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COMPARISON OF DIFFERENT METHODS USED TO PREPARE LIPOSOMES- A REVIEW

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ABSTRACT

Liposomes have received a lot of attention during the past 30 years as pharmaceutical carriers of great potential. More recently, many developments have been seen in the area of liposomal drugs-from clinically approved products to new experimental applications. 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. Liposomes are micro particulate lipid based 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 bio distribution. In this article, basic characteristics, method of preparation and marketed formulations of liposomes have been discussed. The success of liposomes as drug carriers has been reflected in a number of liposome based formulations, which are commercially available, or are currently undergoing clinical trials.

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INTRODUCTION

The name liposome is derived from two Greek words: 'Lipos' meaning fat and 'Soma' meaning body. A liposome can be formed in 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 [1]. Liposomes were first described by British hematologist Dr. Alec D Bangham in 1961, at the Babraham Institute, when he and R.W. Horne were testing the institute's new electron microscope by adding negative stain to dry phospholipids [2]. Liposomes are simple microscopic vesicles in which lipid bilayer structure is present with an aqueous volume entirely enclosed by a membrane, composed of lipid molecules [3]. There are number of components present in liposomes, with phospholipid and cholesterol being the main ingredients. The type of phospholipids includes phosphoglycerides and sphingolipids, together with their hydrolysis products [4]. Classification of liposomes is based on number of lamellae, composition, method of preparation and its size as shown in table 1 and 2. Liposomes can exhibit a range of sizes and morphologies upon the assembly of pure lipids or lipoidal mixtures suspended in an aqueous medium [5]. A common morphology which is analogous to the eukaryotic cellular membrane is the unilamellar vesicles. This is characterized by a single bilayer membrane which encapsulates an internal aqueous solution, thus separating it from the external solution. Both cationic amine head groups and anionic phospholipid head groups can form this single walled vesicle [6]. Vesicle size falls into nanometer to micrometer range: small unilamellar vesicles are 20–200 nm, unilamellar vesicles are 200 nm-1 µm, and giant unilamellar vesicles are larger than 1 µm [7].

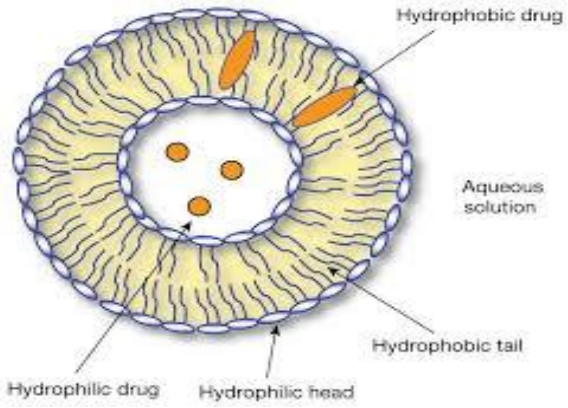

Fig 1: Structure of liposome.

Table 1: Classification of liposomes based on size and lamellarity.

Sr. No	Types	Size
1.	Multilamellar large vesicles(MLV)	(>0.5µm)
2.	Oligolamellar vesicles(OLV)	0.1-1 µm
3.	Unilamellar vesicles(UV)	All sizes
4.	Small unilamellar vesicles(SUV)	20-100 nm
5.	Medium sized unilamellar vesicles(MUV)	_
6.	Large unilamellar vesicles(LUV)	>100 nm
7.	Giant unilamellar vesicles(GU)	>1 µm
8.	Multivesicular vesicles(MVV)	usually >1 µm

Table 2: Classification of liposomes based on the method of preparation.

Sr.No	Types of Liposomes	Method of Preparation
1.	REV(Reverse Evaporation vesicles)	Single or Oligolemellar vesicles made by reverse osmosis
2.	MLV-REV	Multilamellar vesicles made by Reverse Phase Evaporation method
3.	SPLV	Stable Plurilamellar vesicles
4.	FATMLV	Frozen and Thawed MLV
5.	VET	Vesicles prepared by Extrusion method
6.	DRV	Vesicles prepared by Dehydration-Rehydration method

ADVANTAGES OF LIPOSOMES

- Liposomes are biocompatible, completely biodegradable, non-toxic in nature.
- They are suitable for delivery of hydrophobic, amphipathic and hydrophilic drugs [8].
- They protect the encapsulated drug from external environment.
- They reduce toxicity and increase stability-Since therapeutic activity of chemotherapeutic agent can be improved through liposome encapsulation. This reduces deleterious effects that are observed at concentration similar to or lower than those required for maximum therapeutic activity.
- They reduce exposure of sensitive tissue to toxic drugs.

DISADVANTAGES OF LIPOSOMES

- The production cost is high.
- Leakage and fusion of encapsulated drug/molecules can occur.
- It has short half-life. In reticuloendothelial system, particularly the Kupffer cells in the liver remove liposomes from the circulation [9].

