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PHARMACEUTICAL LIPOSOMAL DRUG DELIVERY: A REVIEW OF NEW DELIVERY SYSTEMS

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ABSTRACT

Liposome are most placed acquiring in pharma industries and very useful in the various drug delivery system used to target the drug to particular tissue because of structural similarity between lipid bi-layer and cell membrane, liposome can easily penetrate. Liposome can be encapsulating both hydrophilic and hydrophobic material and are utilizes as a drug carries in drug delivery. Liposomes very useful in certain disease and easily prepare. Liposomes are highly biocompatible, with application ranging from delivering enzymes, antibacterial, antiviral drugs antiparasite drug, transdermal transporters, fungicides, diagnostic tools and adjuvant for vaccines. This paper mainly focus on exclusively scalable techniques and also focus on strength, respectively, limitations in respect to industrial applicability and regulatory requirements concerning liposomal drug formulation based on FDA and EMEA.

KEYWORDS: Liposome, Regulatory requirements, Hydrophilic and hydrophobic.

INTRODUCTION

Liposomes consist of vesicles composed of bilayers or multilayers that contain or have phospholipids and cholesterol surrounding an aqueous compartment. Drug is entrapped within the liposome and is released from the liposome for absorption at the intestinal membrane surface. This dosage form received considerable and this may well relate to their absorption enhancing ability, the feasibility of their use to promote drug absorption is uncertain drugs or chemical entities. Advances in combinatorial chemistry have led to the discovery of a wide number of new chemical entities (NCE) or drugs that have a potential therapeutic action on the biological systems. But most of the NCEs or drugs being discovered provide a challenge or produce most difficulties to the formulation scientist because of their physicochemical properties like poor solubility and permeability. A majority of anti-neoplastic agents, which are highly cytotoxicity to tumor cells in vitro, affect the normal cells also. This is due to their low therapeutic index (TI), i.e., the dose required to produce anti-tumor effect is toxic to normal cells. Such drugs have to be targeted to a specific site (diseased site) in order to reduce their toxic effects to normal tissues. Hence, an efficient drug delivery system is required to present the maximum fraction of administered dose at the target site or valuable for targeted sites. (Amidon et al., 1995).

Liposomes are colloidal carriers, having a size range of 0.01–5.0μm in diameter. Indeed these are bilayer vesicles that are formed when phospholipids are hydrated in excess of aqueous medium or aqueous solution. Liposomes have got a potential advantage of encapsulating hydrophilic as well as hydrophobic drugs and targeting them to the phospholipids are hydrated in excess of aqueous medium.

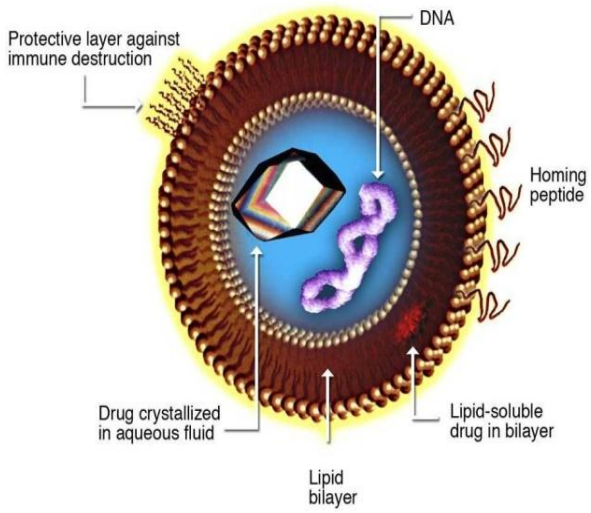

Fig. 1: Liposome for drug delivery.

Size Range of Liposome

Types of liposome	Size range (nm)
Small unilamellar vesicles	20-40
Medium unilamellar vesicles	40-80
Large unilamellar vesicles	100-1000

- **Multilamellar Vesicles (MLV)**

MLV have a size greater than 0.1μm and consists of two or more bilayer. Their method of formulation is simple and very easy to carry which includes thin–film hydration method or hydration of lipids in excess of organic solvent. They are mechanically stable on long storage. Due to the large size, they are cleared early orrapidly by the reticulo-endithelial system (RES) cells and hence can be beneficial for various targeting the organs of RES. MLV have a moderate trapped volume, i.e., amount of aqueous volume to lipid ratio. The drug entrapment or corporate into the vesicles can be enhanced by slower rate of hydration and gentle mixing. Hydrating thin films of dry lipids can also easily enhance encapsulation efficiency. Subsequent lyophilization and rehydration after mixing with the aqueous phase (containing the drug) can yield MLV with good encapsulation efficiency i.e. 40%.

- **Large Unilamellar Vesicles (LUV) (Donaruma Et Al., 1985)**

This class of liposomes particularly large unilamellar vesicles consists of a single bilayer and has a size greater than 0.1μm. They have higher encapsulation efficiency, since they can hold a large volume of solution in their cavity.

