ISSN: 2321-3760

Review Article

International Journal of Current Trends in Pharmaceutical Research

IJCTPR, 2014, Vol. 2(4): 555-561

www.pharmaresearchlibrary.com/ijctpr

A Novel Pharmaceutical Microscopic Vesicle: Liposome

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Received: 15 March 2014, Accepted: 10 June 2014, Published Online: 15 July 2014

Abstract

Liposomes are simple microscopic vesicles in which an aqueous volume is entirely enclosed by a membrane composed of lipid bilayers. Liposomes are defined as structure consisting of one or more concentric spheres of lipid bilayers separated by water or aqueous buffer compartments. The liposomes have emerged as most practically useful carriers for in-vivo drug delivery as majority of reports has concentrated on the use of phospholipid vesicles or liposomes as potential drug carrier systems. The water soluble compounds/drugs are present in aqueous compartments while lipid soluble compounds/drugs and amphiphilic compounds/drugs insert themselves in phospholipid bilayers. The present review focuses upon the advantages, disadvantages, mechanism, classification, method of preparation, characterization and application of liposomes.

Key words: Liposomes, Phospholipid, Stability, Lipid bilayers

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Manuscript ID: IJCTPR2147

PAPER-QR CODE

1. Introduction

The rising number of complications associated with drugs from varied chemical and biological background not only made scientists worldwide to search for newer molecules but also to discover the new ways and means for the proper delivery of molecules. With the help of new delivery systems known as novel drug delivery systems (NDDS) both old and new molecules can be delivered to the site in demand in a defined manner [1, 2].

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 unilamellar or multi-lamellar construction, and its name relates to its structural building blocks, phospholipids, and not to its size. Liposomes were first described by British haematologist Dr Alec D Bangham in 1961, at the Babraham Institute, in 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 [3, 4].

Liposomes are now used to deliver certain vaccines, enzymes and drugs to the body. When used in the delivery of certain cancer drugs, liposomes help to shield healthy cells from the drugs toxicity and prevent their concentration in vulnerable tissues (e.g., kidney, liver), lessening or eliminating the common side effects of nausea, fatigue and hair lose [1, 5, 6].

Advantages of Liposomes [1,4,7]

a. Provide controlled and sustained release
b. Liposomes are biocompatible, completely biodegradable, non-toxic, flexible and nonimmunogenic for systemic and non-systemic administrations.
c. Stabilization of entrapped drug from hostile environment
d. Can carry both water and lipid soluble drugs
e. Drugs can be stabilized from oxidation
f. Can incorporate micro and macro molecules
g. Flexibility to couple with site-specific ligands to achieve active targeting
h. Alter pharmacokinetics and pharmacodynamics of drugs
i. Therapeutic index of drugs is increased
j. Controlled hydration
k. Help to reduce exposure of sensitive tissues to toxic drugs.
l. Targeted drug delivery or site specific drug delivery
m. Can be administered through various routes
n. Act as reservoir of drugs
o. Can modulate the distribution of drug

Disadvantages [5, 6, 8]

1. Problem to targeting to various tissue due to their large size
2. Short half life
3. Less stability
4. Leakage and fusion of encapsulated drug / molecules
5. High production cost
6. Low solubility
7. Quick uptake by cells of R.E.S
8. Sometimes phospholipid undergoes oxidation and hydrolysis like reaction.
9. High production cost
10. Allergic reactions may occur to liposomal constituents

List of Materials used for Liposomes Formulation

1. **Membrane forming components**

   Phospholipids that are the major components of the biological membranes are the building blocks of the liposomes. The phospholipids have tubular shape owning to the presence of two acyl chains attached to a polar head and on hydration, results into a bilayered membrane. Two types of phospholipids are there i.e. phosphodiglycerides and sphingolipids along with their corresponding hydrolysis products.

