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A REVIEW ON LIPOSOMAL DELIVERY SYSTEMS: CONCEPT TO CURRENT APPLICATIONS

Jyotsnarani Gandham, Praveen Sivadasu* and Padmalatha Katamaneni

Vijaya Institute of Pharmaceutical Science for Women, Enikepadu, Vijayawada-521 108 Krishna District Andhra Pradesh, India.

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***Corresponding Author**
Dr. Praveen Sivadasu
Vijaya Institute of Pharmaceutical Science for Women, Enikepadu, Vijayawada-521 108 Krishna District Andhra Pradesh, India.

ABSTRACT

Liposomes have been established as a promising novel drug delivery system with real-time clinical applications. The term liposomes mean lipid body. It has been derived on basis of the name subcellular particles, ribosomes. These bilayer vesicles made up of phospholipids have perceived many advancements in recent times since the first breakthrough by A.D.Bangham in the early 1960s. Their size ranges from 25 to 500nm. 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 liposome's', in which long-circulating liposomes are obtained by modulating the lipid composition, size, and charge of the vesicle. They may act as a solubilizing model for poorly soluble drugs and enhance the penetration of these loaded drugs. Liposomes can encapsulate both hydrophilic and hydrophobic materials and are utilized as drug carriers in drug delivery. This momentous contribution of liposomal technology is widely observed in the healthcare sector. The present review emphasizes on overall methods of preparation characterization and applicability of liposomes in targeted drug action.

KEYWORDS: Liposomes; Drug carriers; Targeted action; Permeation enhancement; Bio-medical application.

INTRODUCTION

Liposomes are microscopic spheres with an aqueous core surrounded by one or more outer shell(s) consisting of lipids arranged in a bilayer configuration. The potential use of

liposomes as drug carriers was recognized more than 40 years ago and, since that time, liposomes have been used in a broad range of pharmaceutical applications. Liposomes are considered as superior carriers due to their capability to encapsulate both hydrophilic and lipophilic molecules.[1] Liposome properties alter with changes in lipid composition, surface charge, size, and method of preparation. Further, the rigidity of a liposome entirely depends on the type of lipid and its surface charge. For example, natural sources like egg give enhanced permeation and less stable bilayers whereas saturated phospholipids form a stable bilayer with decreased permeation.[2-4] The Structure of a typical liposome is depicted in Fig. 1.

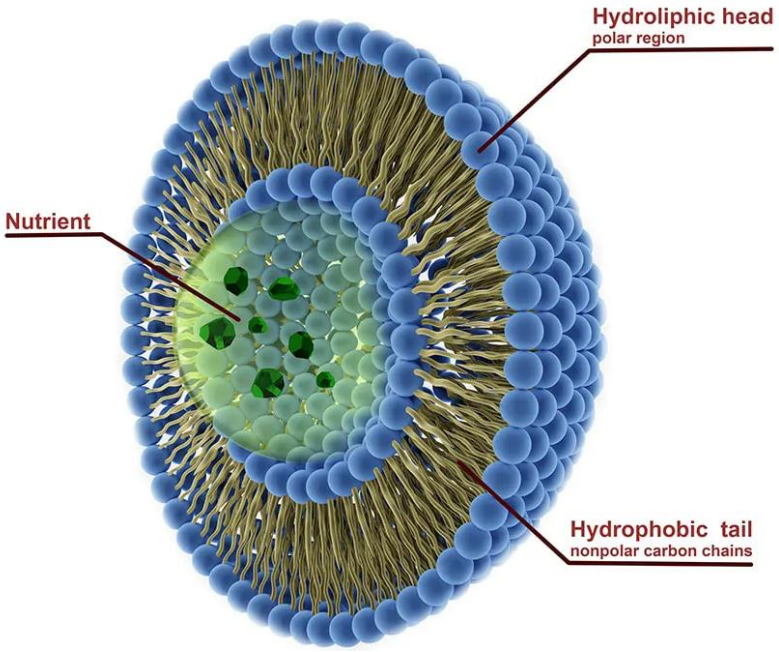

Fig. 1: Structure of liposome.

Liposomes are also found to have important medical, cosmetic, and industrial applications. For an instance in the food industries liposomes are used to encapsulate unstable components like antioxidants, antimicrobials. Further, due to their various advantages like biocompatibility, biodegradability, and ability to load both hydrophilic and hydrophobic drugs liposomes can be used as a commercial drug delivery system.[5-7] The benefits of loading drugs into liposomes are presented in Table 1.

