LIPOSOME: AN OVERVIEW

Bhavana R[1], Jeevak Chander.TR[2], Chandrasekar M[3] , Monisha J[4]*

Abstract:
Liposomes are ball-shaped vesicles made up of one or more phospholipid bilayers. They play an important role in the fields of Pharmaceuticals and Neutraceuticals as well as in Mathematics, Theoretical physics, Biophysics, Chemistry, Colloidal material, Biochem, and Biology. This is a mini-review comprising classification, merits and demerits, method of preparation, applications, marketed products.

Keywords: Liposomes, Lipid, Vesicles, Phospholipids, Aqueous, Solvent

[1,2,3]B. Pharmacy IV year, School of Pharmaceutical Sciences, Vels Institute of Science, Technology and Advanced Studies (VISTAS), Chennai – 600117, Tamil Nadu, India
[4]*Assistant professor, Department of Pharmaceutics, School of Pharmaceutical Sciences, Vels Institute of Science, Technology and Advanced Studies (VISTAS), Chennai – 600117, Tamil Nadu, India.

***Corresponding Author: -** Monisha J
*Assistant professor, Department of Pharmaceutics, School of Pharmaceutical Sciences, Vels Institute of Science, Technology and Advanced Studies (VISTAS), Chennai – 600117, Tamil Nadu, India.

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INTRODUCTION

Liposomes were discovered in 1961 by British haemotologist Dr. Alec D Baghman.In the word liposome, "lipo" means "fat", and "soma" means "body"[1]. These Micro-particulate carriers are formed impulsively when phospholipids are hydrated in aqueous media usually which are in the diameter of 0.05-5.0um. Liposomes have one or several concentric curved lipid bilayer membranes and cholesterol in self closed spherical structure. They entrap both hydrophilic and lipophilic drugs and aim to deliver them to designated sight [2]. Liposomal properties mayvary with the method of preparation, surface charge, and size. There are several drug delivery systems among them liposomal science and technology is a rapidly growing and emerging field in the modern world.[3]

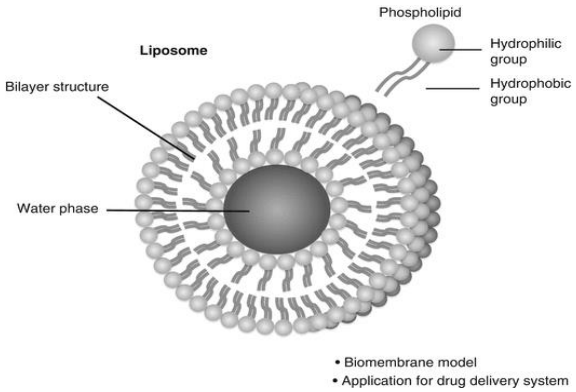

Fig.1; Structure of Liposome
(Source:google.com)

Merits of liposomes:[3,4]

- Biodegradable
- Biocompatible
- Non-toxic and flexible
- Non-immunogenic
- Sustained release
- Given through several routes of administration
- Improved pharmacokinetic effects
- Improved efficacy and therapeutic index
- Have both positively and negatively charged molecules.

Demerits of liposomes:[3,4]

- High production cost
- Solubility is low
- Shorter half-life
- Low stability
- Low therapeutic index
- Less dose effectiveness
- Batch to batch variation.

CLASSIFICATION OF LIPOSOMES

Liposomes are classified on the basis of

1) Based on structural parameters
2) Based on method of preparation
3) Based on composition and its characteristics

LIPOSOMES CLASSIFICATION BASED ON STRUCTURAL PARAMETERS:

i) Small Unilamellar Vesicles (SUV)

These vesicles consist of a single bilayer and have a low ratio of aqueous volume to lipids (0.2:1.5:1 mole lipid). The size ranges from 10-100nm.[5]

ii) Large Unilamellar Vesicles (LUV)

These vesicles, which consist of a single bilayer and have a high ratio of water volume to lipid (7:1 mole lipid), are excellent for transporting hydrophilic medicines. The size spans between 100nm – 1um.[6]

iii) Multilamellar Vesicles (MLVs)

Several bilayers present; lipid-to-water ratio is about 0.5. (1:4:1 mole lipid). It can be anything from 100 nm to 20 um in size.[7]

iv) Oligolamellar Vesicles (OLVs)

Intermediate between LUV and MUV.These are more than one but not as many as MLVs and size ranges between 0.1-1um.[10]

v) Unilamellar Vesicles (ULVs)

It includes all size ranges.

