ABSTRACT
When a self-forming enclosed lipid bi-layer was hydrated, the liposome or lipid vesicle was discovered. Liposome drug delivery systems have played a key role in the development of powerful drugs that have improved therapies. Liposome formulations have recently been aimed at reducing toxicity and increasing accumulation at the target site. The suppression of rapid liposome clearance by regulating particle size, charge, and surface hydration is one of several new liposome manufacturing methods based on lipid drug interactions and liposome disposition mechanisms. Targeting tissue with or without expression of target recognition molecules on the lipid membrane is the most common clinical use of liposomal drug delivery. Physical, chemical, and biological factors are used to characterise the liposomes. Liposome size is another important metric that helps describe the liposome, which is normally done by successive extrusion at different temperatures. Several groups of medications, such as antiviral, antifungal, antibacterial, vaccines, anti-tubercular therapies, and gene treatments, benefit from this route of drug delivery since it increases their safety and efficacy. Liposomes are now used in immunology, dermatology, vaccine adjuvant, eye problems, brain targeting, infectious disease, and cancer therapy. The particular binding properties of a drug-carrying liposome to a target cell, such as a tumour cell, and certain substances in the body (antibodies, proteins, peptides, and so on) are recent breakthroughs in this field; stealth liposomes, which are notably being exploited as carriers for hydrophilic drugs (water soluble) anticancer drugs like doxorubicin, mitoxantrone; and bisphosphonate-liposome mediated depletion of macrophages. This review would be a help to the researchers working in the area of liposomal drug delivery.

KEYWORDS: Liposomes, Advance Drug Delivery, Drug targeting, Nanotechnology

INTRODUCTION
Paul Ehrlich in 1906 started the era of development for targeted delivery system where he envisaged a drug delivery mechanism where he directly target the drug to diseased cells, which he named as magic bullets.

A liposome is a spherical-shaped structure composed of one or more phospholipid bilayers surrounding an equal numbers of aqueous compartments. Liposome are able to encapsulate hydrophilic or lipophilic drugs. Drug encapsulated by liposomes achieve therapeutic level for long duration as drug must first be release from liposome before metabolism and excretion.

PHARMACEUTICAL LIPOSOMAL DRUG DELIVERY: A COMPLETE REVIEW OF NEW DELIVERY SYSTEM

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STRUCTURE OF LIPOSOME

Lipids are amphiphilic molecules with one water-loving (hydrophilic) and one water-hating (hydrophobic). When lipids come into contact with water, the hydrophobic portions of the molecule establish unfavourable interactions with the solvent, resulting in lipid self-assembly in the form of liposomes. By changing medication absorption, lowering metabolism, prolonging biological half-life, and reducing toxicity, liposomes have been utilised to increase the therapeutic index of new and old pharmaceutical features of the carrier, rather than the physico-chemical characteristics of the pharmacological molecule, are therefore used to govern drug distribution.

Liposomes can be made from natural or synthetic lipids, and liposome contents are not limited to lipids; new generation liposomes can also be made from polymers (sometimes referred to as polymersomes) Liposomes, which are made up of natural or synthetic lipids or polymers, are biocompatible and biodegradable, making them ideal for biomedical research. Liposomes are remarkable in that they have the potential to compartmentalise and solubilize both hydrophilic and hydrophobic molecules. Liposomes are particularly appealing as drug delivery vehicles because of their unique characteristic, which is combined with biocompatibility and biodegradability. Hydrophobic drugs place themselves inside the bilayer of the liposome and hydrophilic drugs are entrapped within the aqueous core or at the bilayer interface. Liposomal formulations enhance the therapeutic efficiency of drugs in preclinical models and in humans compared to conventional formulations due to the alteration of bio-distribution.
DRUG ATTACHMENT

Liposome binding drugs, into or onto their membranes, are expected to be transported without rapid degradation and minimum side effects to the recipient because generally liposomes are composed of biodegradable, biologically inert and non-immunogenic lipids. They also have low toxicity and elicit no pyrogenic or antigenic reactions [3-5]. As a result, liposomes are more appealing candidates for employment as drug-delivery vehicles than other drug-carrying systems such as nanoparticles [6, 7] and microemulsions [8, 9] due to all of these qualities, as well as the ease of surface modification to bear the targetable properties.

