A NOVEL DRUG DELIVERY OF LIPOSOMES: A COMPREHENSIVE REVIEW

Miruthula.U.V, Muthukumar.S KMCH college of pharmacy

**ABSTRACT**

Liposomes are little spherical artificial vesicles, which is made up of naturally derived phospholipids or pure surfactants. Liposomes will aid with active targeting because it has flexibility in coupling with site-specific ligands. The components of liposomes are Phospholipids, Cholesterol and other additional excipients. Liposomes was like vesicular in structures consisting of hydrated bilayers. The hydrophilic part was mainly composed of phosphoric acid which is bound to a water-soluble molecule whereas the hydrophobic part which consists of two fatty acid chains. Liposomes are classified into different types like., Based on their structural components, based on method of liposome preparation, based on composition and applications etc. There are some advantages and disadvantages of liposomes formulation.The main goal of an ideal method of liposomes formulation is to obtain efficient drug entrapment, narrow particles size distribution and long-term stability of liposome products. Method of preparation is based on two techniques i.e., active loading technique and passive loading technique. Physio-chemical and biological characteristics of the liposomal formulations, as well as their stability and drug entrapment efficient are controlled by different analytical methods. The pharmacokinetics of liposomes focused on the total body fluids and tissue distribution and their metabolism.Liposomal based formulation is implemented in the clinical fields. Doxil is the anticancer liposome which was first approved in USA.

**KEYWORDS:** Liposomes, characteristics, method of preparation, stability, pharmacokinetics, clinical applications.

**1.INTRODUCTION**

 Rational research in drug delivery began in 1950s with the advent of polyclonal antitumour antibodies developed for tumour targeting of cytotoxic drugs to experimental tumours. Liposomes were first described by Dr Alec D Bangham FRS (British haematologist) at the Babraham Institute, in Cambridge (early 1960s) and R. W. Horne, was the person who tested. According to legend, he was experimenting with new laboratory equipment, and he made a noted observation about phospholipids forming closed multiamellar vesicle spontaneously in aqueous solution which took two years to be proved. The name liposome derived from two Greek words: 'Lipos' - fat and 'Soma' - body. Liposomes are little spherical shaped artificial vesicles, which is made up of naturally- derived phospholipids with mixed liquid chain (like egg phosphatidyl-ethanolamine), or of pure surfactant components like dioleoyl phosphatidyl-ethanolamine. It consists of an internal aqueous compartment entrapped by one or multiple concentric lipidic bilayers.

 The phospholipid bilayer envelope was a cell-like boundary appropriate for affords liposomes a functional scaffold suitable for fundamental cellular functions such as motility and shape change not to mention the ability to mimic the biophysical properties of living cells. Liposomes nanoemulsions were widely used nanoparticles in nanomedicine mainly due to their biocompatibility, stability, ease to synthesize and high drug loading efficiency high bioavailability and their safe excipients used in these formulations. Depending upon the composition and the structure, liposomes can separate hydrophobic or hydrophilic molecules from the solution. When the structural layer of phospholipid is disrupted, they are able to realign themselves into smaller structures. These reassembled bilayer structures are known as liposomes while a monolayer is called micelle. A liposome can be formed at a variety of size. Liposomes can aid with active targeting as it had flexibility in coupling with site-specific. They were biocompatible, completely biodegradable, non-toxic, flexible and nonimmunogenic for systemic and no systemic administrations.

 Liposomes could provide increased therapeutic index and efficacy. Liposomes has many advantages when compared with other methods of drug delivery, but they also have some limitations. The main limitation of the standard liposome delivery system is its fast clearance from circulation due to uptake by the reticuloendothelial system, initially in the liver. The cost of liposome formulation was high than other formulations. Liposome-cell interactions were influenced by a variety of factors, including composition, the diameters of liposomes, surface charge, biological environment and targeting ligand on the liposome surface.