LIPOSOME CLASSIFICATION BASED ON THE METHOD OF LIPOSOME PREPARATION:

- **REV**-Single or olioligolamellar vesicle made by reverse phase evaporation method
- **MLV/REV**-Multilamellar vesicles made by reverse phase evaporation method
- **SPLV**-Stable plurilamellar vesicles
- **FATMLV**-Frozen and thawed MLV
- **VET**-Vesicles prepared by extrusion method
- **FUV**-Vesicles prepared by fusion method
- **FPV**-Vesicles prepared by French press
- **DRV**-Dehydration-rehydration vesicles
- **BSV**-Bubblesomes

LIPOSOMES CLASSIFICATION BASED ON COMPOSITION AND ITS CHARACTERISTICS:

a) Conventional Liposomes

Consisting of phospholipids and cholesterol, both of which have neutral or negative charges.[14] The 'Reticuloendothelial system' is the primary target of these liposomes (RES).[6]

b) PH Sensitive
The material is composed of phospholipids, including phosphatidyl ethanolamine and dioleoyl phosphatidyl ethanolamine.[15] Coated pit endocytosis-sensitive compounds fuse with cell or endosome membranes at low PH, releasing their contents into the cytoplasm.[16]

c) Cationic Liposomes
Cationic lipids make up the structure. Their primary function is to transport macromolecules (negatively charged).[11] Due to their extreme toxicity and potential for causing a short lifespan, these liposomes can only be administered topically.

d) Long Circulating Liposome
In order to create liposomes, the surface of the liposome can be coated with a hydrophilic layer made of oligosaccharides, glycoproteins, or synthetic polymers.[11] Pharmacokinetics up to 10 micromoles of mouse lipid dosage; long circulation half-life (40 hours).[13]

e) Immunolipids
Liposomes, either of the classic or stealth form, that have an antibody or recognition sequence attached to them.[18] Therapeutic action is the result of receptor-mediated endocytosis, cell-specific binding, extracellular content release near the target tissue, and drug diffusion across the plasma membrane.[11,12]

f) Temperature or heat sensitive liposomes
Its made up of dipalmitoyl phosphatidylcholine. Vesicles exhibit their greatest discharge at 41oY. The temperature at which dipalmitoyl phosphatidylcholine transitions into its phase.[18] At the surface of the target cell, liposomes discharged the substances they had captured.[14,15]

METHOD OF PREPARATION OF LIPOSOMES:[8]

Passive loading technique

Involves loading of the entrapped agents before or during the manufacturing process.

Active loading technique

After the production the complete vesicles of some substance With isonisable groups and those that are both lipid and Water soluble can be added to the liposomes

- Mechanical Dispersion Methods
- Solvent Dispersion Methods
- Detergent Removal Methods

➢ **Mechanical Dispersion Methods**
- Lipid film hydration by hand shaking, non-hand shaking or freeze drying
- Microemulsification
- Sonication
- French pressure cell
- Membrane extrusion
- Dried reconstituted vesicles
- Freeze thawed liposomes

➢ **Solvent dispersion methods**
- Ethanol injection
- Ether injection
- Double emulsion vesicles
- Reverse phase evaporation vesicles
- Stable pherilamellar vesicles

➢ **Detergent removal methods**
- Dialysis
- Column chromatography
- Dilution

GENERAL METHOD OF PREPARATION OF LIPOSOME:[15]
Dissolve lipids in organic solvent/co-solvent

↓

Under vacuum organic solvent is removed

↓

Film is deposited

↓

Hydration of solid lipid mixture by using an aqueous buffer

↓

Lipids impulsively swell and hydrate

↓

Form liposomes

The most common methods used in the preparation of liposomes are;

1. Lipid film hydration by hand shaking /non-handshaking

Step-1

i) A lipid mixture including different phospholipid and charge components is prepared in a chloroform: methanol solvent mixture (2:1v/v) and then introduced into the flask, which has a circular bottom and a ground glass neck.[18]

ii) The flask is then rotated at a rate of sixty revolutions per minute (rpm) using a rotary evaporator. Lipids used in this context have a transition temperature of roughly 30 degrees Celsius, at which point the organic solvent evaporates.[19,20]

iii) To eliminate the pressure differential between the inside and outside of the flask, nitrogen is pumped into the evaporator and the pressure at the cylinder head is raised gradually. After the solvents have been removed as much as possible, the flask is removed from the evaporator and attached to the lyophilizer's manifold.[16]

Step-2 Hydration of lipid layer

i) After the vacuum is released and the flask is taken out of the lyophilizer, it is flushed with nitrogen.[8] The absolute to be trapped is then added to 5 ml of saline phosphate buffer.[10]

ii) The flask is then reattached to the evaporator, flushed with N2, and rotated at room temperature and pressure (no more than 60 revolutions per minute).[21] After 30 minutes of agitation, all of the lipid will have been extracted from the flask's wall, and the suspension will be uniform and milky white, with no visible particles.[22,23]

iii) The suspension is left at room temperature (or at a temperature higher than the lipid's transition temperature) for an additional two hours to complete the swelling process and form MLVs.