They have high trapped volume and can be useful for encapsulating hydrophilic drugs. Most useful advantage of LUV is that less amount of lipid is required for encapsulating large quantity of drug. LUV can be formulated by various methods like ether injection, detergent dialysis and reverse phase evaporation techniques. Apart from these methods, freeze thawing of liposomes, dehydration/ rehydration of SUV and slow swelling of lipids in non-electrolyte solution can a prepare LUV.

- **Small unilamellar vesicles (SUV) (Abra et al., 1981)**

SUV are smaller in size (less than 0.1 μm) when compared to MLV and LUV, and have a single bilayer. They have a low entrapped aqueous volume to lipid ratio and characterized by having long circulation half life. SUV can be prepared by using solvent injection method (ethanol or ether injection methods) or alternatively by reducing the size of MLV or LUV using sonication or extrusion process under an inert atmosphere like nitrogen or Argon. The sonication can be performed using either a bath or probe type sonicator. SUV can also be achieved by passing MLV through a narrow orifice under high pressure. These SUV are susceptible to aggregation and fusion at lower or negligible/ no charge.

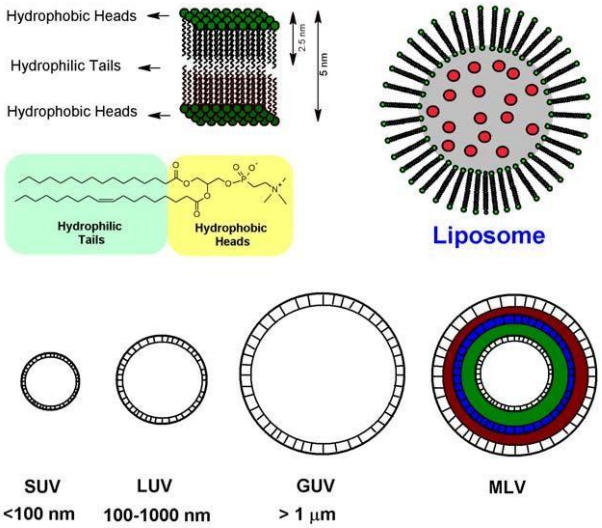

Fig. 3: Basic structures of liposome and their type.

METHODS OF PREPARATION: (ABRA ET AL., 1981)

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. MLV are produced by this method within a size range of 1–5μm. 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. LUV can be prepared by solvent injection, detergent dialysis; calcium induced fusion and reverse phase evaporation techniques. SUV can be prepared by the extrusion or sonication of MLV or LUV. 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. Detailed procedures for liposome preparation can be obtained from literature. (Tirrell et al., 1971) (Sahoo et al., 2003).

General Methods of Preparation (Gabizon Et Al., 1998)

All the methods of preparing the liposomes or formulating the liposomes involve four basic stages:

Drying down lipids from organic solvent.	Dispersing the lipid in aqueous media.
Purifying the resultant liposome.	Analyzing the final product.

Method of liposome preparation and drug loading (Abra et al., 1981)

Following methods are used for the preparation of

liposome:

a) Passive loading techniques b. Active loading technique

Passive loading techniques include three different methods

a. Mechanical dispersion method.
b. Solvent dispersion method.
c. Detergent removal method (removal of non encapsulated material).

Mechanical dispersion method (Tirrell et al., 1971)

The following are types of mechanical dispersion methods:

1	Sonication	5	Micro-emulsification
2	French pressure cell: extrusion	6	Membrane extrusion.
3	Freeze-thawed liposomes.	7	Dried reconstituted vesicles.
4	Lipid film hydration by hand shaking, non-hand, shaking or freeze drying.		

Sonication

Sonication is helpful the most extensively used method for the preparation of SUV. Here, MLVs are sonicated either with a bath type sonicator or aprobe sonicator under a passive atmosphere. 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.(Sunil et al., 2005)

There are two Sonication techniques: (Tirrell et al., 1976)

1. Probe Sonication

The tip of a sonicator is directly engaged 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.

2. Bath sonication

The liposome dispersion in a cylinder is placed into a bath sonicator. Controlling the temperature of the lipid dispersion or scatter is usually easier in this method, in contrast to Sonication by dispersal directly using the tip. The material being sonicate can be protected in a sterile vessel, dissimilar the probe units, or under an inert atmosphere.

French Pressure Cell: (Sahoo Et Al., 2003)

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.[25] 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.

Solvent Dispersion Method (Tirrell Et Al., 1976)

Ether injection (solvent vaporization) A solution of lipids dissolved in diethyl ether or ether-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 (Sahoo Et Al., 2003)

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 difficult because it forms into azeotrope 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 (Sahoo Et Al., 2003)

This method provided a progress in liposome technology, since it allowed for the first time the preparation of liposomes with a high aqueous space-to-lipid 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 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 liposome 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 for 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%).

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).

