Review Article

# A Review on Liposomes

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**ABSTRACT**

Liposomes, sphere-shaped vesicles consisting of one or more phospholipids bilayers. Different types of pharmaceutical carriers are to deliver drug to particular target cell type. They are particulate, polymeric, and macromolecular. Particulate type carrier also known as colloidal carrier system, includes lipid particles (low and high density lipoprotein-LDL and HDL, respectively), microspheres, nanoparticles, polymeric micelles and the vesicular like liposomes, niosomes, pharmacosomes, ribosomes, etc. Liposomes microscopic phospholipid bubbles with a bilayered membrane structure have received a lot of attention during the past 30 years as pharmaceutical carriers of great potential. Liposomes, which are biodegradable and essentially nontoxic vehicles, can encapsulate both hydrophilic and hydrophobic materials, and are utilized as drug carriers in drug delivery systems. As a result, numerous improvements have been made, thus making this technology p potentially useful for the treatment of certain diseases in the clinics. Many liposome based DNA delivery systems have been described, including molecular components for targeting given cell surface receptors or for escaping from the. This review discusses the potential applications of liposomes in drug delivery, their preparation method, targeting, mechanism of formation, liposome component and the problems associated with further exploitation of this drug delivery system. This paper summarizes exclusively scalable techniques and focuses on strengths, respectively, limitations in respect to industrial applicability and regulatory requirements concerning liposomal drug formulations based on FDA and EMEA documents.

**Keywords:** Liposomes, classification, methods of preparation, application.

## INTRODUCTION

Novel drug delivery system aims at providing some control, whether this is of temporal or spatial nature, or both, of drug release in the body. Novel drug delivery attempts to either sustain drug action at a predetermined rate, or by maintaining a relatively constant, effective drug level in the body with concomitant minimization of undesirable side effects. Liposomes are colloidal, vesicular structures composed of one or more lipid bilayers surrounding an equal numbers of aqueous compartments1. The sphere like shell encapsulated a liquid interior which contain substances such as peptides and protein, hormones, enzymes, antibiotic, anti-fungal and anticancer agents. A free drug injected in blood. Stream typically achieves therapeutic level for short duration due to metabolism and excretion. Drug encapsulated by liposome achieve therapeutic level for long duration as drug must first be release from liposome before metabolism & excretion. They are small artificial vesicles of spherical shape that can be created from cholesterol and natural non-toxic phospholipids. Due to their size and hydrophobic and hydrophilic character (besides biocompatibility), liposome's are promising systems for drug delivery It can also localize drug action by spatial placement of controlled release systems adjacent to, or in the diseased tissue or organ; or target drug action by using carriers or chemical derivatization to deliver drug to particular target cell type.1 Different types of pharmaceutical carriers are present. They are – particulate, polymeric, and macromolecular. Particulate type carrier also known as colloidal carrier system, includes lipid particles (low and high density lipoprotein LDL and HDL, respectively), microspheres, nanoparticles, polymeric micelles and the vesicular like liposomes, niosomes pharmacosomes, ribosomes, etc. . The vesicular systems are highly ordered assemblies of one or several concentric lipid bilayers formed, when certain amphiphilic building blocks are confronted with water. Vesicles can be formed from a diverse range of amphiphilic building blocks. The terms such as synthetic bilayers allude to the non-biological origin of such vesiculogenes.

**Liposomes**

Liposomes were first described by British haematologist Dr Alec D Bangham in 1961 (published 1964), at the Babraham institute, Cambridge. They were discovered when Bangham and R. W. Horne were testing the institute's new electron microscope by adding negative stain to dry phospholipids. The resemblance to the plasmalemma was obvious, and the microscope pictures served as the first real evidence for the cell membrane being a bilayer lipid structure.

The name liposome is derived from two Greek words 'Lipid' meaning fat and 'Soma' meaning body. Structurally, liposomes are concentric bilayered vesicles in which an aqueous volume is entirely enclosed by a membranous lipid bilayer mainly composed of natural or synthetic phospholipids.