Lipid film hydration by non-shaking vesicles

i) The technique Reeves and Dowben published in 1996 for producing large unicellular vesicles (LUVs) with a greater volume of entrapment.

ii) The process is different from the hand-shaken approach in that it agitates the sample with a stream of nitrogen rather than rotational movements.

iii) Spread the methanol and mixture of the lipid solution in chloroform over the conical flask with the flat bottom.
By passing nitrogen through the flask, the solution evaporates at room temperature without being disturbed.[24]

iv) Following drying, water-saturated nitrogen is pumped through the flask until the dried film's opaqueness vanishes (15-20 mins)

v) Lipid is inflated by the addition of bulk fluid after hydration.The flask is tilted to one side, 10–20 numbers of 0.2 sucrose in distilled water (degassed) are added, and the flask is slowly brought back to an upright position.[20] The lipid layer at the bottom of the flasks is given a gentle wash by the fluid.

vi) A nitrogen flush is applied, the flask is shut, and it is left to stand for two hours at 37 degrees Celsius. Be careful not to tamper in any way with the flask.

vii) After swell, the vesicles are harvested by agitating the liquid inside to produce a milky suspension.[21,22]

2. Microemulsification/Microfluidizer

i) Small MLVs are made from concentrated lipid dispersion using a microfluidizer.

ii) A microfluidizer forces the fluid through a 5 micrometer hole at an extremely high pressure (10,000 psi).[11]

iii) After that, it is driven along predetermined microchannels that cause two streams of fluid to clash at an angle and at a high speed, thereby transferring energy.

iv) Lipids can enter the fluidizer as big MLVs or as a slurry of a hydrated lipid in an organic media.Up until vesicles with spherical dimensions are formed, the fluid collected can be circulated through the pump and the interaction chamber.[13,15]

v) After a single pass, the vesicles' diameter is decreased to between 0.1 and 0.2um.

3. Sonication method

i) This is the process by which several lamellar vesicles are converted into single-lamellar vesicles. To obtain the SUVs, the MLVs receive ultrasonic irradiation.

ii) Sonication is applied using either the probe method or the bath approach.
iii) The probe is employed for dispersion, which requires a lot of energy in a short amount of space (such a viscous aqueous phase or a lot of lipids), but it is more ideal for huge amounts of diluted liquid.
iii) The probe is used for dispersion, which needs a lot of energy in a short amount of space (such a concentrated lipid solution or an aqueous phase that is viscous), but it is better suited for huge amounts of diluted liquid.
iv) Liposomal dispersion overheating results in lipid breakdown, whereas probe tip sonicators offer high energy input to the liquid dispersion.[11,21]

4. Membrane Extrusion method

i) Both LUVs and MLVs can be processed using the method.
ii) Liposomes are gently run through a membrane filter with a predetermined pore size that is accomplished at a considerably lower pressure (100 psi) to minimise their size.
iii) During this process, phopholipids players rupture and reseal as they move across the polycarbonate membrane, exchanging the contents of the vesicles with the diaper medium.
iv)The liposomes created using this method are known as LUVETs.

5. Freeze-thawed liposomes

i) The unilamellar dispersion freezing method is used in this method (SUV). then thawing by allowing to sit at room temperature for 15 minutes.
ii) The liposome membrane is then briefly subjected to a sonication cycle, which greatly reduces its permeability.
iii) To make GIANT VESICLES with a diameter between 10 and 50 um, the freeze-thaw technique has been changed to add a dialysis phase against hypo-osmolar buffer in place of sonication.
iv) The simple, rapid, and gentle process for the entrapped solute results in a considerable proportion of large unicellular vesicles.[14] For research on the phenomenon of membrane transfer, these vesicles are useful.

6. Ether injection method

i) An aqueous solution of the substance that is to be encapsulated is softly injected at temperatures between 55 and 65 degrees Celsius or under reduced pressure with a solution of lipids that have been dissolved in diethyl ether or a mixture of ether and methanol.
ii) Liposomes are produced when the ether is subsequently removed from the mixture while the container is placed in a vacuum.

