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Liposomal Drug Delivery System: Method of Preparations and Applications

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Abstract

This article is focused on the various aspect of liposomal formulation, which includes its mechanism of formation, characteristics, and application for various therapeutic purposes. Liposomes are most widely investigated by researchers as a drug carrier, because of its biocompatible and biodegradable characteristics, which make it a non-toxic carrier and a good candidate for drug carriers in the treatment of disease. It is composed of phospholipids bilayer vesicles, which favor the encapsulation of both hydrophobic and hydrophilic drugs. Numerous improvements have been done in the development of a liposomal formulation for the use of drug carriers in the treatment of disease, mainly in cancer therapy, gene transfer, immunomodulation, and topical therapy. The liposomal formulation has been a success as a drug carrier, which is reflected by the commercial products available in markets and underlying clinical trials.

Keywords: Liposomes, Phospholipids, Drug delivery, Therapeutic efficacy

INTRODUCTION

Liposome was first discovered by Alec Douglas Bangham, a British hematologist in 1961 at the Babraham Institute, in Cambridge, England. He published his work in 1964. They were discovered when A.D. Bangham and R.W. Horne was testing a new electron microscope in the institute with a dry phospholipid and gram-negative stain. [1] They found the ‘‘Bag Like’’ arrangement formed automatically, which was names as “multilamellar smectic mesophase” or “Banghasomes” by A.D. Bangham. It was his close colleague Gerald Weissman, who suggested these phospholipid bilayer vesicles to be more users friendly, termed as Liposome. [2]

They found that on the hydration of phospholipid, it results in the formation of phospholipid bilayer vesicles, which resemble the structure of the cell membrane. Later on, it becomes a wide research component by scientists for the drug delivery system because of its biocompatibility and its capability to entrap both hydrophilic and lipophilic drugs. In 1974, Gregoriadis et al. proposed the use of liposomes in chemotherapy, and liposome was considered as a good candidate because of safety, size controllability, and easy functionalization. [3] The study of polyethylene glycol long-circulating liposomes begun in 1990. [4] Because PEG-protein conjugation has improved the half-life of protein, so the researcher has begun to conjugate PEG to liposomes to prolong its half-life. [5] The development of long-circulating PEG-liposomes is a major advantage in drug delivery, especially in cancer therapy. Kim et al. has shown that PEG-liposome has improved efficacy, safety, and in-vivo stability as compared to the conventional delivery system. [6] PEG can help its escape from the reticuloendothelial system and reduce its distribution to different organs of the body. Hence, reducing the toxicity of cytotoxic drugs. Liposomes are gaining their popularity due to their contribution to varied areas like drug delivery, cosmetics, and the structure of the biological membrane. [7]

Liposomes are a term derived from the Greek word: where ‘Lipos’ meaning ‘fats’ and ‘Somas’ meaning ‘body’. A. D. Bangham first described liposome in 1964 with his colleagues. His close colleague Gerald Weissman suggested the term “liposomes”, which he defined as “microscopic vesicles composed of one or more lipid layer”. Liposomes are colloidal particles formed when a phospholipid is hydrated in access to water, resulting in the formation of liposomes of size ranging from 0.01-0.5 µm in diameter. The creation of liposomes has been one of the most important novel drug delivery systems and most studied by researchers because of its biocompatibility and biodegradability. Liposomes have gained lots of interest in advanced drug delivery as a carrier, the advancement of liposomal research is also because they mimic biological membrane which makes them more versatile for study in various fields. [8] They consist of an aqueous core entrapped by one or more phospholipid bilayer composed of natural or synthetic phospholipids. Liposomes, which are composed of a natural phospholipid, are non-toxic, non-immunogenic, and biologically inert. Both hydrophilic and lipophilic drugs can be transported therein. Drug targeting can also be achieved by surface modification, making it more localized to target disease tissue. [9]

Liposomes, due to their biphasic environment, can act as a carrier for both hydrophilic and lipophilic drugs. Highly hydrophilic drugs (log P <-0.3) are located exclusively in an aqueous domain, whereas lipophilic drugs (log P>5) are entrapped within the lipid bilayer of the liposomes. Drug with intermediary partition coefficient, i.e.1.7 (log P<4) imposes a problem for drug loading as they equilibrate between the lipid and aqueous layer and are prone to an appreciable degree of leakage on storage. [10]