The polar character of the liposomal core enables polar drug molecules to be encapsulated. A mphiphilic and lipophilic molecules are solubilized within the phospholipid bilayer according to their affinity towards the phospholipids.

Participation of nonionic surfactants instead of phospholipids in the bilayer formation results in niosomes. Channel proteins can be incorporated without loss of their activity within the hy drophobic domain of vesicle membranes, acting as a size-selective filter, only allowing passive diffusion of small solutes such as ions, nutrients and antibiotics. Thus, drugs that are encapsulated in a nanocagefunctionalized with channel proteins are effectively protected from premature degradation by proteolytic enzymes .A liposome is an artificially-prepared spherical vesicle composed of a lipid bilayer. The liposome can be used as a vehicle for administration of nutrients and pharmaceutical drugs3. Liposome's can be prepared by disrupting biological membrane (such as sonication). Liposome's are often composed of phosphatidylcholine-enriched phospholipids and may also contain mixed lipid chains with surfactant properties such as egg or phosphatidylethanolamine. Liposomes have the distinct advantages of being both nontoxic and biodegradable because they are composed of naturally occurring substances. Biologically active materials encapsulated within liposomes are protected to varying extent from immediate dilution or degradation, suggesting drug carrier systems for the transport of drugs and other bioactive capsules to disease-affectedorgans.The unique ability of liposomes to entrap drugs both in anaqueous and a lipid phase make such delivery systems attractive for hydrophilic and hydrophobic drugs. Because of advancements in the methods of preparing an d formulating liposomes, high-entrapment efficiencies are possible for incorporating drugs into liposomes, creating a tremendous pharmaceutical impact. Furthermore, such encapsulation has been shown to reduce drug toxicity while retaining or improving the therapeutic efficacy. Several laboratories have reported the use of liposomes as drug carriers in the treatment of cancer, leishmaniasis, metabolic disorders, and fungal diseases.Innovative research in liposomal drugs has led to commercialization of several anticancer therapeutics such as Doxil, Myocet, two liposomebas ed anticancer drugs; doxorubicin; and an antifungal drug formulation, AmBisome, which is a liposomal formulation of amphotericin B used for systemic therapy. Liposomes may have a use in gene delivery to correct gene-associated disorders or for vaccine therapy.

**Advantages of liposomes**

- Offer targeted drug delivery
- Increase efficacy and therapeutic index of drug
- Non – toxic, flexible, biocompatible, completely biodegradable
- Help reduce exposure of sensitive tissues to toxic drug
- Size can be varied to incorporate smaller or larger drug molecules
- Can incorporate both water and lipid soluble drugs
- Can be administered through various routes

**Disadvantages of liposomes**

- Less stability
- Low solubility
- Short half life
- Phospholipids undergoes oxidation, hydrolysis
- Leakage and fusion
- High production cost
- Allergic reactions may occur to liposomal constituents

**Components of liposomes**

a) **Phospholipids:** Glycerol containing phospholipids are most common used component of liposome formulation and represent greater than 50% of weight of lipid in biological membranes. These are derived from phosphatidic acid. The back bone of the molecule is glycerol moiety. At C3 position OH group is esterified to

phosphoric acid. OH at C1 & C2 are esterified with long chain. Fatty acid giving rise to the lipidic nature. One of the remaining OH group of phosphoric acid may be further esterified to a wide range of organic alcohols including glycerol, choline, ethanolamine, serine and inositol. Thus the parent compound of the series is the phosphoric ester of glycerol. Examples of phospholipids are –

- Phosphatidyl choline (Lecithin) – PC
- Phosphatidyl ethanolamine (cephalin) – PE
- Phosphatidyl serine (PS
- Phosphatidyl inositol (PI)
- Phosphatidyl Glycerol (PG) For stable liposomes, saturated fatly acids are used. Unsaturated fatty acids are not used generally.

