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REVIEW ARTICLE

Lipopharmaceuticals: A Critical Review Focused on Recent Advances in Liposomal Drug Delivery

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ABSTRACT

The first nanoscale drug to be approved for clinical use in 1995 was known as a liposome. Since then, the technology has grown substantially, and current work on liposome drug delivery systems has revoluted remarkable developments with significant clinical implications. Liposomal drug delivery includes long-circulating liposomes, stealth liposomes, nebulized liposomes, and elastic liposomes for topical, oral, and transdermal delivery, and covalent lipid-drug complexes for improved drug plasma membrane crossing and targeting to specific organelles and other recent advancements. This review is based on the liposomal drug delivery system, its introduction, classification, methods of preparation, types of targeting the liposomal drug delivery, applications, recent advancements in liposomes, and its application.

Keywords: Liposomes, Non-PEGylated Liposome technology, Nebulized liposomes, Stimuli-responsive liposomes.

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INTRODUCTION[1-3]

Small artificial vesicles of spherical shape that can be created from cholesterol and natural, nontoxic phospholipids are called liposomes. Liposomes are amphipathic in aqueous media. Their thermodynamic phase properties and self-assembling characteristics influence entropically focused confiscation of their hydrophobic sections into spherical bilayers called as lamellae. Properties of liposomes are different from lipid composition, surface charge, size, and the method of preparation. The choice of bilayer components depends on 'rigidity' or 'fluidity' and the charge of the bilayer. Generally, liposomes are definite spherical vesicles with particle sizes ranging from 30 nm to several micrometers, which consist of one or more lipid bilayers surrounding aqueous units, where the polar head groups are oriented in the pathway of the interior and exterior aqueous phases. Liposomes can be used as carriers for cosmetic and pharmaceutical industries as can be used in encapsulation of unstable compounds as antimicrobials, antioxidants, flavors, and bioactive elements in liposomes form. Because of their biocompatibility, biodegradability, low toxicity, and ability to trap both hydrophilic, lipophilic drugs and simplify site-specific drug delivery to tumor tissues, liposomes have increased rates both as an investigational system and commercially as a drug delivery system.[1]

Unsaturated phosphatidylcholine species obtained from natural sources give much more permeable and less stable bilayers, whereas the saturated phospholipids with long acyl chains form a rigid, rather impermeable bilayer structure. Phospholipids form closed structures when they are hydrated in aqueous solutions. Such vesicles which have one or more phospholipid bilayer membranes can transport lipid drugs, depending on the nature of drugs.[1,2]

Liposomal Encapsulation Technology (LET) is the advanced technique of generating sub-microscopic foams called liposomes, which helps in transmitting drugs that act as remedial promoters to the assured body organs.[1,3] These 'liposomes' form a barrier around their contents, which is resistant to enzymes in the mouth and stomach, alkaline solutions, digestive juices, bile salts, and intestinal flora that are generated in the human body, as well as free radicals. The contents of the liposomes are, therefore, protected from oxidation and degradation. This protective phospholipid barrier remains undamaged until the contents of the liposome are delivered to the exact target.[3]

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CLASSIFICATION OF LIPOSOMES[2-5]

Liposomes are classified based on structure

Unilamellar vesicles

- *Small unilamellar vesicles (SUV):* size ranges from 20–40 nm

- *Medium unilamellar vesicles (MUV):* size ranges from 40-80 nm.
- *Large unilamellar vesicles (LUV):* size ranges from 100 nm-1,000 nm

Oligolamellar Vesicles (OLV)

These are made up of 2-10 bilayers of lipids surrounding a large internal volume

Multilamellar Vesicles (MLV)

They have several phospholipid bilayers. They can compartmentalize the aqueous volume in an infinite number of ways. They differ according to the way by which they are prepared. The arrangements can be onion-like arrangements of concentric spherical bilayers of LUV/ MLV enclosing a large number of SUV etc.[5,6]

METHOD OF PREPARATION[4-6]

Physical dispersion methods

The physical dispersion method is of two types in which aqueous volumes enclosed within the lipid membranes, which is a very small proportion of total volume used for preparation. In these methods, MLVs are formed. So a large amount of water-soluble drug is wasted during preparation. But lipid-soluble drug can be encapsulated to high percentage.[4]

