LIPOSOME: METHOD OF PREPARATION, ADVANTAGES, EVALUATION AND ITS APPLICATION

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Notable research in drug delivery started in 1950's with the advent of polyclonal antitumor antibodies developed for targeting. Bangham et. Al. discovered liposomes in early 1960's. In this review article we are discussing about the liposome, methods of the liposome preparation advantages and their different application of the liposome. Liposomes are artificially prepared vesicles made of lipid bilayer. Liposomes can be filled with drugs, and used to deliver drugs for cancer and other diseases. Liposome is used for the targeted drug delivery system and increase the bioavailability and half life of the any drugs. Liposomes are surfactants, sphingolipids, glycol-lipids, long chain fatty acids and even membrane proteins and drug molecules or it is also called vesicular system. Liposomes have been extensively investigated for drug delivery, drug targeting, controlled release and increased solubility.
Keywords: Liposome, Controlled, Targeted drug delivery system, Classification, Methods

INTRODUCTION

Liposomes are described by British haematologist Dr. Alec D Bangham FRS in 1961 from the Babraham Institute at Cambridge. The name liposome is derived from Greek words 'Lipos' means fat and 'Soma' means body. Liposome means lipid body, spherical microscopic vesicle are composed of one or more concentric lipid bilayer separated by water and aqueous buffer compartment with a diameter ranging from 25nm- 1000nm.[1] Liposome are defined As "Liposome are the simple microscopic vesicles wherein an aqueous volume is entirely enclosed by a membrane composed of lipid molecule." Various amphipathic molecules are used to form liposome. The drug molecules can either be encapsulated in aqueous space or intercalated into the lipid bilayer.[2]

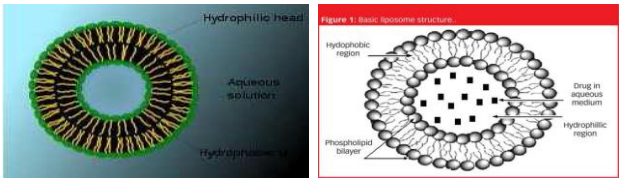

Fig 1 Liposome formed by phospholipids in aqueous solution

ADVANTAGES [3][4]

There are several advantages of the liposome-

- Provides selective passive targeting to tumour tissue (liposomal doxorubicin).
- Liposome are biocompatible, completely biodegradable, non-toxic and non immunogenic.
- Suitable for the delivery of hydrophobic, amphipathic and hydrophilic drugs.
- Site avoidance effect.
- Protect the encapsulated drug from external environment.
- Increased efficacy and therapeutic index.
- Increased stability via encapsulation.
- Reduce exposure of sensitive tissue to toxic drug.
- Improve protein stabilization.
- Provide sustained release.
- Alter pharmacokinetics and pharmacodynamics of drugs.

DISADVANTAGES [3][4]

- Production cost is high.
- Leakage and fusion of encapsulated drug /molecules.
- Sometimes phospholipids undergo oxidation and hydrolysis like reaction.
- Short half-life.
- Low solubility
- Less stability
- Quick uptake by cells of R.E.S
- Allergic reactions may occur to liposomal constituents
- Problem to target various tissues due to their large size

Classification of Liposome-[5]

Liposome may be produced by a wide variety of methods. Their nomenclature also depends upon the method of preparation, Structural parameters or special function assigned

LIPID FILM HYDRATION BY HAND SHACKING:- [7]

Liposome was prepared by physical dispersion technique using different ratio of lipid. In this method the lipids was dissolved in chloroform. This solution of lipid in chloroform is spread over flat bottom conical flask. The solution was then evaporated at room temperature with no disturbing the solution. The hydration of lipid film appearance was carried out with phosphate buffer (pH 7.4) was inclined to one side and aqueous medium containing drug to be entrapped and introduced into

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the side of flask and flask was slowly return to upright orientation. The fluid was allowed to run quietly over lipid layer and flask was allowed to stand for 2 hr at 37 ^{0}C for complete swelling, after swelling, vesicles are harvested by swirl the contents of flask to yield milky white suspension. Then formulations were subjected to centrifugation. Different batches of liposome were prepared in order to select an optimum formula. All batches of liposome were prepared as per the method described above and composition of lipids for the preparation of liposome.