**b) Sphingolipids:** Backbone is sphingosine or a related base. These are important constituents of plant and animal cells. A head group that can vary from simple alcohols such as choline to very complex carbohydrates. Most common Sphingolipids – Sphingomyelin. Glycosphingo lipids. Gangliosides – found on grey matter, used as a minor component for liposome production. This molecule contain complex saccharides with one or more Sialicacid residues in their polar head group & thus have one or more negative charge at neutral pH. These are included in liposomes to provide a layer of surface charged group.

**c) Sterols:** Cholesstrol and derivatives are of ten included in lioposomes for dercresing the fluidity microviscocity of the bialayer .redusing the permeability of the membrane to water solubles molecules.

**d) Synthetic phospholipids:** E.g.: for saturated phospholipids are Dipalmitoyl phosphatidyl choline (DPPC), Distearoyl phosphatidyl choline (DSPC), Dipalmitoyl phosphatidyl ethanolamine (DPPE) ,Dipalmitoyl phosphatidyl serine (DPPS), Dipalmitoyl phosphatidic acid (DPPA), Dipalmitoyl phosphatidyl glycerol (DPPG) E.g.: for unsaturated phospholipids Dioleoyl phosphatidyl choline (DOPC) ,Dioleoyl phosphatidyl glycerol (DOPG)

**e) Polymeric materials:** Synthetic phospholipids with diactylenic group in the hydrocarbon chain polymerizes when exposed to U.V, leading to formation of polymerized liposomes having significantly higher permeability barriers to entrapped aqueous drugs. e.g. for other Polymerisable lipids are – lipids containing conjugated diene, methacrylate.

**Classification of liposomes**

**I. Classification based on structure**

| Class | Specification |
|---|---|
| SUV | <0.1µm |
| LUV | 0.1 – 10 µm |
| MLV | 0.1 – 0.3 µm |
| GUV | >1 mm |
| MVV | >1mm |
| OLV | Intermediate between MLV and LUV |

**II. Classification based on methods of preparation**

| Class | Specification |
|---|---|
| REV | Single or oligolamellar vesicle made by reverse phase evaporation method |
| MLV-REV | Multilamellar vesicle made by reverse phase evaporation method |
| SPLV | Stable plurilamellar vesicle |
| FATMLV | Frozen and thawed MLV |
| VET | Vesicle prepared by extrusion technique |
| DRV | Dehydration rehydration method |

**III. based on Classification composition and application**

| Class | Specification |
|---|---|
| Conventional liposome | Neutral or negatively charged Phospholipid |
| Fusogenic liposome | Reconstitute sendai virus envelop |
| Cationic liposome | Cationic lipid |
| Long circulatory liposome | Neutral high Transition temperature liposome |
| pH sensitive liposome | Phospholipid like Phosphatidyl ethanolamine |
| Immuno liposome | Long circulatory liposome with attached monoclonal antibody |

**Mechanism of formation of liposomes**

Liposomes are vesicular structures consisting of hydrated bilayers. Liposomes structures used for pharmaceutical purposes consist of a phospholipid backbone. But other classes of molecules can form bilayer based vesicular structures as well. On the other hand not all the hydrated phospholipids form bilayer structures. Other forms of self aggregation such as inverted hexagonal phases or micelles with completely different properties can occur. The common feature that all bilayer forming compounds share is their amphilicity. They have defined polar and nonpolar regions. In water the hydrophobic regions tend to self aggregate and the polar regions tend to be in contact with the water phase. Israelachvili and coworkers defined critical packing parameter p by P = v/ a0Ic Where v is the molecular volume of the hydrophobic part, a0 is the

optimum surface area per molecule at the hydrocarbon water interface, and lc is the critical half thickness for the hydrocarbon region which must be less tehn the maximum length of the extended lipid chains. For p < 1/3, spherical micelles are formed. In this category fall single chain lipids with large head group areas.eg lysophosphatidylcholine. For 1/3 < ½ globular or cylindrical micelles are formed. Double chain —fluid state‖ lipids with large head area (1/2< p <1 ) form bilayers and vesicles. This occurs also with double chain —gel state‖ lipids with small head groups and p ~ 1. for p > 1 inverted structures such as the inverted hexagonal phase can be observed. An addtional condition required for bilayer formation is that the compound can be classified as non-swelling amphiphile

**Method of preparation of liposomes**

Liposome may be prepared by two techniques
a) Passive loading technique.
b) Active loading technique.