   Classification of phospholipids

   A. Neutral phospholipids e.g. Sphingomyelin, Phosphatidylethanolamine and Phosphatidylcholine.
   B. Negatively charged phospholipids e.g. Dipalmitoyl phosphatidylcholine, Dipalmitoyl phosphatidyl acid (DDPA), Distearoyl phosphatidyl choline (DSPC), Dioleoyl phosphatidyl choline (DOPC) etc.
   C. Positively charged phospholipids e.g. 1, 2-dihexadecyl-N, N-dimethyl–N-trimethyl amine methyl ethanol amine etc [8, 9].

2. **Membrane Additives (Sterols)**

   Cholesterol is the most commonly used sterol, which is included in the liposomal membranes. It has been called as the ‘motar’ of bilayers because by virtue of its molecular shape and solubility properties, it fills in empty spaces among the phospholipid molecules, anchoring them more strongly into the structure. Cholesterol is an amphipathic molecule and inserts itself into the membrane with its hydroxyl groups oriented towards the aqueous phase and aliphatic chain aligned parallel to acyl chains of the phospholipid molecules. In other words, cholesterol increases the transition temperature of the system by making the

membrane more ordered. Cholesterol reduces this type of interaction to a great extent and provides both physical and biological stability[9, 10].

3. **Charge inducers and Steric stabilizers**
   Stearylamine, dicetylphosphate, solulan C-24 and diacylglycerol are commonly used to impart either a negative or a positive surface charge. Since it is a well-known fact that negatively charged and positively charged liposomes are more rapidly uptaken by the reticulo-endothelial system as compared to neutral liposomes, charge inducers are used to overcome this problem. Also they proved to be useful in reducing aggregation as neutral liposomes show higher tendency to undergo aggregation [2, 11].
4. **Other substances**
   In case, the drug is very prone to oxidation, antioxidants e.g. tocopherol, butylated hydroxy toluene and stabilizers are used. The use of preservatives is very common to increase the shelf-life of liposomal formulations [8, 12].

2. Mechanism of Vesicle Formation

It has been proved that phospholipids spontaneously form closed structures when hydrated in aqueous media. Because phospholipids are amphipathic (both hydrophilic and hydrophobic) in nature, their thermodynamic phase properties and self-assembling characteristics evoke entropically driven sequestration of hydrophobic regions into spherical bilayers. In other words, unfavorable interactions come into play between lipid molecules and water molecules. The self-assembling action of phospholipid molecules into bilayered sheets leads to lowering of unfavorable interaction between the solvent and long hydrocarbon fatty chains thus acquiring a state of lower energy and almost maximum stability. Well known amphiphiles include soaps, detergents and polar lipids (lecithins, kephalins). Further, to gain a completely stable state, bilayer sheets start folding or curl-on itself to form closed sealed bilayered vesicles enclosing a central aqueous core.

This phenomenon can be understood in quantitative terms by considering the critical micelle concentration (CMC) of phosphatidylcholine in water. The CMC is defined as the concentration of the lipid in water (usually expressed as moles per liter) above which the lipid forms either micelles or bilayer structures rather than remaining in solution as monomers. The CMC of dipalmitoyl phosphatidylcholine has been measured by Smith and Tenford and found to be $4.6x10^{-10}$ M in water. This value is in agreement with those obtained for similar amphiphiles. Clearly, this is a very small number indicating the overwhelming preference of this molecule for a hydrophobic environment such as that found in the core of a micelle or bilayer [5,11,13].

Mechanism of Transportation Through Liposomes [3,5,14]

Liposome can interact with cells by four different mechanisms:

1. Endocytosis by phagocytic cells of the reticulo endothelial system such as macrophages and neutrophils.
2. Adsorption to the cell surface either by nonspecific weak hydrophobic or electrostatic forces or by specific interactions with cell-surface components.
3. 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
4. Transfer of liposomal lipids to cellular or subcellular membranes, or vice versa, without any association of the liposome contents. It often is difficult to determine what mechanism is operative and more than one may operate at the same time.
5.

3. Classification

Based on composition and mode of drug delivery

A. Conventional liposomes
These types of liposomes are composed of neutral or negatively charged phospholipids and cholesterol. It is useful for E.E.S targeting; rapid and saturable uptake by R.E.S; short circulation half life, dose dependent pharmacokinetics.

