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A brief review on Liposomal Drug Delivery System

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ABSTRACT: Liposomes are the spherical shape drug a delivery vesicle in which aqueous core is completely encompassed by one or more phospholipid bilayer. Liposome has been used for a number of diseases ranging from cardiovascular disease to bacterial infection whereas; it has also shown its effectiveness in different field like cosmetic and food industry. A liposome is a formulation which has the ability to overcome with the limitation of conventional therapies. A number of liposome formulations are presently in clinical trial however, few of them are in clinical use. Ocular and inhalation route are some of the advanced technology for delivery of liposome. Pulmonary delivery is useful due to their solubilization capacity for poorly water-soluble substances. However liposomes-based vaccines have been demonstrated in clinical trials and further human trials are also in progress. This review discusses the mechanism of liposome formation, method of preparation, evaluation, their application as well as few marketed product. The liposomal approach can be utilized to improve the pharmacokinetics and therapeutic efficacy and simultaneously reducing the toxicity of various highly potent drugs.

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INTRODUCTIONS:

Development of NDDS (new drug delivery system) always requires a considerable attention. Delivery of drug at a rate directed by the need of the body, over the period of treatment and delivery of the drug to the targeted site are the two main criteria for NDDS [1]. The liposomal systems permits a various range of drugs to be enclosed and deliver both hydrophilic and hydrophobic substance because it has the ability to entrap both hydrophilic and lipophilic compounds. Thehydrophobic orlipophilic molecules are inserted into the bilayer membrane; where as hydrophilic molecules can be entrapped in the aqueous center [2].

In the year1965, Alec D. Bangham discovered the term Liposome. It was derived from the Greek word, where lipo means "fatty" constitution and soma means "structure" [3]. Liposomes are comparatively small in size and it ranges from 50 nm to several micrometers in diameter. Liposomes generally have minimal toxicity and are supposed to be pharmacologically inactive, as they tend to be composed of natural phospholipids [4]. These are spherical vesicle in which aqueous core is completely encompassed by one or more phospholipid bilayers. However, an increasing number of studies have shown that liposomes are not as immunologically inert [5].

MATERIALS USED TO PREPARE LIPOSOME:

Phospholipid:

Phospholipids are the basic structural components of a biological membrane such as the cell membrane. Phospholipid consists of a hydrophilic or polar head and hydrophobic or lipophilic tail. Hence hydrophilic drugs can be encapsulated in the aqueous phase whereas lipophilic drug molecule can be incorporated in the lipid layer. However hydrophobic tail consists of 2 fatty acid chain containing 10-20 carbon atoms and 0.6 double bonds in each chain. Whereas hydrophilic head consist phosphoric acid bound to water-soluble molecule [8,9].

Cholesterol:

Cholesterol does not form bilayer structure, but can be incorporated into phospholipid membrane in very high concentration. In order to improve the fluidity of the bilayer membrane and reduce the permeability of the bilayer membrane in the presence of biological fluids such as blood/plasma. Cholesterol appears to reduce the interaction with blood proteins [8,9].

MECHANISM OF FORMATION OF LIPOSOME:

The hydrophilic head are attracted toward water where the head part faces toward the water and the hydrophobic tail are repelled by the water, when the lipid is exposed to aqueous environment, due to its amphipathic nature the phospholipid orient themselves to form bilayer where one layer of the phospholipid faces outside of the cells. Whereas another layer of the phospholipid faces inside the cell to avoid the water phase. The hydrocarbon tail of one layer faces the hydrocarbon tail of another layer and combines to form bilayer this structure is also called as lamella. Upon further hydration, the lipid cake (lamella) swells eventually that curves to form closed vesicles in the form of spheres known as liposome [10].

METHOD OF PREPARATION OF LIPOSOME:

Hand shaking:

It is the most common and simple method used for the preparation of MLVs. In these processes, the lipids are dissolved in solvents which are then transfer to round bottom flask. The RBF containing the mixture is then attached to rotary evaporator and then it was rotated at 60 rpm until a dry thin layer is formed after that it is dried in lyophilizer to remove the last traces of solvent and it is hydrated with phosphate buffer saline containing the material to be entrapped and then it was attached to rotary evaporator at 60 rpm or below it was rotated until the layer adhering on the wall of the RBF is removed and it was kept stand at room temperature for 2 h upon hydration the lamella swells and form myelin figure [11,12].

Non-shaking method:

In these method lipid mixed with chloroform: methanol is spread over the conical flask and the solution is evaporated at room temperature without disturbing by the flow of nitrogen. After the solution gets dried it is hydrated by water-saturated nitrogen which is passed through the conical flask until the opacity of dried lipid film disappears. After hydration, the lipid gets swelled. Then the flask is inclined to one side and 10 to 20 ml of 0.2 M sucrose in distilled water is added to the side of the flask and then the flask is slowly returned to its original position. The fluid gently runs over the lipid layer on the bottom of the flask. Then the flask is flushed with nitrogen and sealed it was then allowed to stand for 2 h at room temperature. After swelling the suspension is centrifuged at 12000 g for 10 min at room temperature [11,12].

Micro- emulsification:

This method is also known as microfluidization. Here microfluidizer is used to prepare small MLVs from concentrated lipid dispersion. The lipid can be introduced into fluidizers, either as a slurry of unhydrated lipids in organic medium or as a dispersion of large MLVs. Microfluidizer pumps the fluid at very high pressure through a 5 µm orifice and then it is forced along defined microchannels, which direct two streams of fluid to collide together at right angles at a very high velocity, thereby affecting an efficient transfer of

Table 1. Advantages and disadvantages of liposome (LPs) [6-8].

Advantages	Disadvantages
Delivery of hydrophobic (e.g. amphotericin B) hydrophilic (e.g. cytarabine) and amphipathic agents.	Once administrated, the liposome cannot be removed.
LPs increases efficacy and therapeutic index of drug (actinomycin-D)	The possibility of dumping, due to faulty administration.
Liposome increase stability via encapsulation	Leakage of the encapsulated drug during storage.
Suitable for controlled release	Low solubility
Suitable to give localized action in particular tissue	Production cost is high.
Suitable to administer via various routes	
LPs reduces the exposure of sensitive tissue to a toxic drug.	

Table 2. Classification of Liposomes [7,12].

Based on structural	Based on method of preparation	Based on the application
Multilamellar large vesicle (MLVs) has several bilayer, and are 0.5 µm in diameter.	REV are single or oligo lamellar vesicle made by reverse phase evaporation	Conventional liposome (CL) are neutral or negatively charged phospholipid and cholesterol
Oligo lamellar vesicle (OLVs) is made up of 2-10 bilayer & Diameter: 0.1-1µm.	MLV-REV multilamellar vesicle made by reverse phase evaporation method	Fusogenic Liposomes (RSVE): Reconstituted sendai virus envelopes
Unilamellar vesicle (UV) are of all size range	Stable plurilamellar vesicle (SPLV).	pH-sensitive LPs: Phospholipids such as PE or DOPE with either CHEMS or OA
Small unilamellar vesicle (SUVs): Composed of a single lipid bilayer, diameter: ranging from 30-70 nm.	FATMLV are multilamellar vesicle made by Frozen and thawed method	Cationic LPs: Cationic lipids with DOPE
Medium unilamellar vesicle (MUV)	VET is the vesicle prepared by the extraction method	Immuno-LPs: immune LPs with antibody on their surface to enhance target site binding.
Large unilamellar vesicle (LUV): > 100 µm in size.	dehydration-rehydration method (DRV)	Long Circulatory (stealth) Liposomes (LCL): Liposome that persist for prolong period of time in the bloodstream
Giant unilamellar vesicle (GUV) > 1 µm in size		
Multivesicular (MV) vesicle > 1 µm in size.		

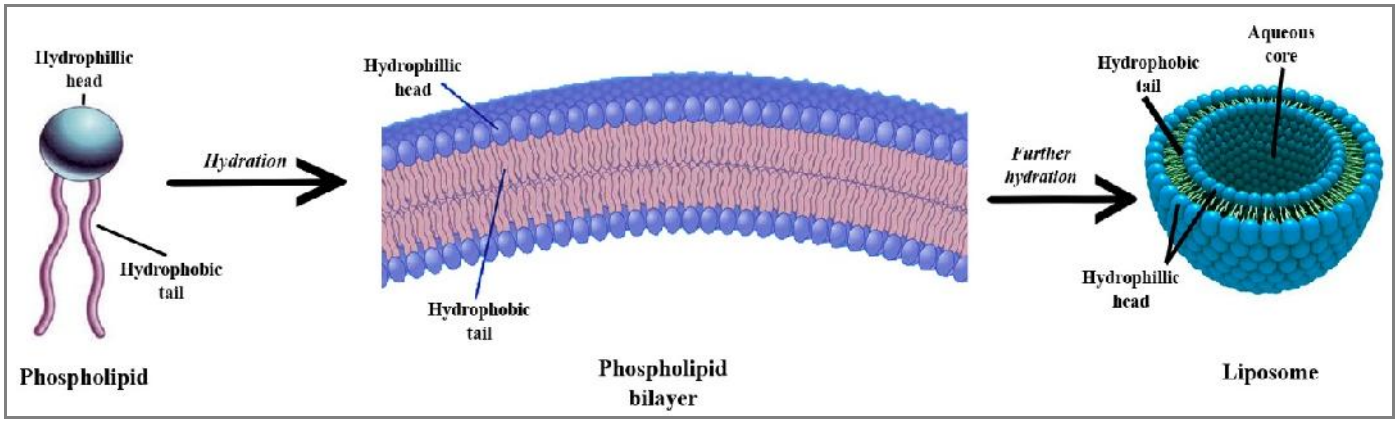

Fig 1. Mechanism of the liposome formulation.

energy. The fluid that is collected to be recycled through spherical dimension is obtained after a single pass; the size of the vesicle is reduced to a size 0.1 and 0.2 µm in diameter [13,14].

Membrane extraction:

This method is carried out either with LUV or MLV. So initially, in these processes, the phospholipids are introduced in the buffered saline solution to form LUV or MLV. After that, the liposome is passed through polycarbonate membrane filter which results in uniform distribution of liposome which is of about 100 nm in diameter [14].

Ultra-sonication:

Probe sonication:

The tip of the sonicator is directly dipped into the LPs dispersion which results in the local heating so the vessel containing the LPs dispersion must be immersed into the ice bath. Sonication upto 1 h may result in de-esterification of more than 5 % lipid. It has the chances of contaminating the preparation with metal which may lead to degradation of the lipid. This method is generally used for the suspension which requires high energy in a small volume [15,16].

***Bath sonicator*:**

These processes are generally used for the large volume of dilute lipids which may not be required to reach the vesicles size. The liposome suspension is kept in a test tube and it placed in 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. Size of the liposome is influenced by temperature, composition and concentration, sonication time and power, the volume of the product [15,16].

French pressure cell:

The liposomes prepared by this technique are less likely to suffer from the structural defect and instability as observed in the sonicated vesicle. Leakage of contents from liposome preparing using French press is slower and slower than sonicated liposome [17].

Dried reconstituted vesicle:

Here the preformed liposomes are rehydrated to an aqueous fluid containing an active ingredient which is followed by dehydration of the mixture [17].

Freeze thawing sonication:

In this method, SUVs are rapidly frozen and then by slow thawing. The aggregated materials are then The pump in interaction chamber until vesicles of the sonicated to form LUV. The formation of ULV is due to the fusion of SUV during the processes of freezing and or thawing. This type of fusion is strongly inhibited by increasing the ionic strength of the medium and by increasing the phospholipid concentration. The encapsulation efficiencies from 20 to 30 % were obtained [18].

Ethanol injection:

In this method, a lipid solution of ethanol is rapidly injected to a vast excess of the buffer as a result MLVs are immediately formed [18].

Ether injection:

A solution of lipids dissolved in diethyl ether or ether and the mixture is slowly injected to an aqueous solution of the material to be encapsulated at 55 to 65 °C or under reduced pressure. The subsequent removal of ether under vacuum leads to the formation of liposomes [19].

Double emulsion: In this method the active ingredient is mixed to the aqueous phase (w_1) and then it was mixed with an organic phase to make a primary emulsion (w_1/o) and then the primary emulsion is mixed with an aqueous phase to make a double emulsion $w_1/o/w_2$. The removal of the solvent leaves microspheres in the aqueous continuous phase, making it possible to collect them by centrifuging or filtering [19].

Reverse phase evaporation vesicle:

In this method at first w/o emulsion is prepared by brief sonication. The liposome is formed when the residual solvent is removed by rotary evaporation under reduced pressure [20].

Dialysis:

The detergents at their CMC have been used to solubilize lipid. As the concentration of detergent is reduced in the aqueous phase the detergent molecule can be washed away from mixed micelle by dialysis. lipoprep a commercial version of the dialysis system is available for the removal of detergent [20].

Other techniques have been used for the removal of detergents [21,22]:

(a) By using Gel Chromatography involving a column of Sephadex G-25.

(b) By adsorption or binding of Triton X-100 (a detergent) to Bio-Beads SM-2.

(c) By binding of octyl glucoside (a detergent) to Amberlite XAD-2 beads.

Pro-liposome:

In this process, the free-flowing granular material is prepared by coating lipid and drug onto a soluble carrier which on hydration forms an isotonic liposomal suspension [24].

EVALUATION OF LIPOSOME [23-25]:

Size and its distribution:

When a liposomeis used for parenteral or inhalation size distribution plays an important role. The varioustechnique used to determine the size of the vesicle are light microscopy, fluorescence microscopy, electron microscopy techniques, scanning electron microscopy (SEM), transmission electron microscopy (TEM), negative staining technique, atomic force microscopy (AFM), cryogenic tem, environmental SEM.

Surface charge:

Vesicle surface charge can be determined by measurement of particle electrophoresis mobility. It is expressed as zeta potential which can be calculated using Henry equation;

$\tau = \mu E 4\pi\eta/\epsilon$ ..... (1)

Where, τ = Zeta potential, μe = Electrophoretic mobility, η = Viscosity of medium and ε = Dielectric constant.

Drug release:

In-vitro drug release of the formulation can be carried out using Franz diffusion cell. In the latter, a known amount of sample was placed in a dialysis membrane at 37 °C. The receptor compartment consists of a buffer solution and the donor compartment consists of the liposomal formulation. At a specific interval of time, the sample was withdrawn from the receptor compartment and the equivalent amount of the fresh solution was replaced to the receptor compartment. The drug release of the sample was assayed by UV spectrophotometer. The drug diffused can be examined using the following formula.

Drug diffused (%) = C_rV_r/ C_dV_d …….. (2)

Where, C_r = Conc. of drug in the receptor compartment (RC), V_r = Volume of the RC, C_d = Conc. of drug in the donor compartment (DC) and V_d = Volume of the DC.

Lamellarity:

^{31}P nuclear magnetic resonance (NMR) analysis:

In this process, the broadening agent such as manganese ions is added and the signals are recorded both before and after adding the broadening agent which give information about outer to inner phospholipid. Other methods for determination include freeze electron microscopy, small angle X-ray scattering.

