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REVIEW ARTICLE

A platform for liposomal drug delivery

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Abstract:
Liposome has been used as a carrier to transport a wide variety of compounds in its aqueous compartment. The clinical potential of liposomes as a vehicle for replacement therapy in genetic deficiencies of lysosomal enzymes was first established in 1970. Liposomes are surfactants, sphingolipids, glycolipids, long chain fatty acids and even membrane proteins and drug molecules or it is also called vesicular system. These are different in size, composition and charge. These are a drug carrier variety of molecules such as proteins, nucleotides and even plasmids. Some drugs are also formulated as liposomes to improve their therapeutic index, stability and compatability. The vesicular drug delivery systems such as liposomes, niosomes, transfersomes, and pharmacosomes were developed. In this paper main focus on liposomal classification, various method of preparation, evaluation parameter , merits, recent status and scope.

Keywords: Liposomes,Vesicles,genedelivery,Vaccine

Introduction

Liposomes were first described by British haematologist Dr Alec D Bangham FRS in 1961 (published 1964), at the Babraham Institute, in Cambridge. The name liposome is derived from two Greek words: 'Lipos' meaning fat and 'Soma' meaning body. Liposomes meaning lipid body are spherical microscopic vesicle composed of one or more concentric lipid bilayer separated by water and aqueous buffer compartment a diameter ranging from 25nm-1000nm. Membranes are usually made of phospholipids, which are molecules that have a hydrophilic head group and a hydrophobic tail group. Phospholipids are found in stable membranes composed of bilayer. In the presence of water, the heads are attracted to water and line up to form a surface facing the water. The tails are repelled by water, and line up to form a surface away from the water. In a cell, one layer of heads faces outside of the cell, attracted to the water in the environment, and another layer of heads faces inside the cell, attracted by the water inside the cell.

The hydrocarbon tails of one layer face the hydrocarbon tails of the other layer, and the combined structure forms a bilayer. When membrane phospholipids are disrupted, they can reassemble themselves into tiny spheres, smaller than a normal cell, either as bilayers or monolayers. The bilayer structures are liposomes. The monolayer structures are called micelles. The lipids in the plasma membrane are chiefly phospholipids like phosphatidyl ethanolamine and phosphatidyl choline. Phospholipids are amphiphilic with the hydrocarbon tail of the molecule being hydrophobic; its polar head hydrophilic. Liposomes can be composed of naturally-derived phospholipids with mixed lipid chains (like egg phosphatidyl ethanolamine), or of pure surfactant components like DOPE (dioleoylphosphatidyl ethanolamie) Liposomes, usually but not by definition, contain a core of aqueous solution; lipid spheres that contain no aqueous material are called micelles, however, reverse micelles can be made to encompass an aqueous environment(fig.1).

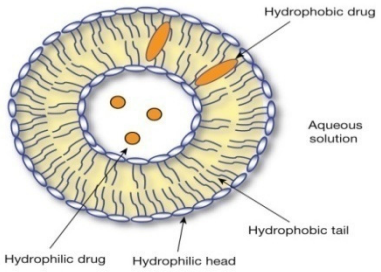
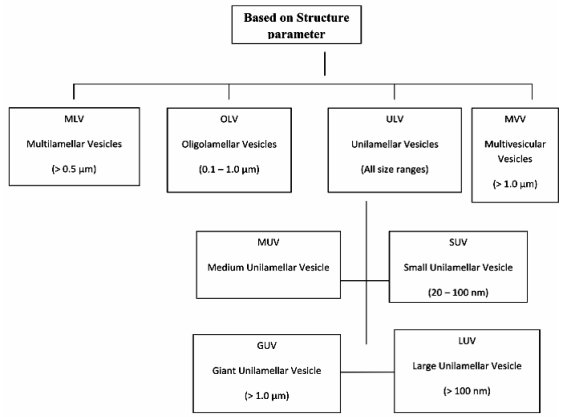


Fig-1

Flow chart of liposomal classification in market-



Methods of liposomal prepration-

(1) Multilamellar Liposomes (MLV)

(i) Lipid Hydration Method :

(a) This is the most widely used method for the preparation of MLV. The method involves drying a solution of lipids so that a thin film is formed at the bottom of round bottom flask and then hydrating the film by adding aqueous buffer and vortexing the dispersion for some time. The hydration step is done at a temperature above the gel-liquid crystalline transition temperature Tc of the lipid or above the Tc of the highest melting component in the lipid mixture. The compounds to be encapsulated are added either to aqueous buffer or to organic solvent containing lipids depending upon their solubilities. MLV are simple to prepare by this method and a variety of substances can be encapsulated in these liposomes. The drawbacks of the method are low internal volume, low encapsulation efficiency and the size distribution is heterogeneous.

