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Research paper

LIPOSOMES AS DRUG CARRIER FOR NOVEL DRUG DELIVERY SYSTEM

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A liposome is a tiny bubble (vesicle), made out ofthe same material as a cell membrane. Liposomes can be filled with drugs, and used to deliver drugs for cancer and other diseases. An artificial microscopic vesicle consisting of an aqueous core enclosed in one or more phospholipid layers, used to convey vaccines, drugs, enzymes, or other substances to target cells or organs. Liposomes characterize an advanced technology to deliver active molecules to the site of action, and at present, several formulations are in clinical use. Research on liposome technology has progressed from conventional vesicles to 'second-generation liposomes', in which long-circulating liposomes are obtained by modulating the lipid composition, size, and charge of the vesicle. Liposomes with modified surfaces have also been developed using several molecules, such as glycolipids or sialic acid. This review discusses the classification, formulation, characterization and potential applications of liposomes in drug delivery.

KEYWORDS: Liposomes, phospholipids, formulation, characterization

INTRODUCTION

The name liposome is derived from two Greek words 'Lipos', which means fat and 'Soma', which means body. A liposome is usually a tiny bubble which is made of the same material as a cell membrane. Drugs can be filled into the liposomes, and hence these liposomes can be used for cancer and the other diseases by delivering those drugs.[1]-Simply it can be said that liposomes are microscopic bubbles in which an aqueous volume is entirely enclosed by a membrane. This membrane is composed of lipidmolecule. Liposomes are formed by using various amphipathic molecules. The drug molecules can either be intercalated into the lipid bilayer or they can be encapsulated in aqueous space. Usually membranes are made of the phospholipids.[2] These phospholipids are the molecules which contain a hydrophobic tail group and a hydrophilic head group. The head is attracted to water, and the tail is repelled by water because the tails is made of a long hydrocarbon chain. Phospholipids can easily get integrated with the skin lipids as the main components of liposomes.[3]The stability of liposomes can be increased by the cholesterol which are incorporated in the lipid membrane. Also, the permeability of the membrane can be reduced with the help of that cholesterol. The fluidity of the bilayer is decreased or the micro viscosity is increased because of the characteristics of the cholesterol. Pure surfactant components like DOPE (dioleoyl phosphatidyl-ethanolamine) or naturally-derived phospholipids with mixed lipid chains are the key components to compose the liposomes.[4]

Advantages of Liposome[1-6]

1. Flexibility to couple with site-specific ligands to

2. achieve active targeting.
3. Biodegradability
4. Liposome is increased stability via encapsulation.
5. Liposome are increased efficacy and therapeutic index of drug

Disadvantages of Liposome[1-6]

1. Short half-life, Low solubility.
2. Leakage and fusion of encapsulated drug /molecules.
3. Production cost is high.
4. Low solubility

Structural parameter of liposomes

The major structural components of liposomes include:

Phospholipids:

A phosphate group is the head of the phospholipids, whereas, two fatty acids are the tail of the phospholipids. The phosphate group contains a phosphorous molecule and 4 oxygen molecules. On the other hand, the fatty acids are the long chains which are usually made of carbon and hydrogen.[2]These two components of the phospholipid are connected by glycerol, which is the third molecule. Cell membrane can be formed by the phospholipids because of the reason that phosphate group head is hydrophilic (water-loving), and the fatty acids tail is hydrophobic (water-hating). Because of these properties, those two components automatically get arranged in a way that they create a certain pattern, and hence the cell membranes are formed.[6]The phospholipids get lined up in such a way that their tails are inside, and their heads are outside. In the same way, the second layer of the phospholipids get arranged in a way that their heads face the inside of the cell, and their tails face away. Hence, a double layer is formed in which the phosphate group heads be on the outside, and fatty acid tails be on the inside. This double layer is known as a lipid bilayer through which the main part of the cell membrane is formed.[7]

Furthermore, the Glycerol containing phospholipids are the most common used components of liposome formulation, and they represent greater than 50% of weight of the lipids in biological membranes. These are derived from Phosphatidic acid. [8]

Below are a few examples of phospholipids:

1. Phosphatidyl choline
2. Phosphatidyl ethanolamine
3. Phosphatidyl serine
4. Phosphatidyl inositol
5. Phosphatidyl Glycerol

Cholesterol: Cholesterol dose not by itself form bilayer structure, but can be incorporated into phospholipid membranes in very high concentration up to 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.[12,13]The high solubility of cholesterol in phospholipid liposome has been attributed to both hydrophobic and specific head group interaction, but there is no unequivocal evidence for the arrangement of cholesterol in the bilayer.[9]

Classification of liposomes:

The liposome size can vary from very small (0.025 µm) to large (2.5 µm) vesicles. Moreover, liposomes may have one or bilayer membranes. The vesicle size is an acute parameter in determining the circulation half-life of liposomes, and both size and number of bilayers affect the amount of drug encapsulation in the liposomes. [9]On the basis of their size and number of bilayers, liposomes can also be classified into one of two categories:

Multilamellar vesicles (MLV) and (2) Unilamellar vesicles. Unilamellar vesicles can also be classified into two categories:

1. large unilamellar vesicles (LUV)
2. Small unilamellar vesicles (SUV).

In unilamellar liposomes, the vesicle has a single phospholipid bilayer sphere enclosing the aqueous solution. In multilamellar liposomes, vesicles have an onion structure. Classically, several unilamellar vesicles will form on the inside of the other with smaller size, making a multilamellar structure of concentric phospholipid spheres separated by layers of water. [4,10]

General method of liposomes

All the methods of preparing the liposomes involve four basic stages:

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

Method of Liposome Preparation and Drug Loading[1,4,10]

Various method used for the preparation of liposome: -

1. Passive loading techniques
2. 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

a. Mechanical dispersion method

- Lipid film hydration by hand shaking, non-handshaking or freeze drying
- Sonication
- French pressure cell
- Freeze-thawed liposomes

b. Solvent dispersion method

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

c. Detergent removal method

- Detergent (cholate, alkylglycoside, Triton X-100) removal form mixed micelles
- Dialysis
- Dilution

Lipid film hydration by hand shaking, non-hand shaking or freeze drying

When preparing liposomes with mixed lipid composition, the lipids must first be dissolved andmixed in an organic solvent to assure a homogeneous mixture of lipids. Usually this process is carried outusing chloroform or chloroform: methanol mixtures. The intent is to obtain a

clear lipid solution for the lipid solution is transferred to containers and frozen by placing the containers on a block of dry ice or swirling the container in a dry ice-acetone or alcohol (ethanol or methanol) bath. The thickness of the lipid cake should not be more than the diameter of the container being used for lyophilization.[3]

Dry lipid films or cakes can be removed from the vacuum pump, the container should be closed tightly and taped, and stored frozen until ready to hydrate. Two membranes fall into an energy well where they adhere and form aggregates. The aggregates settle out of solution as large flocculates which will disperse on agitation but reform upon sitting.[11]

The product of hydration is a large, multilamellar vesicle (LMV) analogous in structure to an onion, with each lipid bilayer separated by a water layer. The spacing between lipid layers is dictated by composition with poly-hydrating layers being closer together than highly charged layers which separates on electrostatic repulsion. Once a stable, hydrated LMV suspension has been produced, the particles can be downsized by a variety of techniques, including sonication or extrusion.[12]

Sonication

Sonication is perhaps the most extensively used method for the preparation of SUV. Here, MLVs are sonicated either with a bath type sonicator or a probe sonicator under a passive atmosphere. The main disadvantages of this method are very low internal volume/encapsulation efficacy, possible degradation of phospholipids and compounds to be encapsulated, elimination of large molecules, metal pollution from probe tip, and presence of MLV along with SUV.[1,3,12]

There are two sonication techniques:

a. Probe sonication. The tip of a sonicator is directly engrossed into the liposome dispersion. The energy input into lipid dispersion is very high in this method. The coupling of energy at the tip results in local hotness; therefore, the vessel must be engrossed into a water/ice bath. Throughout the sonication up to 1 h, more than 5% of the lipids can be de-esterified. Also, with the probe sonicator, titanium will slough off and pollute the solution.[11]
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.[12]

