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Liposomes: An advance tools for novel drug delivery system

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Abstract
Although hundreds of new agents with potential activity against a wide range of therapeutic targets *in vitro* are designed and synthesized, most of these new drugs fail to live up to their potential in the clinic due to their inability to show activity at target site. Liposomes characterize an advanced technology to deliver active molecules to the site of action, and at present, several formulations are in clinical use. They can encapsulate and effectively deliver both hydrophilic and lipophilic drug in aqueous media. Liposomes have been used to deliver anticancer agents in order to reduce the toxic effects of the drugs when given alone or to increase the circulation time and effectiveness of the drugs. This review summarizes the prospect and challenges of this drug delivery system, which hold the potential of delivering drug most effectively to the target site.

Keywords: Liposome, controlled drug delivery, phospholipid, liposome, glycolipids, drug formulations and drug delivery systems

Introduction
For newer drugs, the task of avoiding undesirable drug actions on normal organs and tissues and minimizing side effects of the therapy is very important. Thus, screening of biologically active compounds became necessary, permitting the choice of drug with selective action on the appropriate organs or tissues. Liposome's are colloidal, vesicular structures composed of one or more lipid bilayers surrounding an equal numbers of aqueous compartments [1]. At the sometime, many pharmacologically effective compounds cannot be used as drugs due to their undesirable action on normal tissues. Normally, they are more or less evenly distributed in the whole body and to reach the target zone the drug has to cross many other organs, cells, and intracellular compartments *etc.*, where it can be partially inactivated. To overcome this problem, a high concentration of drug has to bead ministered, which has a potential to cause undesirable complications and is sometimes expensive too. The ideal solution to such problems is the targeting of drugs using suitable carriers like liposomes.
Liposomes are colloidal, vesicular structures [2] composed of a bilayer of phospholipids or any similar amphipathic lipids. The sphere like shell encapsulates a liquid interior, which contain substances such as peptides andprotein, hormones, enzymes, antibiotic, anti-fungaland anticancer agents. Liposomes can be created from cholesterol and natural non-toxic phospholipids. Due to their size (30nm to several micrometers) and hydrophobicand hydrophilic character (besides biocompatibility), liposomes are promising system for drug delivery. The liposome can be used as a vehicle for administration of nutrients and pharmaceutical drugs [3].
Liposome properties differ considerably with lipid composition, surface charge, size, and the method of preparation. Furthermore, the choice of bilayer components determines the rigidity or fluidity and the charge of the bilayer. For instance, unsaturated phosphatidylcholine species from natural sources (egg or soybean phosphatidylcholine) give muchmore permeable and less stable bilayers, where as the saturated phospholipids with long acyl chains. (For example, dipalmitoyl-phosphatidyl choline) form a rigid, rather impermeable bilayer structure [2].

Definition [4]
Liposomes are defined as structure consisting of one or more concentric spheres of lipid bilayers separated by water or aqueous buffer compartments. (OR) Liposomes are simple microscopic vesicles in which an aqueous volume is entirely enclosed by a membrane

composed of lipid bilayers. This represents a multilamellar liposome. It contains many layers of phospholipids with water in between the layers.

Discovery of Liposome

Liposome was discovered about 40 years ago by Bangham and co-workers. This was an accidental discovery, when he dispersed Phosphatidyl choline molecules in water; he found that it was forming a closed bilayer structure containing an aqueous phase entrapped by lipid bilayers. It was defined as microscopic spherical vesicles that form when phospholipids are hydrated or exposed to an aqueous environment. Liposomes are microscopic vesicles composed of a bilayer of phospholipids or any similar amphipathic lipids [5]. They can encapsulate and effectively deliver both hydrophilic and lipophilic insoluble drug, because lipids are amphiphatic (both hydrophilic and hydrophobic) in aqueous media, their thermodynamic phase properties and self-assembling characteristics evoke entropically driven sequestration of their hydrophobic regions into spherical bilayers are referred as lamellar. liposomes vary in charge and size depending on the method of preparation and the lipids used the multi lamellar vesicle [MLV] size range is0.1-5.0micrometres. the small unilamellar vesicle [SUV] size range is 0.02 0.05micrometres,and the large unilamellarvesicle [LUV] size range varies from0.06micrometre and greater [6].