Gel-Permeation Chromatography

In this method, the detergent is depleted by size special chromatography. Sephadex G-50, Sephadex G-l 00 (Sigma-Aldrich, MO, USA), Sepharose 2B-6B, and Sephacryl S200-S1000 (General Electric Company, Tehran, Iran) can be used for gel filtration. 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, pretreatment is necessary. The pre-treatment is done by

Stealth Liposomes and Conventional Liposome

Although liposomes are like biomembranes, they are still foreign objects of the body. Therefore, liposomes are known by the mononuclear phagocytic system (MPS) after contact with plasma proteins. Accordingly, liposomes are cleared from the blood stream. These stability difficulties are solved through the use of synthetic phospholipids, particle coated with amphipathic polyethylene glycol, coating liposomes with chitin derivatives, freeze drying, polymerization, micro-encapsulation of gangliosides. Coating liposomes with PEG reduces the percentage of uptake by macrophages and leads to a prolonged presence of liposomes in the circulation and, therefore, make available abundant time for these liposomes to leak from the circulation through leaky endothelium.

A stealth liposome is a sphere-shaped vesicle with a membrane composed of phospholipid bilayer used to deliver drugs or genetic material into a cell. A liposome can be composed of naturally derived phospholipids with mixed lipid chains coated or steadied by polymers of PEG and colloidal in nature. Stealth liposome are attained and grown in new drug delivery and in controlled release. This stealth principle has been used to develop the successful doxorubicin-loaded liposome product that is presently marketed as Doxil (Janssen Biotech, Inc. Horsham, USA) or Caelyx (Schering-Plough Corporation, Kenilworth, USA) for the treatment of solid tumor. Recently impressive therapeutic improvements were described with the use of corticosteroid loaded liposome in experimental arthritic models. The concerning on the application of stealth liposome has been on their potential to escape from the blood circulation. However, long circulating liposome may also act as a reservoir for prolonged release of a therapeutic agent. Pharmacological action of vasopressin is formulated in long circulating liposome.

Liposomes In Anticancer Therapy (Johnston Et Al., 2007)

Numerous or various type of liposome formulations of numerous anticancer agents were shown to be less toxic than the free drug. Anthracyclines are drugs which stop the growth of dividing cells by intercalating into the DNA and, thus, kill mainly rapidly dividing cells. These cells are not only in tumors but are also in hair,

gastrointestinal mucosa, and blood cells; therefore, this class of drug is very toxic.

The most used and studied is Adriamycin (commercial name for doxorubicin HCL Ben Venue Laboratories, Bedford, Ohio). In addition to the above-mentioned acute toxicities, its dosage is limited by its increasing cardio toxicity. Numerous diverse formulations were tried. In most cases, the toxicity was reduced to about 50%.

These include both acute and chronic toxicities because liposome encapsulation reduces the delivery of the drug molecules towards those tissues. In some cases, such as systemic lymphoma, the effect of liposome encapsulation showed enhanced efficacy due to the continued release effect, i.e., longer presence or long time of therapeutic concentrations in the circulation, while in several other cases, the sequestration of the drug into tissues of mononuclear phagocyte system actually reduced its efficacy. Applications in man showed, in general, reduced toxicity and better tolerability of administration with not too encouraging efficacy. Several different formulations are in different phases of clinical studies and show mixed results which are very useful.

FUTURE PERSPECTIVE

Significant development has been reported on combination of the liposome based technology with temporary depot polymeric-based technology in sustaining drug release over prolonged periods of time. However, combination of both drug delivery technologies into a single model of drug delivery has been reported to be associated with inadequate drug release. Integration of the more advanced types of liposome based technologies such as targeted- or stimuli-sensitive liposomes in this system can enhance therapeutic efficacy. In addition, targeted liposome formulations, with targeted moieties such as antibodies, peptide, glycoprotein, polysaccharide, growth factors, carbohydrate, and receptors may increase liposomal drug accumulation in the tissues/cells via over expressed receptors, antigen, and unregulated selections. Sensitivity of liposome to pH, light, magnetism, temperature, and ultrasonic waves can enhance therapeutic efficacy.

Some polymeric systems have demonstrated some disadvantages in this application such as non-degradability that results in insufficient drug release. The use of a combination liposomal-based system with natural and/or synthetic polymeric biodegradable and/or non-degradable polymers may add strength to the depot while improving liposomal release profile. In summary, the combination system, as model of sustained release of drug-loaded liposome from temporary polymeric depots, has been declared successful but system improvements are demanded. Since this system is implantable, it may be useful in future for the management of chronic diseases such as Aid Dementia Complex, Tuberculosis, Cancer, or Neurodegenerative disorders, such as Parkinson's and Alzheimer's disease, which normally require regular doses over prolonged periods of time.

CONCLUSION

A number of drug candidates or chemical molecules which are highly potent and have low therapeutic indication can be targeted to the required diseased site using the liposomal drug delivery system. Liposomes have been used in a broad range of pharmaceutical applications. 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 phospholipids bilayer 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. Finally, liposomal drugs exhibit reduced toxicities and retain enhanced efficacy compared with free complements. Thus liposomal approach can be successfully utilized to improve the pharmacokinetics and therapeutic efficacy, simultaneously reducing the toxicity of various highly potent drugs. However, property of liposome based on the pharmaceutical applications and available products, we can say that liposomes have not definitely but surely most acquire space in pharma Industry and also established their position in modern de.

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