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A REVIEW ON LIPOSOMES: NOVEL DRUG DELIVERY SYSTEM

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ABSTRACT

The all new potential therapeutics has poor pharmacokinetic and biopharmaceutical properties. Hence there is need to develop a suitable drug system that distributes the therapeuticallyactive drug molecule only to site of action, without affecting healthy tissue or organ. Liposomes are spherical shaped microscopic vesicles consisting of lipid bilayer in structure with phospholipids. and cholesterol being the main ingredients. These are superior carrier and have ability to encapsulate hydrophilic and lipophilic drugs and protect them from degradation. Liposomes are prepared by using various methods hand shaking method,sonication, freeze drying method, ethanol injection method, ether injection method, micro-emulsion method. The different application of liposomes is use for treatment of infection, anti-cancer, vaccination, for human therapy and gene delivery system. After the formulation, the evaluation of liposomes are checked by using physical parameters, chemical parameters, and biologically for the establish the purity and potency of various lipophilic constituents and establish the safety and suitability of formulation for therapeutic application.

KEYWORDS: Liposome, Lipid vesicle, Method of preparation, Evaluation and Applications.

INTRODUCTION

Liposomes were first discovered by Bangham in 1965, the name liposome made of two Greek words first "lipos" means fat and second somas means body.Liposomes are microscopic vesicles composed of one or more lipid bilayers which have the spherical shapeand size of liposome ranging from 20 to 1000 nm.Drug molecules can either be encapsulated in the aqueous space or entrapped into the lipid bilayers. The exact location of a drug in the liposome will depend upon its physicochemical characteristics and the composition of thelipids.[1- 4]

Liposomes membrane is composed of natural and synthetic lipids, which are relatively biocompatible, biodegradable and non-immunogenic material. Because of their unique bilayer structure properties, liposomes are used as carriers for both lipophilic and water-soluble molecules. Liposomes have attractive biological properties, including the biocompatibility and biodegradability. They show promise as active vectors due to their capacity to enhance the entrapment performance by increasing drug solubility, and stability; delivering encapsulated drugs to specific target sites, and providing sustained drug release. The structure of liposome shown in figure 1.[5]

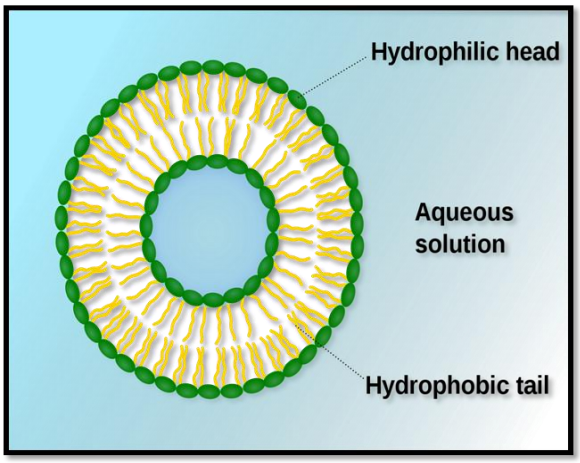


Fig. 1: Structure of liposome.

Mechanism of transportation of liposomes

The main action of liposome is interaction of liposomes with cells is either;

1. Simple adsorption (by specific interactions with cell-surface components, electrostatic forces or by non-specific weak hydrophobic forces) or
2. By following endocytosis (by phagocytic cells of the reticuloendothelial system, for example macrophages and neutrophils).
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, is much rare.

4. The fourth possible interaction is the exchange of bilayer components, for instance cholesterol, lipids, and membrane-bound molecules with components of cell membranes. It is often difficult to determine what mechanism is functioning, and more than one may function at the same time.[6-7]

Advantages

1. Biodegradable, biocompatible, flexible, Non-ionic and non-immunogenic.
2. Suitable for transport of both water soluble and lipid soluble drugs.
3. Increased efficacy.
4. Increased stability via encapsulation.
5. Reduces toxicity of the encapsulated agents.
6. They reduce exposure of sensitive tissues to toxic drugs.
7. They are increase the activity of chemotherapeutic drugs and can improved through liposome encapsulation this reduce deleterious effect that are observed at concentration similar to or lower than those required for maximum therapeutic activity.

