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Review article *Drug Delivery System*

A Review on Liposomes as a Drug Delivery System

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ABSTRACT

Nanoparticle systems have been perceived as the ultimate goal for effective drug delivery for decades. The ideal nanoparticle carries the drug-load safely to a predefined target. There, it is capable of releasing its cargo intracellular or in the extracellular space where the drug can be directly internalized and exert the desired action. Enroute, the nanoparticle prevents unwanted interactions of the drug-load with non-target tissues and where needed, it will enhance the circulation time of the encapsulated drug and enable sustained release. In this context, liposomes, a class of synthetic lipid nanoparticles have been explored in depth. Liposomes are microscopic self-assembling unilamellar or multilamellar vesicles made up of phospholipid bilayer. Both the hydrophilic and hydrophobic drugs can be attached to the lipid bilayer of liposomes and can show their efficacy in the target cell of the human body. Liposomes can significantly alter the pharmacokinetics of drugs. They have been investigated for diverse applications such as treatment of cancer, delivery of gene and vaccine, treatment of lung and skin diseases, treatment of tumours, and imaging tumours at the site of infection. They are leading present-day smart delivery systems due to their flexible biophysical and physicochemical properties, which permit easy control to address different delivery concerns. This review will discuss various advances and updates in liposome-assisted drug delivery and the current clinical use of liposomes for biomedical applications.

Keywords: liposomes**,** nanoparticles, targeted drug delivery, site-specific drug delivery.

INTRODUCTION

Over the past decades, lipid-based nanoparticle Drug Delivery Systems (DDS) have caught the attention of researchers worldwide, encouraging the field to rapidly develop improved ways for effective drug delivery. One of the most prominent examples is liposomes. The name liposome is derived from two Greek words: 'Lipos' meaning fat and 'Soma' meaning body. A liposome can be formed at a variety of sizes as unilamellar or multi-lamellar construction, and its name relates to its structural building blocks, phospholipids, and not to its size. Liposomes were first described by British haematologist Dr Alec D Bangham in 1961 (published 1964), at the Babraham Institute, in Cambridge. They were discovered when Bangham and R. W.

Horne were testing the institute's new electron microscope by adding negative stain to dry phospholipids [1,2].

Liposomes have the ability to deliver a drug to a target site with the least amount of systemic toxicity by overcoming hurdles of cellular and tissue uptake, stabilizing therapeutic compounds, and improving bio distribution. However, despite the evident advantages of drug delivery via liposomes, their clinical application has seen substantial biological barriers to be conquered, such as, rapid clearance from the bloodstream, off-target accumulation in clearance organs, and triggering of the innate immune response [3]. They can be designed to retain their physical properties at body temperature, through proper lipid composition using phospholipids with high phase transition temperature. Besides composition, properties of liposomes are governed by several other factors which include their method of preparation, size, surface charge, firmness of bilayer and surface functionalization [4].

It has been well established that liposomes have an internal volume and can entrap a drug. The ability to incorporate both hydrophilic and hydrophobic drugs in them makes them a valuable drug delivery system. Liposome assisted drug delivery is associated with several advantages including improving the solubility of a drug [5], releasing a drug at the target site in a sustainable manner [6], providing targeted delivery [7], providing protection against drug degradation, reducing toxic side effects of the drug to normal cells, delivering drugs to multidrug resistance tissues by combination therapy [8] and improving the therapeutic index of drugs [9].

An important milestone in liposomal drug delivery was the development of the remote drug loading process based on ammonium sulphate gradient in the 1990s. When remote drug loading was applied to Doxorubicin (DOX), accumulation in the aqueous phase of the liposomes reached a record of 100-fold concentration in the remote loading medium [10].

Other major developments include the introduction of Polyethylene Glycol (PEG)ylated liposomes with enhanced circulation times and reduced reticuloendothelial uptake, which were useful in the treatment of cancers/ tumours. With the advent of clinical translation of liposome-based drugs, it was highly desirable to develop a programmable, automated delivery system to control the physicochemical characteristics of liposomes. A number of methods were developed for automated production of liposomes, amongst them it was a robust platform-microfluidic technology, which provided control over size, lamellarity, membrane composition and internal contents [11]. Significant progress was made in the area of targeting liposomes to specific cells or organelles by active/passive targeting [12,13,14]

.Lipoplexes,which are lipid-based assemblies of non-covalently associated Deoxyribonucleic Acid (DNA) by charge-charge interactions, are a promising alternative in gene therapy [14].

Liposomes, as extraneous substances, are subjected to many obstacles including detection, deactivation and elimination processes from the defence system once injected into the body. To become a successful drug carrier, liposomes should have the ability to overcome all such obstacles and deliver drugs to the target site. This can be achieved by designing liposomes with desirable properties but not restricted to: Targeting ability to various specific organelles, improved circulation times, enhanced skin penetration (ethosomes) and the capacity for pulmonary delivery of drugs (nebulised liposomes).