Naturally, phospholipids occur in two layers (bilayer) where hydrophilic polar heads are attracted to water inside and outside the membrane, whereas the lipophilic hydrocarbon tail is attracted to each other to form a bilayer, as they are repelled by water to form a surface away from water. The hydrocarbon tail of one layer faces the hydrocarbon tail of another layer to form a bilayer. When the phospholipid bilayer disrupts it forms a sphere

smaller than a normal cell, it may be monolayer or bilayer. The monolayer spheres are micelles and the bilayer sphere is liposomes. Liposomes are composed of naturally derived phospholipids such as Egg phosphatidylethanolamine or pure surfactant component like DOPE (Dioleoyl-phosphatidylethanolamine). [11]

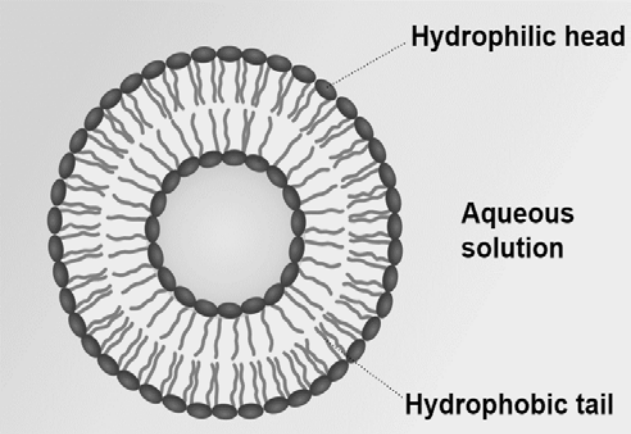

Fig. 1: Structure of liposome

Advantages of liposomes

There are many drugs in the market, which have good therapeutics activities, but they are used in the dearest situation, because of their poor pharmacokinetics and pharmacodynamics activities. Drugs encapsulated in liposomes can be used regularly, as its pharmacokinetics and pharmacodynamics can be controlled. [12] Some of the advantages of the liposome are as follows;

1. Provides selective passive targeting to tumor tissues (Liposomal doxorubicin).
2. Increased efficacy and therapeutic index.
3. Increased stability via encapsulation.
4. Reduction in toxicity of the encapsulated agents.
5. Site avoidance effect.
6. Improved pharmacokinetic effects (reduced elimination, increased circulation lifetimes).
7. Flexibility to couple with site-specific ligands to achieve active targeting.

Disadvantages of liposomes

All drug delivery system has faults, same is the case of liposomes. As liposomes are required to enhance and increase the efficacy of drugs, the cost as well as all the other implications thereof must be taken into account. Cost is an issue when it comes to phospholipid preparation. This preparation is expensive to produce because of the costly raw material and equipment required for preparation. Liposomes are non-toxic but in the case of cationic liposomes, it tends to be toxic at higher concentrations. [13]

Other problems related to liposomes are as following:

1. **Sterilization:** Sterilization of liposomes is a complicated process. Because it is unstable in heat and certain methods of radiation. Sterilizing with chemicals may affect stability problems. The only sterilization method is a membrane filter that is capable to filter liposomes of size <0.2µm. This method does not filter viruses.
2. **Short self-life and stability**: It is very difficult to achieve the stability of liposomal formulation due to chemical and physical degradation. Chemically, they are prone to oxidation and hydrolysis and they can physically fuse forming larger vesicles. It can be prevented by the addition of anti-oxidant such as tocopherol and the addition of cholesterol to avoid fusion.
3. **Entrapment efficacy**: The amount of drug a liposome can entrap is often low and sometimes leakage of drugs takes place.
4. **Removal from circulation by the reticuloendothelial system (RES)**: The major drawback of liposomes as a drug carrier is that they are rapidly cleared by a phagocytic cell of the Mononuclear Phagocytic System (MPS). Larger liposomes are eliminated from circulation faster than smaller liposomes. PEGylation can increase the self-life of liposomes.