Advantages [7]

It provides controlled drug delivery.

a) It should be biodegradable, biocompatible, andmflexible.
b) It should be non-ionic.
c) It can carry both water and lipid soluble drugs.
d) The drugs can be stabilized from oxidation.
e) It should be improve the protein stabilization.
f) It provides controlled hydration.
g) It provides sustained release.
h) It provides targeted drug delivery or site specific drug delivery.
i) Stabilization of entrapped drug from hostile environment.
j) Alter pharmacokinetics and pharmacodynamics of drugs.
k) It can be administered through various routes.

Disadvantages

Less stability, Low solubility, Short half-life and Phospholipids undergoes oxidation, hydrolysis. Leakage and fusion. High production cost. Quick uptake by cells of R.E.S. Allergic reactions may occur to liposomal constituents [7]. Phospholipids impulsively form closed structures when they are hydrated in aqueous solutions. Such vesicles which have one or more phospholipid bilayer membranes can transport aqueous or lipid drugs, depending on the nature of those drugs. Because lipids are amphipathic (both hydrophobic and hydrophilic) in aqueous media, their thermodynamic phase properties and self-assembling characteristics influence entropically focused confiscation of their hydrophobic sections into spherical bilayers. Those layers are referred to as lamellae. Self-aggregation of polar lipids is not limited to conventional bilayer structures which rely on molecular shape, temperature, and environmental and preparation conditions but may self-assemble into various types of colloidal particles.

Liposomes are extensively used as carriers for numerous molecules in cosmetic and pharmaceutical industries. Additionally, food and farming industries have extensively studied the use of liposome encapsulation to grow delivery systems that can entrap unstable compounds (for example, antimicrobials, antioxidants, flavors and bioactive elements) and shield their functionality. Liposomes can trap both hydrophobic and hydrophilic compounds, avoid decomposition of the entrapped combinations, and release the entrapped at designated targets [8]. Because of their biocompatibility, biodegradability, low toxicity, and aptitude to trap both hydrophilic and lipophilic drugs and simplify site-specific drug delivery to tumor tissues. Liposomes have increased rate both as an investigational system and commercially as a drug-delivery system. Many studies have been conducted on liposomes with the goal of decreasing drug toxicity and/or targeting specific cells. One of the main aims of any cure employing drug is to increase the therapeutic index of the drug while minimizing its side effects. The clinical usefulness of most conservative chemotherapeutics is restricted either by the incapability to deliver therapeutic drug concentrations to the target soft tissue or by Spartan and harmful toxic side effects on normal organs and tissues. Different approaches have been made to overcome these difficulties by providing the 'selective' delivery to the target area; the ideal solution would be to target the drug alone to those cells, tissues, organs that are affected by the disease. Selected carriers, for instance colloidal particulates and molecular conjugates, can be appropriate for this determination.

Structure of Liposomes [9]

Liposomes are spherical lipid bilayers from 50nm to 1000 nm diameter that serve as convenient delivery vehicles for biologically active compounds. The field of liposome research has expanded considerably over the last 30 years. It is now possible to wide range of liposomes varying in size, phospholipid composition and surface characteristics to suit the specific application for which they are intended. This paper gives an overview of the main advances in liposome research from a point of view of their applications in medicine.

Classification of Liposomes

Liposomes can be classified either on the basis of their structural properties or on the basis of the preparation method used.

Liposome Classification Based On Structural Features

1. MLV Multilamellar large vesicles
2. OLV Oligolamellar vesicles
3. UV Unilamellar vesicles
4. SUV Small unilamellar vesicles
5. MUV Medium sized unilamellar vesicles
6. LUV Large unilamellar vesicles
7. GUV Giant unilamellar vesicles
8. MVV Multivesicular vesicles

Liposome Classification Based on Method of Liposome Preparation

1. REV Single or oligolamellar vesicle made by reverse phase evaporation method
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. FUV Vesicles prepared by fusion
7. FPV Vesicles prepared by french press
8. DRV Dehydration- rehydration vesicles
9. BSV Bubblesomesa

Mechanical Dispersion Method

The following are types of mechanical dispersion methods:

A. Sonication.
B. French pressure cell: extrusion.
C. Freeze-thawed liposome's.
D. Lipid film hydration by hand shaking, non-hand shaking or freeze drying.
E. Micro-emulsification.
F. Membrane extrusion.
G. Dried reconstituted vesicles.

