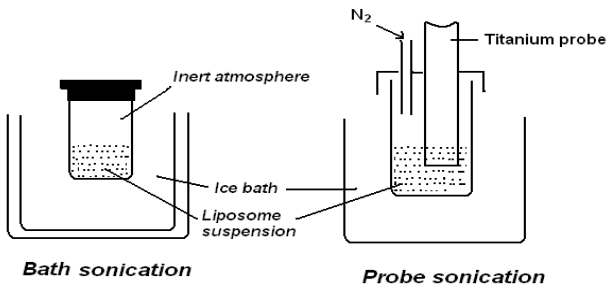

Figure 5: Sonication apparatus [20]

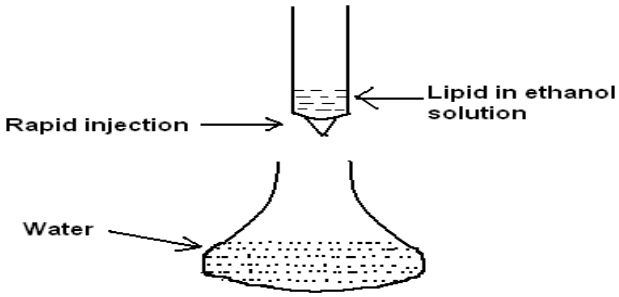

Figure 6: Ethanol injection Technique [20]

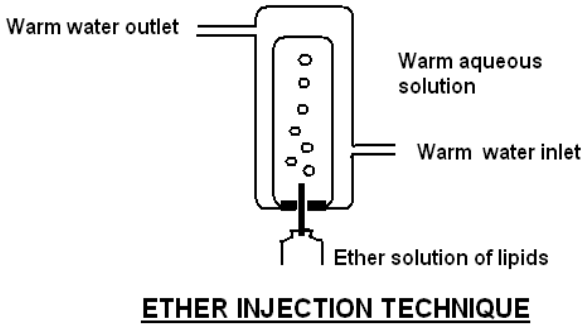

Figure 7: Ether injection technique [20]

CONCLUSION

Liposomes have been realized as extremely useful carrier systems for targeted drug delivery. The flexibility of their behavior can be exploited for the drug delivery through any route of administration and for any drug material irrespective of their solubility properties. The use of liposomes in the delivery of drugs and genes are promising and is sure to undergo further developments in future.

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