ADVANTAGES OF LIPOSOMES

- Liposomes are biodegradable, non-toxic and non-immunogenic.
- Liposomes have ability to carry large drug payloads.
- Liposomes are suitable for delivery of hydrophilic, amphipathic and hydrophobic drugs.
- Liposomes have wide range of physicochemical and biophysical properties that can be modified to control their biological characteristics.

DISADVANTAGES

- Production cost of liposomes are high.
- Leakage of drug may occur.
- Short half-life.

TYPES OF LIPOSOMES

Liposomes are classified on their basis of:

A. BASED ON STRUCTURAL PARAMETER

(1) Unilamellar Vesicles (UV)
   a. Small Unilamellar vesicles (SUV) 20-40nm
   b. Medium Unilamellar Vesicle (MUV) 40-80nm
   c. Large Unilamellar Vesicle (LUV) 100-1000nm

(2) Oligolammellar Vesicle (OV)

Made up of 2-10 bilayers of lipids surrounding a large internal volume.

(3) Multilamellar Vesicle (MV)

Have onion like arrangement of concentric spherical bilayer of LUV/MLV enclosing large number of SUV.

B. BASED ON METHOD OF LIPOSPME PREPARATION

(1) REV: Single or oligolamellar vesicle made by reverse-phase evaporation method

(2) MLV-REV: Multilamellar vesicle made by reverse-phase evaporation method

(3) SPLV: Stable plurilamellar vesicle.

(4) FATMLV: Frozen and thawed MLV.

(5) VET: Vesicle prepared by extrusion technique.

(6) DRV: Dehydration-rehydration method.

C. BASED UPON COMPOSITION AND APLICATION

(1) Conventional liposomes

(2) Fusogenic liposomes
(3) pH sensitive liposomes

(4) Cationic liposomes
(5) Long circulatory liposomes (LCL)
(6) Immuno-liposomes

STRUCTURAL COMPONENT

1) Phospholipids

Glycerol-containing phospholipids are the most frequent component of liposome composition, accounting for more than half of the lipid weight in biological membranes. Phosphatidic acid is used to make these. Phosphatidic acid is used to make these. The glycerol moiety is the molecule's backbone. Phosphoric acid is esterified at the C2OH group. Long chain esterification of OH at C1 and C2. The lipidic character is due to fatty acids. Glycerol, choline, ethanolamine, serine, and inositol are among the organic alcohols that can be esterified to one of phosphoric acid's remaining OH groups. As a result, the series' parent component is the phosphoric ester of glycerol.

• PC- Phosphatidyl choline (Lecithin)
• PE- Phosphatidyl ethanolamine (cephalin)
• (PS) Phosphatidyl serine
• (PI) Phosphatidyl inositol
• (PG) Phosphatidyl Glycerol

To create stable liposomes, saturated fatty acids are used. Unsaturated fatty acids are less commonly utilised.

2) Sphingolipids

Sphingosine or a similar base serves as the backbone. These are essential components of both plant and animal cells. This consists of three distinct construction pieces.

• A head group that can range from basic alcohols like choline to highly complex polysaccharides. A mol of F.A.

Glycosphingolipids are lipids made up of glycine and sphingomyelin. Gangliosides - a small component of liposome synthesis found in grey matter.

At neutral pH, this molecule contains complex saccharides with one or more Sialicacid residues in its polar head group, resulting in one or more negative charges. These are used to create a coating of surface charged groups in liposomes.

3) Sterols:

Cholesterol and its derivatives are widely used in liposomes to:

• limit the permeability of the membrane to water soluble chemicals;
• lower the fluidity or microviscosity of the bilayer.
• In the presence of biological fluids such as plasma, the membrane must be stabilised. (this effect used in formulation of i.v. liposomes)

Albumin, transferrin, and macroglobulin are known to interact quickly with liposomes that are cholesterol-free. These proteins remove bulk phospholipids from liposomes, depleting the vesicles' outer monolayer and causing physical instability. Cholesterol appears to decrease this sort of interaction significantly. Cholesterol is known as the “mortar of bilayers” because, due to its molecular form and solubility, it fills up empty gaps between Phospholipid molecules, firmly attaching them to the structure. The OH group at the third position offers a tiny polar head group, and the hydrocarbon chain at C17 is converted to a non-polar end by these molecules, allowing cholesterol to intercalate in bilayers.
4) Synthetic phospholipids