SONICATION:-[8][17]

The Sonication is most extensively method used for the preparation of SUVs. Here, MLVs are sonicated either with bath type sonicator or probe type sonicator under a passive atmosphere. The main disadvantage of this method is very low internal volume, low encapsulation efficacy, degradation of phospholipids and elimination of large molecules, metal pollution from probes tip, and presence of MLV along with SUV. There are two sonication techniques:-

a) Probe sonication: These sonicator is directly engrossed into the liposome dispersion. In this method energy input into lipid dispersion is very high. The coupling of energy at tip results in local hotness, therefore, the vessels must be engrossed into water or ice bath. Throughout the sonication up to 1 h, more than 5% of the lipids can be deesterified. With the probe sonicator titanium determination slough off and pollute the solution.

b) Bath sonication: The cylinder containing liposomes is placed into a bath sonicator at controlled temperature it is usually easier method, in contrast to sonication by dispersion directly using the tip. The material is being sonicated and can be protected in a sterile vessel, dissimilar the probes unit, or under an inert atmosphere.

Based on structure parameters

SUV- Small Unilamellar vesicles (20-100nm)
MUV- Medium sized Unilamellar vesicles
LUV- large Unilamellar vesicles >100
MLV- Multilamellar large vesicles->0.5mm
OLV- Oligolamellar vesicles-0.1-1mm
UV- Unilamellar vesicles (All size ranges)
GUV- Giant Unilamellar vesicles->1mm
MV-Multivesicular vesicles->1mm

Based on method of liposome preparation

REV- Single or Oligo lamellar vesicle by reverse- phase evaporation method
MLV-REV- Multilamellar vesicles made by reverse- phase evaporation method
SPLV- Stable plurilamellar vesicles
FATMLV- Frozen and thawed MLV
VET- Vesicles prepared by extrusion technique
DRV- Dehydration –rehydration method

Method of liposome preparation

Mechanical dispersion method

- Lipid film hydration by hand shacking,
- Non-hand shacking or freeze drying
- Micro-emulsification
- Sonication
- French- pressure cell
- Membrane extrusion
- Dried reconstituted vesicles
- Freeze- Thawed liposome

Solvent dispersion method

(1) Ethanol injection
(2) Ether injection
(3) Double emulsion vesicles
(4) Reverse phase evaporation vesicles
(5) Stable plurilamellar vesicles

Detergent removal methods

(1) Detergent (chlorate, Aglycoside Triton X-100) Removal from mixed micelles by
- Dialysis
- Column chromatography
- Dilution
- Reconstituted Sendai virus enveloped

MACRO-EMULSIFICATION:-[9]

Micro-fluidizer is used for the preparation of small vesicles from concentrated lipid suspension. The lipids can be introduced into the fluidizer as a suspension of large MLVs. The equipment pumps fluid at very high pressure through 5 mm screen. Then it is forced with long micro channels, which

dried two streams of fluids, collide together at right angles at very high velocity. The collected fluid can be recycled by using pump and in interaction chamber until vesicles of spherical dimensions are obtained.

FRENCH-PRESSURE CELL:- [10][11]

French pressure cell involves the extrusion of MLV though a small orifice in important feature of the French pressure vesicle method is that the protein do not seem to the significantly during the procedure as they are in sonication. The method involves gentle handling of unstable methods. The method has several advantages over sonication method. The resulting liposome are rather larger the sonicated SUVs. The drawbacks of the method are that the high temperature is difficult to achieve and the working volumes are comparatively small (about 50 ml as the maximum).