 Fig.1 Structure of liposome

**2.COMPOSITION OF LIPOSOME**

 The components of liposomes are:

 1.**Phospholipids** 2. **Cholesterol** 3. **Additional excipients**

 2.1. Phospholipids:

Phospholipid id the major structural components of biological membrane, where there are two types of phospholipids were existed – phosphodiglycerides and sphingolipids, together with their corresponding hydrolysis as a product. The most commonly used phospholipid is phosphatidylcholine (PC) which is an amphipathic molecule, that consists of a hydrophilic polar head group, phosphocholine is a glycerol bridge and has a pair of hydrophobic acyl hydrocarbon chains. Glycerophospholipids contains a lipophobic head and a lipophilic tail. Liposome is made up of both natural and synthetic phospholipids.

 The hydrophilic group in the lipids may be negatively, positively charged, or zwitterionic (both negative and positive charge in the same molecule). The charge of the hydrophilic group provides stability through electrostatic repels. The hydrophobic group of lipids varies in the acyl chain length, saturation, and symmetry. To achieve charged vesicles, charged phospholipids like diethyl phosphate and stearyl amine will be used. Binding of sphingomyelin helps in enhancement of water permeability in some kinds of liposomes and proton permeability reduction. Lipids were capable to manipulate the surface charge, bio distribution, permeability, release and clearance of various formulations of liposomes.

2.2. Cholesterol:

 Cholesterol is a fat-like substance and an essential component of the body. Incorporation of sterols in liposomes bilayer can bring about major changes in the preparation of these membranes. Cholesterol does not form bilayer structure by itself but it can be incorporated into the phospholipid membrane in very high concentration. Cholesterol inserts into membrane with its hydroxy group which is oriented towards the aqueous surface and aliphatic chain aligned parallel to acyl chains in the centre of bilayer.

2.3. Additional excipient:

 Polyethylene glycol [PEG] on the liposome surface offers extended circulation property, protects the captured drug from inactivation, enhances stability and improves intracellular intake. PEG may produce stealth liposomes that are undetectable by the body’s reticuloendothelial system. Moreover, PEG assists in decreasing particle’s aggregation and improves the stability on storage. Cellular intake of PEGylated liposomes can be enhanced by ligands such as antibodies, vitamins, proteins and nucleic acids, which exists on the receptor surface of target cells.

**2.4. General Ingredients**

 Generally, liposome composition includes natural and/or synthetic phospholipids (Phosphatidylethanolamine, Phosphatidylglycerol, Phosphatidylcholine, Phosphatidylserine, Phosphatidylinositol) Phosphatidylcholine and phosphatidylethanolamine constitute the two major structural components of most biological membranes. Liposome bilayers may also contain other constituents whereas, cholesterol, hydrophilic polymer conjugated lipids and water. It improves the membrane bilayer stability, fluidity and reduces the permeability of water-soluble molecules through the membrane. An advantage of liposomes was the fact that the lipid membrane was made from physiological lipids which decreases the danger of acute and chronic toxicity.

**3. MECHANISM OF LIPOSOME FORMATION**

Liposomes were vesicular structures consisting of hydrated bilayers. Liposomes were formed by phospholipids (amphiphilic molecules having a hydrophilic head and hydrophobic tail). The hydrophilic part was mainly phosphoric acid bound to a water-soluble molecule whereas the hydrophobic part consists of two fatty acid chains with 10-24 carbon atoms and 0-6 double bonds in each chain. They form lamellar sheets when dispersed in aqueous medium by aligning themselves in such a way that the polar head group faces outwards the aqueous region while fattyacid group face each other forming a spherical, vesicle like structures called as liposomes.

 Fig.2 Formation of liposome

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 The reason for bilayer formation includes:

 • The unfavourable interactions created between hydrophobic and hydrophilic phase which can be minimized by folding into concentric vesicles which is closely packed.

 • The large free energy difference existing between the hydrophobic and hydrophilic environment is reduced by the formation of large vesicle formation. Since spherical structures has minimum surface tension and maximum stability. Hence there was maximum stability of self-assembled structure by forming vesicles.

 Liposomes which were targeted to specific tissues were developed by stabilization of phosphatidylethanolamine into bilayer with antibody derivates of fatty acids like palmitic acid. After binding to the target’s cell surface, immunoglobulin molecules concentration at contact point leads to the destabilization of bilayers. Finally, liposomal content is released at this site. Specific sub-cellular targeting is still in novice and is in vitro research phase.