**1 )Passive loading technique**

**A] Mechanical dispersion method**

- Lipid hydration by hand shaking or freeze drying
- Micro emulsification
- Sonication
- French pressure cell
- Membrane extrusions
- Dried reconstituted vesicle
- Freeze thawed liposome

**B] Solvent dispersion method**

- Ethanol injection
- Ether injection
- Double emulsion vesicle
- Reverse phase evaporation vesicle
- Stable plurilamellar vesicle

**C) Detergent removal method**

- Detergent (cholate, alkylglycoside, Triton x-100) removed from mixed micelles
- Dialysis
- Column chromatography
- Dilution

**2) Active loading technique**

**1) Passive loading technique:** Passive loading techniques include three different group of method working on different principles namely mechanical dispersion, solvent dispersion and detergent solubilization.

**A.) Mechanical dispersion method**

**The following are types of mechanical dispersion methods**

- Sonication.
- French pressure cell: extrusion
- Freeze-thawed liposome's
- Lipid film hydration by hand shaking, non-hand shaking or freeze drying
- Micro-emulsification.
- Membrane extrusion.
- Dried reconstituted vesicles.

**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 atmosphere11. The main disadvantages of this method are very low internal volume/encapsulation efficacy, possible degradation of phospholipids and compounds to beencapsulated, elimination of large molecules, metal pollution from probe tip, and presence of MLV along with SUV. There are two sonication techniques.

**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.

**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 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. 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 (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. The encapsulation efficacies from 20% to 30% were obtained.

**B. Solvent dispersion method**

**Ether injection (solvent vaporization):** A solution of lipids dissolved in diethyl ether or 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 (70 to 200 nm) and the exposure of compounds to be encapsulated to organic solvents at high temperature.

**Ethanol injection:** 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, the removal all ethanol is difficult because it forms into zoetrope 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-tolipid 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 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. 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 80%)

**C. 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. A commercial device called LipoPrep (Diachema AG, Switzerland), 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 (equilibrium dialysis). Detergent (chelate, alkyl glycoside, Triton X- 100) removal of mixed micelles (absorption) Detergent absorption is attained by shaking mixed micelle solution with beaded organic polystyrene absorbers 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 absorbers is that they can eliminate detergents with a very low CMC, which are not entirely depleted.

**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 pretreatment is done by pre-saturation of the gel filtration column by lipids using empty liposome suspensions.

**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 micelle phase boundary, a spontaneous transition from polydispersed micelles to vesicles occurs.

**Purification of liposome:** Liposomes are generally purified by gel filtration chromatography, Dialysis and centrifugation. In chromatographic separation, Sephadex-50 is most widely used. In dialysis method hollow fibre dialysis cartridge maybe used. In centrifugation method, SUVs in normal saline may be separated by centrifuging at 200000 g, for 10-20hours. MLVs are separated by centrifuging at 100000g for less than one hour. Mechanism of transportation through liposome The limitations and benefits of liposome drug carriers lie critically on the interaction of liposomes with cells and their destiny in vivo after administration. In vivo and in vitro studies of the contacts with cells have shown that the main interaction of liposomes with cells is either simple adsorption (by specific interactions with cell- surface components, electrostatic forces, or by non- specific weak hydrophobic) or following endocytosis (by phagocyte cells of the reticulo- endothelial system, for example macrophages and neutrophils). Fusion with the plasma cell membrane by insertion of the lipid bilayer of the liposome into the plasma membrane, with simultaneous release of liposomal content into the cytoplasm, is much rare. The fourth possible interaction is the exchange of bilayer components, for instance cholesterol, lipids, and membrane-bound molecules with components of cell membranes.