Sonication

Sonication is perhaps the most extensively used method for the preparation of SUV. Here, MLVs are sonicated either with a bath type sonicator or a probe 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.

There Are Two Sonication Techniques

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

French pressure cell: 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. 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

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 pressure12. 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

A lipid solution of ethanol is rapidly injected to a huge excess of buffer. The MLVs are at once. Available online: www.uptodateresearchpublication.com May - June 163 formed. The disadvantages of the method are that the population is heterogeneous (30 to 110 nm), liposome's are very dilute, the removal all ethanol is 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

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 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. With this method, high encapsulation efficiency up to 65% can be obtained in a medium of low ionic strength. 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 Nonencapsulated Material)

Dialysis

The detergents at their critical micelleconcentrations (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).

Detergent (Cholate, Alkyl Glycoside, Triton X-100) Removal of Mixed Micelles (Absorption)

Detergent absorption is attained by shaking mixed micelle solution with beaded organic polystyrene adsorbers such as XAD-2 beads (SERVA Electrophoresis GmbH, Heidelberg, Germany) and Bio-beads SM2 (Bio-RadL aboratories, Inc., Hercules, USA). The great benefit of using detergent adsorbers is that they can eliminate detergents with a very low CMC, which are not entirely depleted.

Gel-Permeation Chromatography

In this method, the detergent is depleted by size special chromatography. 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, pre-treatment is necessary. The pre-treatment is done by pre-saturation of the gel filtration column by lipids using empty liposome suspensions.

Dilution

Upon dilution of aqueous mixed micellar solution of detergent and phospholipids with buffer, the micellar size and the polydispersity increase fundamentally, and as the system is diluted beyond the mixed micellar phase boundary, a spontaneous transition from polydispersed micelles to vesicles occurs.

Drug Loading in Liposomes

Drug loading can be attained either passively (i.e., the drug is encapsulated during liposome formation) or actively (i.e., after liposome formation). Hydrophobic drugs, for example amphotericin B taxol or annamycin, can be directly combined into liposomes during vesicle formation, and the amount of uptake and retention is governed by drug-lipid interactions. Trapping effectiveness of 100% is often achievable, but this is dependent on the solubility of the drug in the liposome membrane. Passive encapsulation of water-soluble drugs depends on the ability of liposomes to trap aqueous buffer containing a dissolved drug during vesicle formation. Trapping effectiveness (generally <30%) is limited by the trapped volume delimited in the liposomes and drug solubility. On the other hand, water-soluble drugs that have protonizable amine functions can be actively entrapped by employing pH gradients, which can result in trapping effectiveness approaching 100%.

Freeze-Protectant for Liposomes (Lyophilization)

Natural excerpts are usually degraded because of oxidation and other chemical reactions before they are delivered to the target site. Freeze-drying has been a standard practice employed to the production of many pharmaceutical products. The overwhelming majority of these products are lyophilized from simple aqueous solutions. Water is the only solvent that must be detached from the solution using the freeze-drying process. Freeze-drying (lyophilization) involves the removal of water from products in the frozen state at tremendously low pressures. The process is normally used to dry products that are thermo-labile and would be demolished by heat-drying. The technique has too much potential as a method to solve long-term stability difficulties with admiration to liposomal stability. Studies showed that leakage of entrapped materials may take place during the process of freeze-drying and on reconstitution. Newly, it was shown that liposomes when freeze-dried in the presence of adequate amounts of trehalose (a carbohydrate commonly found at high concentrations in organism) retained as much as 100% of their original substances. It shows that trehalose is an excellent cryoprotectant (freeze-protectant) for liposome.

Purification of Liposome

Liposomes are generally purified by gel filtration chromatography14, Dialysis and centrifugation. In chromatographic separation, Sephadex-50 is most widely used. In dialysis method hollow fibre dialysis cartridge maybe used. In centrifugation method, SUVs in normal saline may be separated by centrifuging at 200000 g, for 10-20hours. MLVs are separated by centrifuging at 100000g for less than one hour.

Mechanism of Transportation through Liposome

The limitations and benefits of liposome drug carriers lie critically on the interaction of liposomes with cells and their destiny *in vivo* after administration. *In vivo* and *in vitro* studies of the contacts with cells have shown that the main interaction of liposomes with cells is either simple adsorption (by specific interactions with cellsurface components, electrostatic forces, or by nonspecific weak hydrophobic) or following endocytosis (by phagocyte cells of the reticuloendothelial system, for example macrophages and neutrophils). 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. The fourth possible interaction is the exchange of bilayer components, for instance cholesterol, lipids, and membrane-bound molecules with components of cell membranes.

Evaluation of Liposomes

Liposomal formulation and processing for specified purpose are characterized to ensure their predictable *in vitro* and *in vivo* performance. The characterization parameters for purpose of evaluation could be classified into three broad Categories, which include physical, chemical and biological parameters. Physical characterization evaluates various parameters including size, shape, surface features, lamellarity phase-behaviour and drug release profile. Chemical characterization includes those studies which establish the purity and potency of various lipophillic constituents. Biological characterization parameters are helpful in establishing the safety and suitability of formulation for therapeutic application. Some of parameters are:

Vesicle Shape and Lamellarity

Vesicle shape can be assessed using electron Microscopic Techniques. Lamellarity of vesicles i.e. number of bilayers presents in liposome's is determined using Freeze Fracture Electron Microscopy and P31 Nuclear Magnetic Resonance Analysis.

Vesicle Size and Size Distribution

Various techniques are described in literature for determination of size and size distribution. These include Light Microscopy, Fluorescent Microscopy, Electron Microscopy (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. 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 [15]. Most of methods used in size, shape and distribution analysis can be grouped into various categories namely microscopic, diffraction, scattering, and hydrodynamic techniques.

Techniques of Liposome Preparation

There are three stages for liposomes preparation

1. Hydration stage

a. **Mechanical Methods:** MLVs were traditionally produced by hydrating thin lipids films deposited from an organic solution on a glass wall by shaking at temperatures above the phase transition temperature of the phospholipid with the highest Tc. The wide size distributions of the produced liposome dispersions were usually narrowed down by (low) pressure extrusion or ultrasoncation [11].
b. **Methods based on replacement of organic solvent by aqueous media:** The lipid constituents are first dissolved in an organic solvent which is subsequently brought in contact with an aqueous phase. The organic solvent is removed later. During the removal of the organic phase, liposomes are formed. Their characteristics (size, organisation of bilayers) depend on the protocol used. If the organiic solvent with the dissolved lipids is not miscible with the aqueous phase (ether, chloroform, freons), then the intrmediate stage is an emulsion (immiscible solvent). Other organic solvents containing the dissolved lipid (s) can be mixedhomogenously with the aqueous phase (ethanol) in the first stage. Then lipoosmes formation occurs when the organic solvent concentration drops below a certain critical value (miscible solvents). The contents of residual organic solvent that is exceptable in the finished product depends on the solvent in question and the route of administration [12].
c. **Methods based on detergent removal:** (Phospho) lipids, lipophilic compounds and amphiphatic proteins can be solubilized by detergents forming mixed micells. Upon removal of the detergent, vesicle formation can occur. This technique is well established for preparation of reconstituted virus envelopes 10 or reconstituted tumor membrane material. Schreier and coworkers described a two-step strategy for insertion of proteins into the outer layer of liposomes. First liposomes were formed by detergent dialysis method and subsequently proteins were inserted by partial resolubalization of the membrane by the detergent (deoxycolate) inthe presence of protein [13].
d. **Method based on size transformation and fusion:** Sonication of phospholipds below their phase transition temperature (Tc) results in vesicles with defects in the bilayers. Heating the dispersion to Tc eliminates these structural defects and causes fusion resulting in large unilamellar liposomes with a wide size distribution [14].

2. Sizing stage

There are two approaches, one without a special sizing step A and one with a special sizing step B.

A-In liposome formation process, circumstances are selected and controlled in such a way that particle size distributions with an acceptable width are produced. High shear homogenization produces a size distribution which depends on operational pressure [15, 16]. B-For small dispersion volumes, the liposome dispersion can be fractionated by centrifugation as liposome density usually differs from the density of the medium. Gel permeation chromatography has also been used for subdividing wide particle size distribution. On an analytical or semi-preparative scale, the selection of the pore size of the chromatographic material provides an opportunity to manipulate the size class resolution within certain limits [17].

3. Removal of Non-Encapsulated Material

Many lipophilic drugs exhibit a high affinity to the bilayer and are completely liposome associated. However, for other compounds, the encapsulation efficiency is less than 100 percent. The non-encapsulated fraction of the active compound can cause unacceptable side effects or physical instability [18]. 10 For removal of non-encapsulated material, the following techniques are used:

A. Dialysis and ultracentrifugation,
B. Gel permeation chromatography,
C. Ion exchange reactions.

Encapsulation Efficiency

Determine amount and rate of entrapment of water soluble agents in aqueous compartment of liposome.

Liposome for Targeted Delivery

Use of liposome-encapsulated enzymes for delivery into cells was first reported in 1971. About the same time, a specific receptor on hepatocytes was demonstrated to mediate clearance of ß-galactose–terminated glycoproteins from circulation. A mannoside-specific receptor was recognized on the cell surface of the RES of rats (including the liver sinusiod and macrophages). By grafting different glycosides on the surface of liposomes, it is possible to direct the latter to different cell types of rat liver [19]. Galactosylated liposomes are mainly taken up by liver hepatocytes, whereas mannosylated liposomes are mainly taken up by nonparenchymal cells [20]. Grafting specific ligands to the liposome surface facilitates a fusion of the liposome with target cells by endocytosis, thus releasing material to be delivered. In cancer chemotherapy, the toxicity of anticancer drugs is of major concern. Liposomes could be used to deliver such drugs and minimize their toxic effects on healthy cells. Targeted delivery to cancer cells could be achieved by coating

monoclonal antibodies (MAbs) raised against tumor-cell specific antigens. *In vitro* and in vivo studies by Ahmad *et al*. of squamous-cell carcinoma in mouse models provided evidence that antibody-coated polyethyleneglycol liposomes containing doxorubicin were more effective and less toxic than free drugs, drugs incorporated into antibody-free liposomes, and
antibodycoated conventional liposomes [21]. The major concern in antibody-grafted liposome use is the induction of immune response to the grafted antibodies. Basten *et al*. suggested a novel approach to overcoming that difficulty [22]. They used 125I-labeled antigen to kill the cells responsible for immune induction (the "antigen suicide" technique). Other possible approaches to overcome the immune-system problem include immunosuppressive drugs and humanized antibodies or establishing neutral immune windows for subsequent injection. Liposomes can be designed to release their entrapped contents under certain controlled conditions:
pH-sensitive and temperature-dependent liposomalsystems [23]. Drug targeting using liposomes as carriers holds much promise, especially in reducing toxicity and targeting delivery to disease sites. The future is bright for liposome research, with a large number of clinical trials ongoing in several countries with liposomal formulations of various anticancer drugs, cytokines, peptides and proteins. In the near future, several more liposome-based drugs will find their way into the pharmaceutical market.

Encapsulation Efficiency
Determine amount and rate of entrapment of water soluble agents in aqueous compartment of liposome.

Applications of Liposomes in Medicine and Pharmacology
Applications of liposomes in medicine and pharmacology can be divided into diagnostic and therapeutic applications of liposomes containing various markers or drugs, and their use as a tool, a model, or reagent in the basic studies of cell interactions, recognition processes, and mode of action of certain substances. Unfortunately, many drugs have a very narrow therapeutic window, meaning that the therapeutic concentration is not much lower than the toxic one. In several cases, the toxicity can be reduced or the efficacy can be enhanced by the use of a suitable drug carrier which alters the temporal and spatial delivery of the drug, i.e., its biodistribution and pharmacokinetics. It is clear from many pre-clinical and clinical studies that drugs, for instance antitumor drugs, parceled in liposome demonstration reduced toxicities, while retentive enhanced efficacy. Advances in liposome design are leading to new applications for the delivery of new biotechnology products, for example antisense oligo-nucleotides, cloned genes, and recombinant proteins. A vast literature define the viability of formulating wide range of conservative drugs in liposomes, frequently resultant in improved therapeutic activity and/or reduced toxicity compared with the free drug. Changed pharmacokinetics for liposomal drugs can lead to improved drug bioavailability to particular target cells that live in the circulation, or more prominently, to extravascular disease sites, for example, tumors. Recent improvements include liposomal formulations of all-*trans*-retinoic acid and daunorubicin, as first-line treatment of AIDS related advanced Kaposi's sarcoma. Examples are vincristine, doxorubicin, and amphotericin B. The benefits of drug load in liposomes, which can be applied as (colloidal) solution, aerosol, or in (semi) solid forms, such as creams and gels, can besummarized into seven categories

Benefits of Drug Load in Liposomes
Liposomes in Parasitic Diseases and Infections
From the time when conventional liposomes are digested by phagocytic cells in the body after intravenous management, they are ideal vehicles for the targeting drug molecules into these. The best known instances of this 'Trojan horse-like' mechanism are several parasitic diseases which normally exist in the cell of MPS [24]. They comprise leishmaniasis and several fungal infections. Leishmaniasis is a parasitic infection of macrophages which affects over 100 million people in tropical regions and is often deadly. The effectual dose of drugs, mostly different antimonials, is not much lower than the toxic one. Liposomes accumulate in the very same cell population which is infected, and so an ideal drug delivery vehicle was proposed. Certainly, the therapeutic index was increased in rodents as much as several hundred times upon administration of the drug in various liposomes. These formulations use mostly ionosphere amphotericin B and are transplanted from very successful and prolific area of liposome formulations in antifungal therapy. The best results reported so far in human therapy are probably liposomes as carriers for amphotericin B in antifungal therapies. This is the drug of choice in dispersed fungal infections which often in parallel work together with chemotherapy, immune system, or AIDS, and is frequently fatal. Unfortunately, the drug itself is very toxic and its dosage is limited due to its ionosphere and neurotoxicity. These toxicities are normally related with the size of the drug molecule or its complex. Obviously, liposome encapsulation inhibits the accumulation of drug in these organs and radically reduces toxicity. Furthermore, often, the fungus exists in the cells of the mononuclear phagocytic system; therefore, the encapsulation results in reduced toxicity and passive targeting. These benefits, however, can be associated with any drug carrier. Certainly, similar improvements in therapy were observed with stable mixed micellar formulations and micro-emulsions. Additionally, it seems that many of the early liposomal preparations were in actual fact liquid crystalline colloidal particles rather than self-closed MLV. Since the lives of the first terminally ill patients (who did not rely to all the conventional therapies) were saved, many patients were very effectively treated with diverse of amphotericin B formulations. Comparable methods can be achieved in antiviral and antibacterial therapies. Most of the antibiotics, however, are orally available; liposome encapsulation can be considered only in the case of very potent and toxic ones which are administered parenterally. The preparation of antibiotic-loaded liposomes at sensibly high drug-to-lipid ratios may not be easy because of the interactions of these molecules with bilayers and high densities of their aqueous solutions which often force liposomes to float as a creamy layer on the top of the tube [25]. Several other ways, for instance, topical or pulmonary (by inhalation) administration are being considered also. Liposome-encapsulated antivirals (for example ribavirin, azidothymidine, or acyclovir) have also shown to reduce toxicity; currently, more detailed experiments are being performed in relation to their efficacy.

Liposomes in Anticancer Therapy
Numerous different 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 [26]. 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. 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 encapsulation reduces the delivery of the drug molecules towards those tissues. For the same reason, 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. In some cases, such as systemic lymphoma, the effect of liposome encapsulation showed 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 mononuclear phagocytic system actually reduced its efficacy.

Other Applications

1. Gene therapy
2. Liposomes as carriers for vaccines
3. Liposomes as carrier of drug in oral treatment
4. Liposomes for topical applications
5. Liposomes for pulmonary delivery
6. Against Leishmaniasis
7. Lysosomal storage disease
8. Cell biological application
9. Metal storage disease
10. Ophthalmic delivery of drugs.

Therapeutic Applications of Liposomes

Liposome as drug/protein Delivery Vehicle

a) Controlled and sustained drug release insitu.
b) Enchaned drug solubilization, Altered and biodistribution.
c) Enzyme replacement therapy and lysosomal disorders.

Liposome in Antimicrobial, Antifungal and Antiviral Therapy

a) Liposomal drugs
b) Liposomal biological response modifier

Liposomes in Tumour Therapy

a) Carrier of small cytotoxic molecule.
b) Vehicle formacromolecule as cytokines or genes.

Liposome in Gene Therapy

Gene and antisence therapy, Genetic (DNA) vaccination

Liposome in Immunology

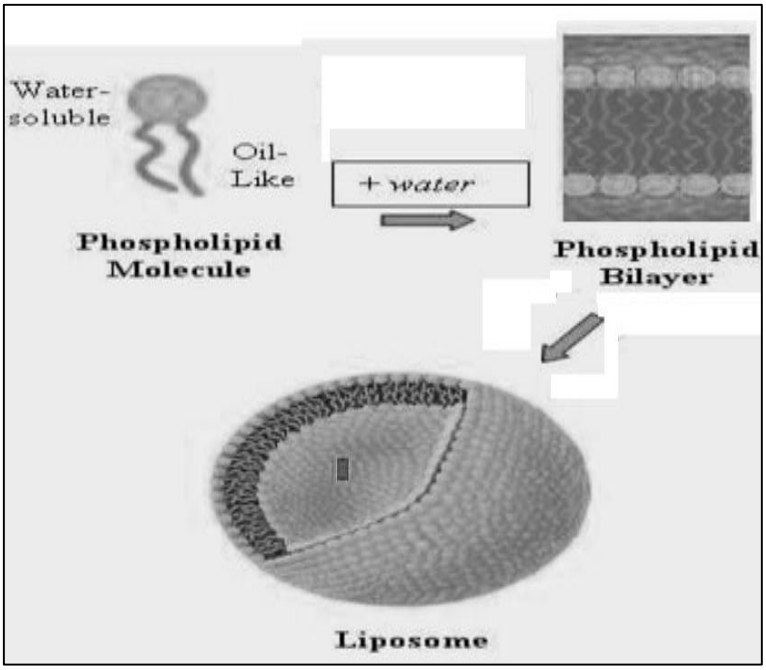
a) Immunoadjuvant.
b) Immunomodulator and Immunodiagnosis,
c) Liposome as artificial blood surrogates,
d) Lipososmes as radiopharmaceutical and radio-diagnostic carrier.
e) Liposomes in cosmetics and dermatology.
f) Liposomes in enzyme immobilization and bioreactor technology.

Limitation in liposome technology

1. Stability
2. Sterilization
3. Encapsulation efficiency
4. Active targeting
5. Gene therapy
6. Lysosomal degradation

Conclusion

Liposomes have been used in a broad range of pharmaceutical applications including intracellular delivery systems for anti-sense molecules, ribosomes, proteins/peptides, and DNA. Liposomes with enhanced drug delivery to disease locations, byability of long circulation residence times, are now achieving clinical acceptance. In addition, liposomes promote targeting of particular diseased cells within the disease site. Finally, liposomal drugs exhibit reduced toxicities and retain enhanced efficacy compared with free complements. Based the pharmaceutical applications and available products, we can say that liposome's have definitely established their position in modern delivery systems. The use of liposomes in the delivery of drugs and genes are promising and is sure to undergo further developments in future.



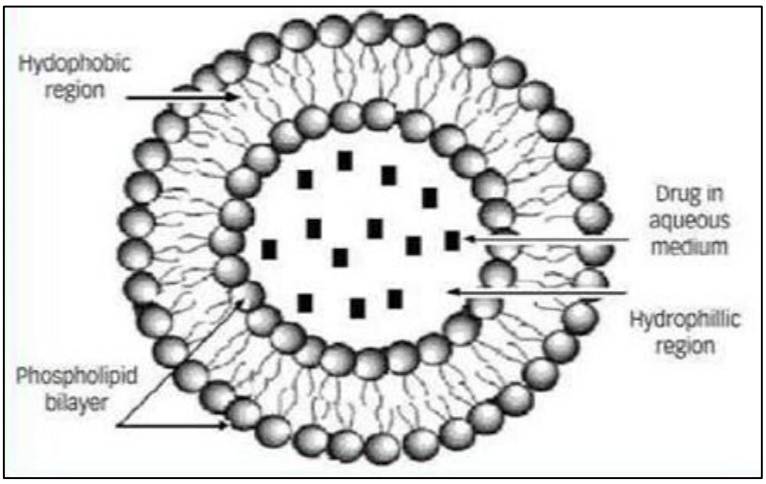


Fig: Structure of Liposome

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