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Liposomal drug delivery system

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Abstract
Liposomes are drug vesicles nowadays used for a promising drug delivery. As compared to other traditional drug delivery system liposomes have better properties which includes site targeting. sustained release also controlled release, liposomes also protects the drug from getting degrade and from clearance. Liposomes shows superior therapeutic effects and also lower toxic effects. In this review the

Keywords: Liposomes, Phospholipids, Drug delivery, Cholesterol, Bilayer stability

Introduction
Liposomes are self assembled phospholipid based drug vesicles which forms a bilayer or a series of multiple bilayers which enclose a central aqueous compartments. [1] The liposomes size ranges from 30nm to micrometer range. which has a phospholipid bilayer of 4-5 nm thickness. [2]

Lipids used in the formulation of liposomes
The importance of liposomes as a drug carrier has been established well over these years. It is very important to understand the composition of liposomes specially with the lipids components used in the formulation. The structure of the liposomes consists of a lipid bilayer which surrounds and aqueous core with the size ranging from 20 to 1000nm. [3]

Phospholipids
Phospholipids are highly found in the structural composition of the biological membranes and therefore are extensively used in the preparation of liposomes. [4] The biocompatibility and the ampiphilic properties of phospholipids contribute to the formation of liposomes and thus potentiate their profitability as drug carriers. [5] Liposomes made with phospholipids are characterise into multifunctional properties which includes stimuli sensitive liposomes. targeted liposomes. pH sensitive liposomes and thermo sensitive liposomes. [6]
Phospholipids are been categorised into glycerophospholipids and sphingomyelins based on the alcohol groups present in their structures. [7]
The mostly used glycerophospholipids in the formation of liposomes are phosphotidylcholine (PC).
Phosphotidylethanolamine (PE), phosphotidylserine (PS). Phosphatidylinositol (PI) and phosphotidylglycerol (PG). [8,9] Hydrogenated soy phosphotidylcholine (HSPC) which is a_glycerophospholipid is a saturated phospholipid which is hydrogenated and also possesses greater physico chemical stability compared to unsaturated lipids. [10]
The degree of unsaturation. fatty acid side chain and phase transition temperature of phospholipids are used to determine the stability of the liposomes. [11]
The phospholipids. tightly packed in the bilayer region of the liposomes starts loosening up and becomes more permeable when the phase transition temperature of the phospholipids is reached. [12] These changes causes more empty spaces between the phospholipids which leads in the film formation step of liposome preparation. [12] The phase transition temperature of HSPC is 53^0C and the phospholipids with high transition temperature provides enhanced physical stability at room temperature. [13, 14]

Cholesterol
Cholesterol is often used as an additive to rigidify the lipid bilayer and to stabilize the liposome structure. Cholesterol molecules can be integrated in the membrane to literally fill in any gaps. In addition, liposome size and lamellarity are parameters that can be controlled. In terms of size, liposomes are categorized as small, large, or giant vesicles. Though the precise classification varies amongst publications, overall small vesicles have a size ranging between 20 and 100 nm, large vesicles between 100 and 1000 nm and giant vesicles are typically larger than 1000 nm. [15]
An increase in cholesterol can affect a variety of processes, including the compression of lipid chains and bilayer organization, modification of membrane fluidity/rigidity, progression of the effect on medication release, and solidity of liposomes. [16]

Types of Liposomes
Based on their compositions and applications, liposomes can be classified into conventional liposomes, [17] charged liposomes [18], stealth stable liposomes [19], actively targeted liposomes [20]. stimuli-responsive liposomes [21]. and bubble liposomes [22].

Conventional liposomes
Conventional liposomes were synthesized from natural or synthetic phospholipids with or without cholesterol as a liposomes first generation. [23]
Conventional liposome formulations are mainly comprised of natural phospholipids or lipids such as 1, 2-distearoryl-sn-glycero- 3-phosphatidyl choline (DSPC), sphingomyelin, egg phosphatidylcholine and monosialoganglioside. [24]

Charged liposomes

Oleic acid and N- [1(2,3-dioleoyloxy) propyl]-N, N, N-trimethylammonium chloride (DOTAP) are usually used to prepare anionic and cationic liposomes, respectively. Charged liposomes showed higher liposomal stability during the storage, as charged particles repel each other and reduce aggregation abilities. Cationic liposomes are used in gene therapy due to their ability to successfully encapsulate nucleic acids by electrostatic attractions. [25]

Cationic liposomes are suitable for delivering various negatively charged macromolecules such as DNA, RNA, and oligonucleotides because their negative charge and rather a large size restrict their passive diffusion into cells. [26]

Stealth stable liposomes

Stealth liposomes are important in cancer treatment for their passive targeting effect, which may lead to preferential accumulation in tumor tissue, but this phenomenon is not fully understood: Stealth liposomes are able to lodge in the interstitial spaces among tumor cells but, once in the tumor area, they locate in the extracellular fluid surrounding the tumor cell without entering it. Thus, to deliver the active form of an anticancer agent, such as doxorubicin or cisplatin, the drug must be released from the liposomes into the tumor extracellular fluid and then diffuse into the cell. As a result, the ability of liposomes to carry the anticancer agent to the tumor (which depends on their stability) and to release it into the tumor extracellular fluid (depending on membrane composition and fluidity) are equally important factors in determining the anti-tumor effect of liposome-encapsulated anticancer agents. [27]

Actively targeted liposomes

Stimuli Responsive liposomes

The stimuli can be either internal (e.g. enzyme activity, pH changes, or the presence of reducing agents) or they can be external stimuli applied from outside (e.g. temperature, light, magnetic field, or ultrasound (US)). [28]

Bubble liposomes

Bubble liposomes (gas-encapsulated liposomes) are expected to create new applications in the field of gene delivery and drug delivery systems. [29] Recently, liposomes have been used to encapsulate bioactive gases and/or drugs for ultrasound-controlled drug release with enhanced drug delivery [30]. Nitric oxide (NO) bubble liposomes offer a distinguishing NO intravenous therapeutics option overcome common microbubbles, in which liposomes shield NO from haemoglobin rummaging *in vitro* as usually occurred by free NO. Oxygen bubble liposome (OBL) enables high oxygen fixations with high pO2 conditions of the lungs. This separates OBL from great fluorocarbon and haemoglobin-based oxygen transporters and keeps their utilization as supported oxygen conveyance stages. [31]

Structure of Liposomes

Liposomes can be classified as unilamellar vesicles (ULVs), oligolamellar vesicles (OLVs), multi lamellar vesicles (MLVs), and multivesicular liposomes (MVLs) depending on the compartment structure and lamellarity (Fig 1) [32]. OLVs and MLVs show an ion-like structure but present 2–5 and >5 concentric lipid bilayers, respectively. Different from MLVs, MVLs include hundreds of non-concentric aqueous chambers bounded by a single bilayer lipid membrane and display a honeycomb-like structure [33]. Based on the particle size, ULVs can be further divided into small uni-lamellar vesicles (SUVs, 30–100 nm), large uni-lamellar vesicles (LUVs, >100 nm), and giant uni-lamellar vesicles (GUVs, >1000 nm) [34]. Different size range of ULVs was reported, i.e., SUVs with a size of less than 200 nm and LUVs with a size of 200–500 nm [35].

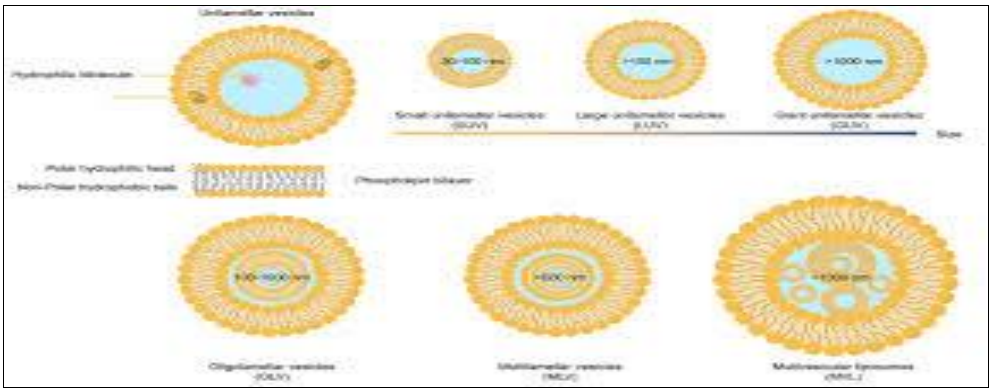

Fig 1: Methods of preparation of liposomes:

A variety of methods have been used in the preparation of liposomes. The selection of method of liposome preparation is based on the type of drug loading, i.e. Passive or Active loading. Passive loading includes two methods of preparation: [36] Mechanical dispersion method and Solvent dispersion method.

Mechanical dispersion method includes lipid film hydration, extrusion, sonication, French pressure cell method, freeze thaw. Solvent dispersion method includes ether injection, ethanol injection, reverse phase evaporation and supercritical fluids in the preparation of liposomes [36,37].

Among the mechanical dispersion methods, sonication and lipid film hydration are most widely used in preparation of liposomes [36].

In sonication, the multilamellar vesicles (MLV) are sonicated with use of bath or probe sonicator to form unilamellar vesicles. The method has disadvantages such as non-uniformity in size, low drug encapsulation efficiency [36].

Lipid film hydration along with extrusion can be used to overcome the disadvantages of sonication while achieving passive drug loading [37,38].

Lipid Film hydration method (Bangham method)

The Bangham method is the first commonly used method for liposome preparation [39,40]. This method utilizes an organic solvent (dichloromethane, chloroform, ethanol and chloroform–methanol mixture) to dissolve lipids; further the organic solvent can be removed by evaporation under vacuum at a temperature of 45–60^{0}C to form a thin lipid film. Subsequently, the thin lipid film gets hydrated in aqueous media by continuous agitation up to 2 h at a temperature of 60–70^{0}C where it swells to produce round closed liposomes [41].

Injection methods

1. Ether Injection
2. Ethanol injection

Ether Injection: In ether injection method a solution of lipids is dissolved in ether or diethyl ether/methanol mixture which is slowly injected to an aqueous solution of the material to be capsulated. The subsequent removal of the organic solvent under reduced pressure leads to the formation of liposomes [42,43]. The main disadvantage of the method is heterogeneous population and the exposure of compounds to be encapsulated to organic solvents or high temperature [44].

Ethanol Injection: In ethanol injection method the ethanolic lipid solution is rapidly injected to a vast excess of preheated distilled water [42] or TRIS-HCl buffer [45]. The incorporation of the drug in liposomal vesicle depends on its hydrophilic/hydrophobic character. Nimesulide as lipid soluble component incorporates better in liposomes than 5-fluorouracil which migrates to external aqueous phase [42,45]. The main advantage of ethanol injection method is including of non-harmful solvent as ethanol, as well as easy scale up of the method. The possibility of formation of azeotrope with water reduces its applicability [42,43].

Sonication method

Methods for the preparation of sonicated SUVs have been reviewed in detail by Bangham *et al* [46]. Classically the MLV dispersion is placed in test tubes and sonicated either in a bath sonicator or by tip sonication. Normally a 5–10-min sonication procedure (above Tc) is suffi cient to prepare SUVs with radii < 50 nm. With some lipids, radii < 20 nm are also possible while some diacyl cationic lipids (including 1- [2-(oleoyloxy)-ethyl2-oleoyl-3-(2-hydroxyethyl) imidazolinium chloride (DOIC) and dioctadecylamidoglycylspermine (DOGS) can even form micelles. Dioctadecyldiammonium bromide (DOBAD) neutral lipid liposomes cannot be sized <130nm [47,48].