DPPC (Dipalmitoyl phosphatidyl choline), DSPC (Distearoyl phosphatidyl choline), DPPE (Dipalmitoyl phosphatidyl ethanolamine), DPPS (Dipalmitoyl phosphatidyl serine), DPPA (Dipalmitoyl phosphatidic acid), DPPG (Dipalmitoyl phosphatidyl glycerol)

Above are some examples of saturated phospholipids

DOPC (Dioleoyl phosphatidyl choline), DOPG (Dioleoyl phosphatidyl glycerol)

Above are some examples of unsaturated phospholipids

5) Polymeric materials

When exposed to ultraviolet light, synthetic phospholipids with a diactylenic group in the hydrocarbon chain polymerize, resulting in polymerized liposomes with much greater permeability barriers to entrapped aqueous pharmaceuticals. Other polymerisable lipids include, for example, lipids containing conjugated diene, Methacrylate, and so on. Also generated are a number of polymerisable surfactants.

6) Polymer bearing lipids

The durability of repulsive interactions with macromolecules is primarily due to repulsive electrostatic forces. This repulsion may be caused by coating liposome surfaces with charged polymers.

Polyvinyl alcohol, polyethylene oxide, and polyoxazolidines are non-ionic and water-friendly polymers with greater solubility. However, because adsorption of such copolymers with hydrophilic segments and hydrophobic parts results in liposome leakage, the greatest results are obtained by covalently attaching polymers to phospholipids. For example, PEG polymer connected to a carbon at the succinate bond in Diacyl Phosphatidyl ethanolamine.

7) Cationic lipids

For example, DODAB/C (dioctadecyl dimethyl ammonium bromide or chloride), DOTAP (dioleoyl propyl trimethyl ammonium chloride) an analogue of DOTAP, and a variety of additional compounds such as DOTMA analogues and cationic cholesterol derivatives.

8) Additional Substances

Liposomes are made up of a variety of lipids and surfactants.

• Non-ionic lipids • A range of Polyglycerol and Polyethoxylated mono and dialkyl amphiphiles utilised mostly in cosmetic preparations • Single and double chain lipids containing fluoro-carbon chains can produce extremely stable liposomes when mixed with cholesterol

PREPARATION OF LIPOSOMES

Generally two methods are followed

(1) Passive loading &
(2) Remote loading

1. PASSIVE LOADING

This method involve the loading of entrapped agents before or during the manufacturing procedure.

Passive loading can be done by three method:

A. Mechanical / Physical dispersion
B. Solvent dispersion
C. Detergent solubilisation
A. MECHANICAL/PHYSICAL DESPERSION:

(1) HAND SHAKEN METHOD

Method involves

- Dissolution of the lipid mixture and charge components in chloroform:methanol (2:1) solvent.
- Evaporation of solvent in rotary evaporator or by hand shaking to form a film.
- Drying of film by attaching the flask to the manifold of the lyophilizer.
- Casted film is then dispersed in an aqueous medium.
- Upon hydration, lipid swell and peel off the wall of the flask and vesiculate forming multi-lamellar vesicle (MLVs).

(2) NON SHAKEN METHOD

- In this method nitrogen is used to provide agitation rather than rotationary movements.
- For 15-20 mins the lipid film is exposed to water saturated nitrogen.
- After hydration addition of bulk fluid (10-20ml of 0.2 M sucrose dissolve in water is added). By which lipid get swelled up.
- The flask is then flushed with nitrogen, sealed and allow to stand for two hours at 37°C
- After swelling, vesicles are harvested by swirling the content of the flask two yield milky suspension.

(3) PRO-LIPOSOMES:

To increase the surface area of dried lipid film, it is dried over a finely support, such as powdered NaCl, sorbitol or other polysaccharides. These dried coated lipids are known as pro-liposomes.

(4) FREEZE DRIING METHOD:

In this method lipids are freeze dried in suitable organic solvent before adding to any aqueous medium. Ideal solvent for this method is tertiary butanol.