Hand Shaken Method

Simplest and widely used method in which mixture of lipids and charged components are dissolved with chloroform and methanol mixture in a ratio of 2:1 ratio, and then this mixture is introduced into a round-bottomed flask. A rotary evaporator is fixed with a round bottom flask connected with a vacuum pump, which will rotate at 60 RPM.[5] The organic solvents will evaporate after some time, and dry residue will get formed at the walls of the round bottom flask. The evaporator will get replaced by nitrogen from the vacuum pump. The flask is then removed from the evaporator and fixed onto the lyophilizer` to remove residual solvent. Then the flask is again flushed with nitrogen, and 10 mL of phosphate buffer is added. The flask is attached to the evaporator again and rotated at about 60 RPM speed for 30 minutes or until all lipid has been removed from the wall of the flask. Finally, the milky white suspension will get formed then allowed to stand for two hours to complete the swelling process to give MLVs.[4]

Non-Shaking method

This method I quite the same as the hand-shaken method except swelling procedure. The solution of lipid in chloroform and methanol mixture is spread over the flat bottom of the conical flask. By the flow of nitrogen, the solution will get evaporated at room temperature through the flask without disturbing the solution. Until the opacity of the dried film gets disappear, water-saturated nitrogen is passed through the flask. After drying, a lipid is swelled by the addition of bulk liquid. Then the flask is maintained at an inclined position. 10 to 20 mL sucrose solution will introduce down the side of the flask, and then the flask is returned to an upright position. The solution is allowed to run gently over the lipid layer on the bottom of the flask; then, the flask is flushed with nitrogen sealed and allowed to stand for swelling purpose at 37°C forms milky suspension, then the milky suspension is centrifuged at 1200 rpm for 10 minutes. From this method, MLV's and LUV's will form. The layer of MLVs floating on the top of the surface is removed. From the remaining fluid, LUVs can be produced.[5]

Solvent Dispersion Methods

In this method, lipids first get dissolved in an organic solvent and then contacted with an aqueous phase containing materials to be entrapped within the liposome. At the interface between the organic and the aqueous phases, the phospholipids form a monolayer; this is an important step to form the bilayer of the liposome.

Ethanol injection method

This method is the simplest method for the formation of liposomes. In this method, an ethanol solution of the lipids is directly injected rapidly to an excess of saline or another aqueous medium with a fine needle. The ethanol is diluted in water, and phospholipids molecules are dispersed evenly through the medium. This procedure helps in the formation of SUV's (about 25 nm diameter).

Ether injection

The immiscible organic solution injected very slowly into an aqueous phase through a fine needle at a temperature at which organic solvent can vapourize. In this method, the lipids are treated carefully should have less risk of oxidative degradation. A long time is required for the process and careful control is needed for the introduction of lipid solution is the major disadvantage of ether injection method.[6]

DETERGENT SOLUBILIZATION TECHNIQUE

In this method, the micelles form when phospholipids come in contact with detergents, which get associated with phospholipid molecules. The concentration of detergent in water at which micelles start to form is called CMC. Below CMC, the detergent molecule exists in a free solution. Micelle forms in large amounts when a detergent molecule is dissolved in water at a higher concentration than the CMC.[3,5]

TARGETING OF LIPOSOMES[7-9]

Passive Targeting

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 IV administration of liposomal antigen elicited spleen phagocyte mediated antibody response whereas the non-liposome associated antigen failed to elicit an antibody response.

As a means of passive targeting, such usually administered liposomes have been shown to be rapidly cleared from the bloodstream and taken up by the RES in the liver spleen. Thus the capacity of the macrophages can be exploited when liposomes are to be targeted to the macrophages. This has been demonstrated by the successful delivery of liposomal antimicrobial agents to macrophages.[7]

Active Targeting

The liposome physically prepared such that the lipophilic part of the adapter is anchored into the membrane during the formation of the membrane.[8] The hydrophilic part on the surface of the liposome, to which the targeting agent should be held in a satirically correct position to bond to the receptor on the cell surface. The active targeting can be brought about using:

- *Immuno liposomes:* These are conventional or stealth liposomes with attached antibodies or another recognition sequence [e.g., Carbohydrate determinants like glycoprotein] the antibody-bound, direct the liposome to specific antigenic receptors located on a particular cell. Glycoprotein or Glycolipid cell surface component that plays a role in cell-cell recognition and adhesion.[7,8]
- *Magnetic liposomes:* Contain magnetic iron oxide. An external vibrating magnetic field can direct these liposomes in their delivery sites.[8]
- *Temperature or heat-sensitive liposomes:* heat-sensitive liposomes are composed of the transition temperature, which is just above body temperature. After reaching the site, externally heat the site for the release of the drug.[9]

APPLICATIONS OF LIPOSOMES IN PHARMACOLOGICAL ASPECTS[10-13]

Liposomes in Parasitic Diseases and Infections

When Conventional liposomes were digested by phagocytic cells in the body after intravenous management, so liposomal drug delivery system is ideal for targeting drug molecules into these macrophages.' Trojan horse-like' parasitic diseases which normally exist in the cell of MPS. They consist of leishmaniasis and several fungal infections.

Liposomes accumulate in cell population which is infected, so liposomal drug delivery was proposed. There are several continuing studies with various anti-parasitic liposome formulations in humans. These formulations use mostly ionosphere amphotericin B and are transplanted from very successful and prolific area of liposome formulations in antifungal therapy.[10]

Liposomes in Anticancer Therapy

Anticancer agents when administered in the form of liposomes were shown to be less toxic than the free drug. Anthracyclines resists the growth of dividing cells by intercalating into the DNA and kill mainly rapidly dividing cells. These cells are found in tumors and hair, gastrointestinal mucosa, and blood cells; but the class of this drug is toxic.[11] Liposomal encapsulation reduces the delivery of the drug molecules towards these tissues which causes acute and chronic toxicities. The efficiency was in many cases compromised due to the reduced bioavailability of the drug, especially if the tumor was not phagocytic or located in the organs of mononuclear phagocytic system. 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 of therapeutic concentrations in the circulation, while in several other cases, the sequestration of the drug into tissues of a mononuclear phagocytic system reduced its efficacy.[12]

RECENT ADVANCEMENTS

Stealth liposomes[13-15]

First-generation liposomes were based on lipids bilayer membranes, which have shown poor stability and rapid clearance after administration due to affected by physical interactions with circulating protein in blood and protein adsorption.

In order to overcome from the shortcoming, longer circulating liposomes were formulated by coating the liposomal shell with inert lipophobic polymers as propylene glycol (PEG) are known as stealth liposomes. Dose-independency, increased bioavailability, non saturability are some of the advantages of stealth liposomes. This type of liposome can be formulated by various long chains of PEG which will be covalently attached to hydrocarbon chain anchors.

Stealth Liposomes are suitable drug delivery vehicles for active targeting to targeted cells with the help of prolonged circulation time and a protective hydrophilic layer. Active targeting ligand coupled with the carriers

has included small molecule ligands peptides and monoclonal antibodies.

Non-PEGylated Liposome Technology[14]

Non-PEGylated Liposomal Technology (NPLT) is a recent drug-delivery system that introduced in cancer therapy which eliminate the side effects associated with PEG such as hand-foot syndrome (HFS). The NPLT Doxorubicin (NPLD) injection provides a better safety profile, which reduces the cardiac toxicity associated with DOX, and dose-limiting toxicity linked with the use of Doxil®, such as Hand-Foot Syndrome(HFS). This is achieved by a combination of specific composition and a unique manufacturing process of the NPLD liposome, which gives it the desired physicochemical properties. NPLD does not have a PEG coating, so they are not associated with the painful HFS.

DepoFoam™ Liposome Technology[15,17]

In combination with cyclophosphamide, Myocet® is a NPLD manufactured by Elan Pharmaceuticals, Princeton, NJ, approved in Europe and Canada for the treatment of metastatic breast cancer. Extended-release drug delivery technology as DepoFoam™ Liposome Technology is a proprietary, introduced by Pacira Pharmaceuticals, Inc., Parsippany, NJ, USA which consists of microscopical spheroids (3–30 μm) with granular structure and single-layered lipid particles which composed of a honeycomb of numerous nonconcentric internal aqueous chambers containing the bounded drug. Each particle contains numerous non-concentric aqueous chambers bounded by a single bilayer lipid membrane. In this technology, the drug get encapsulated in its multivesicular liposomal platform without modification of their molecular structure. The multivesicular liposomes releases drug(s) over a required period of time, ranging from one to thirty days. It has improved patient care by providing a remarkable solution for medications that require frequent multiple injections and have a short period of action or side effects.