(b) MLVs with high encapsulation efficiency can be prepared by hydrating the lipids in the presence of an immiscible organic solvent (petroleum ether, diethyl ether). The contents are emulsified by vigorous vortexing or sonication. The organic solvent removed by passing a stream of nitrogen gas over the mixture. MLVs are formed immediately in the aqueous phase after the removal of organic solvent .The main drawback of this method is the exposure of the materials to be encapsulated to organic solvent and to sonication.

(ii) Solvent Spherule Method: A method for the preparation of MLVs of homogeneous size distribution was proposed. The process involved dispersing in aqueous solution the small spherules of volatile hydrophobic solvent in which lipids had been dissolved. MLVs were formed when controlled evaporation of organic solvent occurred in water bath.

(2) Small Unilamellar Liposomes (SUV)

(i) Sanitation Method: Here MLVs are sonicated either with a bath type sonicator or a probe sonicator under an inert atmosphere. The main drawbacks of this method are very low internal volume/ encapsulation efficiency, possibly degradation of phospholipids and compounds to be encapsulated, exclusion of large molecules, metal contamination from probe tip and presence of MLV along with SUV.

(ii) French Pressure Cell Method: The method involves the extrusion of MLV at 20,000 psi at 4°C through a small orifice. The method has several advantages over sonication method. The method is simple rapid, reproducible and involves gentle handling of unstable materials. The resulting liposomes are somewhat larger than sonicated SUVs. The drawbacks of the method are that the temperature is difficult to achieve and the working volumes are relatively small about 50 ml maximum.

(iii) A new method for the preparation of SUV. They deposited egg phosphatidyl cholinc mixed with 1.5 %w/v of cetyl tetramethyl ammonium bromide (a detergent) in CHCI3/CH3OH on various supports for example silica gel powder, zeolite X, zeolite ZSM5. After the removal of organic phase, the system was resuspended by shaking or stirring in distilled water or 5 mM NaCl. There was some loss of phospholipid (about 10-20%) due to adsorption on the supports. The loss was 70% and 95% in the case of silica gel and zeolite ZSMS respectively. A homogenous population of vesicle with average diameter of 21.5 nm was obtained when zeolite X (particle size of 0.4 mm) was used as a support.

3) Large Unilamellar Liposomes(LUV)- They have high internal volume/ encapsulation efficiency and are now days being used for the encapsulation of drugs and macromolecules.

(i) Solvent Injection Methods

(a) Ether Infusion Method: A solution of lipids dissolved in diethyl ether or ether/methanol mixture is slowly injected to an aqueous solution of the material to be encapsulated at 55-65°C or under reduced pressure. The

subsequent removal of ether under vacuum leads to the formation of liposomes. The main drawbacks of the method are that the population is heterogeneous (70-190) nm and the exposure of compounds to be encapsulated to organic solvents or high temperature.

(b) Ethanol Injection Method: A lipid solution of ethanol is rapidly injected to a vast excess of buffer. The MLVs are immediately formed. The drawbacks of the method are that the population is heterogeneous (30-110 nm), liposomes are very dilute, it is difficult to remove all ethanol because it forms azeotrope with water and the possibility of various biologically active macromolecules to inactivation in the presence of even low amounts of ethanol .

(ii) *Detergent Removal Methods:* The detergents at their critical micelles concentrations have been used to solubilize lipids. As the detergent is removed the micelles become progressively richer in phospholipid and finally combine to form LUVs. The detergents were removed by dialysis. The advantages of detergent dialysis method are excellent reproducibility and production of liposome populations, which are homogenous in size. The main drawback of the method is the retention of traces of detergents within the liposomes. A commercial device called LIPOPREP which is a version of dialysis system, is available for the removal of detergents.