**4.CLASSIFICATION OF LIPOSOMES**

 Liposomes are classified into different types:

1. Based on their structural components

* Multilamellar large vesicles (MLV)
* Oligolamellar vesicles (OLV)
* Unilamellar vesicles (UV)
* Medium sized unilamellar vesicles (MUV)
* Large unilamellar (LUV)
* Giant unilamellar vesicles (GUV)
* Multivesicular vesicles (MVV)

 2. Based on method of liposome preparation Fig.3 Size and lamellarity of liposomes

* Single or oligolamellar vesicle made by reverse phase evaporation method (REV)
* Multilamellar vesicles made by reverse phase evaporation method (MLV / REV)
* Stable plurilamellar vesicles (SPLV)
* Frozen and thawed MLV (FATMLV)
* Vesicles prepared by extrusion method (VET)
* Vesicles prepared by fusion (FUV)
* Vesicles prepared by French press (FPV)
* Dehydration‐ rehydration vesicles (DRV)
* Bubblesomes (BSV)

3.Based on composition and applications

* Conventional liposomes
* Fusogenic liposomes
* pH sensitive liposomes
* Stealth liposomes
* Immune liposomes

**5. ADVANTAGES OF LIPOSOMES**

* Liposome increased efficacy and therapeutic index of drug.
* Liposome provide controlled and sustained release.
* Suitable for delivery of hydrophobic, hydrophilic and amphipathic drugs and agents.
* Site avoidance effect.
* Flexibility to couple with site specific ligands to achieve active targeting
* Liposome increase stability via encapsulated drug.

**6. DISADVANTAGES OF LIPOSOMES**

* Sometimes, they are less stable.
* Short half-life.
* Difficult in large scale manufacture and sterilization.
* Very high production cost
* Low solubility and oxidation off bilayer phospholipid.
* Low therapeutic index and dose effectiveness.

**7. METHODS OF LIPOSOME PREPARTION**



The main goal of an ideal method of liposome formulation is to have an efficient drug entrapment, narrow particles size distribution and long-term stability of liposome products.

* 1. ACTIVE LOADING TECHNIQUE
1. Prollposome:

 Lipid and active substances(drug) were covered onto a solvent transporter to shape free-streaming granular material in supportive of liposomes which structure an isotonic liposomal suspension of hydration.

1. Lyophilization:

 The expulsion of water from items in a solidified state at incredibly decreased weight is known as lyophilization (freeze-drying).

* 1. PASSIVE LOADING TECHNIQUE

 I.MECHANICAL DISPERSION METHOD:

1. Lipid film hydration

 The Bangham method was the first commonly used method for liposome preparation. In this method, first preparation of homogenous mixture of lipids takes place. By dissolving and mixing a lipid component in an organic solvents like ethanol, chloroform etc, the solvent was removed to yield a film of lipid. This lipid

 Fig.4 Lipid film hydration method

film was thoroughly dried by placing the vial or flask on a vacuum pump by removing the residual organic solvent. Lipid solution was frozen by placing the container on a block of dry ice or swirling the container in dry ice- acetone or alcohol.

 Advantages: Disadvantage:

1. Simple process i. Difficulty in scaling up.
2. Straightforward approach ii. Time consuming method.
3. Micro emulsification:

 In this method, small vesicles were prepared by micro emulsifying lipid composition using high shearing stress generated from high-pressure homogenizer. These methods were used to prepare the small lipids vesicles on a commercial scale.

1. Sonication:

 Sonication was perhaps the most extensively used method for the preparation of SUV. The MLVs were sonicated either with a bath type sonicator or a probe sonicator under a passive atmosphere. The main disadvantage of the sonication method are very low internal volume and efficiency of encapsulation is also low and possible degradation of phospholipids and compounds to be encapsulated and the presence of MLV along with SUV lead to metal pollution.

1. French pressure cell:

 Extrusion French pressure cell involves the extrusion of MLV through a small orifice. The important feature of the French press vesicle is that the protein does not seem to be important pretentious during the procedure as they are in sonication.

1. Membrane extrusion:

 In this method, the processed liposome has a narrow size distribution and selected average sizes less than about 0.4 microns.