REVERSE PHASE EVAPARATION:-[11]

The lipid mixture is added to round bottom flask and the solvent is removed under pressure by a rotary evaporation. The system is purge with nitrogen and lipids are re-dissolved in the organic phase, vesicles will form. Diethyl ether and isopropyl ether are the usual solvent of choice after the lipids are re-dissolved the emulsion are obtained and then the solvent is evaporated from an emulsion by evaporation to a semisolid gel under reduced pressure and non encapsulated material is then removed. The resulting liposome are called reverse phase evaporation vesicle (REV). This method is the preparation of large macro-molecules with high efficiency.

ETHER INJECTION:-[12][13]

Ether injection, a solution of lipids is dissolved in ether or diethyl ether or methanol mixture which is slowly injected in aqueous solution of the material to be capsulation.The successive removal of the organic solvent under reduced pressure leads to the formation of liposome. The main disadvantage of the method is heterogeneous population and the exposure of compound to be encapsulating to organic solvents or high temperature.

ETHANOL INJECTION:-[12][14][15]

The ethanol injection method is ethanolic lipid solution is rapidly injected to a vast excess of preheated distilled water or TRIS-HCl buffer. The incorporation of the drug in liposomal vesicle depends on its hydrophilic/hydrophobic character. Nimesulide is lipid soluble component incorporates better in liposomes than 5-fluorouracil which migrates to external aqueous phase. The main advantage of ethanol injection method is including of non harmful solvent as ethanol method. The possibility formation of azeotrope with water reduces its applicability.

MECHANISM OF LIPOSOME FORMATION

In the order to understand why liposome is formed when phospholipids are hydrated, it requires basic understanding physiochemical phospholipids. Phospholipid is amphipathic molecules as hydrophobic tail and hydrophilic polar head. The hydrophobic tail is composed two fatty acid chain containing 10-24 carbon atoms and 0-6 double bonds in each chain. The polar end molecules are mainly phosphoric acid bound to a water- soluble molecule. The hydrophilic and hydrophobic domain/segment molecular geometry of amphiphilic lipid orient and self organize in ordered supramolecular structure confronted with solvent.

Structural Components:-

Phospholipids- [4][3]

These are derived from the phosphatidic acid. The back bone of the molecules is glycerol moieties. Glycerol containing phospholipids are the most common used component of liposome formulation and represent the greater than 50% of weight of lipid in biological membranes. At C_3 OH group is esterified to phosphoric acid.

Example of phospholipids ar

- Phosphatidyl choline (Lecithin)
- Phosphatidyl ethanolamine (Cephalin)
- Phosphatidyl serine (PS)
- Phosphatidyl inositol (PI)
- Phosphatidyl glycerol (PG)

For stable liposomes, saturated fatty acids are used. Unsaturated fatty acids are not used generally.

Sphingolipids-[4][3]

It is Backbone of sphingosine or a related base. These are the important constituents of plant and animal cells. This contains 3 characteristic building blocks.

- A mole of Fatty acids
- A mole of sphingosine
- A head group can vary from simple alcohol such as choline to very complex carbohydrates.

Most common Sphingolipids- Sphingomyelin.

- Glycosphingo lipids.
- Gangliosides- Found on grey matter, used as miner component for liposome production.

This molecule contain complex saccharides with the residues Sialic acid and their polar head group and thus have one or more negative charge at neutral pH.The liposome added in to provide a surface charged layer group.

Sterols- [4][3]

Cholesterol & derivative are often included in liposomes for-

- declining the fluidity or microviscosity of the bilayer
- falling the permeability of the membrane to water soluble molecules
- stabilize the membrane in the presence of biological fluids such as plasma (This effect used in the formulation of i.v. liposomes)

Liposome without cholesterol interacts with plasma proteins viz. albumin, transferrine, and macroglobuline. These proteins tend to extract bulk phospholipids from liposome, thus depleting the monolayer of the vesicles leadings to physical instability.