This review will discuss the advances in liposome assisted drug delivery, including developments in the design of liposomes for controlling rapid clearance, remote drug loading and drug release. The steps forward towards improved circulation times through stealth liposomes are deliberated. It will cover the progress in dermal delivery of drugs through ethosomes and pulmonary delivery by nebulised liposomes. Further, it will discuss the targeting of liposomes to specific organelles, an overview of Nucleic Acid Therapeutics (NATs) and wrap up with a section on the current clinical use of liposomes for biomedical applications [15].

It has been found that glycerol is the backbone of a molecule that's why phospholipid containing glycerol were found to be an essential component of liposomal formulation and it represents of lipid weight It is use as vehicle for administration of nutrients as well as pharmaceutical drugs. It shows both characteristics.

1) Hydrophilic head
2) Lipophilic tail.

Structural component of liposome

Liposomes are composed lipid bilayer size:

50-1000 nm in diameter that serves as targeted delivery vehicle that contain active biological compound. Liposome most often composed of phospholipid and cholesterol.

Phospholipids

Phospholipids are the major structural components of liposome. The most common phospholipids used in liposomal preparation are Phosphatidylcholine. Phosphatidyl-choline is an amphiphatic molecule consist of-

A hydrophilic polar head group, phosphocholine

- A glycerol bridge
- A pair of hydrophobic acyl hydrocarbon chains

The chemical structure of naturally occurring Phosphatidylcholine has a glycerol moiety attached to two acyl chains which may be saturated or unsaturated. The stability of liposome membrane depends on the packing of hydrocarbon chains of the lipid molecules [16]. The nature of the fatty acid in lipid molecule, such as number of double bonds in the chain, is responsible for bilayer properties such as elasticity and phase behaviour Phospholipids are Very abundant in nature and which contains choline is used for the preparation of liposomes.

Examples of phospholipids are

1. Phosphatidyl choline (Lecithin) PC
2. Phosphatidyl ethanolamine (Cephalin)-PE
3. Phosphatidyl serine (PS)
4. Phosphatidyl Glycerol (PG)
5. Phosphatidyl inositol (PI)

Cholestrol

Cholesterol is another important structural component of liposome. It is a commonly used sterol. The addition of sterols modulates the function of stability and rigidity. It does not by itself form a bilayer structure. It gets incorporated into phospholipids in a very high concentration up to 1:1 or 2:1 molar ratio of cholesterol to phosphatidyl choline. The presence of cholesterol in the lipid bilayer enhances the

stability and form highly ordered and rigid membrane structure. Cholesterol reduces the permeability of water soluble molecules and improves the fluidity and stability of biological membrane.The interaction and destabilization of liposomes was prevented by cholesterol [17].

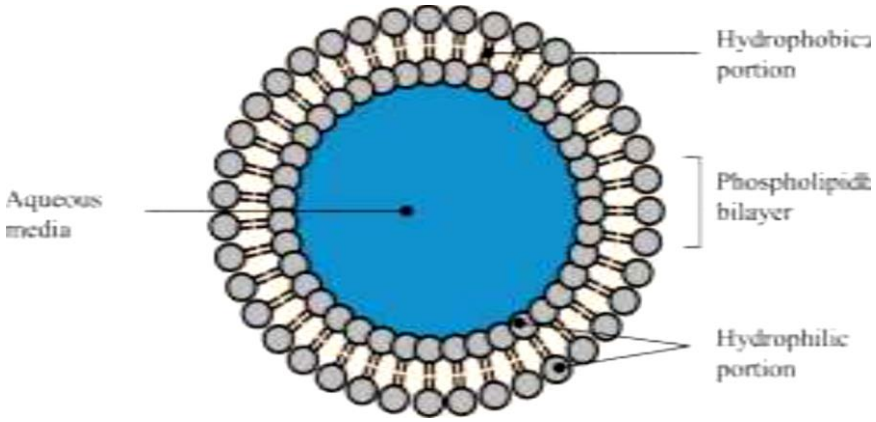

Fig 1: Structure of Liposome

Mechanism of Liposome Formation

Phospholipids are amphipathic having affinity for both aqueous and polar moieties molecules as they have a hydrophobic tail and a hydrophilic or polar head. The hydrophobic tail is composed of two fatty acid chain containing 10-24 carbon atom and 0-6 double bonds in each chain. The macroscopic structures most often formed include lamellar, hexagonal or cubic phases dispersed as colloidal nanoconstructs ie., artificial membranes referred to as liposomes, hexasomes or cubosomes (Fig. 2)

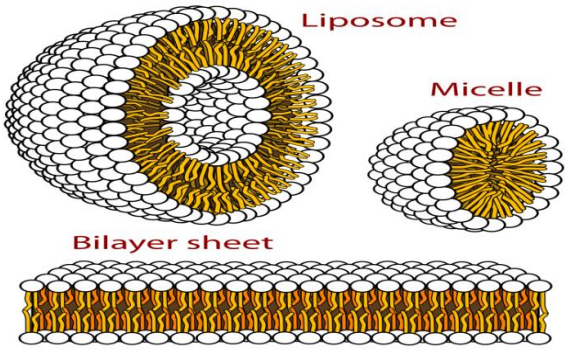

Fig 2: Cross-sectional view of the structures that can be formed by phospholipids in an aqueous solution.