French pressure cell: extrusion

French pressure cell involves the extrusion of MLV through a small orifice. 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.[2,12]

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

Freeze-thawed liposomes

The method of freezing and thawing is introduced for increasing the trapped volume of liposomal preparations. The freeze-thaw method is dependent on the ionic strength of the medium and the phospholipid concentration. It influences to a physical disruption of lamellar structure leading to formation of unilamellar vesicles. The unilamellar vesicles are rapidly frozen followed by slow thawing while the freeze and thawing cycles are repeated.[4]The preparation of MLV propranolol liposomes by freeze-thaw method is described in the literature. The liposomal propranolol formulation is prepared by using distearoylphosphatidyl choline and dimyristoylphosphatidyl choline as phospholipids in phosphate buffered saline buffer, followed by six freeze-thaw cycles.[13]

Solvent dispersion method

Ether Injection Method

A solution of lipids dissolved in diethyl ether or ether/methanol mixture is slowly injected to an aqueous solution of the material to be encapsulated at 55-65°C or under reduced pressure. The subsequent removal of ether under vacuum leads to the formation of liposomes. The main drawbacks of the method are population is heterogeneous (70-190 nm) and the exposure of compounds to be encapsulated to organic solvents or high temperature.[12,15]

Ethanol injection method

A lipid solution of ethanol is rapidly injected to a huge excess of buffer. The MLVs are at once formed. The disadvantages of the method are that the population is heterogeneous (30 to 110 nm), liposomes are very dilute,[15] 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.[16,17]

Reverse phase evaporation method

First water in oil emulsion is formed by brief sonication of a two-phase system containing phospholipids in organic solvent (diethyl ether or isopropyl ether or mixture of isopropyl ether and chloroform) and aqueous buffer. The organic solvents are removed under reduced pressure, resulting in the formation of a viscous gel. The liposomes are formed when residual solvent is removed by 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 0.01M NaCl. The method has been used to encapsulate small and large macromolecules. The main disadvantage of the method is the exposure of the materials to be encapsulated to organic solvents and to brief periods of sonication.[18]

Detergent removal method

Detergent absorption is attained by shaking mixed micelle solution with beaded organic polystyrene adsorbers such as XAD-2 beads and Bio-beads SM2. The great benefit of using detergent adsorbers is that they can eliminate

detergents with a very low CMC, which are not entirely depleted.[2,18]

Dilution

Upon dilution of aqueous mixed micellar solution of detergent and phospholipids with buffer, the micellar size and the polydispersity increase fundamentally, and as the system is diluted beyond the mixed micellar phase boundary, a spontaneous transition from polydispersed micelles to vesicles occurs.[4,14]

Dialysis

The detergents at their critical micelle concentrations (CMC) have been used to solubilize lipids. As the detergent is detached, the micelles become increasingly better-off in phospholipid and lastly combine to form LUVs. The detergents were removed by dialysis. A commercial device called LipoPrep, which is a version of dialysis system, is obtainable for the elimination of detergents. The dialysis can be performed in dialysis bags engrossed in large detergent free buffers.[9]

Active loading technique

Industrial Production of Liposomes

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.[6,10]

Detergent dialysis

The production capacity at higher lipid concentration (80 mg/ml) is 30 ml liposomes/minute. But when lipid concentration is 10-20 mg/ml then up to many liters of liposomes can be produced.[1]

Micro-fluidization

A method based on micro-fluidization i.e. micro-emulsification is used for the large-scale manufacture of liposomes. The preparation of antibiotic liposomes by thin-layer hydration method followed by sonication with a bath-type sonicator and micro-fluidization. The process of micro-fluidization is reproducible and yield liposomes with good aqueous phase encapsulation.[10]

Characterization of liposomes

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.[3]

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, 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

(phase contrast) can detect liposome> 0.3 µm and contamination with larger particles.[7]