Table 1: Benefits of loading drug into liposome.[8]

Sl. No	Benefits of loading drug into a liposome	Examples
1.	Improves solubility of hydrophobic drugs	Amphotericin B, Doxorubicin
2.	Passive targeting to the cells	Immunodilatorss, Vaccines
3.	As a sustained release systems	Cortisones, Vasopresin
4.	Site-avoidance mechanism	Amphotericin B, Doxorubicin
5.	Site-specific targeting	Anti-cancer and anti-infection drugs
6.	Improved transfer of charged molecules	Antibiotics and chelators
7.	Improved penetration into tissues	Corticosteroids and anesthetics

Liposomal encapsulation technology (LET) is a new technique that has been developed in recent times by scientists to transport the loaded drugs to the targeted site in the body. Liposomes formulated using this technology form a barrier around the components loaded and protects them from enzymes, digestive juices, alkaline solutions, bile salts, and intestinal flora which are generated in the human body. Further, this protective phospholipid barrier remains intact until the liposome reaches the targeted site in the body where the contents have to be released.[9]

The main aim of any cure by using a drug is to enhance the therapeutic index with minimized adverse effects. The traditional approach of drug delivery is restricted by their incompatibility to deliver the drugs to the targeted organ or by causing adverse effects. Different alternative approaches are developed to overcome these limitations among which liposomes were extensively studied. Their attractiveness lies in composition wherein aqueous core is entrapped by one or more bilayers which are of natural or synthetic origin. Further, drugs with different polarities can be encapsulated into the bilayers by effortlessly partitioning between the lipid and aqueous phases.[10-12]

The present review will briefly explain various characteristics of liposomes with a special emphasis on formulation techniques, evaluations, biomedical applications, and recent advancements in the field of application.

***Advantages*[13]**

- Liposomes are biodegradable, biocompatible, and non-toxic.
- Liposomes are suitable for the delivery of hydrophilic, hydrophobic drugs.
- Reduce exposure of sensitive tissues to toxic drugs.
- Protect the encapsulated drug from the external environment.
- Reduced toxicity and increased stability.

Disadvantages[13]

- Production cost is high.
- Short half-life.
- Leakage and fusion of encapsulated drugs.
- Sometimes phospholipids undergo oxidation and hydrolysis like reactions.
- Leakage and fusion.

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. Based on their size and number of bilayers, liposomes can also be classified into one of two categories: (1) multi-lamellar vesicles (MLV) and (2) uni-lamellar vesicles. Uni-lamellar vesicles can also be classified into two categories: (1) large uni-lamellar vesicles (LUV) and (2) small uni-lamellar vesicles (SUV) in uni-lamellar liposomes, the vesicle has a single phospholipid bilayer sphere enclosing the aqueous solution. In multi-lamellar liposomes, vesicles have an onion structure. Classically, several uni-lamellar vesicles will form on the inside of the other with a smaller size, making a multi-lamellar structure of concentric phospholipid spheres separated by layers of water.[14, 15]

METHODS OF LIPOSOME PREPARATION

General methods of preparation

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

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

Method of liposome preparation and drug loading

The following methods are used for the preparation of liposome:

A) Passive loading techniques

B) Active loading technique.

A) Passive loading techniques include three different methods:

1. Mechanical dispersion method.

2. Solvent dispersion method.

3. Detergent removal method (removal of non-encapsulated material).

1. Mechanical dispersion method

The following are types of mechanical dispersion methods:

1.1. Sonication.

1.2. French pressure cell: extrusion.

1.3. Freeze-thawed liposomes.

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

1.5. Micro-emulsification.

1.6. Membrane extrusion.

1.7. Dried reconstituted vesicles.[16]

Sonication

Sonication of LMV dispersion is accomplished by placing a test tube containing the suspension in a bath sonicator (or placing the tip of the sonicator in the test tube) and sonicated for 5-10 minutes above the melting point of the lipid. The lipid suspension should begin to clarify to yield a slightly hazy transparent solution. The haze is due to light scattering induced by residual large particles remaining in the suspension. These particles can be removed by centrifugation to yield a clear suspension of SUV. Mean size and distribution is influenced by composition and concentration, temperature, sonication time and power, volume, and sonicator tuning. Since it is nearly impossible to reproduce the conditions of sonication, size variation between batches produced at different times is not uncommon. Also, due to the high degree of curvature of these membranes, SUV is inherently unstable and will spontaneously fuse to form larger vesicles when stored below their phase transition temperature.[16]

There are two sonication techniques

a) Probe sonication: The tip of a sonicator is directly immersed in 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.