When dried lipid obtained in a foam like structure, water or saline is added to produce MLVs.

B. SOLVENT DISPERSION METHOD:

In these method lipids are first dissolved in an organic solvent and then brought into contact with aqueous phase that contains material to be entrapped with liposomes. Phospholipids form a monolayer between organic phase and aqueous phase.

(1) Ethanol injection:

Method involve preparation of SUVs without sonication.
Ethanol solution of lipids are introduced in aqueous or saline medium via needle

(2) Ether injection:

In this method immiscible organic solvent are injected in an organic solvent via narrow needle at vaporizing temperature.

(3) Rapid solvent exchange vesicle (RSEVs):

In this method lipid’s organic solvent is passed into tube containing aqueous buffer through orifice of blue-tipped under vacuum. Tube is mounted on a vortexer.
Lipid mixture in quickly transferred between pure solvent environment and pure aqueous environment. Excess solvent gets vaporize before reaching to the aqueous medium.

C. DETERGENT SOLUBLIZATION:

Phospholipids are brought in contact with aqueous phase with the help of detergent, which associate with phospholipid molecule to produce the screen of hydrophobic portion of molecule. The structure formed is known as micelles.

The concentration of detergents increases therefore the size of the micelles decreases until become saturated. Removal of detergents is performed.
2. REMOTE (ACTIVE) LOADING:

The drug loading into performed liposomes is achieved using pH gradients and potential difference across the liposomal membrane.

MECHANISM OF LIPOSOMES FORMATION 8-10

Lipids that may form liposomes have two chemical properties. Their fatty acyl chains are hydrophobic while their head groups are hydrophilic.

Each Zwitter ionic head group of Phosphatidyl choline is thought to have on the order of 15 molecules of water weakly linked to it, explaining its strong affinity for the water phase. The hydrocarbon fatty acid chains, on the other hand, prefer the companionship of one another over that of H2O. When the CMC of P.C is considered, this becomes clear. In water, the CMC of Dipalmitoyl P.C was determined to be 4.6–10 M, which is a modest number indicating that this molecule prefers a hydrophobic environment like that found in the centre of a micelle or bilayer.

Dipalmitoyl PC has a free energy of transfer from water to micelle of 15.3K cal/mol, while Dimyristoyl PC has a free energy of transfer of 13.0K cal/mol. These findings clearly demonstrate the hydrophobic effect, which is the thermodynamic foundation for bilayer construction.

The significant free energy difference between a water and a hydrophobic environment explains why normal lipids prefer to assemble in bilayer structures with as much water as possible from the hydrophobic core in order to attain the lowest energy level and hence the best aggregate structure stability.

PURIFICATION OF LIPOSOMES 10, 11

Gel filtration chromatography, dialysis, and centrifugation are commonly used to purify liposomes. Sephadex-50 is the most frequently used chromatographic separation material. A hollow fibre dialysis cartridge can be used in the dialysis technique. SUVs in normal saline can be separated by centrifuging at 200000 g for 10-20 hours using the centrifugation technique. MLVs are separated by centrifuging for less than an hour at 100000 g.

LIPOSOMES EVALUATION 11-15

The formulation and processing of liposomes for a specific application are described in order to assure predictable in vitro and in vivo performance. Physical, chemical, and biological characteristics are the three major types of characterisation parameters for evaluation purposes.

- Size, shape, surface characteristics, lamellarity, phase behaviour, and drug release profile are all evaluated during physical characterisation.
- Chemical characterisation refers to tests that determine the purity and potency of certain lipophillic components.
- Biological characterisation factors aid in determining the formulation's safety and appropriateness for therapeutic use.

Some parameters are:

1. Vesicle shape and lamellarity: Vesicle shape may be examined using electron microscopy. The lamellarity of vesicles, or the number of bilayers present in liposomes, is determined using P-31 nuclear magnetic resonance and freeze-fracture electron microscopy analysis.

