

A Concise Review on Liposomes: A Novel Approach

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Abstract: *Liposomes the novel drug delivery system which was firstly introduced by Bangham et al in 1961. Liposomes are a very stable system that are made from phospholipids and cholesterol molecules entrapping the drug in between and forms a bilayer system. This drug delivery system aims to deliver the drug directly at the site of action and provides a controlled and sustainable release. The liposomal formulation has an excellent property to entrap both hydrophilic as well as hydrophobic drugs. Due to these excellent characteristics liposomes finds applications in tumour targetting, genetic transfer, immunomodulator, topical, cosmetics, in vaccine delivery as well as in industries. This review aims to outline the distinct features of the liposomal formulation, their updated method of preparation, and various aspects of liposomal drug delivery systems.*

Keywords: Liposomes, novel drug delivery, sustained and targeted drug delivery

1. Introduction

The liposomes were concentric circular vesicles is derived from two Greek words “lipos” meaning fat and “soma” means the body. Liposome was first developed by Bangham et al in 1961, it was an accidental discovery they distributed phosphatidyl choline molecule in water, during this time he discovered that the molecule formed a closed bilayer building with an aqueous section enclosed by lipid bilayer. Liposome is very helpful as it act as a carrier of various drugs, having a potential therapeutic action or other properties. Liposomes are colloidal carriers, with a size range of 0.01-5.0µm in width. Liposome-induced drug has high therapeutic effect of as the release of medicament must be first from the liposome before metabolism and extraction. It is a small artificial vesicle with a spherical shape that can be developed with cholesterol and phospholipids. Due to their size and hydrophobic as well as hydrophilic character (without compliance) liposomes are promising drug delivery systems. The unique ability of liposomes to bind both types of drugs i.e. aqueous and lipid phase makes them attractive hydrophilic and hydrophobic drug delivery systems.

Liposomes are novel drug delivery systems that deliver the drug at the site of action directly. They have the ability to encapsulate both hydrophilic and lipophilic compounds to protect the drug from damage from the external environment and deliver the active medicament in a controlled manner. Glycerol has been found to be the core of the molecule hence the phospholipid containing glycerol have been an important part of liposomal formation.

2. Structure of the Liposomes

The liposomes are composed of:

1) Phospholipids

Phospholipids are key components of the composition of liposome. Common phospholipids used in Liposomal

preparation are Phosphatidylcholine (PC). Phosphatidylcholine is an amphipathic molecule that contains –

- Hydrophilic polar head group, phosphocholine
- Glycerol bridge
- Hydrophobic acyl hydrocarbon chains

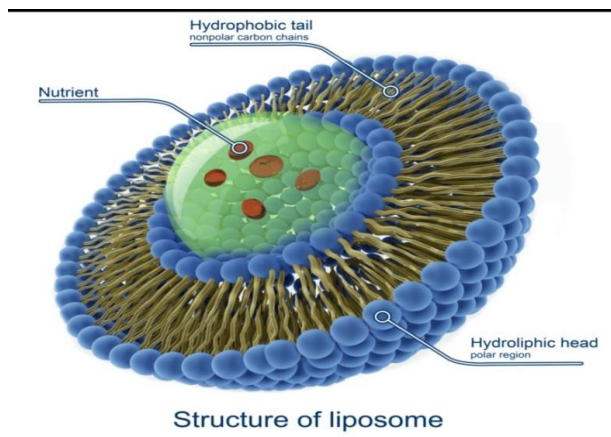
The stability of the liposome membrane depends on the packaging of hydrocarbon chains of lipid molecules. Type of fatty acid in the lipid molecule, such as number of double bond in chains, is responsible for bilayer properties such as elasticity and phase behaviour. Phospholipids are very natural and contains choline which is used for the preparation of liposomes.

Examples of phospholipids are:

- Phosphatidyl choline (Lecithin)
- Phosphatidyl ethanolamine (Cephalin) –PE
- Phosphatidyl serine (PS)
- Phosphatidyl Glycerol (PG)

2) Cholesterol

Cholesterol is one of the most important components in the formation of liposome. It is the most widely used sterol. Addition of sterols does the job of firmness and firmness. Cholesterol itself does not form a bilayered structure but can do so if it is incorporated into phospholipids membrane at very high concentrations along with PC (1:1 or 2:1 molar ratio). The presence of cholesterol in the lipid bilayer improves stability and the most ordered form and severity of disease. Cholesterol decreases the penetration of soluble water molecules also promotes fluidity and stability membrane biology.