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.[17]

French pressure cell: extrusion

French pressure cell method involves extrusion of liposomes through a small orifice at high pressure. Further, by applying this method liposomes are formed with fewer structural defects when compared to the sonication method. The disadvantages of this technique are it is difficult to attain high temperature and working samples are comparatively small.[18-20]

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. The encapsulation efficacies from 20% to 30% were obtained.[21-23]

Solvent dispersion methods

Ether injection (solvent vaporization)

A solution of lipids dissolved in diethyl ether or ether-methanol mixture is gradually injected into 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 (70 to 200 nm) and the exposure of compounds to be encapsulated to organic solvents at high temperatures.[24, 25]

Ethanol injection

A lipid solution of ethanol is rapidly injected into a huge excess of the 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, 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.[26]

Reverse phase evaporation method

This method provided 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 a 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.[13]

Detergent removal method (removal of non-encapsulated material)

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.[27, 28]

Detergent (cholate, alkyl glycoside, Triton X-100) removal of mixed micelles (absorption)

Detergent absorption is attained by shaking mixed micelle solution with beaded organic polystyrene adsorbers such as XAD-2 beads (SERVA Electrophoresis GmbH, Heidelberg, Germany) and Bio-beads SM2 (Bio-RadLaboratories, Inc., Hercules, USA). The great benefit of using detergent adsorbers is that they can eliminate detergents with a very low CMC, which are not entirely depleted.[29]

Characterization of Liposomal Formulation

Size distribution

Prepared liposomal batches were monitored for their morphological attributes using an optical microscope. Mean vesicle size and size distribution profile of liposome was determined by using the Malvern particle size analyzer model SM 2000, which follows Mie's

theory of light scattering. Diluted liposome suspension was added to the sample dispersion unit containing stirrer and stirred at 2000 rpm to reduce the interparticle aggregation, and the laser obscuration range was maintained between 10-20%. The average particle size was measured after experimenting triplicate.[29]

Entrapment efficiency

Drug associated with liposome was separated from an un-entrapped drug using the centrifugation method. Liposomes were centrifuged at 20000 rpm for 1 h at a controlled temperature of 4 °C. The supernatant containing un-entrapped drug was withdrawn and measured UV spectrophotometrically against phosphate buffer saline (pH 7.4).[30]

Zeta potential (z) determination

Charge on empty and drug-loaded vesicle surface was determined using Zetasizer 300HSA (Malvern Instruments, Malvern, UK). Analysis time was kept for 60 sec and average zeta potential and charge on the liposome was determined.[31]

Rheological studies

While considering the stable liposome dispersion or any other delivery system they usually need to be incorporated into convenient dosage to obtain formulation with desired semisolid consistency for ease in topical and transdermal application. It is important and controls the flow properties to ensure product quality and effectiveness of the production. It helps in the selection of dermatological formulation that will progress to clinical efficacy. Rheological analysis of liposome was performed using a stress control rheometer (Viscotech Rheometer, Rheological Instruments AB, Lund, Sweden), equipped with stress rheologic basic software, version 5, using cone-plate 387 geometry with a diameter of the cone is 25 mm and a cone angle of 10, operating in the oscillation and static mode. The rheological analysis was performed at room temperature. The following parameters were carried out for rheology measurement.[32, 33]

Oscillation stress sweep

Dynamic oscillation stress sweep was performed to determine the linear viscoelastic region (LVR). LVR is the region where the elastic modulus (G') was independent of applied stress because destruction in the structure of gels occurs at high shear stress. Analysis of viscoelastic material was designed not to destroy the structure so that measurement can provide information about intermolecular and inter-particle forces in the material. This test

gives an idea about the critical stress beyond which the sample may show significant structural changes, and therefore the consequent choice of the stress value to be used in other oscillation tests. The samples were exposed to increasing stress (0.5 to 150 Pa) at a constant frequency of 0.1 Hz. The three main parameters determined in this test were the storage modulus G', loss modulus G" and loss tangent tan! The endpoint of the linear viscoelastic region was determined as stress when the G' value was dropped 10% from the linear level that indicated a significant change in the structure gel samples.[34, 35]

Oscillation frequency sweep

The samples were exposed to stepwise increasing frequency (0.1 to 100 Hz) at constant stress in the field of LVR and elastic moduli (G'), as well as viscous modulus (G"), were recorded against frequency.[36]

Drug content and content uniformity

The liposome sample (100 mg) was withdrawn and drug content was determined using a UV spectrophotometer. Similarly, the content uniformity was determined by analyzing drug concentration in liposome taken from 3 to 4 different points from the container. In the case of liposomal gel, it was shaken with a sufficient quantity of methanol to extract the drug and then analyzed by using a UV spectrophotometer.[37]

In vitro drug release

The mechanism of drug release from the formulated liposomes was evaluated by a well-established dynamic dialysis technique. A typical procedure involves keeping 2 ml of the formulated liposomes in a dialysis membrane with a molecular weight within the range of 8000-14000 Da and the system is immersed in a fixed quantity of selected buffer. Further, the system is maintained at a temperature of 37 °C with continuous magnetic stirring at 100 RPM. A fixed quantity of aliquot was withdrawn and replenished with fresh buffer at pre-determined time intervals and analyzed by either UV spectrophotometer or HPLC and % drug release was calculated.[38]