B. Cationic Liposomes
These types of liposomes are composed of cationic lipids. These are mainly suitable for delivery of negatively charged macromolecules (DNA, RNA); ease of formation, structurally unstable; toxic at high dose, mainly restricted to local administration

C. Temperature or heat sensitive liposomes
These types of liposomes are composed of dipalmitoyl phosphotidyl choline. These are vesicles showed maximum release at 41˚C, the phase transition temperature of dipalmitoyl phosphotidyl choline. Liposomes release the entrapped content at the target cell surface upon a brief heating to the phase transition temperature of the liposome membrane.

D. pH sensitive liposomes

These types of liposomes are composed of phospholipids such as phosphatidyl ethanolamine, dioleoyl phosphatidyl ethanolamine. These are subjected to coated pit endocytosis at low pH, fuse with cell or endosomes membrane and release their contents in cytoplasm; suitable for intra cellular delivery of weak base and macromolecules. Biodistribution and pharmacokinetics similar to conventional liposomes.

E. Long circulating or stealth liposomes

These types of liposomes are composed of neutral high transition temperature lipid, cholesterol and 5-10% of PEG-DSPE. These are subjected to hydrophilic surface coating, low opsonisation and thus low rate of uptake by R.E.S. So, it has long circulating half life (40 hrs) and dose independent Pharmacokinetics.

F. Immuno liposomes

These are conventional or stealth liposomes with attached antibody or recognition sequence. These are subjected to receptor mediated endocytosis. It has cell specific binding (targeting) and can release contents extra cellularly near the target tissue and drugs diffuse through plasma membrane to produce their effects.

G. Magnetic Liposomes

These types of liposomes are composed of phosphotidyl choline, cholesterol and small amount of a linear chain aldehyde and colloidal particles of magnetic iron oxide.These are liposomes that indigenously contain binding sites for attaching other molecules like antibodies on their exterior surface. These can be made use by an external vibrating magnetic field on their deliberate, on site, rapture and immediate release of their components.

1. Based on Size and Number of Lamellae

A. Small Unilamellar Vesicles (S.U.V.)

Single bilayer, homogeneous in size, thermodynamically unstable, susceptible to aggregation and fusion at low or no charge, limited capture of macro molecules, low aqueous volume to lipid ratio (0.2 : 1.5 : 1 mole lipid) prepared by reducing the size of M.L.V. or L.U.V. using probe sonicator or gas extruder or by active loading or solvent injection technique.

B. Large Unilamellar Vesicles (L.U.V.)

Large unilamellar vesicles have single bilayer, high aqueous volume to lipid ratio (7: 1 mole lipid), useful for hydrophilic drugs, high capture of macro molecules; rapidly cleared by R.E.S. Prepared by detergent dialysis, ether injection, reverse phase evaporation or active loading methods.

C. Multi Lamellar Vesicles (M.L.V)

Multi lamellar vesicles have more than one bilayer; moderate aqueous volume to lipid ratio 4: 1 mole lipid. Greater encapsulation of lipophilic drug, mechanically stable upon long term storage, rapidly cleared by R.E.S, useful for targeting the cells of R.E.S, simplest to prepare by thin film hydration of lipids in presence of an organic solvent.

- **a.** Oligo lamellar vesicles or Paucilamellar vesicles: Intermediate between L.U.V. & M.L.V.
- **b.** Multi vesicular liposomes: Separate compartments are present in a single M.L.V.
- **c.** Stable Pluri lamellar vesicles: Have unique physical and biological properties due to osmotic compression.