Disadvantages

1. Production cost is high.
2. Leakage and fusion of encapsulated drug/molecules.
3. Liposomes have short half-life.[8]

Types of Liposome[9]

Types	Size
Small Unilamellar vesicles (SUV)	20-100nm
Large Unilamellar vesicles (LUV)	>100nm
Oligolamellar vesicles (OLV)	100-500nm
Multilamellar vesicles (MLV)	>500nm

Properties of Liposome

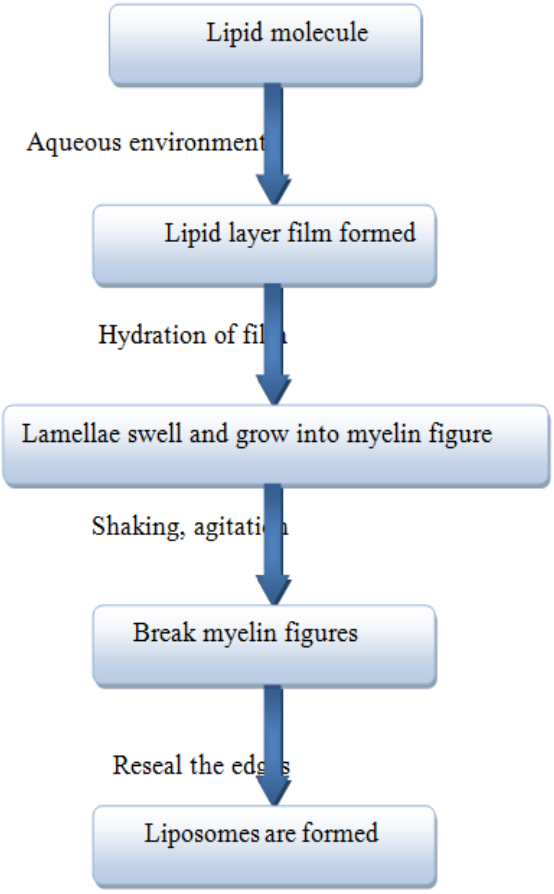
1. Liposomes are biocompatible, nontoxic, biodegradable and physicochemically stable.
2. Liposomes can entrap water-soluble (hydrophilic) pharmaceutical agents in their internal water compartment and water-insoluble (hydrophobic) pharmaceuticals into the membrane.
3. Liposome-incorporated pharmaceuticals are protected from the inactivating effect of external conditions; yet do not cause undesirable side reactions.
4. Liposomes provide a unique opportunity to deliver pharmaceuticals into cells or even inside individual cellular compartments.
5. Size, charge and surface properties of liposomes can be easily changed simply by adding new ingredients to the lipid mixture before liposome preparation.[10]

METHOD OF PREPARATION

1. Hand-shaken multilamellar vesicles method (MLVs).
2. Freeze-drying.
3. Sonication
4. French pressure cell method
5. Ethanol injection.
6. Ether injection.
7. Reverse phase evaporation method
8. Micro-emulsification method

1. Handshaking Method

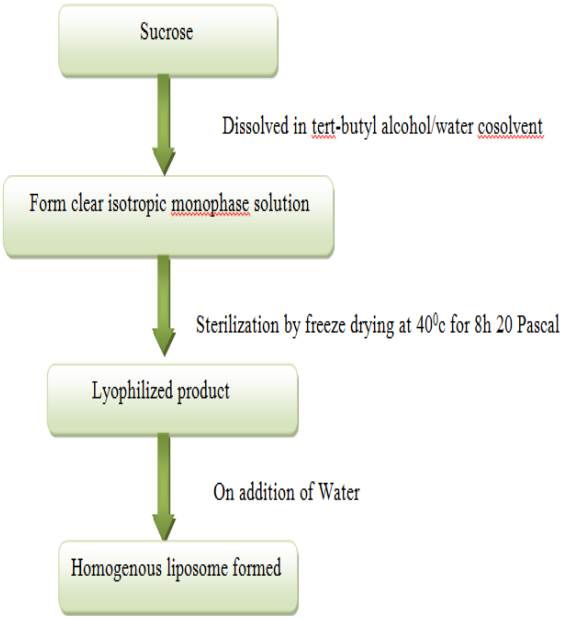
In order to produce liposome lipid molecules must be introduced into an aqueous environment. When dry lipid layer film is hydrated the lamellae swell and grow into myelin figures. Only mechanical agitation provided by vortexing, shaking, swering or pipetting causes myelin figures to break and reseal the exposed hydrophobic edges resulting in the formation of liposome can be made by hand shaken method.