(iii) Reserves Phase Evaporation Method: First water in oil emulsion is formed by brief sonication of a two-phase system containing phospholipids in organic solvent (diethylether or isopropylether or mixture of isopropyl ether and chloroform) and aqueous buffer. The organic solvents are removed under reduced pressure, resulting in the formation of a viscous gel. The liposomes are formed when residual solvent is removed by continued rotary evaporation under reduced pressure. With this method high encapsulation efficiency up to 65% can be obtained in a medium of low ionic strength for example 0.01 M NaCl. The method has been used to encapsulate small, large and macromolecules. The main disadvantage of the method is the exposure of the materials to be encapsulated to organic solvents and to brief periods of sonication. These conditions may possibly result in the denaturation of some proteins or breakage of DNA strands. We get a heterogeneous sized dispersion of vesicles by this method.

(iv) Microfluldization Method: The technique of microfluidization / microemulsification / homogenization for the large-scale manufacture of liposomes. The reduction in the size range can be achieved by recycling of the sample. The process is reproducible and yields liposomes with good aqueous phase encapsulation. The prepared liposomes consisting of egg yolk, cholesterol and brain phosphatidyl serin disodium salt (57:33:10) were prepared by this method. First MLV were prepared were passed through a Microluidizer at 40 psi inlet air pressure. The size range was 150-160 nm after 25 recylces. In the Microluidizer, the interaction of fluid streams takes place at high velocities (pressures) in a precisely defined micro channel, which are present in an interaction chamber. In the chamber pressure reaches up to 10,000 psi this can be cause partial degradation of lipids.

4) Giant Liposomes :

(i) The procedure for the formation of giant liposomes involves the dialysis, of a methanol solution of phosphatidylcholine in the presence of methyl glucoside detergent against an aqueous solution containing up to 1 M NaCl. The liposomes range in diameter from 10 to 100 mm.

(ii) A method for the formation of giant single lamellar liposomes with size in the range of 10 to 20 prn by the removal of sodium trichloroacetate by dialysis.

5) Multivesicular Liposomes:

(i) The formation of multivesicular liposomes has been reported by Kim et al. The water in oil emulsion was converted to organic solvent spherules by the addition of the emulsion to across solution. The evaporation of organic solvent resulted in the formation of multivesicular vesicles. The diameter of liposomes ranges from 5.6 to 29 pm. The materials which can be encapsulated include glucose, EDTA, human DNA. These liposomes have very high encapsulation efficiency up to 89%.

(ii) Cullis et al. found that when MLV preparations were subjected to five cycles of freeze on liquid nitrogen)-thaw and followed by thawing in warm water, the liposomes of high encapsulation efficiency up to 88%could be obtained. Freeze fracture electron micrographs revealed vesicles within vesicles.

6) Assymetric Liposomes :

It has been shown that the phospholipid distribution in natural membranes is asymmetric. For example phosphatidytcholine and sphingomyelin concentrate at the outer half of lipid bilayer whereas phosphatidyl ethanolamine, phosphatidyl inositol and, phosphatidylserine are mainly localized in the inner half of bilayer. Due to this, attempts have been made to prepare LUVs in which phospholipid distribution in both halves of bilayer is different. It appears that as model membranes the asymmetric liposomes are nearer to natural membranes than the conventional unilamellar liposomes. In the latter the phospholipids distribution is symmetrical in bilayer.

Merits of liposomal drug delivery-

- It can be used both hydrophilic and lipophilic drugs without chemical modification.
- It has the ability to protect labile compound.
- Increased stability via encapsulation.
- Reduce the toxicity of the encapsulated agents.
- Provides selective passive targeting to tumor tissues (Liposomal doxorubicin).
- Reduced elimination, increased circulation life times.
- Provide flexibility to couple with site specific ligands.