1. Dried reconstituted vesicle:

 This method starts with freeze drying of a dispersion of empty SUVs and then rehydrating it with the aqueous fluid containing the material to be entrapped

 Fig.5 Dried reconstituted vesicle method

 However, this leads to dispersion of solid lipids in finely subdivided form. Liposomes obtained from this method are usually uni- or oligo- lamellar of the order of 1.0 µm or less in diameter. Entrapment yield can vary, but 40% is fairly standard compared with 2-10% for MLVs prepared by hand-shaking method.

1. 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.

II.SOLVENT DISPERSION METHOD:

1. Ethanol injection:

 An ethanol solution of lipid is injection rapidly into an excess of saline or another aqueous medium, through a fine needle. The force of the injection was unusually sufficient to achieve complete mixing, so that the ethanol was diluted almost instanteously in water, and phospholipid molecules were dispersed evenly throughout the medium.

 Fig. 6 Ethanol injection method

1. Ether injection:

 A solution of lipids dissolved in diethyl ether or ether-methanol mixture was gradually injected to an aqueous solution of the material to be encapsulated. The consequent removal of ether under vacuum leads to the creation of liposomes. The main disadvantages of the technique were that the population is heterogeneous and the exposure of compounds to be encapsulated to organic solvents at high temperature.

1. Double emulsion

 Double emulsion method is prepared firstly by dissolving the drug in the aqueous phase (W1), which is then emulsified in an organic solvent like ethanol, chloroform of a polymer is called primary emulsion (W/O). after the primary emulsion it is mixed in an emulsifier-containing aqueous solution (W2) to make a W1/O/W2 which is double emulsion.

1. Stable plurilamellar vesicles:

 In this method, water in oil dispersion is prepared earlier with excess lipid, but drying process is accompanied by continued bath sonication with a stream of nitrogen.

 III.DETERGENT REMOVAL METHOD:

 Detergents were used to solubilize the lipids at their critical micellar concentrations. LUVs are shaped by eliminating the detergent by dialysis and combining the micelles. In this method, the liposomes are formed in homogenous size and the retention of detergent contaminants is the drawback of this method.

**8.CHARACTERISATION OF LIPOSOMES**

 Physio-chemical and biological characteristics of the liposomal formulations, as well as their stability and drug entrapment efficiency are controlled by different analytical methods. The characteristics parameters of liposomes are based on different categories:

1. Physical characterisation
2. Chemical characterisation
3. Biological characterisation

**8.1. Physical characterisation:**

The control of physical parameters was based on measuring vesicle shape, surface morphology, mean vesicle size and size distribution, surface charge, lamellarity, phase behaviour, percent of free drug/percent capture and drug release.

a). Size and size distribution

 The vesicle size was crucial to determine in the vivo release of drug- loaded liposomes. The average size of the liposome is depended on the method of preparation and phospholipids. The particle size and distribution are critical parameters especially when liposomes are intended for therapeutic use by inhalation or parenteral route. Liposomes have sizes of 30nm to several micrometres. The size of deformable liposomes in the size range of 300-350nm has been reported to maintain the drug in the skin layer for topical use and avoid its absorption into the systemic circulation.. So, other methods used d to evaluate the size and size distribution such as:

 i). Microscopic techniques:

 Microscopic techniques such as optical microscopy, scanning electron microscopy (SEM), negative stain TEM and freeze-fracture TEM. SEM and TEM techniques were used for imaging of liposomes and also provides information about bilayer thickness and inter-bilayer distance of liposome.

 ii). Hydrodynamic techniques:

 Hydrodynamic techniques such as ultracentrifugation, field flow fractionation and gel exclusion chromatography and analytical centrifugation procedures were used to estimate the molecular mass of compound and it also used for comparison of size distribution, elution characteristics and uniformity of the liposomes. Large vesicles of 1-3 µm diameter usually fail to enter the gel and are retained on the top of the column.

 iii). Diffraction light scattering techniques:

 Proton correlation spectroscopy (PCS) is the analysis of the time dependence of intensity fluctuation in scattered laser light due to the Brownian motion of particles in solution/suspension. Since small particles diffuse more rapidly than large particles, the rate of fluctuation of scattered light intensity varies accordingly. Thus, the transitional diffusion coefficient (D) can be measured, which in tur can be used to determine the mean hydrodynamic radius (Rh) of the particles using Stokes-Einstein equation. Diffraction light scattering techniques such as laser light scattering, quasi-elastic light scattering and photon correlation spectroscopy give information about the size of the lipid vesicles.