Synthetic phospholipids- [4][5]

Example: For saturated phospholipids are-

- Dipalmitoyl Phosphatidyl choline (DPPC)
- Disteroyl Phosphatidyl choline (DSPC)
- Dipalmitoyl Phosphatidyl ethanolamine (DPPE)
- Dipalmitoyl Phosphatidyl serine (DPPS)
- Dipalmitoyl phosphatidic acid (DPPA)
- Dipalmitoyl phosphatidyl glycerol (DPPG)

Example: For unsaturated phospholipids are-

- Dioleoyl Phosphatidyl choline (DOPC)
- Dioleoyl Phosphatidyl glycerol (DOPG)

Polymeric material-[4]

Synthetic phospholipids with diactylenic group in hydrocarbon chain polymerizes when exposed to U.V. leading to the formation of polymerized liposome having significantly higher permeability barriers to entrapped aqueous drugs. Ex. For other polymerisable lipids are- lipids containing conjugated dienes, Methacrylate etc**.** Also several polymerisable surfactants are also synthesized.

Polymer bearing lipids-[3]

Stability of repulsive interaction with macromolecules is governed mostly by repulsive electrostatic forces. This repulsion can be induced with coating liposome surfaces by charge polymers.Non- ionic and water compatible polymer like polyethylene oxide; Polyvinyl alcohol and polyoxazolidine confer higher solubility. But adsorption of such copolymer containing hydrophilic segments with hydrophobic part lead to the liposome leakage, so best results can be achieved by covalently attaching polymers to phospholipids. Ex. Diacyl phosphatidyl ethanolamine with PEG polymer linked via a carbon at or succinate bond.

Cationic lipids- [3]

E.g.: Dioctadecyl dimethyl ammonium bromide or chloride (DODAB/C)

DOTAP- Dioleoyl propyl trimethyl ammonium chloride- This is analogue of DOTAP and various other including various analogues of DOTMA and cationic derivatives of cholesterol.

Other substances-

- Variety of other lipid of surfactants is used
- Many single chain surfactants can form liposome mixing with cholesterol
- Non ionic lipid.
- Variety of polyglycerol and polyethoxylated mono and dialkyl amphiphiles used many in cosmetics preparation.
- Single and double chain lipids having fluoro carbon chain can form very stable liposome.
- Sterylamine and Dicetyl phosphate.
- Incorporated into liposomes so as to impart either a negative or positive surface charge to these structures

GENERAL METHOD OF PREPARATION AND DRUG LOADING [5]

Liposomes are the manufactured in majority using various procedures in which the water soluble (hydrophilic) materials are entrapped by using aqueous solution of these material as hydrating fluid or by the addition of drug/drug solution at some stage during manufacturing of the liposome. The lipid soluble (lipophilic) materials are solubilized in the organic solution the constitutive lipid and then evaporated dry drug containing lipid film followed by its hydration. These methods involve the loading the entrapped agents before or during manufacturing procedure (passive loading). Certain type of compounds by can be introduced the liposomes after the formation of intact vesicles.

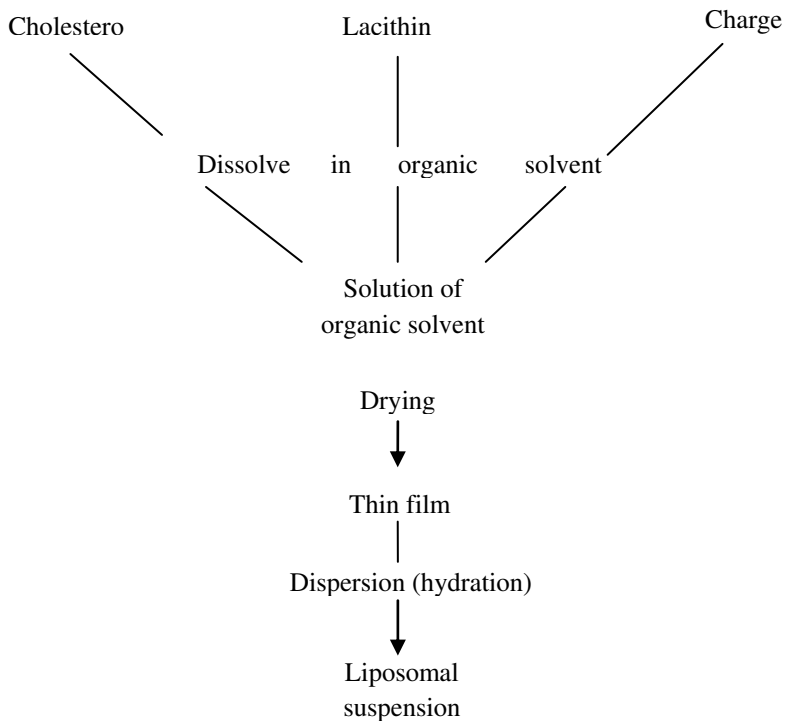

Fig: - general method of liposome preparation

EVALUATION OF LIPOSOMES [11]