2. Size distribution

When liposomes are intended for inhalation or parenteral administration, the size distribution is of primary consideration, since it influences the in vivo fate of liposomes along with the encapsulated drug molecules. Various techniques of determine the size of the vesicles include microscopy (optical microscopy, negative stain transmission electron microscopy cryo-transmission electron microscopy, freeze fracture electron microscopy and scanning electron microscopy, diffraction and scattering techniques (laser light scattering and photon correlation spectroscopy) and hydrodynamic techniques (field flow fractionation, gel permeation and ultracentrifugation).[9]

3. 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.[1,6]

4. High Performance Liquid Chromatography (HPLC)

The HPLC method has been widely applied to the determination of drugs in liposome formulation. Some of these methods involve the analysis of encapsulated drug in liposome or drugs encapsulated in liposomes in biological samples such as plasma3 and liver. Solid-phase extraction (SPE) is of great interest in the separation of liposomal and non-liposomal drug forms. Separation is based on the property of liposomes to cross reversed-phase C18 silica gel cartridges without being retained, while a non-liposomal drug is retained on the stationary phase which are subsequently determined by high-pressure liquid chromatography (HPLC) or electrophoresis.[10,22]

5. In Vitro Drug Release Study

In vitro release studies are usually based on dialysis method. These studies are developed in validated diffusion cell across either Cellophane or dialysis membrane. An aliquot of prepared formulation is placed in a dialysis membrane at the temperature of 37 ºC and the sample is assayed for drug realizing by UV spectrophotometer. The receptor medium consists of acetate buffer6 at pH 5, citrate-phosphate buffer, phosphate buffer7 at pH 7.4 and the donor medium consists of liposomal formulation. Drug released in the case of using dialysis membrane is monitored. for 30 min or 8 hours. The diffusion studies are carried by using Franz diffusion cell also.[3,9,12]

6. Stability

During the development of liposomal drug products, the stability of the developed formulation is of major consideration. The therapeutic activity of the drug is governed by the stability of the liposomes right from the manufacturing steps to storage to delivery. A stable dosage forms is the one which maintains the physical stability and chemical integrity of the active molecule during its developmental procedure and storage. A well-

designed stability study includes the evaluation of its physical, chemical and microbial parameters along with the assurance of product's integrity throughout its storage period. Hence a stability protocol is essential to study the physical and chemical integrity of the drug product in its storage.[9,20]

Physical stability

Liposomes are bilayer vesicles that are formed when phospholipids are hydrated in water. The vesicles obtained during this process are of different sizes.[23] During its storage, the vesicles tend to aggregate and increase in size to attain thermodynamically favourable state. During storage, drug leakage from the vesicles can occur due to fusion and breaking of vesicles, which deteriorates the physical stability of the liposomal drug product. Hence morphology, size and size distribution of the vesicles are important parameters to assess the physical stability. In order to monitor this, a variety of techniques like light scattering and electron microscopy can be used to estimate the visual appearance (morphology) and size of the vesicles.[6,21]

Therapeutic Application of Liposome[1,3,6,14,19,24]

- Liposome as drug/protein delivery vehicles
- Controlled and sustained drug release
- Enhanced drug solubilization
- Altered pharmacokinetics and bio distribution

2. Liposome in antimicrobial, antifungal and antiviral therapy

- 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

6. Liposome as artificial blood surrogates

7. Liposome as radiopharmaceutical and radio diagnostic carriers

8. Liposome in cosmetics and dermatology

CONCLUSION

Liposomes are one of the classical specific drug delivery system, which can be of potential use in controlling and targeting drug delivery. These systems can be administered through oral, parenteral as well as topical route. Nowadays liposomes are used as versatile carriers for targeted delivery of drug. The success of liposomes as drug carriers has been reflected in a number of liposome-based formulations due to delivering of higher drug concentration. The new formulation offers safety over the existing formulation. The success of liposomes as drug carriers has been reflected in a number of liposome-based formulations due to delivering of higher drug concentration. Although their potential applications require specific condition for their preparation, as well as sterility, they are promising candidates in the drug delivery because of the ability of targeting to specific cells

or tissues, increasing efficacy and therapeutic index and reducing the toxicity of the encapsulated drugs. liposomal approach can be successfully utilized to improve the pharmacokinetics and therapeutic efficacy, simultaneously reducing the toxicity of various highly potent drugs.

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