METHODS OF LIPOSOMAL PREPARATION:

Passive Loading Techniques

Mechanical dispersion methods:

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

Solvent dispersion methods:

- Ethanol injection
- Ether injection
- Double emulsion
- Reverse phase evaporation vesicles
- Stable pluri- lamellar vesicles c. Detergent removal methods:
- Dialysis
- Column chromatography
- Dilution
- Reconstituted Sendai virus enveloped

Detergent removal method

Active loading technique

The conventional methods for preparing liposomes include solubilizing the lipids in organic solvent, drying down the lipids from organic solution, dispersion of lipids in aqueous media, purification of resultant liposomes and analysis of the final product. Of all the methods used for preparing liposomes, thin-film hydration method is the most simple and widely used one. Multi lamellar large vesicles (MLV) are produced by this method within a size range of 1 – 5 µm. If the drug is hydrophilic it is included in the aqueous buffer and if the drug is hydrophobic, it can be included in the lipid film. But the drawback of this method is poor encapsulation efficiency (5 – 15% only) for hydrophobic drugs. By hydrating the lipids in presence of organic solvent, the encapsulation efficiency of the MLV can be increased [10]. Large Unilamellar vesicles (LUV) can be prepared by solvent injection, detergent dialysis; calcium induced fusion and reverse phase evaporation techniques. Small Unilamellar vesicles (SUV) can be prepared by the extrusion or sonication of multi lamellar vesicles or large unilamellar vesicles. All these preparation methods involve the usage of organic solvents or detergents whose presence even in minute quantities can lead to toxicity. In order to avoid this, other methods like polyol dilution, bubble method and heating method have been developed without using any organic solvents or detergents [11].

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 ways to modify their ultimate properties. The post hydration treatments include vortexing, sonication, freeze thawing and high- pressure extrusion [12].

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 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.

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 [13]. 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 from 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 unilamellar vesicle formed spontaneously.

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 trans membrane gradient, such as electric, ionic (pH) or specific salt (chemical potential) gradient [14]. 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 methods 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 [15].
- 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 [16].

MARKETED PRODUCTS:

In clinical applications, liposomal drugs have been proven to be most useful for their ability to passively accumulate at site of increased vasculature permeability, when their average diameter is in the ultra-filterable range, and for their ability to reduce the side effects of the encapsulated drugs relative to free drugs. This has resulted in an overall increase in therapeutic index, which measures efficacy over toxicity. Liposomes have diverse applications in the treatment of infections, vaccine and gene delivery, cancer treatment, lung diseases and skin conditions as shown in table 3.

Table 3: Marketed Products.

NAME	TRADE NAME	COMPANY	INDICATION
Liposomal Amphoterecin B	Abelcet	Enzon	Fungal infection
Liposomal Amphoterecin B	Ambisome	Gilead Sciences	Fungal and Protozoal infection
Liposomal cytarabine	Depocyt	Pacira(Skye Pharma)	Malignant lymphomatous meningitis
Liposomal Daunorubicin	Daunoxome	Gilead Sciences	HIV-related Kaposi's Sarcoma
Liposomal doxorubicin	Myocet	Zeneus	Combination therapy with cyclophosphamide in metastatic breast cancer
Liposomal IRIV	Epaxal	Crucell	Hepatitis A
Liposomal IRIV Vaccine	Inflexal V	Berna Biotech	Influenza
Liposomal morphine	DepoDur	Skye Pharma, Endo	Post-surgical analgesia
Liposomal verteporfin	Visudyne	QLT, Novartis	Age-related macular degeneration, pathologic myopia, ocular histoplasmosis
Liposome- Proteins SP-B and SP-C	Curosurf	Chiesi Farmaceutici, S.p.A	Pulmonary Surfactant for Respiratory Distress Syndrome (RDS)
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
Liposomal vincristine	Marqibo	Spectrum pharmaceuticals	Acute Lymphoblastic Leukemia (ALL) and Melanoma

CONCLUSION

A number of drug candidates which are highly potent and have low therapeutic indication can be targeted to the required diseased site using the liposomal drug delivery system. Drugs encapsulated in liposomes can have a significantly altered pharmacokinetics. The efficacy of the liposomal formulation depends on its ability to deliver the drug molecule to the targeted site over a prolonged period of time, simultaneously reducing its (drug's) toxic effects. The drugs are encapsulated within the phospholipid bilayers and are expected to diffuse out from the bilayer slowly. Various factors like drug concentration, drug to lipid ratio, encapsulation efficiency and *in- vivo* drug release must be considered during the formulation of liposomal drug delivery systems. The development of deformable liposomes and ethosomes along with the administration of drug loaded liposomes through inhalation and ocular route are some of the advances in the technology. Thus liposomal approach can be successfully utilized to improve the pharmacokinetics and therapeutic efficacy, simultaneously reducing the toxicity of various highly potent drugs.

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