1. Freeze Drying

It is based on the formation of a homogenous dispersion of lipids in water-soluble carrier materials. Liposome-forming lipids and water-soluble carrier materials such as sucrose were dissolved in tert-butyl alcohol/water cosolvent systems in appropriate ratio to form a clear isotropic monophase solution. Then the monophase solution was sterilized by filtration and filled into freeze-drying vials. In recent study, a laboratory freeze drier was used and freeze-drying process was as follows: freezing at -40^0C for 8 h; primary drying at 25^0C for 10 h.The chamber pressure was maintained at 20 Pascal during the drying process. On addition of water, the lyophilized product spontaneously forms homogenous liposome preparation. After investigation of the various parameters associated with this method it is found that

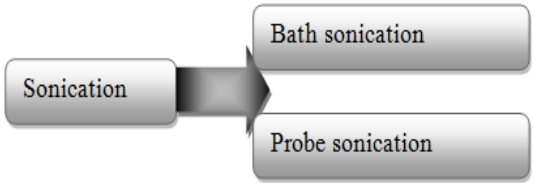
the lipid/carrier ratio is the key factor affecting the size and the polydispersity of the liposome preparation.[11]



2. Sonication

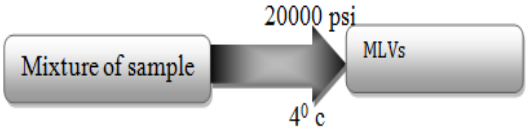
Sonication is the most preferable method for the preparation of SUVs. The MLVs are sonicated either with a bath type or probe type Sonicator under a passive atmosphere. In that bath Sonicator method liposome dispersion in a tube is placed in Sonicator. Control of temperature of lipid dispersion is easier in this method than sonicationthe dispersion directly using tip. Materials which are sonicated can be protected by kept in sterile container under an inert atmosphere. While in probe sonication the tip of sonicator is directly immersed into the liposome dispersion. The dispersion energy at the tip result in local overheating and therefore the vessel must be immersed into an ice bath. During the sonication in one hour more than 5% of the lipid can be de-esterified.The main disadvantages of this method are very low internal volume/encapsulation efficacy, possible degradation of phospholipids and compounds to be encapsulated, elimination of large molecules, metal pollution from probe tip, and presence of MLV along with SUV.[12]

Types of sonication



3. French pressure cell method

This method is simple rapid and reproducible and involves gentle handling of unstable materials. This method involves the extrusion of multilamellar vesicles at 20000 psi at 4^0 C through a small orifice. The advantage of this method is liposomes are somewhat larger than sonicated small lamellar vesicles. The disadvantage of this method is difficult to achieve the temperature.[13]



4. Ethanol injection method

In this method an ethanol solution of lipids is directly inject into an excess of saline rapidly and other aqueous medium through a fine needle. The ethanol is diluted in water and phospholipids molecules are dispersed evenly through the medium. From this method yields a high proportion of SUVs ranging of size 70-190nm only.

5. Ether injection

This method involves injecting the immiscible organic solution very slowly into an aqueous phase through a narrow needle at temperature of vaporizing of organic solvent. There is less risk of oxidative degradation and forms liposome of size only from 30-110nm.[14-16]

6. Reverse phase evaporation method

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 removal 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 byreverse 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 brief 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.

Advantages

1. With this method, high encapsulation efficiency up to 65% can be obtained in a medium of low ionic strength for example 0.01 M NaCl.
2. The method has been used to encapsulate small, large, and macromolecules. And the main benefit of this method is high encapsulation efficiency (about 80%).

Disadvantages

1. The contact of the materials to be encapsulated to organic solvents and to brief periods of sonication.
2. These conditions may possibly result in the breakage of DNA strands or the denaturation of some proteins.[17]

1. Micro-emulsification method

This method is also called asmicro-fluidizerwhich is used to prepare small vesicles from the concentrated lipid suspension. The lipid suspension is introduced into the fluidizer which pumps the fluid at very high pressure through 5 micrometer screen.Then it is force through long micro channels, which dried two streams of fluids and collides together at right angles at very high velocity. The fluid is collected and recycled through the pump or interaction chamber. The vesicles of spherical dimension are obtained.[18]

EVALUATION OF LIPOSOMES

The characterization parameters for purpose of evaluation could be classified into 3 broad categories which include physical, chemical, and biological parameters.