 b). Surface charge

 A technique has been developed that separates extruded vesicles on the basis of their surface charge by electrophoresis on a cellulose acetate plate in a sodium borate buffer pH 8.8. The lipid samples (5nmoles) are applied to the plate and electrophoresis is carried out at 4°C on a flatbed apparatus for 30 min at 18 V/cm. The plate is dried and the phospholipids are visualized by the molybdenum blue reagent. Liposomes upto 0.2 µm diameter can migrate on this support and with this technique as little as 2 moles % of charged lipids can be detected in a liposome bilayer. This sensitive assay should prove valuable for examining the charge heterogeneity in liposome preparation for following fusion between two populations of vesicles with different charge and for determining the presence of charge impurities e.g., fatty acids in liposome.

 Liposome characterization is the surface charge of the liposome measured by the zeta potential. The zeta potential is the key factor that affects the cellular uptake and targeted drug delivery. The particles move in an electrical of known strength in the interference pattern of two laser beams and produce scattered light which depends on the speed of the particles. However, if the zeta potential value of a particle was too small, there would be a force of attraction greater than the repulsion force so that it causes coagulation and flocculation which indicate colloidal instability.

 c). Lamellarity

 Lamellarity was defined as the number of lipid bilayers present around the lipid vesicles. Liposomal lamellarity can be measured by using cryo-electron microscopy, 31P-nuclear magnetic resonance (NMR) and small angle X-ray scattering (SAXS) technique that provide information about size, homogeneity and lamellarity of liposomes. Liposome lamellarity is often accomplished by methods that are based on the visible or fluorescence signal change of lipids marker upon reagents addition. This approach is reviewed in more detail, since it is a relatively simple procedure that can be easily carried out in a standard lab. Several lipids can be used and results rely on the comparison of the total signal to the signal achieved from the reaction between the lipids marker and the specified reagents. To confirm the lamellarity results by an imaging method, freeze fracture technique with subsequent transmission electron microscopy was used.

 d). Phase behaviour of liposomes

 An important feature of lipid membrane is the existence of a temperature dependent reversible phase transition, where the hydrocarbon chains of the phospholipid undergo a transformation from an ordered(gel) state to more disordered fluid (liquid crystalline) state.

 e). Percent capture (Entrapment)

 The efficiency of entrapment is a key parameter in developing liposome-based delivery. High absorption efficiency can reduce cost and increase efficacy. For longer term stability test and for developing new liposome formulation or method of preparation, a technique was needed for separating free from entrapped material. In general, two methods may be used i.e., mini column centrifugation and protamine aggregation.

 f). In vitro Drug release studies

 The in vitro drug release studies are assessed at 370°C through in vitro diffusion cell or by using dialysis bag. The cell or bag must be wet through receptor medium containing pH 7.4 buffer with constant stirring under sink conditions, which mimics the in vivo conditions. Some millilitres aliquot of liposome suspension is placed in the dialysis bag, hermetically tied and dropped in the receptor compartment containing the dissolution medium. The entire system is kept at 37°C under continuous magnetic stirring and the receptor medium is closed to avoid evaporation of the dissolution medium.

**8.2. Chemical characterisation**

 Chemical analysis of liposomes was important to determine the purity and potency of various liposomal constituents. The most common chemical parameters which are analysed are:

1. Phospholipid concentration
2. Cholesterol concentration.
3. Phospholipid peroxidation.
4. Phospholipid hydrolysis.
5. Cholesterol auto-oxidation
6. Osmolarity.

 Phospholipid peroxidation is quantitatively determined using UV absorbance, iodometry (for hydroperoxidase) and GLC techniques. Phospholipid hydrolysis as well as cholesterol autooxidation can be determined using HPLC and TLC. pH of the liposomal dispersion can be determined using pH meter.

**8.3. Biological characterisation**

The importance of determining biological parameters was very helpful in determining the safety of formulation for therapeutic applications. Sterility, pyrogenicity and animal toxicity were determined during the biological characterisation of the liposomes.