The most common natural polar phospholipids are phosphatidylcholine. These are amphipathic molecules in which a glycerol bridge links to a pair of hydrophobic acyl hydrocarbon chains with a hydrophilic polar head group, phosphocholine. The amphipathic nature of phospholipids and their analogues render them the ability to form closed concentric bilayers in presence of water. Liposomes are formed when thin lipid films or lipid cakes are hydrated and stacks of lipid crystalline bilayers become fluid and swell. The hydrated lipid sheets detach during agitation and self-close to form large, multilamellar vesicles prevent interaction of water with the hydrocarbon core of the bilayer at the edges [18].

Mechanism of action of liposomes

Liposome performs their action by four different mechanism, they are as follows:

1. Endoytosis: This take place by phagocytic cells of reticuloendothelial system such as neutrophills.

2. Adsorption: It occurs to the cell surface by non- specific electrostatic forces or by interaction with cell surface components.

3. Fusion: It occurs by the insertion of liposomal bilayer into plasma membrane with continuous release of liposomal content into the cytoplasm.

4. Lipid exchange: In this transfer of liposomal lipids to the cellular membrane without association of liposomal contents.

Advantages of liposomes

Provide selective passive targeting to tumour tissue (liposomal doxorubicin).

- Liposomes are increased efficacy and therapeutic index of drug (Actinomycin-D).
- Liposomes are increased stability via encapsulation.
- Liposomes are biocompatible, completely biodegradable, non-toxic, flexible and non-immunogenic for systemic and non-systemic administrations.
- Liposome is reduction in toxicity of the encapsulated agent (Amphotericin B, Taxol).

- Liposomes help to reduce exposure of sensitive tissues to toxic drugs.
- Site avoidance effect.
- Flexibility to couple with site-specific ligands to achieve active targeting.

Disadvantages of liposome [19]

- Production cost is high.
- Leakage and fusion of encapsulated drug /molecules.
- Sometimes phospholipid undergoes oxidation and hydrolysis like reaction.
- Short half-life.
- Low solubility.
- Fewer stables.

Properties of liposomes [20]

The system is composed of structures of bimolecular sheets intercalated by aqueous space.

- They are permeable to water.
- They are osmotically sensitive.
- Positively charged membranes are impermeable to cations and negatively charged ones are relatively permeable to anions.

Modes of liposome action

The preceding discussion shows that liposomes exhibit different biodistribution and pharmacokinetics when compared to free drug particles. In some cases, this can be used to improve the therapeutic effectiveness of the encapsulated drug molecules. The benefits of drug-loaded liposomes, which can be applied as colloidal solution, aerosol, or in semi-solid structures, such as creams and gels, can be classified into seven categories:

(i) Enhanced solubility of amphiphilic and lipophilic drugs:

Furthermore, in some cases, hydrophilic drugs, such as the anticancer agent Doxorubicin or Acyclovir, can be encapsulated in the liposomal interior at concentrations several fold above their aqueous solubility. This is possible because of the precipitation of the drug or gel configuration inside the liposome with appropriate substances encapsulated [21].

(ii) Inactive objective to the cells of the immune system:

Instances are antimonials, porphyrins, Amphotericin B, and also vaccines, immune modulators or (immune) suppressors [22, 23]

(iii) Maintained free system of systemically or locally administered liposomes

Cases in points are doxorubicin, cortisones cytosine, arabinose, biological proteins or peptides such as vasopressin [24].

(iv) Site-avoidance mechanism:

Liposomes do not dispose in certain organs, such as heart, kidneys, brain, and nervous system and this decreases cardio-, nephro-, and neuro-toxicity. Characteristic examples are reduced nephrotoxicity of Amphotericin B, and reduced cardiotoxicity of Doxorubicin liposomes [25, 26].

(v) Precise targeting of Location:

In certain cases, liposomes with surface attached ligands can bind to target cells, or can be delivered into the target tissue by local anatomical conditions such as leaky and badly formed blood vessels, their capillaries and basal lamina. Instances include anti-cancer, anti-disease and anti-provocative drugs [27].

(vi)Improved transfer of hydrophilic, electric molecules such as antibiotics, chelators, plasmids and genes, into cells:

(vii) Improved penetration into tissues, particularly in the case of dermally functional liposomal dosage forms

Usually, liposome encapsulation is done carefully when the drugs are very potent, toxic and have very short life times in the blood circulation or at the sites of local administration.