1. Vesicle shape and lamellarity

Various electron microscopic techniques can be used to assess the shape of the vesicles. The number of bilayers present in the liposome, i.e., lamellarity can be determined using scanning electron microscopy and P^{31}-Nuclear magnetic resonance analysis. Apart from knowing the shape and lamellarity, the surface morphology of liposomes can be assessed using freeze-fracture and freeze-etch electron microscopy.

2. Vesicle size and size distribution

Various techniques are described in literature for determination of size and size distribution. These include Light Microscopy, Electron Microscopy (especially Transmission Electron Microscopy), Laser light scattering Photon correlation Spectroscopy, Field Flow Fractionation, Gel permeation and Gel Exclusion. The most precise method of determine size of liposome is Electron Microscopy Since it permit one to view each individual liposome and obtain exact information about profile of liposome population over the whole range of sizes. Unfortunately, it is very time consuming and require equipments that may not always be immediately to hand. In contrast, laser light scattering method is very simple and rapid to perform but having disadvantage of measuring an average property of bulk of liposomes. Another more recently developed microscopic technique known as atomic force microscopy has been utilized to study liposome morphology, size, and stability.[19]

3. Zeta potential

To measure the zeta potential, a laser is used to provide a light source illuminating particles within the samples. The incident laser beam passes through the centre of the sample cell and the scattered light at an angle of about $13^0$is detected. When an electric field is applied to the cell, any particles moving through the measurement volume will lead to fluctuation of the detected light with a frequency proportional to the particle speed. This information is passed to a digital signal processor, then to a computer and hence potential zeta is calculated.[20-21]

4. Drug release study

In vitro drug release can be performed using the dialysis tube diffusion technique. The dialysis bag membrane should be selected following screening of various membrane, no drug adsorption may occur and the membrane should be freely permeable to the active ingredient (the cut off molecular weight shouldn't be a limiting step in the diffusion process). Some milliliters aliquot of liposome suspension is placed in the dialysis bag, hermetically tied and dropped in the receptor compartment containing the dissolution medium. The entire system is kept at 37^0C under continuous magnetic stirring and the receptor medium is closed to avoid evaporation of the dissolution medium. The kinetic experiments are carried out respecting the sink conditions in the receptor compartment. Samples of the dialysate are taken at various time intervals and assayed for the drug by HPLC, spectrophotometer or any other convenient method. The sample volume is replaced with fresh dissolution medium so as the volume of the receptor compartment remains constant. Every kinetic experiment is performed in triplicate and the average values are taken to establish the release profile of the drug from the liposome suspension.[22]

5. Fourier Transforms Infrared Spectroscopy (FT-IR)

Infrared spectra were obtained in a Spectrum One (FTIR with ATR, Perkin Elmer, USA), with scans in the frequency range of 4000 to 600 cm-1 at room temperature, with a 4 cm-1 spectral resolution. For each spectrum, 16 scans were co-added. Data was analyzed using the software FTIR Spectrum (Perkin Elmer).[23]

6. Entrapment Efficiency (EE)

The liposome suspension was ultra centrifuged at 5000 rpm for 15 minutes at 4°C temperature by using remicooling centrifuge to separate the free drug. A supernant containing liposomes in suspended stage and free drug at the wall of centrifugation tube. The supernant was collected and again centrifuged at 15000 rpm at 4°C temperature for 30 minutes. A clear solution of supernant and pellets of liposomes were obtained. The pellet containing only liposomes was resuspended in

distilled water until further processing. The liposomes free from unentrapped drug were soaked in 10 ml of methanol and then sonicated for 10 min. The vesicles were broken to release the drug, which was then estimated for the drug content. The absorbance of the drug was noted at 222 nm.[24]

