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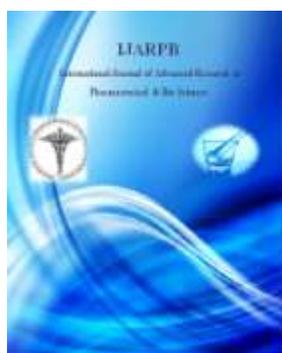
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A REVIEW ON LIPOSOME

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ABSTRACT

Liposomes are acceptable and superior carriers and have ability to encapsulate hydrophilic and lipophilic drugs and protect them from degradation. Liposomes are microparticulate lipoidal vesicles which are under extensive investigation as drug carriers for improving the delivery of therapeutic agents. Due to new developments in liposome technology, several liposome-based drug formulations are currently in clinical trial, and recently some of them have been approved for clinical use. Reformulation of drugs in liposomes has provided an opportunity to enhance the therapeutic indices of various agents mainly through alteration in their biodistribution. This review discusses the potential applications of liposomes in drug delivery with examples of formulations approved for clinical use, their preparation method, targeting, mechanism of formation, liposome component and the problems associated with further exploitation of this drug delivery system.

KEY WORDS: Liposome, classification, preparation method, application, limitation

(Review Article)**INTRODUCTION****LIPOSOME**

Liposomes are concentric bilayered vesicle in which an aqueous volume is entirely enclosed by a membranous lipid bilayer mainly composed of natural or synthetic phospholipids.

The name liposome is derived from two Greek words: 'Lipos' meaning fat and 'Soma' meaning body. A liposome can be formed at a variety of sizes as uni-lamellar or multi-lamellar construction, and its name relates to its structural building blocks, phospholipids, and not to its size. A liposome does not necessarily have lipophobic contents, such as water, although it usually does.

Liposomes are artificially prepared vesicles made of lipid bilayer. Liposomes can be filled with drugs, and used to deliver drugs for cancer and other diseases. Liposomes can be prepared by disrupting biological membranes, for example by sonication.

Liposomes are micro particulate or colloidal carriers, usually 0.05- 5.0 μm in diameter which form spontaneously when certain lipid are hydrated in aqueous media. Liposomes are composed of relatively biocompatible and biodegradable material, and they consist of an

aqueous volume entrapped by one or more bilayer of natural and/or synthetic lipids. Drug with widely varying lipophilicities can be encapsulated in liposomes, either in the phospholipids bilayer, in the entrapped aqueous volume or at the bilayer interface

Manufacturing

The correct choice of liposome preparation method depends on the following parameters

- 1) The physicochemical characteristics of the material to be entrapped and those of the liposomal ingredients.
- 2) The nature of the medium in which the lipid vesicles are dispersed.
- 3) The effective concentration of the entrapped substance and its potential toxicity.
- 4) Additional processes involved during application/delivery of the vesicles.
- 5) Optimum size, polydispersity and shelf-life of the vesicles for the intended application.
- 6) Batch-to-batch reproducibility and possibility of large-scale production of safe and efficient liposomal products.

(Review Article)**Table: 1** classification of liposome¹

TYPE-1	SPECIFICATION
BASED ON STRUCTURE PARAMETER	
MLV	Multilamellar large vesicle->0.5µm
OLV	Oligolamellar vesicle-0.1-0mm
UV	Unilamellar vesicle (all range size)
SUV	Small sized unilamellar vesicle
MUV	Medium sized unilamellar vesicle
LUV	Large unilamellar vesicle-> 100nm
GUV	Giant unilamellar vesicle- >1µm
MV	Multivesicular vesicle >1µm
TYPE-2	
BASED ON LIPOSOME PREPARATION	
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
TYPE-3	
BASED UPON COMPOSITION AND APPLICATION	
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

(Review Article)**METHOD OF LIPOSOME PREPARATION AND DRUG LOADING²**

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
- Reconstituted sendai virus enveloped vesicle

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 of passive loading

All method covered under this category begin with a lipid solution in organic solvent and end up with lipid dispersion in water. The various components are typically combined by co-dissolving the lipid in organic solvent and organic solvent is then removed by film diposition under vacuum. When all solvent is removed, the solvent dispersion mixture is hydrated using aqueous buffer. The film spontaneously swell and hydrate to form liposomes. At this point method incorporate some diverge processing parameters in various way to modify their ultimate properties. The post hydration treatments include vortexing, sonication, freeze thawing and high- pressure extrusion.

B) Solvent dispersion method of passive loading

In solvent dispersion method, lipid a is first dissolved is an organic solution, which is then brought into contact with an aqueous phase containing materials to be entrapped within the liposome. The lipid align themselves at the interface of organic and aqueous phase forming monolayer of phospholipids, which form the half of the bilayer of the liposome method employing solvent dispersion can be categorized on the basis of the miscibility of the organic solvent and aqueous solution. These include condition where the organic solvent is miscible with aqueous

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phase, the organic solvent is immiscible with the aqueous phase, the latter being in excess and the case where the organic solvent is in excess, and immiscible with the aqueous phase.