Classification of liposomes [28,29,30,31]

Based on structural parameters

a. MLV: Multi lamellar vesicle (0.5 µm)
b. OLV: Oligolamellar vesicle (0.1-1 µm)
c. UV: Unilamellar vesicle (All size range)
d. SUV: Small unilamellar vesicle (30-70 nm)
e. MUV: Medium sized unilamellar vesicle
f. LUV: Large unilamellar vesicle(µm)
g. GUV: Giant unilamellar vesicle (µm)

Based on the method of preparation

a. REV: Reverse phase evaporation vesicles
b. MLV-REV: Multi lamellar vesicle by REV
c. DRV: Dehydration- rehydration method
d. VET : Vesicle prepared by extraction method
e. SPLV: Stable plurilamellar vesicles
f. FATMLV: Frozen and thawed MLV

Based on composition of application

a. Conventional liposome
b. Fusogenic liposomes
c. Ph sensitive liposomes
d. Cationic liposomes
e. Long circulatory liposome
f. Immuno liposomes

Method of preparation

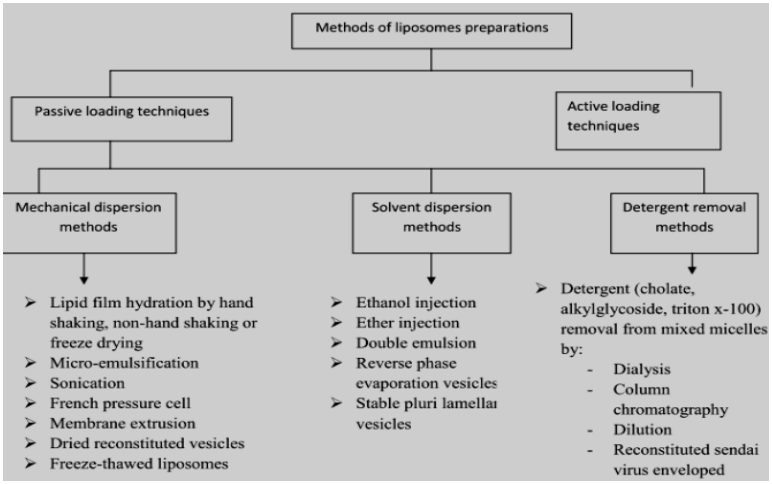

Fig 3: Different methods for liposome preparation.

Passive loading techniques

Hand shaking MLVs

It is the most common and simple method used for the preparation of MLVs. In these processes, the lipids are dissolved in solvents (choloroform: methanol) which are then transfer to a round bottom flask. The RBF containing the mixture is then attached to rotary evaporator and then it was rotated at 60 rpm until a dry thin layer is formed after that it is dried in lyophilizer to remove the last traces of solvent and it is hydrated with phosphate buffer saline containing the material to be entrapped and then it was attached to rotary evaporator at 60 rpm or below it was rotated until the layer adhering on the wall of the RBF is removed and it was kept stand at room temperature for 2 h upon hydration milky white disperse appear.

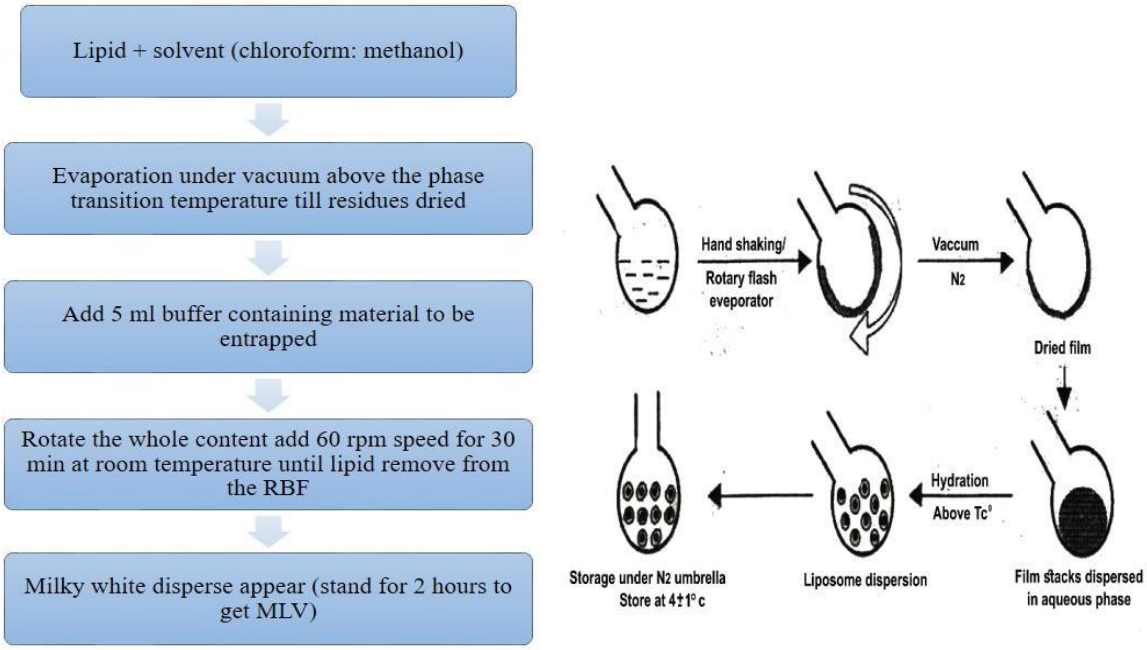

Fig 4: Method of handshaking

Non-hand shaking

In these methods lipid mixed with solvent is spread over the conical flask and the solution is evaporated at room temperature without disturbing by the flow of nitrogen. After the solution gets dried it is hydrated by water-saturated nitrogen which is passed through the conical flask until the opacity of dried lipid film disappears. After hydration, the lipid gets swelled. Then the flask is inclined to one side and 10 to 20 ml of 0.2 M sucrose in distilled water is added to the side of the flask and then the flask is slowly returned to its original position. The fluid gently runs over the lipid layer on the bottom of the flask. Then the flask is flushed with nitrogen and sealed it was then allowed to stand for 2 h at room temperature. After swelling the suspension is centrifuged at