**Characterization of liposomes**

**a) Physical properties**

| Size and its distribution | Microscopy. Laser light scattering |
|---|---|
| Surface charge | Gel electrophoresis |
| Entrapped volume | NMR |
| Lamellarity | Freeze electron microscopy, 31P-NMR |
| Phase behavior of liposomes | DSC |
| Drug release | In vitro diffusion cell |
| Encapsulation efficiency(% capture) | Mini column centrifugation, protamine aggregation |

**b) Chemical properties**

| Quantitative determination of phospholipids | Barlett assay, Stewart assay, TLC |
|---|---|
| Phospholipid hydrolysis | HPLC |
| Phospholipid oxidation | UV, GLC,TBA |

**Drug loading in liposomes**

Drug loading can be attained either passively (i.e., the drug is encapsulated during liposome formation) or actively (i.e., after liposome formation). Hydrophobic drugs, for example amphotericin B taxol or annamycin, can be directly combined into liposomes during vesicle formation, and the amount of uptake and retention is governed by drug-lipid interactions. Trapping effectiveness of 100% is often achievable, but this is dependent on the solubility of the drug in the liposome membrane. Passive encapsulation of water-soluble drugs depends on the ability of liposomes to trap aqueous buffer containing a dissolved drug during vesicle formation. Trapping effectiveness (generally <30%) is limited by the trapped volume delimited in the liposomes and drug solubility. On the other hand, water-soluble drugs that have protonizable amine functions can be actively entrapped by employing pH gradients, which can result in trapping effectiveness approaching 100%.

**EVALUATION OF LIPOSOMES**

- **Vesicle shape and lamellarity**
- **Vesicle size and size distribution**
- **Microscopic Techniques**
- **Cryo-Transmission Electron Microscopy Techniques (cryo-TEM)**
- **Diffraction and Scattering Techniques Laser Light Scattering Photon correlation**
- **Hydrodynamic Techniques**
- **Encapsulation Efficiency**

**Application of liposomes**

1) Liposome as drug/protein delivery vehicle:
   - Controlled and sustained drug release in situ

- Enchaned drug solubilization
- Altered pharmacokinetic and biodistribution
- Enzyme replacement therapy and lysosomal disorders

2) Liposome in antimicrobial, antifungal and antiviral therapy
- Liposomal drugs
- Liposomal biological response modifier

3) Liposomes in tumour therapy
- Carrier of small cytotoxic molecule
- Vehicle for macromolecule as cytokines or genes

4) Liposome in gene therapy
- Gene and antisence therapy
- Genetic (DNA) vaccination

5) Liposome in immunology
- Immunoadjuvant
- Immunomodulator
- Immunodiagnosis s

6) Liposome as artificial blood surrogates
7) Lipososmes as radiopharmaceutical and radiodiagnostic carrier
8) Liposomes in cosmetics and dermatology
9) Liposomes in enzyme immobilization and bioreactor technology

**Limitation in liposome technology**

• Stability
• Sterilization
• Encapsulation efficiency
• Active targeting
• Gene therapy
• Lysosomal degradation

## CONCLUSION

Liposomes have been used in a broad range of pharmaceutical applications. Liposomes are showing particular promise as intracellular delivery systems for anti-sense molecules, ribosomes, proteins/peptides, and DNA. Liposomes with enhanced drug delivery to disease locations, by ability of long circulation residence times, are now achieving clinical acceptance. Also, liposomes promote targeting of particular diseased cells within the disease site. Finally, liposomal drugs exhibit reduced toxicities and retain enhanced efficacy compared with free complements. However, based on the pharmaceutical applications and available products, we can say that liposome's have definitely established their position in modern delivery systems. The use of liposomes in the delivery of drugs and genes are promising and is sure to undergo further developments in future.

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