TYPES OF LIPOSOMES

Liposomes are classified based on their structural properties, methods of preparation and composition, and application. Their properties such as the size of liposomes, number, the position of lamellae depend widely on the method of preparation, types of lipids used, and preparation condition of liposomes. This parameter, influence the in-vitro and in-vivo characteristics of liposomes. The classification of liposomes based on structural properties is mentioned in Table 1, classifications based on liposomes preparation are mention in Table 2, and based on composition and application are mentioned in Table 3. [14, 15]

Table 1: Based on the structural parameter

MLV	Multilamellar vesicles, >0.5 µm
OLV	Oligolamellar vesicles, 0.1-1 µm
UV	Unilamellar vesicles, all size ranges
SUV	Small Unilamellar vesicles, 20-100 nm
MUV	Medium-sized Unilamellar vesicles.
LUV	Large Unilamellar vesicles, >100 nm
GUV	Giant Unilamellar vesicles, >1 µm
MV	Multivesicular vesicles, >1 µm

Table 2: Based on the method of preparation

REV	Single or Oligolamellar vesicles are made by the reverse-phase evaporation method.
MLV-REV	Multilamellar vesicles are made by a reverse-phase evaporation method.
SPLV	Stable plurilamellar vesicles.
FATMLV	Frozen and Thawed MLV.
VET	Vesicles prepared by the extrusion method.
DRM	Dehydration-rehydration method.

Table 3: Based on composition and application

Conventional liposomes (CL)	Neutral and negatively charged phospholipid and cholesterol.
Fusogenic liposomes	Reconstituted Sendai virus envelopes (RSVE)
pH-sensitive liposomes	Phospholipids such as PE and DOPE with either CHEMS or OA.
Cationic liposomes	A cationic lipid with DOPE.
Long-circulatory(stealth) liposomes (LCL)	Neutral high Tc^{o}, cholesterol and 5-10% of PEG-DSPE or GM1
Immuno-liposomes	CL or LCL with attached monoclonal antibodies or recognition sequences

MECHANISM OF LIPOSOMES FORMATION

A basic understanding of the physicochemical properties of phospholipids is needed to understand the liposome formation. Phospholipids are amphiphilic (having both aqueous and polar moiety affinity), it has two fatty acid chains containing 10-24 carbon atoms and 0-6 double bond in each chain, which are the non-polar tail of phospholipids. The polar end is mainly phosphoric acid bond to the water-soluble molecule when phospholipids are hydrated, they are arranged in such an orientation that the polar portion of the phospholipids remain in contact with the polar environment and at the same time shield the non-polar part. The most common natural polar phospholipids are phosphatidylcholine (PC). [16]

METHOD OF PREPARATION

The conventional method for the preparation of liposomes includes the solubilization of lipids in the organic solvent, drying down the lipids from organic solution, dispersion of lipids in aqueous media, purification of resultant liposomes, and analysis of the final product. [17]

All the method for the preparation of liposomes involves four steps:

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

Techniques used for the preparation of liposomes are described below;

1. **Hand Shaking method**: In this method, the lipid is solubilized in an organic solvent (mainly ethanol) in a round bottom flask with constant shaking in a circular manner, when the organic solvent evaporates, it forms a thin film of lipid on the RBF which on hydrated with purified water, with constant shaking, form a liposome. This method is useful for the preparation of MLV liposomes. Nowadays, a Rotary evaporator machine is used for the formation of lipid film and hydration as it is more reliable than the handshaking method. [18]
2. **Sonication Method**: This is the most widely used method for the preparation of SUV from MLV, prepared from the handshaking method and rotary evaporator method. There are two types of sonication methods used in the preparation of SUVs. [19]

a) **Probe Sonication method**: In this method, the tip of the titanium probe is directly dispersed into liposome dispersion for the production of SUVs. In this method, the energy input is high due to which there is the generation of heat. For controlling heat, liposome dispersion is kept in the ice bath. The main disadvantage of this method is that the titanium fragment is sludge in a solution and contaminate it.

b) **Bath sonication**: In this method, liposome dispersion in a container is placed on the sonication bath. This method is more convenient as compared to probe sonication for the production of SUVs because the temperature can be controlled easily. The sterilized liposome can be obtained, there is no titanium contamination. [20]