Stability studies

The ability of vesicles to retain the drug (i.e., drug retentive behaviour) was assessed by keeping the liposomal suspensions and liposomal gel at two different temperature conditions, i.e., 4-8 °C (Refrigerator; RF), 25±2 °C (Room temperature; RT), for 60 days. Samples were withdrawn periodically and analyzed for the drug content and particle size for liposomal

suspension and drug deposition for liposomal gel in the manner described under entrapment efficiency and particle size distribution studies.[39]

BIO-MEDICAL APPLICATIONS

Treatment for Leishmaniasis

Leishmaniasis is a parasitic infection of macrophages which affects over 100 million people in tropical regions and is often deadly. The effectual dose of drugs, mostly different antimonial, is not much lower than the toxic one. Liposomes accumulate in the very same cell population which is infected, and so an ideal drug delivery vehicle was proposed.[40] Further, Kevin J Peine et al have developed liposomes loaded with resiquimod for the treatment of leishmaniasis and the results suggested that treatment with liposomal resiquimod significantly decreased the parasite load in the liver, spleen and bone marrow. Besides, resiquimod treatment increased interferon-γ and interleukin-10 production in an antigen recall assay.[41]

Amphotericin B in treating fungal infections

Liposomes act as carriers for amphotericin B in antifungal therapies. This is the drug of choice in dispersed fungal infections which often in parallel work together with chemotherapy, immune system, or AIDS, and is frequently fatal. Unfortunately, the drug itself is very toxic and its dosage is limited due to its ionosphere and neurotoxicity. These toxicities are normally related to the size of the drug molecule or its complex. Liposome encapsulation inhibits the accumulation of the drug in these organs and radically reduces toxicity.[42]

Doxorubicin in treating cancer

Doxorubicin dosage is limited by its increasing cardiotoxicity. Numerous diverse formulations were tried. In most cases, the toxicity was reduced to about 50%. These include both acute and chronic toxicities because liposome encapsulation reduces the delivery of the drug molecules towards those tissues.[43] Further, Bahareh Sabetiet al have formulated liposomes loaded with doxorubicin, and results suggested that formulated liposomes showed improved cellular uptake with lower IC50.[44]

Gene therapy

Liposomes can also be used to deliver genetic materials to the cells. Rationality is the ability of formulated liposomes to increase the accumulation in cells and facilitate the transfer of these large and heavily charged molecules across the cellular membrane.[45] Further, Joanna

Rejman et al have studied the effect particle size of liposome in delivering the genetic materials and the results suggested that With increasing size, a shift to a mechanism that relied on caveolae-mediated internalization became apparent, which became the predominant pathway of entry for particles of 500 nm in size.[46] Some of the recent research works carried out on liposomes as drug carriers are reported in Table 2.

Table 2: Recent research works that carried out on liposomes as drug carriers.

Drug	Category	Experimental model	Reference
Pilocarpine Nitrate	Miotic agent	*In-vitro* dissolution studies using modified USP XXI dissolution rate model and pharmacokinetic studies in albino rabbits	[47]
Quinoxaline derivative (LSPN331)	Anti-infective agent	*In-vitro* cytotoxicity assay using J774.A1and Vero cells	[48]
Fenofibrate	Antilipemic	*In-vitro* release studies using USP Type- II (Paddle) dissolution apparatus	[49]
Nimesulide	Non-steroidal anti-inflammatory drug (NSAID)	*In-vitro* release studies using diffusion cell across cellophane membrane	[50]
Ketoprofen	Non-steroidal anti-inflammatory drug (NSAID)	*In - vitro* release studies using Dialysis membrane	[51]
Glimepiride	Anti-diabetic	*In - vitro* release studies using Dialysis membrane	[52]
Curcumin	Anti-cancer	*In - vitro* release studies using fresh human plasma and *In-vitro* cytotoxicity assay using MTT cells	[53]

CONCLUSION

Liposomes have some potential advantages over the traditional approach as a drug carrier. First, they are variable concerning size and surface properties, and second, they can act as sustained release depots, releasing encapsulated drugs of half-lives ranging from 0.6 to 11 days. Moreover, a new generation of liposomes, the so-called “collagen modified liposomes” can moderate the liposome- liposome and the liposome-cell interaction due to their collagen surface properties. Liposomes with enhanced targeted drug delivery which increased the residence time in the targeted organ are now getting clinical acceptance. Further, liposomes show reduced toxicities and enhanced efficiency when compared with the traditional approach. However, based on the products available in the market and various

pharmaceutical applications we can establish that liposomes as a potential alternative approach to traditional drug delivery systems.

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CONFLICT OF INTEREST

The author confirms that this article content has no conflict of interest.

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