Transferosomes[15,16]

Liposomes can be sued as topical applications also in the form of transferases. The major advantages of topical liposomal formulations as they have less side effects due to undesirable high systemic absorption of a drug and enhance the accumulation of drug at the site of administration as a result of high substantively of liposomes with biological membranes, avoid first-pass metabolism, the improved utility of short half-life drugs, improves physiological and pharmacological response as well as avoid of fluctuations in drug level and convenient to patients.

Ethosomes[14]

Ethosomes improves the penetration efficiency of liposomes on skin. Ethosomes are phospholipid based elastic nanovesicles containing high content of ethanoll (25–45%). Ethanol can interact with the polar head group region of lipids, which increases the fluidity of lipids and enhance the cell membrane permeability. Ethanol may also provide vesicles with soft, flexible characteristics that allow them to penetrate more easily in to deep layers of skin.

Pharmacosomes[16]

Pharmacosomes are amphiphilic phospholipid complex of drug that binds to phospholipids through covalent, electrostatic or hydrogen bonding. Pharmacosomes improve the bioavailability of poorly water-soluble as well as poorly lipophilic drugs. Based on chemical structure, pharmacosomes are of two types ultrafine micelle or hexagonal aggregates. Commonly used Pharmacosomes are formulated by the solvent evaporation method followed by drug complexation step.

Thermosensitive Liposomes[12,18]

The thermosensitive liposome can be formulated by using lysophosphatidylcholine and is intended for the treatment of cancerous diseases. Currently, this type of liposomes is under clinical trials(Phase II/III). It also increases the chances of the performance of drugs.

Nebulized Liposomes & Stimuli-responsive liposomes[1,4,13,17]

Nebulized liposomes are formed by formulating with sustained-release liposomes of phospholipids and cholesterol encapsulating amikacin. The clinical trials for the use of nebulized liposomes for the treatment of cystic fibrosis patients with chronic lung infection is under process.

Two main types of triggers are involved in nebulized and stimuli-responsive liposomes as remote triggers such as heat, ultrasound and light, and local triggers such as enzymes and pH. Stimuli-responsive liposomes have been studied despite a growing body of work that has been fairly disappointing in practice. There have been some systems that have progressed to clinical trials as thermosensitive liposomes as ThermoDox contains lysophosphatidylcholine and is intended for the treatment of various cancers including primary liver cancer, recurrent chest wall breast cancer, colorectal, pancreatic and metastatic liver cancer, is recently in various stages of human clinical trials (Phase II/III) with studies ongoing for further improvement in the performance of the drug and increase its chance of clinical trial success. The clinical trials for the use of these

liposomes for the treatment of cystic fibrosis patients with chronic Pseudomonas aeruginosa lung infection are complete, and the formulation is currently undergoing clinical trials for the treatment of M. avium infections.

Targeting Liposomes to Specific Organelles[19,20]

Active targeting discussed in the section on stealth liposomes, an area of interest, is the ability to target drugs to specific organelles. While specific subcellular targeting is still a challenge, efforts have been most successful with targeting drugs to lysosomes or mitochondria. Most of these systems are still at the in vitro research phase. In one such in vitro demonstration, drugs encapsulated in liposomes modified with various lysosomotropic ligands, such as octadecyl-rhodamine B (RhB), were successfully delivered to lysosomes. Elsewhere, mitochondria-targeting was achieved in vitro with the polymer (Rh123)-PEG-DOPE (rhodamine 123-polyethylene glycol-1,2-dioleoyl-sn-glycero-3-phosphoethanolamine) which contains mitochondriotropic dye rhodamine.

CONCLUSION

Liposomes have been perceived as useful carrier systems for targeted drug delivery. The flexibility of their behavior can be used for drug delivery via any route of administration and for any drug material irrespective of their solubility properties. The use of liposomes in the delivery of drugs and genes is promising and is sure to undergo further developments in the future.

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