Recent status of liposomal drug delivery-

1. Phototriggering liposomes nano drug delivery **:** these liposome formulation is used in visudyne theraphy
2. Photodynamic drug liposome formulation**:** these liposome are used in all type of cancer

Scope of liposomal drug delivery-

1) Cationic liposomes for gene delivery: Among various synthetic carriers currently in use in gene therapy, cationic liposomes are the most suitable transfecting vectors. Gene encapsulation in liposomal vesicles allows condensation of DNA plasmid into a highly organized structure, and protects DNA against degradation during storage and in the systemic circulation of the gene encoding a therapeutic protein. Moreover, structural organization of the gene-delivery system must bypass the cell membrane and facilitate endosomal escape avoiding DNA degradation in the lysosomal compartment. Among different mechanism of intracellular uptake of liposomes, endocytosis of targeted liposomes is exemplified. Numerous cationic lipids have been tested in the formulation of liposomes for gene delivery. Transfection efficiency is strongly affected by the presence of three components in the structure of these lipids: a positively charged head-group that interacts with negatively charged DNA, a linker group (which determines the lipid's chemical stability anDiodegradability), and a hydrophobic region to anchor the cationic lipid into the bilayer. Among these, the most often used are N-[1-dioleyloxy)propyl]-N,N,N-trimethylammonium (DOTMA) and dioleoylphophatidyl ethanolamine (DOPE) in a 1:1 phospholipid mixture (fig.-2).

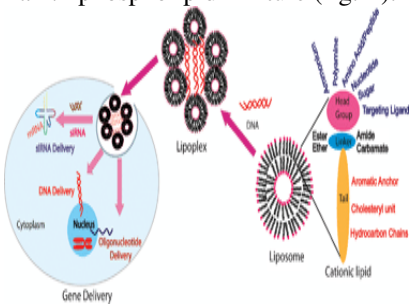


Fig-2

2) Liposomes for diagnostic imaging: Actively or passively targeted liposomes can be used as carriers for contrast agents to increase the signal difference between areas of interest and background, and to specifically localize the contrast moieties in the target tissues or organs. The versatility of liposomal vesicles to carry different types of compound in the bilayer or in the aqueous compartment makes them suitable for all contrast procedures, including gamma-scintigraphy, magnetic resonance imaging (MRI), computed tomography imaging (CTI), and sonography. Using liposomes in diagnostic imaging leads to several advantages, owing to their capability to incorporate multiple contrast moieties, to specifically delivery the agent to the target area, and to enhance the contrasting signal. In order to incorporate diagnostic agents in liposomes, metals can be complexed with a soluble chelating agent (such as DTPA) that will be encapsulated in the aqueous core of the vesicles. Alternatively, the chelating compound complexing with the metal can be derivatized with a hydrophobic group for insertion in the lipid bilayer. Gd-DTPA complexes were the first to be incorporated in the aqueous core. Among the various lipophilic DTPA–conjugates that have been synthesized, DTPA-sterylamine (DTPA-SE) and DTPA-phosphatidyl ethanolamine (DTPA-PE) show reduced leakage and toxicity of potentially toxic metals.

3) Liposomes for vaccines: Genetic vaccination-encoding antigens from bacteria, virus, and cancer have shown promise in protecting humoral and cellular immunity. The success of liposomes-based vaccines has been demonstrated in clinical trials and further human trials are also in progress. Liposomes are of interest as carriers of antigens, especially because they act as effective adjuvants for the immune system response, without causing granulomas at the injection site and producing no hypersensitivity reactions. Liposome formulations would also protect their DNA content from deoxyribonuclease attack. Moreover, their transfection efficiency could be improved by modulating surface charge, size, and lipid composition of the vesicle and entrapping additional adjuvant or immune stimulator compounds in the antigen formulation. Several strategies have been followed to target liposomes to cell receptors, such as antibodies or branched chain mannose moieties. Cationic or pH-sensitive liposomes that are able to release their contents into the cytoplasm following endocytosis have also been developed. Two commercial vaccines based on virosome technology are currently on the market. A vaccine, has inactivated hepatitis A virus particles adsorbed on the surface of the immune potentiating reconstituted influenza virosomes (IRIV). The virosome components themselves are the vaccine protective antigens. Virosomes are liposomal formulations that have viral envelope proteins anchored to their lipid membrane. The lipid bilayer is acomposed of PC intercalated with the virus-derived protein hemagglutinin and neuraminidase. These virus-

like particles have proven to be effective immunogens with unique adjuvant properties. Liposome-encapsulated malaria vaccine contains monophosphoryl lipid A as adjuvant in the bilayer and the formulation is adsorbed on aluminum hydroxide.(fig.-3)