1. **STABILITY OF LIPOSOMES**

Liposomes face a number of chemical and physical destabilisation processes. Liposomes can be degraded chemically through oxidation and hydrolysis.  The aspects in stability of liposomes are of two types:

* **Physical stability**
* **Chemical stability**
	1. **Physical stability:**

  The leakage rate strongly depends on the bilayer composition and the physiochemical nature of the drug. Aggregation is the formation of larger units of liposome material, these units are still composed of individual liposomes. This process can also undergo reversibly, e.g., by applying mild shear forces, or by changing the temperature or by binding metal ions that initially induced aggregation.   Bilayer permeability is not necessarily a constant parameter.

* 1. **Chemical stability:**

 The two acyl ester bonds are most liable to hydrolysis.  The glycerophosphate and phosphocholine ester bonds are more stable. The polyunsaturated acyl chains of phospholipids are sensitive to oxidation via free radical reactions. Phospholipids are chemically unsaturated fatty acids, prone to oxidation and hydrolysis, which may alter the stability of the drug products. Cyclic peroxides, hydroperoxides, malondialdehyde, alkanes are the major degradation products.

* 1. **Stability protocols:**

 The liposomal stability can be determined by storing it under some conditions. The conditions are:

1. Visual or microscopic examination.
2. Highest and lowest temperatures likely to be encountered (1 month)
3. Room temperature (12-24 months)
4. 2-3 freeze-thaw cycles. (20-25°C)
5. 6-8 heat-cool cycle (5-45°C)
6. **CLINICAL APPLICATION OF LIPOSOMES**

 Liposomes can be formulated in liquid (suspension), solid (dry powder) or semi-solid (gel, cream) forms. In vivo, they can be administered topically or via parenteral route.

* 1. . Cancer treatment:
* liposomal daunorubicin and pegylated liposomal doxorubicin versions has greatly prolonged circulation.
* Doxil was designed as a polyethylene glycol coated doxorubicin (DOX) liposome intended for the treatment of Kaposi's sarcoma.
* LipoDox is FDA approved PEGylated liposomal formulation encapsulating DOX manufactured by Sun Pharma.
* Daunorubicin was the second anthracycline antineoplastic drug loaded in liposomes to treat acute myeloid leukaemia (AML) under the generic name DaunoXome.
* Pegylated liposomal doxorubicin has shown substantial efficacy in breast cancer treatment both as monotherapy and in combination with another chemotherapeutics.
* Lipoplatin is developed recently by using cisplatin as a carrier for treating cancer.
* The thermo­-sensitive liposomal formulation ThermoDox, which contains Lys phosphatidylcholine and is employed in the treatment of various cancers like liver cancer, breast cancer, pancreatic cancer.
	1. . Liposomes in vaccinations:
* Liposome formulations could protect DNA/RNA and proteins payload from biodegradation.
* To enhance the immune response of diphtheria toxoid, liposomes were first used as immunological adjuvants.
* Two commercial vaccines based on virosome technology are currently on the market, Epaxal and Inflexal are hepatitis A vaccine.
	1. . Ophthalmic treatment
* So many drugs are used for the treatment of eye disorders like dry eye syndrome, corneal ulcer etc.
* The pharmaceutical preparations may be suspension form or ointment for topical application as well as in solution forms but those preparations will have poor ocular bioavailability. To omit this barrier, liposomal formulation is used.
* The ciprofloxacin/ciprocin is used widely in eye drops which is more effective in gram bacteria (+ and -)
	1. . Pain relievers management
* DepoDur is a formulation of morphine which is formulated in sustained release formula using DepoFoam Technology with extending the time of clinical effect.
* Exparel release Bupivacaine, uses the DepoFoam technology for sustained pain relief.

**CONCLUSION**

 In conclusion, liposomes have a diverse range of uses ever since it was first noted that it is able to self-assemble into vesicles. Crucial progress has been made in the long circulating liposome process which was not recognized immediately and they are removed by the cells of mononuclear phagocyte system. Liposomes with increased drug delivery to the desired disease locations, by the ability of long circulating residence time. Now they achieved the acceptance from clinical sector. Liposomes also promote the particular diseased cell to target within the disease site. The fact that all issues are associated with scaleup, stability and increasingly world wise lipid-based therapeutics in future.

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