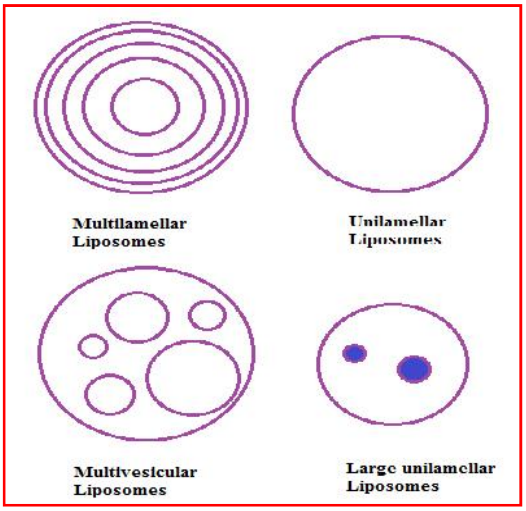

Figure 1. Schematic Representation of Different Liposomes

Method of Preparation [3,4,8,17]

The preparation of all types of vesicular systems requires the input of energy. Generally all the methods of liposome preparation involve three basic stages:

1. Dissolved cholesterol and lecithin in organic solvent.
2. Dispersion of lipids in aqueous media.
3. Separation and purification of resultant liposomes.

Various methods used for the preparation of liposome:

1. Passive loading techniques

Passive loading techniques include three different methods:

I. Mechanical dispersion method

a. Lipid film hydration by hand shaking, non-hand shaking or freeze drying
b. Micro-emulsification
c. Sonication
d. French pressure cell
e. Membrane extrusion
f. Dried reconstituted vesicles
g. Freeze-thawed liposomes

II. Solvent dispersion method

a. Ether injection
b. Ethanol injection
c. Double emulsion vesicles
d. Reverse phase evaporation vesicles
e. Stable plurilamellar vesicles

III. Detergent removal method

a. Detergent (cholate, alkylglycoside, Triton X-100) removal form mixed micelles
b. Dialysis
c. Column chromatography
d. Dilution

2. Active loading

Characterization of Liposomes [13,15,17]

Liposome prepared by one of the preceding method must be characterized. The most important parameters of liposome characterization include visual appearance, turbidity, size distribution, lamellarity, concentration, composition, presence of degradation products, and stability.

1. Visual Appearance

Liposome suspension can range from translucent to milky, depending on the composition and particle size. If the turbidity has a bluish shade this means that particles in the sample are homogeneous; a flat, gray color indicates that presence of nonliposomal dispersion and is most likely disperse inverse hexagonal phase or dispersed microcrystallites. An optical microscope (phase contrast) can detect liposome > 0.3 μm and contamination with larger particles.

2. Determination of Lamillarity

The lamellarity of liposomes is measured by electron microscopy or by spectroscopic techniques. Most frequently the nuclear magnetic resonance spectrum of liposome is recorded with and without the addition of a paramagnetic agent that shifts or bleaches the signal of the observed nuclei on the outer surface of liposome. Encapsulation efficiency is measured by encapsulating a hydrophilic marker.

3. Entrapped Volume

The entrapped volume of a population of liposome (in μL/ mg phospholipid) can often be deduced from measurements of the total quantity of solute entrapped inside liposome assuring that the concentration of solute in the aqueous medium inside liposomes is the same after separation from unentrapped material. For example, in two phase method of preparation, water can be lost from the internal compartment during the drying down step to remove organic solvent.

4. Determination of Liposomal Size Distribution

Size distribution is normally measured by dynamic light scattering. This method is reliable for liposomes with relatively homogeneous size distribution. A simple but powerful method is gel exclusion chromatography, in which a truly hydrodynamic radius can be detected. Sephacryl-S100 can separate liposome in size range of 30-300nm. Sepharose -4B and -2B columns can separate SUV from micelles.

5. Surface Charge

Liposomes are usually prepared using charge imparting constituting lipids and hence it is imparting to study the charge on the vesicle surface. In general two method are used to assess the charge, namely

freeflow electrophoresis and zeta potential measurement. From the mobility of the liposomal dispersion in a suitable buffer, the surface charge on the vesicles.

6. Liposome Stability

Liposome stability is a complex issue, and consists of physical, chemical, and biological stability. In the pharmaceutical industry and in drug delivery, shelf life stability is also important. Physical stability indicates mostly the constancy of the size and the ratio of lipid to active agent. The cationic liposomes can be stable at 4°C for a long period of time, if properly sterilized.