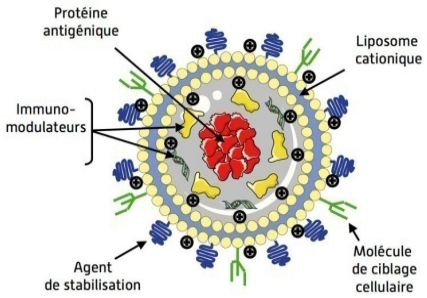


Fig-3

4. Targeting for anticancer drug doxorubicin and donorubicin can be prepared in form of tumor cell.

5. Liposome increase penetration of transdermal patch to skin.

6. Targeting of antiviral drug.

7. Site specific delivery of antibiotic.

Evalution parameter for liposomal drug delivery-

1) Entrapment efficiency: Drug entrapped is determine by complete description using 15% N propanol or 0.1% triton 100 analyzing the sample in u,v.

I.E= Total number of drug entrapped * drug

2) Vessical diameter: It can be determined by optical microscopy laboratory and TEM.

3) In-vitro release study: It is performed by dialysis tube a dialysis is washed and socked distilled water. The vesicle suspension is placed into dialysis bag and shield. It is placed in 1200 ml phosphate buffer wit constant shaking at 37c at various time in interval 5ml buffer is removed and analyzed for drug content by u.v.

Clinically approved liposomal drugs-

12 drugs with liposomal delivery systems have been approved and five additional liposomal drugs were in clinical trials (table- 1).

Conclusion-

In the development of liposomes has the various applications, which are utilized as a carrier for therapeutic molecules.. In case of tumor development, certain anticancer drugs such as doxorubicin (Doxil) and daunorubicin are provided through liposomes. Liposomal cisplatin has received orphan drug designation for pancreatic cancer from EMEA. Further advances in liposome research have been able to allow liposomes to avoid detection by the body's immune system, specifically, the cells of reticulo endothelial system (RES). These liposomes are known as "stealth liposomes", and are constructed with PEG (Polyethylene Glycol) studding the outside of the membrane. The PEG coating, which is inert in the body, allows for longer circulatory life for the drug delivery mechanism. However, research currently seeks to investi at what amount of PEG coating the PEG actually hinders binding of the liposome to the delivery site. In addition to a PEG coating, most stealth liposomes also have some sort of biological species attached as a ligand to the liposome in order to enable binding via a specific expression on the targeted drug delivery rate.

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Table-1: Clinically Approved Liposomal Drug Products

Name	Trade name	Company	Indication	LiposomalExcipients
Liposomal amphotericin B	Abelcet	Enzon	Fungal infections	DMPC and DMPG
Liposomal amphotericin B	Ambisome	Gilead Sciences	Fungal and protozoal infections	HSPC, Cholesterol, DSPG
Liposomal cytarabine	Depocyt	Pacira (formerly SkyePharma)	Malignant lymphomatous meningitis	DOPC, Cholesterol, DPPG
Liposomal daunorubicin	DaunoXome	Gilead Sciences	HIV-related Kaposi's sarcoma	DSPC, Cholesterol
Liposomal doxorubicin	Myocet	Zeneus	Combination therapy with cyclophosphamide in metastatic breast cancer	LIPOVA-E120, Cholesterol
Liposomal IRIV vaccine	Epaxal	Crucell	Hepatitis A	LECIVA-S70
Liposomal IRIV vaccine	Inflexal V	Berna Biotech	Influenza	LECIVA-S90
Liposomal morphine	DepoDur	SkyePharma, Endo	Postsurgical analgesia	DOPC, Cholesterol, DPPG
Liposomal verteporfin	Visudyne	QLT, Novartis	Age-related macular degeneration, pathologic myopia, ocular histoplasmosis	Egg PG, DMPC
Liposome-proteins SP-B and SP-C	Curosurf	Chiesi Farmaceutici, S.p.A.	pulmonary surfactant for Respiratory Distress Syndrome (RDS)	Leciva-S90
Liposome-PEG doxorubicin	Doxil/Caelyx	Ortho Biotech, Schering-Plough	HIV-related Kaposi's sarcoma, metastatic breast cancer, metastatic ovarian cancer	MPEG-DSPE, HSPC, Cholesterol
Micellular estradiol	Estrasorb	Novavax	Menopausal therapy	Soybean oil, Polysorbate80
Liposomal vincristine	Marqibo	Spectrum Pharmaceuticals	Acute Lymphoblastic Leukemia (ALL) and Melanoma	Cholesterol and egg sphingomyelin

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