7. Ethanol Injection Method

i) A large quantity of buffer is rapidly mixed with a lipid solution of ethanol in a short length of time. The MLVs instantly form.
ii) The method's drawbacks include the population's heterogeneity (30-110 nm), the liposomes' diluted state, the difficulty of totally removing ethanol since it forms an azeotrope with water, and the possibility that a number of biologically active macromolecules could become inactive in the presence of even minute levels of ethanol.[21,30]

8. Reverse phase evaporation vesicles

i) A two-phase solution including phospholipids in an organic solvent (diethylether, isopropylether, or a combination of isopropyl ether and chloroform), as well as an aqueous buffer, is briefly sonicated to create the first water in oil emulsion.
ii) A thick gel is produced when the organic solvents are eliminated at reduced pressure.
iii) Liposomes are created when leftover solvent is eliminated using continuous rotational evaporation while operating at a lower pressure.
iv) By utilising this method, it is feasible to accomplish a high encapsulation effectiveness of up to 65% in a medium with a low ionic strength, such as 0.01M NaCl.
v) Both tiny and big macromolecules have been encapsulated using the technique.[21]

SEM OF LIPOSOMES:

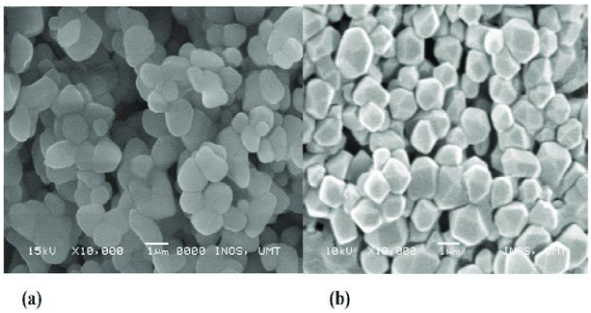

Fig.2; SEM of Liposome (Source: google.com)

- The architecture of the liposomes were examined using scanning electron microscopy (SEM). On SEM stubs with carbon tape, the liposomes that did not go through lyophilization were air-dried. To prevent electron charging, these samples needed to be

sputter-coated with iridium at a thickness of 20 nm..[16,17]

TEM OF LIPOSOMES:

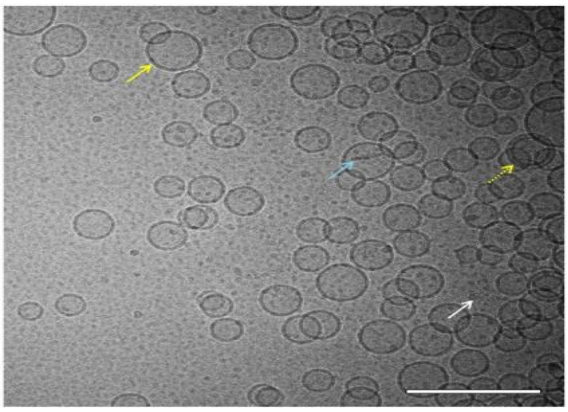

Fig.3; TEM of Liposome (Source: google.com)

- TEM can directly see individual particles and even their internal structure, making it a crucial tool for determining the size and form of nanoparticles.[23]

Applications of Liposomes:

Applications of liposomes in medicine and pharmacology can be divided into diagnostic and therapeutic applications of liposomes containing various markers or drugs, and their use as a tool.[22]

- Liposomes as Vehicles for Drug and Protein Delivery
- Liposomal medicine has applications in bacterial, fungal (lung therapies), and viral (anti-HIV) treatment.
- Liposomes are used in tumor therapy
- Liposomes increase bioavailability
- Liposomes increase the therapeutic index
- It protects active materials by membrane barrier from metabolism or degradation
- Liposomes are utilized as models for artificial cells.

MARKETED PRODUCTS OF LIPOSOMES:

S.NO	PRODUCT	DRUG	COMPANY	INDICATIONTARGET
1.	Atragen	Tretinoin	Anorex Pharmaceuticals Inc.	Acute promyelocyticleukemia
2.	Depocyt	Cytarabine	Pacira Pharmaceuticals Inc.	Treatment of lymphomatous meningitis
3.	Doxil	Doxorubicin	Sequus Pharmaceuticals Inc.	Kaposi sarcoma inAIDS
4.	VincaXome	Vincristine	NeXstar Pharmaceuticals Inc.	Solid Tumors
5.	Nyotran	Nystatin	Anorex Pharmaceuticals Inc.	Systemic fungal infection

Fig.2; (Source: google.com)

CONCLUSION:

Liposomal drug delivery systems are well known forachieving effective concentration at the desired site for various pathological conditions. The liposomal membrane made up of phospholipids also play an important role by giving good strength and protection from mechanical impacts. There are many researchers researching on different targeted drug delivery system among which liposomes are one paving way for study and improvement in targeted drug delivery.

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