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Liposome- as drug carriers

Himanshu Anwekar*, Sitasharan Patel and A.K Singhai
Lakshmi Narain College of Pharmacy, Bhopal, (M.P) - India

Abstract

Liposomes are artificially prepared vesicles made of lipid bilayer. Liposomes can be filled with drugs, and used to deliver drugs for cancer and other diseases. Amongst the various carrier, few drug carrier reached the stages of clinical trails where phospholipid vesicles (liposome) show strong potential for effective drug delivery to the site of action. Liposomes can be composed of naturally-derived phospholipids with mixed lipid chains or other surfactants. Liposome have covered predominantly medical, albeit some non-medical areas like bioreactor, catalysts, cosmetics and ecology. However,their predominance in drug delivery and targeting has enabled them to be used as therapeutics tool in fields like tumour targeting, gene and antisense therapy etc.

Key-Words: Liposome, MLV, LUV, Application, Drug carriers

Introduction

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 uni-lamellar 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]

The resemblance to the plasmalemma was obvious, and the microscope pictures served as the first real evidence for the cell membrane being a bilayer lipid structure.

Liposome are defined As "Liposome are simple microscopic vesicles in which an aqueous volume is entirely enclosed by a membrane composed of lipid molecule." Various amphipathic molecules have been used to form liposome. The drug molecules can either be encapsulated in aqueous space or intercalated into the lipid bilayer. (Fig. 1)

*** Corresponding Author:**
E-mail: himanshu.anwekar@yahoo.com
Mob. 07898213986

A liposome is a spherical vesicle with a membrane composed of a phospholipid bilayer used to deliver drug or genetic material into a cell. Liposomes can be composed of naturally-derived phospholipids with mixed lipid chain like egg phosphatidylethonalimine or of pure components like DOPE (dioleolylphosphatidylethanolamine).[3]

Advantages of Liposome

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

1. Liposome are increased efficacy and therapeutic index of drug (Actinomycin-D).
2. Liposome is increased stability via encapsulation.
3. Liposomes are biocompatible, completely biodegradable, non-toxic, flexible and nonimmunogenic for systemic and non-systemic administrations.
4. Liposome are reduction in toxicity of the encapsulated agent (Amphotericin B, Taxol).
5. Liposomes help to reduce exposure of sensitive tissues to toxic drugs.
6. Site avoidance effect.
7. Flexibility to couple with site-specific ligands to achieve active targeting.

Disadvantages of Liposome

1. Production cost is high.
2. Leakage and fusion of encapsulated drug / molecules.
3. Sometimes phospholipid undergoes oxidation and hydrolysis like reaction.
4. Short half-life.
5. Low solubility.

6. Fewer stables.

Structural Components of Liposome

There are number of the structural and nonstructural components of liposomes, major structural components of liposomes are:

a. Phospholipids

Phospholipids are the major structural component of biological membranes, where two type of phospholipids exit- PHOSPHODIGLYCERIDES AND SPHINGOLIPIDS. The most common phospholipid is phosphatidylcholine (PC) molecule. Molecule of phosphatidylcholine are not soluble in water and in aqueous media they align themselves closely in plannar bilayer sheets in order to minimize the unfavorable action between the bulk aqueous phase and long hydrocarbon fatty chain. (Fig. 2)

The 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.

Examples of phospholipids are:

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

b. Cholesterol

Cholesterol dose not by itself form bilayer structure, but can be incorporated into phospholipid membranes in very high concentration upto 1:1 or even 2:1 molar ration of cholesterol to phosphatidylcholine. Cholesterol inserts into the membrane with its hydroxyl group oriented towards the aqueous surface and aliphatic chain aligent parallel to the acyl chains in the center of the bilayer. The high solubility of cholesterol in phospholipid liposome has been attributed to both hydrophobic and specific headgroup interation, but there is no unequivocal evidence for the arrangement of cholesterol in the bilayer.[4-5]

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 (artificial membranes) referred to as liposomes, hexasomes or cubosomes (Fig. 3)

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.[6]

Classification of Liposomes

Liposome may bo produced by variety of methods. Their nomenclature also depends upon the method of preparation, structural parameters or special functions assigned to them (table 1)

General Methods of Preparation

All the method of preparing liposomes involve four basic stages:

1. Drying down lipids from organic solvent.
2. Dispersion of lipid in aqueous media.
3. Purification of resultant liposome.
4. Analysis of final product. (Fig 4)

Method of Liposome Preparation and Drug Loading [7]

Various method used for the preparation of liposome:-

1. Passive loading techniques

Passive loading techniques include three different methods:

a. Mechanical dispersion method

- Lipid film hydration by hand shaking, non-hand shaking or freeze drying
- Micro-emulsification
- Sonication
- French pressure cell
- Membrane extrusion
- Dried reconstituted vesicles
- Freeze-thawed liposomes

b. Solvent dispersion method

- Ether injection
- Ethanol injection
- Double emulsion vesicles
- Reverse phase evaporation vesicles
- Stable plurilamellar vesicles

c. Detergent removal method

- Detergent (cholate, alkylglycoside, Triton X-100) removal form mixed micelles
- Dialysis
- Column chromatography
- Dilution
- Reconstituted sendai virus enveloped vesicles

2. Active loading technique

Industrial Production of Liposomes [7-8]

The several preparation methods described in the literature, only a few have potential for large scale manufacture of liposomes. The main issues faced to formulator and production supervisor are presence of organic solvent residues, physical and chemical stability, pyrogen control, sterility, size and size distribution and batch to batch reproducibility.

Liposomes for parenteral use should be sterile and pyrogen free. For animal experiments, adequate sterility can be achieved by the passage of liposomes through up to approximately 400 nm pore size Millipore filters. For human use, precautions for sterility must be taken during the entire preparation process: that is,

1) the raw materials must be sterile and pyrogen free,
2) preparation in sterile system: working areas equipped with laminar flow and
3) use of sterile containers

Some issues related to phospholipids need attention. The liposomes based on crude egg yolk phospholipids are not very stable. The cost of purified lipids is very high. Recently, liposomes have been prepared using synthetic and polymerizable lipids . The liposomes prepared from polymerizable phospholipids are exposed to UV light. The polymerization process takes place in the bilayer(s). Such liposome preparations usually have better storage stability. It should be noted that such materials usually are phospholipid analogues and their metabolic fates have yet to be established.

(i) Detergent Dialysis

A pilot plant under the trade name of LIPOPREPR II-CIS is available from Diachema, AG, Switzerland. The production capacity at higher lipid concentration (80 mg/ml) is 30 ml liposomes/minute. But when lipid concentration is 10-20 mg/ml 100 mg/ml then up to many litres of liposomes can be produced. In USA, LIPOPREPR is marketed by Dianorm-Geraete.

(ii) Microlluidization

A method based on microemulsiftcation/homogenization was developed for the preparation of liposomes. MICROFLUIDIZERR is available from Micro0udics Corporation, Massachusetts, USA. A plot plant based on this technology can produce about 20 gallon/minute of liposomes in 50-200 nm size range. The encapsulation efficiency up to 75% could be obtained.

(iii) Aqueous dispersions of liposomes often have tendency to aggregate or fuse and may he susceptible to hydrolysis and or oxidation. Two solutions have been proposed:

(a) Proliposomes

In proliposomes, lipid and drug are coated onto a soluble carrier to form free-flowing granular material which on hydration forms an isotonic liposomal suspension. The proliposome approach may provide an opportunity for cost-effective large scale manufacture of liposomes containing particularly lipophilic drugs.

(b) Lyophilization

Freeze-drying (lyophilization) involves the removal of water from products in the frozen state at extremely low pressures. The process is generally used to dry products that are thermolabile and would be destroyed by heat-drying. The technique has a great potential as a method to solve long term stability problems with respect to liposomal stability. It is exposed that leakage of entrapped materials may take place during the process of freeze- drying and on reconstitution. Recently, it was shown that liposomes when freeze-dried in the presence of adequate amounts of trehalose (a carbohydrate commonly found at high concentrations in organism) retained as much as 100% of their original contents. It shows that trehalose is an excellent cryoprotectant (freeze-protectant) for liposomes. Freeze-driers range in size from small laboratory models to large industrial units are available from Pharmaceutical Equipment Suppliers. Recently Schrier et al. (1994) have studied the in vitro performance of formulations prepared from lyophilized liposomes.

Characterization of Liposomes [9-11]

Liposome prepared by one of the preceding method must be characterized. The most important parameters of liposome characterization include visual appearance, turbidity, size distribution, lamellarity, concentration, composition, presence of degradation products, and stability.

1. 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, gray color indicates that presence of a nonliposomal dispersion and is most likely a disperse inverse hexagonal phase or dispersed microcrystallites. An optical microscope (phase contrast) can detect liposome> 0.3 μm and contamination with larger particles.

2. Determination of Liposomal 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.

3. Determination of Lamillarity

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

4. Liposome Stability

Liposome stability is a complex issue, and consists of physical, chemical, and biological stability. In the pharmaceutical industry and in drug delivery, shelf-life stability is also important. Physical stability indicates mostly the constancy of the size and the ratio of lipid to active agent. The cationic liposomes can be stable at 4°C for a long period of time, if properly sterilized.

5. Entrapped Volume

The entrapped volume of a population of liposome (in µL/ mg phospholipid) 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.

6. Surface Charge

Liposome are usually prepared using charge imparting constituting lipids and hence it is imparting to study the charge on the vesicle surface. In general two method are used to assess the charge, namely free-flow electrophoresis and zeta potential measurement. From the mobility of the liposomal dispersion in a suitable buffer, the surface charge on the vesicles.

Stability of Liposomes [12-13]

1. Physical stability

The stability of a pharmaceutical product usually is defined as the capacity of the delivery system to remain within defined or pre-established limits during the self life of the product. There is no established protocol for either accelerated or long–term stability studies for the liposomal formulation. Classical models from colloidal science can be used to describe liposome stability. Colloidal systems are stabilized electrostatically, sterically or electrosterically. In addition the self-assembling colloids can undergoes fusion or phase change after aggregation. Liposome exhibit both physical and chemical stability characteristics. Generally, the physical characteristic describes the preservation of liposome structure and the chemical characteristic refers to molecular structure of liposomal components. (hydrolysis and oxidation of phospholipid) Physically stable formulations preserve both liposome size distribution and the amount of material encapsulated. The stability problem overcomes by using appropriate techniques like freezing, lyophilization and osmification. It is also prevented by using fresh solvents and freshly purified lipid, using inert nitrogen gas, avoid high temperature and include anti-oxidants α, β, γ and δ-tocopherol.

2. Plasma Stability

Although liposomes resemble biomembranes, they still are foreign objects for the host. Therefore, liposomes are recognized by the mononuclear phagocytic system (MPS) after interaction with plasma proteins. As a result, liposomes are cleared from the blood stream. These stability problems solve by using synthetic phospholipids, gangliosides, polymerization, coating liposomes with chitin derivatives, freeze drying, microencapsulation and particle coated with amphipathic polyethylene glycol.

Application in Medicine:

As of 2008, 11 drugs with liposomal delivery systems have been approved and six additional liposomal drugs were in clinical trials. List of clinically-approved liposomal drugs: [14] (Table 2)

Therapeutic Application of Liposome [15]

1. Liposome as drug/protein delivery vehicles
   - Controlled and sustained drug release
   - Enhanced drug solubilization
   - Altered pharmacokinetics and biodistribution
   - Enzyme replacement therapy and biodistribution
   - Enzyme replacement therapy and lysosomal storage disorders
2. Liposome in antimicrobial, antifungal and antiviral therapy
   - Liposmal drugs
   - Liposomal biological response modifiers
3. Liposome in tumour therapy
   - Carrier of small cytotoxic molecules
   - Vehicle for macromolecules as cytokines or genes
4. Liposome in gene delivery
   - Gene and antisense therapy
   - Genetic (DNA) vaccination
5. Liposome in immunology
   - Immunoadjuvant
   - Immunomodulator

- Immunodiagnosis

6. Liposome as artificial blood surrogates
7. Liposome as radiopharmaceutical and radio diagnostic carriers
8. Liposome in cosmetics and dermatology
9. Liposome in enzyme immobilization and bioreactor technology.

Conclusion

Twenty five year of research into the use of liposome in drug delivery. Liposomes are one of the unique drug delivery system, which can be of potential use in controlling and targeting drug delivery. Liposomes are administrated orally, parenterally and topically as well as used in cosmetic and hair technologies, sustained release formulations, diagnostic purpose and as good carriers in gene delivery various drugs with liposomal delivery systems have been approved. Nowadays liposomes are used as versatile carriers for targeted delivery of drug.

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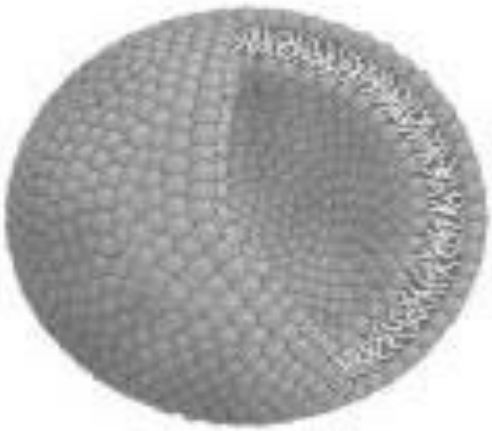

Fig 1. Spherical Formation of Liposomes

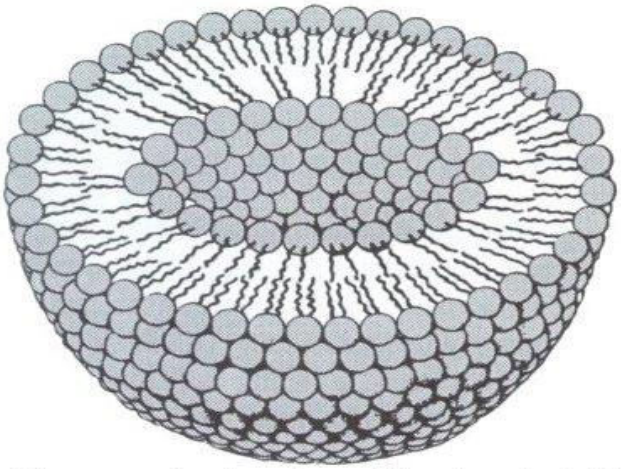

Fig 2. Liposome structure formed by phospholipids

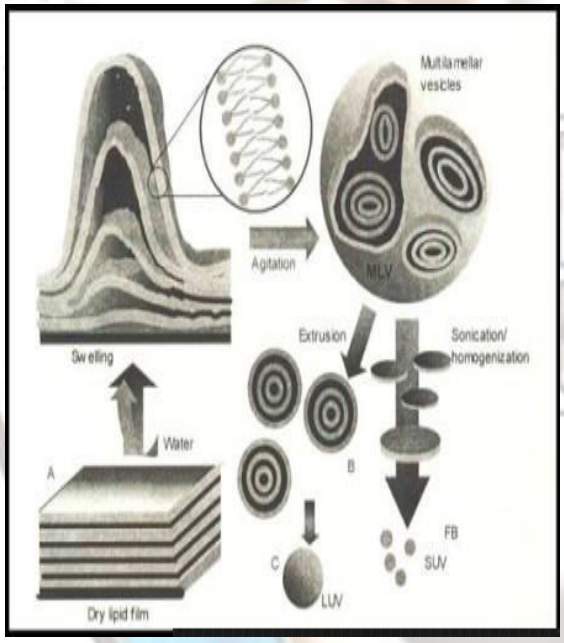

Fig 3. Mechanism and processing steps to generate various types of vesicles

Fig 4: Common stages of all method of preparation of liposome

Table 1: Liposome classification based on pharmaceutical and therapeutical aspects

Type	Specification
MLV	Multilamellar large vesicles- >0.5µm
OLV	Oligolamellar vesicles- 0.1-1µm
UV	Unilamellar vesicles (all size range)
SUV	Small Unilamellar vesicles- 20-100nm
MUV	Medium sized Unilamellar vesicles
LUV	Large Unilamellar vesicles- >100
GUV	Giant Unilamellar vesicles- >1µm
MV	Multivesicular vesicles- 1µm

Table 2: List of clinically-approved liposomal drugs

Name	Trade name	Company	Indication
Liposomal amphotericin B	Abelcet	Enzon	Fungal infections
Liposomal amphotericin B	Ambisome	Gilead Sciences	Fungal and protozoal infections
Liposomal cytarabine	Depocyt	Pacira (formerly SkyePharma)	Malignant lymphomatous meningitis
Liposomal daunorubicin	DaunoXome	Gilead Sciences	HIV-related Kaposi's sarcoma
Liposomal doxorubicin	Myocet	Zeneus	Combination therapy with cyclophosphamide in metastatic breast cancer
Liposomal IRIV vaccine	Epaxal	Berna Biotech	Hepatitis A
Liposomal IRIV vaccine	Inflexal V	Berna Biotech	Influenza
Liposomal morphine	DepoDur	Skye Pharma, Endo	Postsurgical analgesia
Liposomal verteporfin	Visudyne	QLT, Novartis	Age-related macular degeneration, pathologic myopia, ocular histoplasmosis
Liposome-PEG doxorubicin	Doxil/Caelyx	Ortho Biotech, Schering-Plough	HIV-related Kaposi's sarcoma, metastatic breast cancer, metastatic ovarian cancer
Micellular estradiol	Estrasorb	Novavax	Menopausal therapy