2. Vesicle size and distribution: The literature describes a number of approaches for determining vesicle size and dispersion. Light Microscopy, Electron Microscopy (mainly Transmission Electron Microscopy), Fluorescent Microscopy, Laser light scattering Photon correlation Spectroscopy, Field Flow Fractionation, Gel Permeation, and Gel Exclusion are just a few of the methods that may be used. Electron microscopy is the most precise approach for determining liposome size because it allows you to see each individual liposome and acquire precise information about the profile of the liposome population over a wide range of sizes. Unfortunately, it takes a long time and requires equipment that isn't always readily available. The laser light scattering method, on the other hand, is very easy and quick to use, but it has the disadvantage of assessing the average property of the bulk of liposomes. All of these approaches necessitate the purchase of expensive equipment. Gel exclusion chromatography methods are indicated if just a rough notion of size range is required, as the sole costs are buffers and gel material. Liposome shape, size, and stability have been studied using atomic force microscopy, a more recently developed microscopic method. The majority of techniques employed in size, shape, and distribution study fall into one of four categories: microscopic, diffraction, scattering, and hydrodynamic.
a) Microscopic Techniques

i) Optical Microscopy:
This microscopic technique uses a Bright-Field, Phase Contrast Microscope, and Fluorescent Microscope to assess the size of big vesicles.

ii) Negative stain TEM:
Negative stain TEM and scanning electron microscopy are the most common electron microscopic techniques used to examine liposome structure and size. The latter method is less popular. Bright spots against a dark backdrop are observed via Negative Stain Electron Microscopy (hence termed as negative stain) Ammonium molybdate, Phosphotungstic acid (PTA), or uranyl acetate are employed as negative stains in TEM examination. PTA and ammonium molybdate are both anionic compounds, but uranyl acetate is cationic.

iii) Cryo Transmission Electron Microscopy Techniques (cryo-TEM):
This method has been utilised to determine the shape and size of vesicles on their surfaces.

b) Diffraction and Scattering Techniques

Photon correlation spectroscopy (PCS) is an examination of the time dependence of intensity fluctuations in scattered laser light caused by Brownian motion of particles in solution/suspension. Small particles disperse faster than big particles, therefore the rate of change in scattered light intensity varies correspondingly. The translational diffusion coefficient (D) may then be calculated using the Stoke-Einstein equation to obtain the mean hydrodynamic radius (Rh) of particles. This method may be used to detect particles in the 3nm range.

c) Hydrodynamic Techniques: Gel Permeation and Ultracentrifuge are two examples of hydrodynamic techniques. To distinguish SUVs from radial MLVs, exclusion chromatography on large pure gels was used. Large vesicles with a diameter of 1-3 μm, on the other hand, frequently fail to enter the gel and remain on top of the column. A thin layer chromatography system based on agarose beads has been developed as a convenient and quick method for estimating the size distribution of liposome preparations. However, it was not stated if this technique was susceptible to a physical obstruction of agarose gel pores, as is the case with more traditional column chromatography.

3) Encapsulation Efficiency and Trapped Volume: These factors influence the amount and rate of water soluble substances entrapped in liposomes' aqueous compartment.

a) Encapsulation Efficiency: This term refers to the percentage of the aqueous phase, and therefore the percentage of water soluble medication, that becomes entrapped during liposome production, and is generally represented as percent entrapment/mg lipid. The effectiveness of encapsulation is measured using two methods: minicolumn centrifugation and Protamine aggregation. On a small scale, minicolumn centrifugation is commonly employed for liposome purification and separation. The hydrated gel is placed in a 1ml syringe barrel without a plunger and plugged with a Whatman filter pad in the minicolumn centrifugation technique. In a centrifuge tube, this barrel is resting. To remove extra saline solution from the gel, spin this tube at 2000 rpm for 3 minutes. The gel column should be dry and have come away from the barrel's side after centrifugation. After that, the eluted saline is drained from the collecting tube. Liposome suspension (0.2ml) is added dropwise to the top of the gel bed, and the column is spun for 3 minutes at 2000 rpm to expel the void volume containing the liposomes into the centrifugation tube. After that, the elute is extracted and placed away for analysis. For neutral and negatively charged liposomes, the protamine aggregation technique can be utilised.

b) Trapped volume: This is an essential feature that controls vesicle morphology. The aqueous entrapped volume per unit quantity of lipids is referred to as the trapped or internal volume. This can range between 0.5 and 30 microlitres per micromol. To estimate trapped/external volume, different materials such as spectroscopically inert fluid, radioactive markers, and fluorescent markers are employed. The simplest approach to determine internal volume is to measure the quantity of water directly by substituting a spectroscopically inert fluid (deuterium oxide) for the external medium (water) and then detecting the water signal using NMR by distributing lipid in an aqueous solution containing a non-permeable radioactive solute, the trapped volume may also be measured experimentally. The proportion of solute trapped is measured by centrifugation to remove external radioactivity, and then residual activity per lipid is determined.