7. Sterilization

Sterility of liposome maintained by using membrane filtration method. The liposomes are sensitive to high temperatures, as well as certain methods of radiation. Sterilizing with chemicals is not a viable option either, as it may affect the stability of the liposomes. The only method for creating sterile liposomes is by filtering the liposomes through a 0.22 µm membrane filter after production. This method is only suitable if the liposomes are smaller than 0.2 µm in diameter. This method does not remove viruses. Another option is filtering the initial solutions through 0.45 µm regenerated cellulose filters and glass fiber filters before starting production, thereafter the entire production process must be done under aseptic conditions.[25]

- Physical characterization evaluates various parameters including size, shape, surface features, lamellarity, phase behaviors and drug release profile.[26]

Parameters	Instruments for analysis
Vesicles shape and surface morphology	TEM and SEM
Vesicle size and size distribution	Dynamic light scattering TEM
Surface charge	Free flow electrophoresis
Electrical surface potential and surface pH	Zeta potential measurement and pH sensitive probes
Lamellarity	p31 NMR
Phase behavior	DSC , freeze fracture electron microscopy
Drug release	Diffusion cell/ dialysis

- Chemical Characterization includes those studies which establish the purity and potency of various lipophilic constituents.[27]

Parameters	Instruments for analysis
Drug interaction	FTIR
Phospholipids concentration	HPLC / Barrlet assay
Cholesterol concentration	HPLC / Cholesterol oxide assay
Phospholipids per oxidation	U.V observation
pH	pH Meter
Osmolarity	Osmometere

- Biological Characterization parameters are helpful in establishing the safety and suitability of formulation for therapeutic application.[28]

Parameters	Instruments for analysis
Sterility	Aerobic anaerobic culture
Pyrogenicity	Rabbit fever response
Animal toxicity	Monitoring survival rats

APPLICATIONS OF LIPOSOMES

1. Drug Targeting

The approach for drug targeting through liposomes involves the use of ligands like antibodies, sugar residues, apoproteins or hormones which are encapsulated in lipid vesicles. (e.g., antibodies, sugar residues, apoproteins or hormones), which are tagged on the lipid vesicles. The ligands recognizes specific site of receptor and thus causes the lipid vesicles attachment to such target site.

2. Treatment of human immunodeficiency virus (HIV) infections

Several antiretroviral nucleotide analogues have been developed for the treatment of patients suffering from the acquired immunodeficiency syndromes (AIDS). These include antisense oligonucleotide, which is a new antiviral agent that has shown potential therapeutic application against HIV-1

3. Enhanced antimicrobial efficacy/ safety

Antimicrobial agents have been encapsulated in liposomes for two reasons. First, they protect the entrapped drug against enzymatic degradation. For instance, the penicillins and cephalosporin are sensitive to the degradative action of β-lactamase, which is produced by certain microorganisms. Secondly, the lipid nature of the vesicles promotes enhanced cellular uptake of the antibiotics into the microorganisms, thus reducing the effective dose and the incidence of toxicity as exemplified by the liposomal formulation of amphotericin B.[29]

4. Liposomal Anticancer Therapy

Drug is encapsulated into the liposome to reduce toxic side effects of the drug and used for delivery of anticancer agents. Liposomes are passively targeted through intravenous administration. Liposomes in anticancer therapy Based on the early studies that showed that encapsulation of a drug inside of liposomes reduces its toxic side effects; the liposomes were

considered as attractive candidates for the delivery of anticancer agents.[30]

5. Immunological adjuvants in vaccines

Immune response can be enhanced by delivering antigens encapsulated within liposomes. Depending on the lipophilicity of antigens, the liposome can accommodate antigens in the aqueous cavity or incorporate within the bilayers. In order to enhance the immune response to diphtheria toxoid, liposomes were first used as immunological adjuvants.

6. Sustained release drug delivery

Liposomes can be used to provide a sustained release of drugs, which require a prolonged plasma concentration at therapeutic levels to achieve the optimum therapeutic efficacy. Drugs like cytosine Arabinoside can be encapsulated in liposomes for sustained release and optimized drug release rate in vivo.

7. Intraperitoneal administration

Tumors that develop in the intra-peritoneal (i.p.) cavity can be treated by administering the drug to i.p. cavity. But the rapid clearance of the drugs from the i.p. cavity results in minimized concentration of drug at the diseased site. However, liposomal encapsulated drugs have lower clearance rate, when compared to free drug and can provide maximum fraction of drug in a prolonged manner to the target site.[31]

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