3. **French Press method**: In this method, unstable MLVs are converted to SUVs and LUVs bypassing then through a small orifice of equipment. Liposomes produced through this method are more reliable, as it has good stability as compared to those prepared by sonication method. The drawback of this method is that it has a small working volume of a maximum of 50 ml and a high temperature is hard to manage. [21]
4. **Freeze Thawed liposomes**: Here, SUVs formed by the sonication method is frozen and thawed slowly and continuously, resulting in the formation of LUVs due to aggregation of SUVs during the thawing process. By this method, the encapsulation efficacies increase by 20%-30%. [22, 23]
5. **Solvent Dispersion method:**

a) **Ether injection (solvent evaporation)**: In this method, lipid dissolved in a diethyl-ether or ether-methanol mixture is gradually injected in an aqueous medium containing drug at the temperature of 50 to 65 ^{o}c or reduced pressure. The removal of ether under vacuum results formation of liposomes. The main drawback of this technique is the formation of a heterogeneous population of liposomes (70-200 nm) and exposure of liposomes in high temperatures during encapsulation which can hamper the stability of liposomes. [24]

b) **Ethanol injection**: To a buffer a solution of lipid and ethanol is injected, resulting in the formation of MLVs. The drawback is the formation of a heterogeneous population of liposomes (30-110 nm). It is also difficult to remove ethanol from a solution consequently increasing the chances for the inactivation of biologically active macromolecules. [25]

c) **Reverse Phase evaporation method**: This method has brought a breakthrough in the history of liposomes. The aqueous and lipid ratio used in this method is high, about four times higher than the handshaking method or MLVs. This method is based on the formation of reverse micelle where an aqueous medium is sonicated. The aqueous medium contains a water-soluble molecule to be encapsulated, lipids, and an organic phase. The slow elimination of organic solvent results in the formation of a gel-like consistency. At a critical

point, the gel-like structure collapses to form liposomes. [26]

6. **Detergent removal method (removal of non-encapsulated material)**

a) **Dialysis**: In this method, detergent is used to dissolve lipids at Critical Micelles Concentration (CMC). When it is removed by dialysis, using a commercial device such as LipoPrep (Diachema AG, Switzerland) which is a version of the dialysis method. [27]

b) **Detergent (cholate, alkyl glycoside, Triton X-100) removal of mixed micelles (absorption)**: In this method, removal of detergent in achieved by shaking mix micelle with beaded organic polystyrene absorbers such as XAD-2 beads (SERVA Electrophoresis GmbH, Heidelberg, Germany) and Bio-beads SM2 (Bio-Rad Laboratories, Inc., Hercules, USA). [27]

c) **Gel-permeation chromatography**: Sephadex G-50, Sephadex G-l 00 (Sigma-Aldrich, MO, USA), Sepharose 2B-6B, and Sephacryl S200-S1000 (General Electric Company, Tehran, Iran) can be used for gel filtration as packing material for a column. Liposomes cannot penetrate through this packing which makes it easier to obtain liposomes. [27]

The physical and chemical characteristics of liposomes have a direct impact on the properties of a liposome, in-vivo, and in-vitro. The characterization of liposomes should be done immediately after the formation of liposomes by analysis methods (such as GLC, TLC, and HPTLC). Size, number of lamellae, internal morphology charges, and bilayer fluidity play a direct role in in-vivo properties. Sterilizing of liposomes is difficult as it is sensitive to heat. [28] Hence, it has to be prepared aseptically. Various techniques to determine the size, number of lamellae, charges, and bilayer fluidity are listed in Table 4.

Table 4: Techniques for physical characterization of liposomes

Parameters	Techniques
Size	Electron microscopy, Light scattering microscopy, Ultracentrifugation, Coulter counters method.
Number of lamellae	NMR spectroscopy, Small-angle X-ray scattering microscopy.
Bilayer Fluidity	Fluorescence polarization.
Charge	Microelectrophoresis.