12000 g for 10 min at room temperature. The remaining fluid add iso-osmolar glucose solution then LUVs formed.

Freeze drying method

In these method lipid and solvent are mixed and evaporate at room temperature by flow of nitrogen for drying. Then add some water saturated nitrogen until opacity disappears. Add water fluid and 10-20 ml of 0.2 M sucrose solution to swell. After that stand for 2 hour at 37 c then centrifuged at 12000rpm for 10 min at room temperature and reaming fluid are add to iso-osmolar glucose solution to formed LUVs.

Membrane extrusion

In this technique vesicles contents are exchanged with dispersion medium during breaking and resealing of phosphate lipid bilayer as they pass through polycarbonate membrane and

less pressure is required here as compare to French pressure cell then use to process MLVs and LUVs. At last tortuous and nucleation trach membrane are formed.

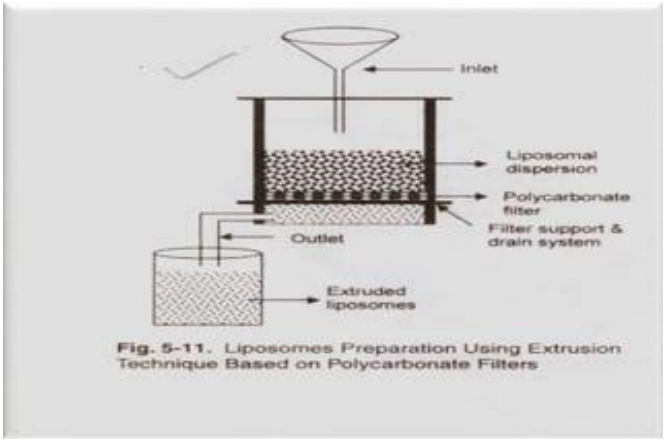

Fig 5: method of membrane extrusion

Dehydration rehydration method

In these technique, liposomal suspension was prepared by THF are frozen in liquid nitrogen then freeze-dried overnight. After hydration with water, the liposome is prepared.

Sonication method

In this method surfactant and cholesterol are mixed in 2 ml of the aqueous phase in a vial and then the mixture is sonicated for 3 min at 60 c using titanium probe sonicator after that unilamellar vesicle is formed (Fig. 6).

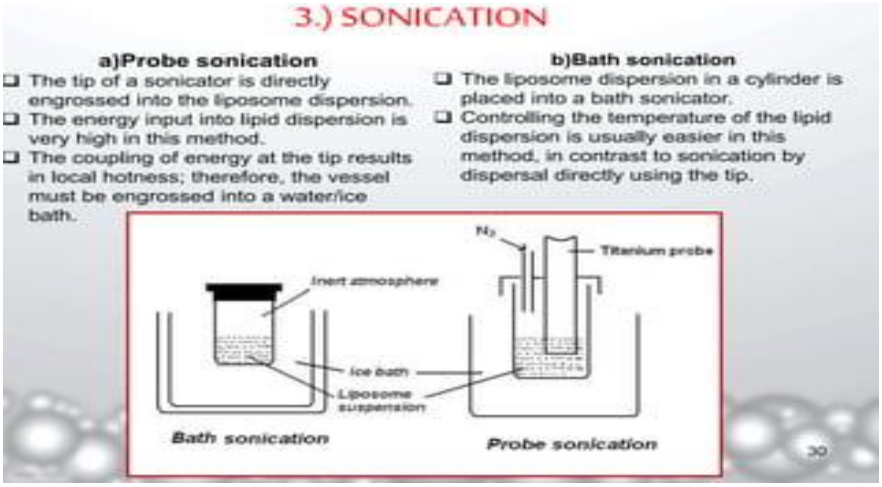

Fig 6: Method of sonication

Freeze-thaw Method

In this method the SUVs are quickly solidified, followed by moderate defrosting.

Solvent dispersion method

1. Ethanol injection method

In this method MLVs are formed by a lipid solution of ethanol are rapidly injected into an excess of a buffer. but some drawbacks of this method are the particles may be with heterogeneous size distribution (30-110).

2. Ether infusion method

In this method, liposomes are prepared by dissolving a lipid solution in diethyl or ether methanol, and then the mixture is slowly injected into an aqueous solution of a drug, to be encapsulated at a temp of 55-65°C under the reduced pressure.