Probe sonication: These sonicator is directly engrossed into the liposome dispersion. In this method energy input into lipid dispersion is very high. The coupling of energy at tip results in local hotness, therefore, the vessels must be engrossed into
water or ice bath. Throughout the sonication up to 1 h, more than 5% of the lipids can be deesterified With the probe sonicator titanium determination slough off and pollute the solution. [49]

Bath sonication: The cylinder containing liposomes is placed into a bath sonicator at controlled temperature it is usually easier method, in contrast to sonication by dispersion directly using the tip. The material is being sonicated and can be protected in a sterile vessel, dissimilar the probes unit, or under an inert atmosphere. [49]

French pressure cell method: 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. The resulting liposomes are somewhat larger than sonicated SUVs. The drawbacks of the method are that the temperature is difficult to achieve and the working volumes are relatively small (about 50 mL maximum). [49]

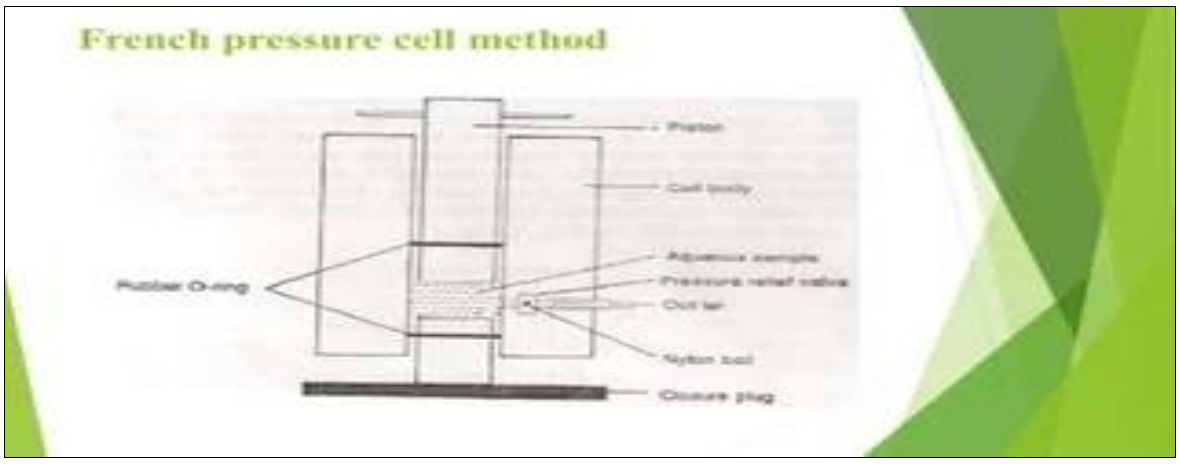

Fig. French pressure cell

Freeze Thaw Method

Liposomes prepared by freeze-thaw extrusion underwent a similar procedure as the standard extrusion protocol, but with an additional step at the beginning. First, the post resuspension solution in its glass test tube was rapidly frozen with liquid nitrogen (196 ^{0} C) for 5 min and then thawed with a lukewarm water bath (30 ^{0} C) for 5 min. This freeze-thaw cycle was conducted 5 times. After the freeze-thaw cycles, the standard extrusion protocol was followed.

Evaluation of liposomes

Characterization of Liposomes

Size and Size Distribution

The vesicle size is crucial to determine the *in vivo* release of drug-loaded liposomes. The average size of liposomes depends on the method of preparation and phospholipid composition. Various methods are used to evaluate the size and size distribution such as:

1. Microscopic techniques such as optical microscopy, scanning electron microscopy (SEM), negative stain TEM and freeze-fracture TEM. SEM and TEM techniques are used for imaging of liposomes and also provide information about bilayer thickness and inter-bilayer distance of liposome. One of the newly established microscopic methods is atomic force microscopy (AFM), which is a very high-resolution scanning probe microscopy that produce 3D micrographs through resolution of nano-meter and A^0 scale to evaluate the liposome morphology, stability, size and dynamic process of lipid nano-capsules.
2. Hydrodynamic techniques such as ultracentrifugation, field flow fractionation and gel exclusion chromatography and analytical centrifugation procedures are used to estimate molecular mass of compound and also used for comparison of size distribution, elution characteristics and uniformity of the liposomes.
3. Diffraction light scattering techniques such as laser light scattering, quasi-elastic light scattering and photon correlation spectroscopy give information about the size of the lipid vesicles.

Lamellarity Determination

Lamellarity is defined as the number of lipid bilayers present around the lipid vesicles. Liposomal lamellarity can be measured by using cryo-electron microscopy, 31P-nuclear magnetic resonance (NMR) and small angle X-ray scattering (SAXS) technique that provide information about size, homogeneity and lamellarity of liposomes.

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