EVALUATION OF LIPOSOME

Evaluation is very important to ensure that the formulated liposome has the character required by the formulator. There are various evaluation test mentioned below

1. Drug-excipient interaction study: This test is very important to ensure the compatibility of drugs and excipient in the formulation. This test is performed by the FTIR spectroscopy method. Here drug and excipient are mixed with KBr to form a sample pellet at the ratio of 100:1. In preparation of pellets, a 5.5 metric ton of pressure is required to prepare a pellet. This pellet is scanned at the range of 4000-400 cm^{-1}. [29]

2. **Drug loading study**: Drug loading was determined spectrophotometrically. The drug quantification was confirmed by HPLC. [30]

3. Lipid Quantification and chemical stability: Concentration and purity of phospholipids and cholesterol in the liposomes are determined by the HPLC method or enzymatically through cholesterol oxidase. TLC is also used to determine the hydrolysis of lipids at various stages of liposome preparation and storage. [31]

4. Drug release determination: Drug release study of liposome is determined using the dialysis method. In a 250ml conical flask, 100ml of phosphate-buffered saline was taken.5mg lyophilized sample suspended in 1ml of PBS was taken into a dialysis bag. The bag is tight from both ends with threads and hanged inside a conical flask containing phosphate buffer which is stirred with the help of a magnetic stirrer under controlled temperature. The sample is taken out with the help of a micropipette, which is analyzed at the spectrophotometer at the wavelength of 290 nm. [32]

APPLICATION OF LIPOSOMES AS DRUG DELIVERY SYSTEM

To obtain a desired therapeutic efficacy and safety of drugs, a new drug delivery system has been formulated by a researcher. Liposomes are a new drug delivery system to achieve therapeutic efficacy and safety of drugs. Various application of liposomal formulation is mentioned below:

1. **Site avoidance delivery:** Many drugs are cytotoxic. On exposure of such drugs to a normal cell may cause an adverse effect, due to a low therapeutic index (TI). Such drugs when formulated to liposomes give a therapeutic effect with low toxicity. For example, Doxorubicin causes cardiac toxicity, which subsides on the liposomal formulation. [33]
2. **Site-specific targeting:** Site-specific targeting of drugs can be achieved by liposomal formulation. Here, drugs are encapsulated in a liposomal formulation, to which specific ligands are attached. This ligand attached liposomes are very specific to a targeted cell. Which is very important for achieving a therapeutic effect at a desired site of action. [34]
3. **Intra-cellular drug delivery:** Cytosol delivery of drugs can be achieved by liposomal formulation. N-(phosphonacetyl)-L-aspartate (PALA) is normally poorly taken up into cells. Such drugs when encapsulated within liposomes, showed greater activity against ovarian tumor cell lines in comparison to free drugs. [35]
4. **Sustained release drug delivery:** Drugs can be retained in a system for a prolonged time for the sustain release effect of drugs in a body. Drugs like cytosine Arabinoside can be encapsulated in liposomes for sustained release and optimized drug release rates *in-vivo.* [36]
5. **Reduced toxicity:** Liposome formulation releases drugs for a prolonged period within the therapeutics index. The toxic effects of drugs can be reduced by

50% in the case of liposome formulation. In anticancer drugs like doxorubicin, with cardiac toxicity, it can be reduced by the liposomal formulation of doxorubicin. [37]

Table 5: The various liposomal formulation for commercial use [38]

Drug	Product	Indication
Ambisome™	Amphoteracin B	Fungal infection
DaounoXome™	Daunorubicin	Kaposi's sarcoma
Doxil™	Doxorubicin	Refractory Kaposi's sarcoma, recurrent breast cancer, and ovarian cancer
Visudyne®	Verteporfin	Age-related macular degeneration, pathologic myopia, and ocular histoplasmosis
DepoCyt®	Cytarabine	Neoplastic meningitis and lymphomatous meningitis
Myocet®	Doxorubicin	Recurrent breast cancer
Lipoplatin®	Cisplatin	Epithelial malignancies

CONCLUSION

There are many drugs molecule which is having good pharmacological action but their use are limited due to the toxicity, they posses. Such drugs can be brought into use by reducing its toxicity and enhancing its pharmacological action. Liposome formulation is an appropriate approach to achieve the therapeutic action of such drugs. Liposome composition has made it more reliable as it is inert and resembles a cellular membrane which makes it an interesting field of research for scientists. A liposome is a good carrier of drugs in the treatment of cancer and it is gaining popularity in the field of chemotherapy. Researchers are developing liposomal technology for improving its therapeutics and pharmacokinetics efficacy, at the same time reducing the toxicity of potent drugs.

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Conflict of Interest: None

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