3. Methods of Liposome Preparation

General methods of preparation

The general method of preparation involves the following four stages:

- 1) Drying down lipids from organic solvent.
- 2) Dispersing the lipid in aqueous media.
- 3) Purifying the resultant liposome.
- 4) Analysis of the final product.

Method of liposome preparation and drug loading

Liposome preparation involves the following methods:

- a) Passive loading technique
- b) Active loading technique

Passive loading techniques include three different methods:

- 1) Mechanical dispersion method.
- 2) Solvent dispersion method.
- 3) Detergent removal method (removal of non-encapsulated material).

Mechanical dispersion method

This method further involves following types:

- Sonication
- French pressure cell: extrusion
- Freeze thawed liposomes
- Lipid film hydration by hand shaking, non hand shaking
- Microemulsification
- Membrane extrusion
- Dried reconstituted vesicles

Sonication

SUV liposomes are most commonly prepared by this method. Here, MLVs has been sonicated either with a bath type sonicator or a probe sonicator. SUVs of minimal size are produced by this method. The production of the SUVs by this method is done by further two types of sonicators:

- a) **Probe sonication:** The tip of the sonicator is directly immersed into the liposome dispersion. The production by this method is very fast. It uses very high energy resulting in thermal rise of the tip so it must be dipped into water/ice bath.
- b) **Bath sonication:** The liposome dispersion in a cylinder is placed into a bath sonicator. Controlling the temperature of the lipid dispersion is usually easier in this method, in contrast to sonication by dispersal directly using the tip. The material being sonicated can be protected in a sterile vessel, dissimilar the probe units, or under an inert atmosphere.

French pressure cell: extrusion

French pressure cell involves the extrusion of MLV through a narrow orifice under high pressure. An important feature of the French press vesicle method is that the proteins do not seem to be significantly pretentious during the procedure as they are in sonication. An interesting comment is that French press vesicle appears to recall entrapped solutes significantly longer than SUVs do, produced by sonication or detergent removal.

The method involves gentle handling of unstable materials. The method has several advantages over sonication method.

Advantages of liposome

- The encapsulation of the drug provides protection from the external environment.
- They provide site specific delivery and release the drug in controlled manner.
- Reduced toxicity of the drug.
- Liposomes help reduce the exposure of sensitive tissues to toxic drugs
- Target site delivery with sustained release can be obtained.
- Liposomes are flexible, non-toxic, biocompatible, completely biodegradable, and non-immunogenic for systemic and non-systemic administrations
- Active targeting can be achieved by the site specific delivery.
- Suitable for incorporation of hydrophobic as well as hydrophilic drugs.
- Can be made into variety of sizes.

Disadvantages of liposome

- Sometimes leakage and fusion of incorporated drug
- Less stability
- Less soluble
- Production cost is high
- Short half life
- Sometimes phospholipids undergoes oxidation and hydrolysis-like reaction

Classification of liposomes

The liposomes are classified on the following basis:

- 1) Based on structural parameters

Types	Size range
a) Multilamellar large vesicles(MLV)	>0.5 um
b) Oligolamellar vesicles(OLV)	0.1-1 um
c) Small unilamellar vesicles(SUV)	20-100
d) Unilamellar vesicles(UV)	All size ranges

- 2) Based on method of preparation

- Single or oligolamellar vesicles made by reverse phase evaporation method
- Multilamellar vesicles made by REV(MLV-REV)
- Stable plurilamellar vesicles (SPLV)
- Frozen and thawed MLV(FAT-MLV)
- Vesicles prepared by extrusion technique(VET)
- Dehydration rehydration method(DRV)

The resulting liposomes are rather larger than sonicated SUVs. The drawbacks of the method are that the high temperature is difficult to attain, and the working volumes are comparatively small (about 50 mL as the maximum).

Freeze-thawed liposomes

SUVs are rapidly frozen and thawed slowly. The short-lived sonication disperses aggregated materials to LUV. The creation of unilamellar vesicles is as a result of the fusion of SUV throughout the processes of freezing and thawing. This type of synthesis is strongly inhibited by increasing the phospholipid concentration and by increasing the ionic strength of the medium.

Solvent dispersion method

Ether injection method

A solution of lipids dissolved in diethyl ether or ether-methanol mixture is gradually injected to an aqueous solution of the material to be encapsulated at 55°C to 65°C or under reduced pressure. The consequent removal of ether under vacuum leads to the creation of liposomes. The main disadvantages of the technique are that the population is heterogeneous and the exposure of compounds to be encapsulated to organic solvents at high temperature.

Ethanol injection method

A lipid solution of ethanol is rapidly injected to a huge excess of buffer. The MLVs are formed. The disadvantages of the method are that the population is heterogeneous (30 to 110 nm), liposomes are very dilute, the removal all ethanol is difficult because it forms into azeotrope with water, and the probability of the various biologically active macromolecules to inactivate in the presence of even low amounts of ethanol is high.