The 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 stapes physical, chemical and biological parameters. Physical characterization evaluate parameter includes size, shape, surface, and drug release profile. Chemical characterization includes studies in establish the purity and potency of various lipophillic constituents. Biological characterizations parameters are helpful in establish the safety and suitability of formulation for therapeutic application. Some of parameters are:-

Vesicle shape and lamellarity

The Vesicle shape assessed using electron Microscopic Techniques. Lamellarity of vesicles is determined by Freeze Fracture Electron Microscopy and P31 Nuclear Magnetic Resonance Analysis.

Vesicle size and size distribution

The liposome size distribution was determined by the photon correlation spectroscopy

Microscopic Techniques Optical Microscopy[11]

The microscopic method includes use the Bright Field, phase-Contrast Microscope and Fluorescent Microscope and is useful in evaluating vesicle size of large vesicles.

CRYO-Transmission Electron Microscopy Techniques (CRYO-TEM)

This technique has been used to elucidate the surface morphology and size of vesicles.

Diffraction and Scattering Techniques:-[11]

Laser Light Scattering Photon correlation spectroscopy (PCS):- The analysis of time dependence of intensity fluctuation in scattered laser light due to Brownian motion of particles in solution or suspension since small particles diffuse more than large particles, the rate of fluctuation scattered light intensity varies accordingly. The translational diffusion coefficient (D) can be measured and can be used to determine the mean hydrodynamic radius (RHSS) of particles using the Stoke-Einstein equation using this technique one can measure particles in range of about 3nm.

Hydrodynamic Techniques:-

This technique includes gel Permeation and Ultracentrifuge. Exclusion chromatography on large pure gels was introduced to separate SUVs from radial MLVs.The large vesicles of 1-3µmdiameter.usually fail to enter the gel and is retained on top of column. A thin layer chromatography system using agarose beads has been introduced as a convent, fast technique for obtaining a rough estimation of size distribution of liposome preparation. However, it was not reported if this procedure was

sensitive to a physical blockage of pores of the agars gel as is the more conventional column chromatography.

Zeta potential determination- [16]

The zeta potential was evaluated by the determination of electro mobility of the 90c angle. The measurement was performed in triplicate using the 3000 HS zeta-seizer equipment. The sample was diluted with suitable diluents for the potential determination.

APPLICATIONS OF LIPOSOMES

Systemic Liposomal Drugs [18]

After systemic (usually intravenous) administration, liposomes are typically recognized as foreign particles and consequently endocytose by the mononuclear phagocyte system cells mostly fixed Kuppfer cells, in the liver and spleen. Liposome can serve as an excellent drug-delivery vehicle cells. sterically stabilized liposome is not avidly taken up by mononuclear phagocyte cells, have different bio distributions properties and have shown enhanced accumulation in sites of trauma, tumour, infection and inflammation. This accumulation is simply due to their prolonged circulation and small size; enabling them to extravasate

Topical Liposomal Drugs [19]

The skin treatment applications of liposomes are based on the similarity between the lipid vesicles bilayer structure and natural membrane are include the ability of lipid vesicle with specific lipid composition, to alter cell membrane fluidity and to fuse in the dermatological field, liposomes were initially used because of their moisturizing and restoring action.