3. Double emulsification

This method firstly prepared the emulsion by dissolving the drug in the aqueous phase(W1), which is then emulsified in an organic solvent of a polymer is called primary emulsion(W/O). After that this primary emulsion further mixed in an emulsifier-containing aqueous solution (W2) to make a W1/O/W2 double emulsion .And after than microspheres are obtained by removal of the solvent and filtration process.

Reverse phase evaporation method (REV)

The lipid mixture is taken in a round bottom flask followed by removal of the solvent under reduced pressure by a rotary evaporator. The system is purged with nitrogen and the lipids are re-dissolved in the organic phase. The reverse-phase vesicles will form in this phase. The usual solvents used are diethyl ether and isopropyl ether. An aqueous phase which contains the drug to be encapsulated is added after the lipids are re-dispersed in this phase. The system is kept under continuous nitrogen and the two-phase system is sonicated until the mixture becomes clear one-phase dispersion. The mixture is then placed on the rotary evaporator and the removal of organic solvent is done until a gel is formed followed by the removal of non-encapsulated material. The resulting liposomes are called reverse-phase evaporation vesicles (Fig. 7)

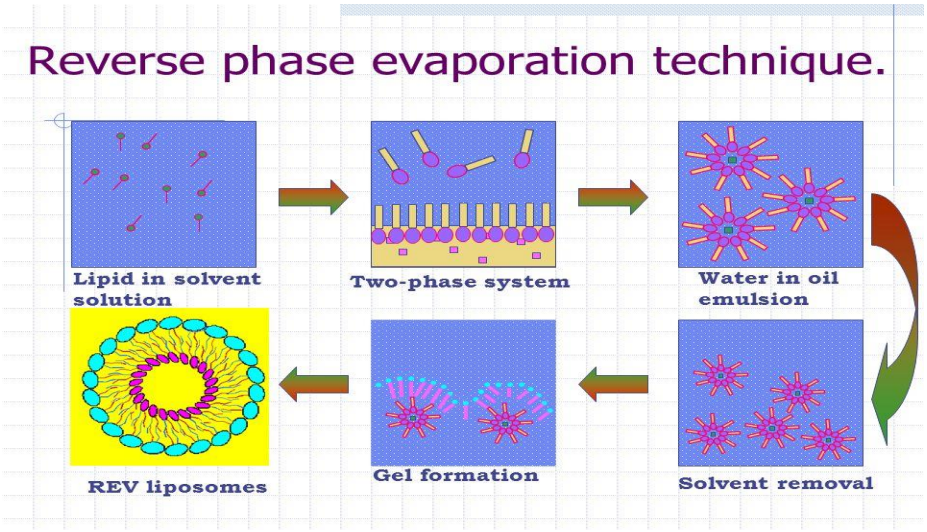

Fig 7: Reverse phase evaporation method

Dried reconstitute vesicle

Here the preformed liposomes are rehydrated to an aqueous fluid containing an active ingredient which is followed by dehydration of the mixture.

French pressure cell

The liposomes prepared by this technique are less likely to suffer from the structural defect and instability as observed in the sonicated vesicle. Leakage of contents from liposome preparing using French press is slower and slower than sonicated liposome. It has used to reduce the heterogeneity of populations of proteoliposomes obtained by detergent dialysis technique. The method has several advantages over the sonication method. The method is simple rapid, reproducible and involving gentle handling of unstable materials. The resulting liposome is somewhat larger than sonicated SUVs. The main drawback of the method is that the temperature is difficult to achieve and the Working volume is relatively small.

Detergent removal

Detergents are used to solubilize the lipids at their critical micellar concentrations. LUVs are shaped by eliminating the detergent by dialysis and combining the micelles. In this method, the liposomes are formed in homogenous size. And the retention of detergent contaminants is the drawback of this method.

Active loading technique [32,33]

1. **Prollposome:-** lipid and active substances (drug) are covered onto a solvent transporter to shape free-streaming granular material in supportive of liposomes which structure an isotonic liposomal suspension on hydration. The favourable to pro- liposome approach may give a chance for cost-effective large scale manufacturing liposomes containing particularly lipophilic drugs.
2. **Lyophilization:-** the expulsion of water from items in a solidified state at incredibly decreased weight is called lyophilisation (freeze-drying). The cycle is commonly used to dry items that are thermolabile which might be annihilated by heat-drying. This method has an incredible potential to unravel long haul steadiness issues as for liposomal solidness. Spillage of entangled materials may occur during the cycle of freeze-drying and on reconstitution.

Evaluation of liposomes

The purpose of the evaluation of liposome to ensure the in vivo and in vitro performance of liposomes. Evaluation parameters are categories into three broad categories are-

Physical characterization

Vesicle shape & morphology (Visual Appearance)

Liposome suspension can range from translucent to milky, depending on the composition and particle size. If the

turbidity has a bluish shade this means that particles in the sample are homogeneous; a flat, grey colour indicates that presence of a non -liposomal dispersion and is most likely a disperse inverse hexagonal phase or dispersed micro crystallites. An optical microscope can detect liposome of size greater than 0.3 μm as well as contamination with larger particles.