Reverse phase evaporation method

This method provided a progress in liposome technology, since it allowed for the first time the preparation of liposomes with a high aqueous space-to-lipid ratio and a capability to entrap a large percentage of the aqueous material presented. Reverse-phase evaporation is based on the creation of inverted micelles. These inverted micelles are shaped upon sonication of a mixture of a buffered aqueous phase, which contains the water-soluble molecules to be encapsulated into the liposomes and an organic phase in which the amphiphilic molecules are solubilized. The slow elimination of the organic solvent leads to the conversion of these inverted micelles into viscous state and gel form. At a critical point in this process, the gel state collapses, and some of the inverted micelles were disturbed. The excess of phospholipids in the environment donates to the formation of a complete bilayer around the residual micelles, which results in the creation of liposomes. Liposomes made by reverse phase evaporation method can be made from numerous lipid formulations and have aqueous volume-to-lipid ratios that are four times higher than hand-shaken liposomes or multilamellar liposomes.

Briefly, first, the water-in-oil emulsion is shaped by brief sonication of a two-phase system, containing phospholipids in organic solvent such as isopropyl ether or diethyl ether or

a mixture of isopropyl ether and chloroform with aqueous buffer. The organic solvents are detached under reduced pressure, resulting in the creation of a viscous gel. The liposomes are shaped when residual solvent is detached during continued rotary evaporation under reduced pressure. With this method, high encapsulation efficiency up to 65% can be obtained in a medium of low ionic strength for example 0.01 M NaCl. The method has been used to encapsulate small, large, and macromolecules. The main drawback of the technique is the contact of the materials to be encapsulated to organic solvents and to brief periods of sonication. These conditions may possibly result in the breakage of DNA strands or the denaturation of some proteins. Modified reverse phase evaporation method was presented by Handa et al., and the main benefit of the method is that the liposomes had high encapsulation efficiency (about 82%).

Detergent removal method

Gel-permeation chromatography

In this method, the detergent is depleted by size special chromatography. The liposomes do not penetrate into the pores of the beads packed in a column. They percolate through the inter-bead spaces. At slow flow rates, the separation of liposomes from detergent monomers is very good. The swollen polysaccharide beads adsorb substantial amounts of amphiphilic lipids; therefore, pre-treatment is necessary. The pre-treatment is done by pre-saturation of the gel filtration column by lipids using empty liposome suspensions.

Evaluation of Liposomes

- **Entrapment efficiency:** the entrapment efficiency can be described as ratio of entrapped drug and total drug. The percent entrapment efficiency can be calculated as entrapped drug divided by total drug multiplied by 100.
- **Zeta potential:** for the determination of zeta potential a Dynamic light scattering instrument is used.
- **Particle size:** Laser light scattering and Transmission electron microscopy (TEM) is used for the particle size as well as particle size distribution.
- **Drug release rate:** In vivo assays are used to determine drug release rate for the pharmacokinetics and bioavailability of the drug.

4. Applications of Liposomes

The liposomes find many applications in the industry as well as in clinics.

Liposomes as drug carriers

These are used as carriers for the various drugs and decrease the toxicity due to the encapsulation of the drug which provides protection from the external environment. They are biodegradable and biocompatible which makes the delivery of the drug very safe.

Liposomes as anticancer agents

The liposomes provide high efficacy of the drug and also show reduced toxicity in the treatment of cancer compared to the conventional treatment methods. The chemotherapeutic therapy provides high toxicity. The

enhanced bioavailability and pharmacokinetic profile is observed by using liposomes as anticancer drugs.

Liposomes in Vaccine delivery

They provide enhanced cellular uptake, controlled release of antigens, improved antigen specific immunity and also protect from degradation which makes them a suitable carrier in vaccine delivery systems.

Liposomes as antimicrobial, antifungal agents

The liposomes have been extensively used as antimicrobial agents as the MLVs provide a large space for the encapsulation of the drug.

Liposomes in gene therapy

Gene therapy provides the transfer of genetic material into cells to alter their functions. Liposome is an excellent vector to deliver the genetic molecules such as DNA for the therapy. For this liposome based vectors are designed to obtain the target site delivery.

Liposomes in immunology

They are used as immunoadjuvant, immunomodulator, and immunodiagnosis.

Liposomes in respiratory disorders

Liposomes are found very effective in respiratory disorders as they possess excellent characteristics of sustained release which increases the therapeutic index and reduced toxicity and provides higher stability.

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