Cosmetic Applications [20] [21] [22]

The properties of liposomes utilized also in the delivery of ingredients in cosmetics. Liposomes offer advantages because lipids are hydrated and reduce the dryness of the skin which is a primary cause for ageing. Also, liposomes supply replenishes lipids and importantly linolenic acid to the skin. The first liposomal cosmetic product to appear on market was the anti-ageing cream Capture launched by Christian Dior in 1986.Liposomes have been also used in the treatment of hair loss minoxidil a vasodilator in the active ingredient in products like “Regaine” that claim to prevent or slow hair loss. The skin care preparations with empty or moisture loaded liposome reduce the transdermal water loss and suitable for the treatment of dry skin. They also enhance the supply of lipids and water to the stratum corneum.

Food application [23] [24]

The majority of microencapsulation techniques at present used in the food industry are based on biopolymer matrices composed of sugar, starch, gum, protein, synthetic, dextrin and alginates. Nevertheless, liposomes have recently begun to gain in importance in food products.

Liposomes in anticancer therapy [25] [26] [27]

The different liposome formulations of various anticancer agents were shown to be less toxic than the free drug. Anthracyclines are drugs stop the growth of dividing cells by intercalating the DNA and therefore kill predominantly quickly dividing cells. These cells in tumours, but also gastrointestinal mucosa, hair, and blood cells and therefore this class of drugs are very toxic. The most used and studied is Adriamycin. In addition to the above mentioned acute toxicities its dosage is limited by cumulative cardio toxicity. Different formulations were tried. In most cases the toxicity was reduced about 50% includes both, short term and chronic toxicities because liposome encapsulation reduces the distribution of drug molecules towards tissues. For the same reason, on the other hand, the efficacy was in many cases compromised due to reduced bioavailability of the drug, especially if the tumour was not phagocytic or located organs of mononuclear phagocytic system. In cases, such as systemic lymphoma, the effect of liposome encapsulation show enhanced efficacy the sustained release effect. Ex longer presence of therapeutic concentrations in the circulation while in several other cases the sequestration of the drug tissues of mononuclear phagocytic system actually reduced its efficacy and applications in man show in general reduced toxicity, better tolerability of administration with not too encouraging efficacy. The different formulations are different phases of clinical studies and show mixed results.

Liposomes in medicine & pharmacology [28] [29] [30]

The liposomes in medicine and pharmacology can be divided into diagnostic and therapeutic application of liposome containing various marker drugs and their use as a tool, model, or reagent in the basic studies of cell interaction, recognition process, and mode of action of certain substance. Unfortunately, drugs are the very narrow therapeutic window, meaning that the therapeutic concentration is not lower than the toxic one. In cases, the toxicity can be reduced the efficacy can be enhanced the used of suitable drug carrier which alters the temporal and spatial delivery of the drug, i.e. it biodistribution and pharmacokinetics is clear from many pre-clinical and

clinical studies that drug, for the instance antitumor drug, parceled in liposome demonstration reduced toxicities, while retentive enhanced efficacy.

Advances in liposome design are the leading in new applications for the delivery of new biotechnology products, example for the antisense oligonucleotide, cloned gene, and recombinant protein. A vast literature define the viability of formulating wide range conservative drugs in liposome, frequently resultant in improved therapeutic activity and reduced toxicity compared with free drug. Changed pharmacokinetics for liposomal drugs can lead to improved drug bioavailability to the particular target cells and live in the circulation, or more prominently, to extravascular disease sites, example for tumors recent improvement consist of liposomal formulation of all-*trans*-retinoic acid.

CONCLUSION

The present study demonstrated that Liposomes are one of the unique drug delivery system, They can be use in controlling and targeting drug delivery. These are also used in the cosmetic and hair Technologies, diagnostic purpose and good carrier in gene delivery. Liposome carriers, well known for their potential application. Liposome is giving a good and encouraging result in the anticancer therapy and human therapy. Liposomes are acceptable and superior carriers and have ability to encapsulate hydrophilic as well as lipophillic drugs and protect them from degradation. As a novel carrier system liposomes provide controlled and sustained release.

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Received	9th May 2015
Revised	21st May 2015
Accepted	9th June 2015

J. App. Pharm. Res., 3 (3); 2015: 01 – 08