Particle size & size distribution

Size distribution is normally measured by dynamic light scattering. This method is reliable for liposomes with relatively homogeneous size distribution. A simple but powerful method is gel exclusion chromatography, in which a truly hydrodynamic radius can be detected. Sephacryl-S100 can separate liposome in size range of 30-300nm. Sepharose -4B and -2B columns can separate SUV from micelles.

Entrapement efficiency

The entrapped volume of liposome (in μL/ mg phospholipids) can often be deduced from measurements of the total quantity of solute entrapped inside liposome assuring that the concentration of solute in the aqueous medium inside liposomes is the same after separation from unentrapped material. For example, in two phase method of preparation, water can be lost from the internal compartment during the drying down step to remove organic solvent.

$$\% \text{ Entrapment Efficiency} = \frac{\text{Entrapped drug}}{\text{Total added drug}} \times 100$$

Determination of Lamellarity

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

Surface charge

Liposomes are usually prepared using charge imparting /constituting lipids and hence it is imparting to study the charge on the vesicle surface. The two methods used in general to assess the charge are free flow electrophoresis and zeta potential measurement.

Zeta potential (z) determination [34]

Charge on empty and drug loaded vesicles surface was determined using Zetasizer. Analysis time was kept for 60 s and average zeta potential and charge on the liposome was determined.

Chemical characterization

Biological characterization

Drug release

The evaluation of the in vitro drug release profile can be performed using dialysis conditions. The selection of dialysis bag membrane should be in accordance with the drug specifications. It must be freely permeable to the drug and should not occur drug adsorption [35]. Liposomal sample is placed into the dialysis bag with specific molecular weight cut off, hermetically tied. The tubing membrane system is put into a simulated physiological fluid means release medium, usually a buffered saline at pH 7.4. The full system is kept at 37 °C to mimic an in vivo environment, and under continuous stirring. At defined time points, an aliquot of sample is taken and analyzed by the conventional methods used for drug quantification. The volume of samples needs to keep constant. Thus, an equal volume of fresh release medium is placed again in the system [36]. The data are used to establish the release profile by plotting the cumulative release percentage against the select time points. As extrapolation to in vivo performance of liposomes as drug delivery system, the results obtained from the in vitro release study are widely considered in the development of liposomes for the controlled release of drugs [37].

Table 1: Biological characterization

Parameters	Analytical method/ instrument
Sterility	Aerobic or anaerobic cultures
Pyrogenicity	Limulus Amebocyte Lysate (LAL) test
Animal toxicity	Monitoring survival rates, histology and pathology

Stability of liposomes

Stability is defined as capacity of particular formulation in a specific container/ closure system to remain with in physical, chemical, microbiological, therapeutic and toxicological specifications.

Stability testing of liposomes: Liposomal stability can be tested by storing under following six conditions. 1. Highest & lowest temp. for 1 month. 2. Room temp. for 12-24 months 3. 2-3 freeze thaw cycles (20-25oC) 4. 60 cycles/min in a reciprocating shaker for 24-48 hr 5. 6-8 heat cool cycles (5-45oC, 48 hrs at each temp.) 6. Visual/ microscopic examination. After storage liposomes are evaluated for vesicle size, shape, no. of vesicles/ cubic mm & residual drug content.

Entrapment of drugs into liposome bilayers

Liposomes, because of their biphasic character, can act as carrier for both lipophillic and hydrophilic drugs. Depending upon their solubility and partitioning characteristics, the drug molecules are located differently in the liposomal

environment and exhibit different entrapment and release properties [38].

Therapeutic applications of liposomes

- **Drug targeting:** An ideal targeted drug delivery delivers for 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 ligand s recognizespecific sites so cause targeting of liposomal drug at those target sites. Ligands are 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 [39]. E.g., MT1-MMP (Membrane type - 1 matrix metalloproteins).
- **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.[40]. 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 [41]..
- **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 [42].
- **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 [43].
- **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.
- **Prophylaxis:**-Butyl cholinesterase encapsulating bio adhesive liposomes may prophylaxis against organophosphate poisoning by preventing loss of intracellular enzyme activity [44].
- **Local therapy:** Antioxidants like calabash SOD delivered via anionic liposomes may provide better targeted treatment in chronic inflammation of colonic epithelium like ulcerative colitis.[45]
- **Pulmonary Application**
  Pulmonary delivery of liposomes has been explored as a target selective alternative to systemic administration of antiasthamatic and antiallergic compounds and for antibiotics used against pulmonary infections. Liposomes are useful tools for pulmonary delivery of drugs due to their solubilization capacity for poorly water soluble substances rendering them more practical for aerolisation. Their biodegradability allows for prolonged pulmonary residence times without danger of allergic or other side effects. The targeting capacity to infected or immunologically impaired alveolar macrophages is a unique feature of liposomes. The toxicity of liposomes aerosols has been investigated systematically [46].
- **Liposomes in Cosmetics**
  Consumers are more focused on their health and appearance than ever before. This trend creates an increasing demand for functional cosmetics ingredients and efficient delivery systems. Known for high performance and efficient development in liposome delivery systems, Creative Biostructure has extended the applications into the cosmetic industry.

Other Clinical Applications

- Gene therapy-The genetic material can be placed in the liposomes in order to increase the DNA uptake in tissue culture.
- Against leishmaniasis.
- Metal storage disease.
- Cell biological application.
- Used in food industry.

Novel liposomes

Archaeosomes

These are the novel generation liposomes in which lipids are obtained from *archaebacteria*. *Archaebacteria* is a domain of prokaryotes. The method used for obtaining lipids from *archaebacteria* is solvent extraction method. The membranes of *archaebacteria* contains diether/ tetraether linkages which promotes the generation of lipid layers of archaeosomes. The major advantage is that they show greater stability even in harsh conditions [47,48]. Archaeosomes shows good biocompatibility compared to liposomes.

Transferosomes

The name transferosomes implies a "carrying body". Transferosomes penetrates through the skin by getting squeezed through the lipids present in the cells of stratum corneum. These are generally composed of amphipathic molecule like phosphatidyl choline which acts as a vesicle forming agent and also contains bilayer softening agent like surfactant which is responsible for flexibility of the transferosomes [49].

Transferosomes offers higher entrapment efficiency and also provides protection to the drugs from metabolic degradation. They have ability to penetrate through small pores. These are biodegradable and biocompatible.

Ethosomes

Ethosomes are the vesicles comprising of phospholipids along with high concentration of alcohol. The alcohols may be either ethanol or isopropyl alcohol. The phospholipids may be phosphotidylcholine, phosphatidic acid, phosphatidylethanolamine etc. Ethosomes enables the drugs to permeate through the stratum corneum. These are considered to be safe drug delivery system which is approved for cosmetic and pharmaceutical use. These are more stable than conventional liposomes. They enhance the delivery of active agents.

Stealth liposomes

These are the spherical vesicles with a membrane composed of phospholipid bilayer, polymers and are used for delivering drugs or genetic materials into the cells. In this, the liposomes are coated with hydrophilic polymer (polyethylene glycol) [50]. They serve as reservoir for extended drug release.

Pharmacosomes

Pharmacosomes are the molecules having positive and negative charges and possess hydrophilic and hydrophobic properties. They improve the permeation rate and possess broader stability profile. Pharmacosomes are widely used because of their greater shelf life.

Immunoliposomes

These are the liposomal drug delivery systems in which antibody molecules are conjugated to the surface of liposomes. They play a major role in treatment of cancer cells [51].

Virosomes

These are the reconstituted viral envelopes which serves as vaccines and as vehicles for cellular delivery of various types of macromolecules. These are non-toxic, biocompatible and biodegradable. These are used to encapsulate various pharmacologically active substances. *Influenza virus* is most commonly used to prepare virosomes [52].

Various Marketed Liposomal Formulations (Payne, 1986)

Trade name	Generic name	Application	Company
Ambisome TM	Amphotericin B	Antifungal activity	NeXstar Pharmaceuticals, Inc., CO
Abelcet TM	Amphotericin B	Antifungal activity	The Liposome Company, NJ
Amphotec TM	Amphotericin B	Antifungal activity	Sequus Pharmaceuticals, Inc., C.A.
Doxil	Doxorubicin	Metastatic ovarian cancer and advanced Kaposi's sarcoma	Sequus Pharmaceuticals, Inc., C.A.
Dauno Xome TM	Daunorubicin	Cancers	NeXstar Pharmaceuticals, Inc., CO
MiKasome	Amikacin	Bacterial infections	NeXstar Pharmaceuticals, Inc., CO
DC99	Doxorubicin	Metastatic breast cancer	Liposome Co., NJ, USA
Epaxal	Hepatitis A Vaccine	Hepatitis A	Swiss Serum Institute,Switzerland
Myocet™	Doxorubicin	Metastatic breast cancer	zeneus
Depocyt	Cytarabine	Neoplastic and lymphomatous meningitis	enzon pharmaceuticals

CONCLUSION

Liposomes have gained extensive attention as drug delivery system for numerous kinds of drugs. The direct application of liposomes in medicine encourages the researchers to create novel liposomes for treatments and diagnosis in a wide range of diseases as well as in a variety of therapeutic applications. It was concluded from the review that liposomes can be a promising carrier for improving targeted delivery of a large number of drugs: Antimicrobial agents, drugs against cancer, antifungal drugs, peptide hormones, enzymes, vaccines, and genetic materials. Liposomes are administrated orally, parenterally and topically as well as employed in a broad range of pharmaceutical and pharmacology applications with therapeutic and diagnostic purposes and as good carriers in gene delivery various drugs. Liposomes are one of the best choices of nano carrier in drug delivery, site-specific drug delivery, specific organ, and receptor targeting. Liposomal delivery systems have been approved as a suit carrier for therapeutic effectiveness in terms of duration of action and decrease in dose frequency and delivering drugs at higher efficiency and lower toxicity.

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CONFLICT OF INTEREST

The authors declare no conflict of interest

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