Applications [13,14,18]

1. Liposome in antimicrobial, antifungal and antiviral therapy
    A. Liposomal drugs
    B. Liposomal biological response modifiers
2. Liposome in tumor therapy
    A. Carrier of small cytotoxic molecules
    B. Vehicle for macromolecules as cytokines or genes
3. Liposome as drug/protein delivery vehicles
    A. Controlled and sustained drug release
    B. Altered pharmacokinetics and biodistribution
    C. Enhanced drug solubilization
    D. Enzyme replacement therapy and biodistribution
    E. Altered pharmacokinetics and biodistribution
4. Liposome in immunology
    A. Immunoadjuvant
    B. Immunodiagnosis
    C. Immunomodulator
5. Liposome in gene delivery
    A. Gene and antisense therapy
    B. Genetic (DNA) vaccination
6. Liposome in enzyme immobilization and bioreactor technology
7. Liposome as radiopharmaceutical and radio diagnostic carriers
8. Liposome in cosmetics and dermatology
9. Liposome as artificial blood surrogates

Future Challenges for Liposomes [2, 17, 18]

Although liposomes have proved their potential as drug delivery vehicles, only few products have come up with the stage of commercial production. Some to mention are Daunoxome, Ambisome, Doxil, Epaxel, etc. There are basically three major problems that we come across with the liposomal delivery systems i.e. uptake by reticulo-endothelial system, large-scale production and instability of phospholipids that pose as a hurdle in their commercial development.

1. Large scale production of liposomes

Preparation of liposomes involves various steps like evaporation of solvent system under reduced pressure, preparation of thin lipid film, sonication etc. These steps are difficult to carry out at large scale level especially the preparation of thin film. So, it is difficult to scale up liposome production from laboratory level to large-scale production level. Also adding organic solvents such as chloroform, methanol etc to solubilize and mix lipids is not recommended in such a high concentrations as per the regulatory norms.

2. Uptake of liposomes by Reticulo-endothelial system

For drug delivery, liposomes can be formulated as a suspension, as an aerosol, in a semisolid form such as a cream, gel or a dry powder and these can be administered .After systemic administration, which seems to be the most promising route for these carrier systems, liposomes are typically recognized as foreign particles and consequently endocytosed by cells of the mononuclear phagocyte system (MPS), mostly fixed Kupffer cells in the liver and spleen. This fate is very useful for delivering drugs to these cells but, in general, excludes other applications, including site-specific drug delivery by using ligands expressed on the liposome surface in order to bind to receptors over-expressed on the diseased cells. For this reason, a search for liposomes that could evade rapid uptake by the MPS started and few lipid compositions that prolonged liposome blood-circulation times have been discovered. PEG-coated or sterically stabilized liposomes are good examples in this regard.

3. Stability of liposomes

a. Physical stability

The stability of a pharmaceutical product usually is defined as the capacity of the delivery system to remain within defined or pre-established limits during the shelf life of the product. There is no established protocol for either accelerated or long–term stability studies for the liposomal

formulation. Classical models from colloidal science can be used to describe liposome stability. Colloidal systems are stabilized electrostatically, sterically or electrosterically. In addition the selfassembling colloids can undergoes fusion or phase change after aggregation. Liposome exhibit both physical and chemical stability characteristics. Generally, the physical characteristic describes the preservation of liposome structure and the chemical characteristic refers to molecular structure of liposomal components. (Hydrolysis and oxidation of phospholipid) Physically stable formulations preserve both liposome size distribution and the amount of material encapsulated. The stability problem overcomes by using appropriate techniques like freezing, lyophilization and osmification.

b. Plasma Stability

Although liposomes resemble biomembranes, they still are foreign objects for the host. Therefore, liposomes are recognized by the mononuclear phagocytic system (MPS) after interaction with plasma proteins. As a result, liposomes are cleared from the blood stream. These stability problems solve by using synthetic phospholipids, gangliosides, polymerization, coating liposomes with chitin derivatives, freeze drying, microencapsulation and particle coated with amphipathic polyethylene glycol.

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