C) Detergent removal method of passive loading

In this method the phospholipids are brought into intimate contact with the aqueous phase via detergent, which associate with phospholipids molecule and serve to screen the hydrophobic portion of the molecule from water. The structure formed as result of this association is known as micelles, and can be composed of several hundreds of component molecule. Their size and shape depend on the chemical nature of detergent, the concentration and other lipid involved. The concentration of detergent of in water at which micelles just start to form is known as 'critical micelle concentration'. Below the critical micelle concentration, micelle the detergent molecule exists entirely in free solution. As detergent is dissolved in water in concentration higher than the CMC, micelle form in more and more numbers, while the concentration of detergent in the free form remain essentially the same as it is at the CMC. Micelle containing other participating component in addition to detergent (or composed of two or more detergent in their formulation known as "mixed micelle".

Invariably in all method, which employed detergent in the preparation of liposome, the basic feature is to remove the detergent from preformed mixed micelle containing phospholipids, where upon uni lamellar vesicle formed spontaneously.

2) Active loading technique

The utilization of liposomes as drug delivery system is stimulated with the advancement of efficient encapsulation procedures. The membrane from the lipid bilayer is in general impermeable to ions and larger hydrophilic molecules. Ions transport can be regulated by the ionophores while permeation of neutral and weakly hydrophobic molecule can be controlled by concentration gradients. Some weak acid or bases however, can be transported through the membrane due to various transmembrane gradient, such as electric, ionic (pH) or specific salt (chemical potential) gradient. Several method exist for improved loading of drugs, including remote (active) loading method which load drug molecules into preformed liposome using pH gradient and potential difference across liposomal membrane. A concentration difference in proton concentration across the membrane of liposomes can drive the loading of amphipathic molecule. Active loading method have the following advantages over passive encapsulation technique:

- A high encapsulation efficiency and capacity.
- A reduced leakage of the encapsulated compounds.
- "bed side" loading of drugs thus limiting loss of retention of drugs by diffusion, or chemical degradation during storage.
- Flexibility of constitutive lipid, as drug is loaded after the formation of carrier unit.
- Avoidance of biological active compounds during preparation step in the dispersion thus reducing safety hazards.

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- The transmembrane pH gradient can be developed using various method depending upon the nature of drug to be encapsulated.

Advantage of liposome³

- Non ionic
- Can carry both water and lipid soluble drugs
- Biodegradable drugs can be stabilized from oxidation
- Improve protein stabilization
- Controlled hydration
- Provide sustained release
- Targeted drug delivery or site specific drug delivery
- Stabilization of entrapped drug from hostile environment
- Alter pharmacokinetics and pharmacodynamics of drugs
- Can be administered through various routes

- Can incorporate micro and macro molecules
- Act as reservoir of drugs
- Therapeutic index of drugs is increased
- Site avoidance therapy
- Can modulate the distribution of drug
- Direct interaction of the drug with cell
- Biodegradable and flexible

Disadvantages³

- Less stability
- Low solubility
- Short half life
- Phospholipids undergoes oxidation, hydrolysis
- Leakage and fusion
- High production cost
- Quick uptake by cells of R.E.S
- Allergic reactions may occur to liposomal constituents
- Problem to targeting to various tissues due to their large size

Table: 2 LIST OF CLINICALLY- APPROVED LIPOSOMAL DRUGS⁴

Name	Trade name	Company	Indication
Liposomal amphotericin B	Abelcet	Enzon	Fungal infections
Liposomal amphotericin B	Ambisome	Gilead Sciences	Fungal and protozoal infections
Liposomal cytarabine	Depocyt	Pacira (formerlySkyePharma)	Malignant lymphomatous meningitis
Liposomal daunorubicin	DaunoXome	Gilead Sciences	HIV-related Kaposi's sarcoma

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Liposomal doxorubicin	Myocet	Zeneus	Combination therapy with cyclophosphamide in metastatic breast cancer
Liposomal Vaccine	Epaxal	Berna Biotech	Hepatitis A
Liposomal Vaccine	Inflexal V	Berna Biotech	Influenza
Liposomal morphine	DepoDur	SkyePharma, Endo	Postsurgical analgesia
Liposomal verteporfin	Visudyne	QLT, Novartis	Age-related macular degeneration, pathologic myopia, ocular histoplasmosis
Liposome-PEG doxorubicin	Doxil/Caelyx	Ortho Biotech, Schering-Plough	HIV-related Kaposi's sarcoma, metastatic breast cancer, metastatic ovarian cancer
Micellular estradiol	Estrasorb	Novavax	Menopausal therapy

Targeting of liposomes⁵

Two types of targeting.

1) Passive targeting

As a mean of passive targeting, such usually administered liposomes have been shown to be rapidly cleared from the blood stream and taken up by the RES in liver spleen. Thus capacity of the macrophages can be exploited when liposomes are to be targeted to the macrophages. This has been demonstrated by successful delivery of liposomal antimicrobial agents to macrophages.

Liposomes have now been used for targeting of antigens to macrophages as a first step in the index of immunity. For e.g. in rats the i.v. administration of liposomal antigen elicited spleen phagocyte mediated antibody response where as the non liposome associated antigen failed to elicit antibody response.

2) Active targeting

A pre requisite for targeting is the targeting agents be positioned on the liposomal surface such that the interaction with the target i.e., the receptor is tabulated such as a plug and socket device. The liposome physically prepared such that the lipophilic part of the connector is anchored into the membrane during the

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formation of the membrane. The hydrophilic part on the surface of the liposome, to which the targeting agent should be held in a sterically correct position to bond to the receptor on the cell surface.

Mechanism of formation of liposomes⁶

In order to understand why liposomes are formed when phospholipid are hydrated, it requires a basic understanding of physiochemical features of phospholipid.

Phospholipids are amphipathic (having affinity for both aqueous and polar moieties) molecules as they have a hydrophobic tail and a hydrophilic or polar head. The hydrophilic tail composed of two fatty acid chains containing 10-24 carbon atoms and 0-6 double bonds in each chain. The polar end of molecule is mainly the phosphoric acid bound to a water soluble molecule. The hydrophilic and hydrophobic domain/segment within the molecular geometry of amphiphilic lipid orient and self organize in ordered supramolecular structure when confronted with solvent.

In aqueous medium the molecule in self assembled structure is oriented in such a way that the polar portion of molecule remain in

contact with the polar environment and at the same time shield the non-polar part. Among the amphiphiles used in drug delivery, such as soap, detergent, polar lipid, the latter (polar lipid) are often employed to form concentric bilayer structure. However, in aqueous medium these molecules are able to form various phases, some of them are stable and others remain in the metastable state. At high concentration of these polar lipids, liquid-crystalline phases are formed that upon dilution with an excess of water can be dispersed into relatively stable colloidal particles. The macroscopic structure most often formed include lamellar, hexagonal or cubic phases dispersed as colloidal nanoconstruct (artificial membrane) referred to as liposomes, hexosomes or cubosomes respectively.

The most common natural polar phospholipids are phosphatidylcholine. These are amphipathic molecules in which a glycerol bridge links to a pair of hydrophobic acyl chains with a hydrocarbon chains with a hydrophilic polar head group, phosphocholine.

Thus the amphipathic (amphiphilic) nature of the phospholipid and their analogues render them the ability to form closed concentric bilayers in the presence of water.

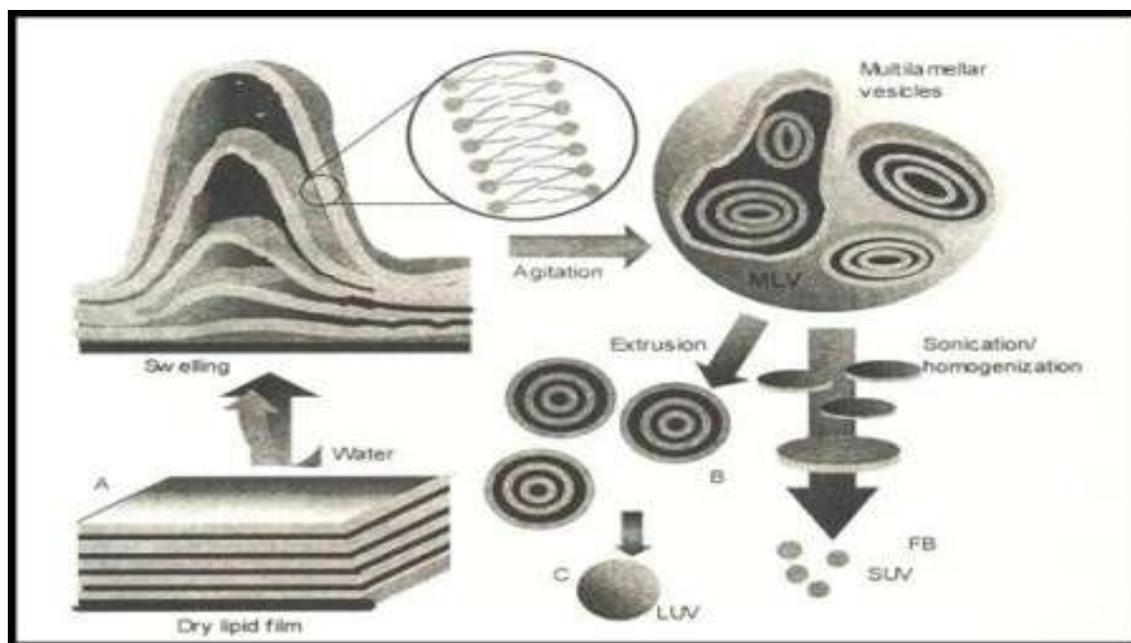
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Fig.1: Mechanism of liposome formulation

Pharmacokinetics of liposomes⁷

- Liposomal drugs can be applied through various routes, but mainly i.v. and topical administration is preferred. After reaching in the systemic circulation or in the local area, a liposome can interact with the cell by any of the following methods.
- Endocytosis by phagocytotic cells of the R.E.S such as macrophages and Neutrophils
- Adsorption to the cell surface either by non specific weak hydrophobic or electrostatic forces or by specific interaction with cell surface components
- Fusion with the plasma cell membrane by insertion of lipid bilayer of liposome into plasma membrane with simultaneous release of liposomal contents into the cytoplasm.
- Transfer of liposomal lipids to cellular or sub cellular membrane or vice versa

without any association of the liposome contents.

- It is often difficult to determine what mechanism is operative and more than one may operate at the same time.

Pharmacodynamics of liposome encapsulated drugs⁸

To continue the action of drugs to a particular site in the body, the general approach is to deposit drug bearing liposome directly into the site where therapy is desired. Since liposomes are large and do not easily cross epithelial or connective barriers, they are likely to remain at the site of local administration. The liposomes would then slowly released into the target site or perhaps create a local drug level higher than the systemic level. Alternatively the drug loaded liposomes might interact directly with cells in the target site, without producing release. The goal of this approach is to maximize the amount of effective drug at the target site, while minimizing the drug

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levels at other sites and thus decreasing systemic toxicity.

For e.g. SUV injected into the skin can persist interact at the site for 600 hrs. And release of entrapped markers from the liposomes occurs only after cellular uptake and intracellular space remain intact.

Structural components^{9,10}**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 backbone 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 fatty 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 Sialic acid 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

- Cholesterol & its derivatives are often included in liposomes for
- decreasing the fluidity or microviscosity of the bilayer
- reducing the permeability of the membrane to water soluble molecules
- Stabilizing the membrane in the presence of biological fluids such as plasma. (This effect used in formulation of i.v. liposomes)

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

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- Dioleoyl phosphatidyl choline (DOPC)
- Dioleoyl phosphatidyl glycerol (DOPG)

Polymeric materials

Synthetic phospholipids with diacylenic 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 etc..

Other Substances

- Variety of other lipids of surfactants are used to form liposomes.
- Many single chain surfactants can form liposomes on mixing with cholesterol.
- Non ionic lipids.
- A variety of polyglycerol and polyethoxylated mono and dialkyl amphiphiles used mainly in cosmetic preparations.
- Single and double chain lipids having fluoro carbon chains can form very stable liposomes.
- Sterylamine and dicetyl phosphate.
- Incorporated into liposomes so as to impart either a negative or positive surface charge to these structures.
- A number of compounds having a single long chain hydrocarbon and an ionic head group found to be capable of forming vesicles. These include quaternary ammonium salts of dialkyl phosphates.

Therapeutic application of liposomes¹¹

- 1) Liposome as drug/protein delivery vehicle:
 - Controlled and sustained drug release in situ
 - Enhanced 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 antisense therapy
 - Genetic (DNA) vaccination
- 5) Liposome in immunology:
 - Immunoadjuvant
 - Immunomodulator
 - Immunodiagnosis
- 6) Liposome as artificial blood surrogates
- 7) Liposomes as radiopharmaceutical and radiodiagnostic carrier
- 8) Liposomes in cosmetics and dermatology
- 9) Liposomes in enzyme immobilization and bioreactor technology

(Review Article)**Limitation in liposome technology**

- 1) Stability
- 2) Sterilization
- 3) Encapsulation efficiency
- 4) Active targeting
- 5) Gene therapy
- 6) Lysosomal degradation

CONCLUSION:

Liposome carriers, well known for their potential application. Liposomes are acceptable and superior carriers and have ability to encapsulate hydrophilic and lipophilic drugs and protect them from degradation. There are a number of methods available by which liposomes can be manufactured separately depending on the property of molecule. The liposomes containing drugs can be administered by many routes (intravenous, oral inhalation, local application, ocular) and these can be used for the treatment of various diseases. A number of problems associated with drug molecule such as bioavailability, degradation, stability, site effect can be overcome by incorporating it into liposome. As a novel carrier system liposomes provide controlled and sustained release.

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