Phospholipid (PL) concentration:

The PL concentration in a LPs, estimated by Bartlett assay, Stewart assay and thin layer chromatography.

***Bartlet assay*:**

This process is very sensitive to inorganic. Here the phospholipid phosphorus in the sample is first hydrolyzed to inorganic phosphate which is converted to phosphomolybdic acid on the addition of ammonium molybdate. Phosphomolybdic acid is then quantitatively reduced to a blue color compound by amino naphthylsulphonic acid and the intensity of the bluecolor is measured spectrophotometrically which is then compared with the standard curve of phospholipid.

Stewart assay:

In this method PL with ammonium ferrothiocynate form a complex in organic solution. The absorbance of samples is compared with a standard curve of phospholipids. The standard curve is prepared by adding 0.1M ammonium ferrothiocynate solution with different concentrations of PL in chloroform. The samples are treated and the optical density of the solutions is measured at 485 nm. Here the presence of inorganic phosphates does not interfere with the assay. Whereas, it is not applicable to samples where mixtures of unknown PL may be present.

***Thin layer chromatography*:**

This process is employed for estimating the purity and concentration of lipids. If the compound is pure, it should run as a single spot in all elution. Whereas if the compound is degraded a long smear with a tail trailing to the origin is observed when compared to the pure material, which runs as one clearly defined spot.

Cholesterol analysis:

Qualitative estimation of cholesterol can be done by using a capillary column of flexible fused silica. However quantitative analysis can be done by measuring the absorbance of purple complex produced with a combined reagent containing ferric perchlorate, ethyl acetate and sulphuric acid at 610 nm.

Biological characterization:

LPs formulation for parenteral use should be pyrogen-free and sterile which can be analyzed by LAL test and aerobic or anaerobic test.

APPLICATIONS [26,27]:

Pharmaceutically:

- Cancer chemotherapy.
- Liposomes as a carrier of the drug in the oral treatment.
- Liposomes for pulmonary delivery.
- Against Leishmaniasis.
- Lysosomal storage disease.
- Cell biological application.
- Metal storage disease and ophthalmic delivery of drug.
- Cationic liposomes for gene delivery.
- Liposomes for diagnostic imaging.
- Liposomes for vaccines.
- Systemic liposomal drug.
- Topical Liposomal Drugs.

Cosmetics:

The liposome used in cosmetic can reduce the dryness of the skin which is the primary cause of the aging. "Capture" was a first liposomal cosmetic product in the year 1986 launched by Christian Dior which was used as an anti-aging cream. Liposome has also been used for aftershaves, hair conditioners, lipstick, treatment of hair loss etc.

Food:

The liposomes also gain its importance in a food product. Food scientist has utilized liposome for controlled delivery of Enzymes, Vitamins, Proteins and Antioxidants.

Table 3. Marketed product approved by FDA [27,28].

Drug	Disease	LBDS
Verteporfin	Molecular degeneration	Cationic
Vincristine	Non-Hodgkin lymphoma	Conven
Amphotericin B	Anti-fungal prophylaxis	Conven
Daunorubicin	Leukemia and solid tumors	Conven
Cytarabine or cytosine arabinoside	Neoplastic meningitis and lymphomatous meningitis	Conven
Morphine sulphate	Pain Management	Conven
Doxorubicin and bortezomib	Relapsed or refractory multiple myeloma	PEG
Doxorubicin	Leukemia, breast cancer, bone cancer, lung cancer, brain cancer	PEG

LBDS – Types of liposomal-based delivery system. PEG – PEGylated. Conven – Conventional.

CONCLUSION:

Liposomes have been explored for various diseases ranging from cancer treatment to pain Management. It is a formulation which hasthe ability to overcome with the limitation of conventional therapies. This approach can be utilized to improve the pharmacokinetics and therapeutic efficacy and simultaneously reducing the toxicity of the various highly potent drug.

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