5) Drug Release: A well-calibrated in vitro diffusion cell can be used to analyse the process of drug release from liposomes. Before conducting costly and time-consuming in vivo investigations, in vitro tests can be used to estimate pharmacokinetics and bioavailability of a liposome-based formulation. The pharmacokinetic
performance of liposomal formulations was predicted using dilution-induced drug release in buffer and plasma, and the bioavailability of the drug was determined using an assay that measured intracellular drug release induced by liposome degradation in the presence of mouse-liver lysosome lysate.

LIPOSOMES TARGETING 15-20

Types of Targeting

1. Passive targeting such commonly given liposomes have been demonstrated to be swiftly removed from the bloodstream and taken up by the RES in the liver and spleen as a passive targeting method. When liposomes are addressed to macrophages, the capability of macrophages may be taken advantage of. Successful delivery of liposomal antimicrobial drugs to macrophages has proven this. As an initial stage in the development of immunity, liposomes have been utilised to target antigens to macrophages. For example, in rats, i.v. injection of liposomal antigen evoked a spleen phagocyte-mediated antibody response, but non-liposome related antigen elicited no antibody response.

MECHANISM OF ACTION

Active targeting is the targeting agents must be positioned on the liposomal surface in such a way that the interaction with the target, i.e., the receptor, can be tabulated, similar to a plug and socket mechanism. The liposome is constructed physically in such a way that the lipophilic portion of the connection is fixed into the membrane during membrane formation. The hydrophilic portion of the liposome's surface on which the targeting agent must be maintained in a sterically correct position to bind to the cell's receptor. The use of active targeting can be achieved.

Antibodies or other recognition sequences [e.g. carbohydrate determinants like glycoprotein] are added to these standard or stealth liposomes in immuno liposomes. The antibody binds to the liposome and directs it to certain antigenic receptors on a cell.

- Glycoproteins and glycolipids are cell surface components that aid in cell-cell recognition and adhesion.
- Magnetic liposomes: Magnetic iron oxide is present in these liposomes. An external oscillating magnetic field in the delivery location can guide these liposomes.
Temperature- or heat-sensitive liposomes: These are made with a transition temperature that is just above body temperature. After arriving at the spot, the medication was released by heating it from the outside.

APPLICATIONS

1. Lysosomal storage illness
2. Cell biology application
3. Metal storage disease
4. Against Leishmaniasis
5. Medicines delivered through the eyes
6. Liposomes as vaccine carriers
7. Liposomes as medicine carriers in oral therapy
8. Liposomes for topical use
9. Liposomes for pulmonary administration

LIPOSOMAL PREPARATION

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CONCLUSION

Liposomes have shown to be highly effective carriers for targeted medication delivery. The versatility of their behaviour may be used to administer drugs by any route of administration and to any drug material, regardless of solubility characteristics. The use of liposomes in the transport of medicines and genes is promising and will almost certainly continue to evolve in the future.

The road from the discovery of liposomes' therapeutic value to their acceptance as a mainstream drug delivery technology has been long and convoluted in the last decade. Liposome systems have been studied in the clinic for a variety of applications, including infection sites and imaging, vaccines, gene delivery, and tiny molecular pharmaceuticals, infection and cancer treatment, lung illness and skin diseases, and so on. Several liposomal formulations are already on the market, and many more are in the works for illness therapy. Traditional liposome preparation and size reduction techniques are still widely used since they are simple to use and do not require specialised equipment. However, not all laboratory-scale procedures are easily scaled up to produce industrial liposomes Many traditional methods for making small and big unilamellar vesicles use water miscible/immiscible organic solvents or detergent compounds. The need to improve the design and stability of liposomal diagnostic and therapeutic systems will continue to drive the development of new and more efficient manufacturing methods.
REFERENCES: