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A study on liposomes: Classification techniques and importance

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Abstract
Liposome are small vesicle of spherical shape that can be produced from cholesterol, nontoxic surfactants, sphingolipids, glycolipids, long chain fatty acid and even membrane protein Almost from the time of their discovery in 1960's and the demonstration of their entrapment potential, liposome vesicle have drawn attention of researchers as potential carriers of various bioactive molecules that could be used for therapeutic application in humans and animal. liposome solubilize lipophilic drug candidates that would otherwise be difficult to administer i.v. the encapsulated drug is inaccessible to metabolizing enzyme; conversely, body compartment such as erythrocyte and tissue injection site are not directly exposed to full dose of the drug.

Keywords: liposome, cholesterol, nontoxic surfactants, sphingolipids, glycolipid, protein, UV, nonencapsulated, chromatography

Introduction
Liposome has been widely investigated since 1970s as drug carries for improving the delivery of therapeutic agent to specific sites in the body. When phospholipids are dispersed in water, they spontaneously form closed structure with internal aqueous environment bounded by phospholipids bilayer membrane, this vesicular system is called as liposome [1]. Liposome are small vesicle of spherical shape that can be produced from cholesterol, nontoxic surfactants, sphingolipids, glycolipids, long chain fatty acid and even membrane protein [2]. Liposome is the drug carrier loaded with great variety of molecule such as small drug molecules, proteins nucleotides and even plasmids. Liposome were discovered about 40 yr ago by A.D. Bangham which has versatile tool in biology biochemistry, medicine today [3].

Classification of liposome
Liposome are classified on the basis of
1. Structure
2. Method of preparation
3. Composition and application.
4. Conventional liposome.
5. Specialty liposome.

1. Classification based on structure
- Vesicle type with their size and number of lipid layers

2. Based on method of preparation
- Different preparation method and the vesicle formed by these methods

3. Based on composition and application
- **Different liposome with their composition**

4. Based upon conventional liposome
1. Stablize natural lecithin (PC) mixture
2. Synthetic identical, chain phospholipids
3. Glycolipid containing liposome

5. Based upon specialty liposome [4]
1. Bipolar fatty acid
2. Antibody directed liposome
3. Methyl /methylene x-linked liposome
4. Carbohydrate coated liposome
5. Lipoprotien coated liposome
6. Multiple encapsulated liposome.

Material and Method
Raw materials for formation of liposomes
Liposome that is used as carriers for drug or diagnostic agents should be prepared from constituents that are safe for use in human. Although limited experience is available on the safety of liposome. Phosphatidylcholines and phosphatidylglycerol from natural sources, semi synthetically or fully synthetically produced and cholesterol and PEG-ylated phosphati-diethanolamine, are frequently encountered in liposomes designed as drug carries for parentral administration or for *in vivo* diagnostic purposes, phosphatidylcholine (PC) is routinely used as bulk neutral phospholipid. As a negatively charged lipid phoshatidyl glycerol is often selected. Finally, if it is desirable to reduce the permeability of "flued crystalline state" bilayers, cholesterol are added to bilayer structure. Sometimes lipid with a special affinity for target cells in the body is deliberately inserted in bilayer. This was, for instance, the case when hepatocytic delivery was aimed for and lactosylcermide, ligand with a special affinity for hepatocytes, was included in the liposomal bilayer.

Five groups of phospholipids that can be used for the liposomal preparation can be disc erned [7]
1. Phospholipids from natural sources
2. Modified natural phospholipids

3. Semisyntheyic phospholipids
4. Fully synthetic phospholipids
5. Phospholipids with non-natural head groups

Phospholipids from natural sources

The sources for natural phospholipids mainly PC, but also phosphatidylethanolamine (PE) phosphatidylinositol (PI), and sphingomyelin (SPM), are egg yolks and soybeans. These PC's are mixed acyl ester phospholipids. Apart from source dependent differences in acyl chain type, considerable inter batch variation has been observed for egg PC the esterified acyl chains of egg PC are different from those of soybean PC.

Modified Natural Phospholipids

Natural phospholipids can be modified. Because of their degree of unsaturation, which makes them sensitive to oxidation, PC from natural sources can be catalytically hydrogenated. Partially or fully hydrogenated natural PCs are readily available. The iodine value of these lipids is reduced as the number of unsaturated C=C bonds drops. Dependent on the degree of unsaturation left after the hydrogenation process, phase transition temperatures can be identified for liposomal dispersions of the partially hydrogenated PCs. Head group modifications can be performed by using phospholipase. With this enzyme one can convert PC into PG, PE or phosphatidylserine (PS).

Semi synthetic Phospholipid

These are acyl chains that are attached to phospholipids from natural sources are often unsaturated. This makes them liable to oxidation reactions, which may limit liposome shelf life. Moreover as mentioned above reproducibility of the quality of the batches in terms of acyl chains may be poor as well, which may cause variation in stability or liposome properties. Removal of the original acyl chain and within certain limits, replacement by a chosen acyl chain is possible. Phospholipase A2 which cuts the acyl chain at the C2 position of glycerol can be used if only replacement of the C2 acyl chain is required.

Fully Synthetic Phospholipids [8]

Eibyl reviewed different completely chemical pathways for phospholipid synthesis.

Phospholipids with Non-natural (Head) Groups [9, 10, 11]

The idea of maintaining the fate of liposomes in the body by selecting the appropriate bilayer characteristics has led to modified phospholipids.

The circulation time of liposomes in the blood compartment can be considerably prolonged when polyethleneglycol chains are attached to bilayer constituents. Alternatively, for active targeting purposes ligands for cell surface receptors can be attached. These ligands can be chemically and physically widely different structures, such as monoclonal antibodies or just a simple peptide. PEG has been linked to PE for the preparation of long circulating liposomes. Various reactions schemes have been developed. Molecular weights fractions for maximum prolongation of circulation times for PEG vary between 1900 and 5000 allen and coworkers described the synthesis of a PEG-carbonate derivative of PE. Klibanv *et al.* used a succinidyl conjugation method, while blume and Cevc adopted the procedure that Abuchowski and coworkers described for the preparation of PEG- albumin conjugates (via cyanuric chloride).

Mechanism of liposome formation

Liposomes are vesicular structures consisting of hydrated bilayers. Liposomes structures used for pharmaceutical purposes consist of a phospholipid back bone. But other classes of molecules can form belayed based vesicular structures as well. On the other hand not all the hydrated phospholipid form belayed structures. Other forms of self-aggregation such as inverted hexagonal phases or micelles with completely different properties can occur. The common feature that all bilayer forming compounds share is their amphilicity. They have defined polar and nonpolar regions. In water the hydrophobic regions tend to self-aggregate and the Polar Regions tend to be in contact with the water phase. Israelachvili and coworkers defined critical packing parameter p by

P = v/ a0lc

Where **v** is the molecular volume of the hydrocarbon part a0 is the optimum surface area per molecule at the hydrocarbon water interface, and 1c is the critical half thickness for the hydrocarbon region which must be less than the maximum length of the extended lipid chains. For $p<1/3$, spherical micelles are formed. In this category fall single chain lipids with large head group areas. E.g. lysophosphatidyl choline.

When $p>1/3$ globular or cylindrical micelles are formed

Techniques of Liposome Preparation [12, 13]

Liposome preparation techniques have been described extensively in a number of review articles. In different preparation procedures a general pattern can be discerned.

1. The lipid must be hydrated, then
2. liposomes have to be sized, and finally
3. None capsulated drug has to be removed. In some preparation schemes the hydration and sizing steps are combined. Sometimes all drugs are liposomes associated and no free drug can be found after stage2 then stage 3 is lacking.

1. Hydration stage

a) Mechanical Methods

MLVs were traditionally produced by hydrating thin lipids films deposited from an organic solution on a glass wall by shaking at temperatures above the phase transition temperature of the phospholipid with the highest Tc. The wide size distributions of the produced liposome dispersions were usually narrowed down by (low) pressure extrusion or ultrasoncation.

b) Methods based on replacement of organic solvent by aqueous media

The lipid constituents are first dissolved in an organic solvent which is subsequently brought in contact with an aqueous phase. The organic solvent is removed later. During the

removal of the organic phase, liposome is formed. Their characteristics (size, organization of belayed) depend on the protocol used. If the organic solvent with the dissolved lipids is not miscible with the aqueous phase (ether, chloroform, Freon'), then the intermediate stage is an emulsion (immiscible solvent). Other organic solvents containing the dissolved lipid (s) can be mixed homogenously with the aqueous phase (ethanol) in the first stage. Then liposomes formation occurs when the organic solvent concentration drops below a certain critical value (miscible solvents). The content of residual organic solvent that is exceptable in the finished product depends on the solvent in question and the route of administration. Apart from evaporation, techniques similar to those used to remove non-encapsulated material can be selected: gel permeation, ultracentrifugation, and dialysis. Organic solvent may contain impurities with a high affinity for bilayers; they may be enriched in the belayed and cause safety or stability problems. Diethyl ether for instance, can be contaminated with peroxide that accumulates in the bilayer. Freshly (from bisulphite) distilled ether should therefore be used.

c) Methods based on detergent removal [14, 15]

Phospholipids, lipophilic compound and amphipathic protein can be solubilized by detergent, forming mixed micelles. Upon removal of the detergent, vesicle formation can occur. This technique is well established for preparation of reconstituted virus envelopes or reconstituted tumor membrane material. Schreier and coworkers described by two step strategy for insertions of proteins into the outer layer of liposomes. First liposomes were formed by detergent dialysis method and subsequently proteins were inserted by partial resolubalization of the membrane by the detergent (deoxycolate) in the presence of protein.

d) Method based on size transformation and fusion [16]

Sonication of phospholipids below their phase transition temperature (Tc) result in vesicles with defects in the bilayers. Heating the dispersion to Tc eliminates these structural defects and causes fusion resulting in large unilamellar liposomes with a wide size distribution. Main disadvantage of this process is the limited number of bilayer composition reacts as the main compounds which have low and the poor reproducibility of the particle size distribution of the liposome dispersion that is formed.

2) Sizing Stage [17, 18, 19]

There are two approaches, one without a special sizing step [A] and one with a special sizing step [B] and [A] In liposome formation process, circumstances are selected and control-led in such a way that particle size distributions with an acceptable width are produced. High shear homogenization produces a size distribution which depends on operational pressure. [B] for small dispersion volume, liposome dispersion can be fractionated by centrifugation as liposome density usually differs from the density of the medium. Gel permeation chromat- ography has also been used for subdividing wide particle size distribution. On an analytical or semi-preparative scale, the selection of the pore size of the chromatographic material provides an opportunity to manipulate the size class resolution within certain limits.

Removal of non-encapsulated Material [20]

Many lipophilic drugs exhibit a high affinity to the bilayer and are completely liposome associated. However, for other compounds, the encapsulation efficiency is less than 100 percent. The non-encapsulated fraction of the active compound can cause UN acceptable side effects or physical instability. For removal of non-encapsulated material, the following techniques are used:

a) Dialysis and ultracentrifugation,
b) Gel permeation chromatography,
c) Ion exchange reactions.

Incorporating Drugs [21]

To understand the performance characteristics of liposomal systems it is important to understand the mechanisms of introducing drugs into liposomes. That process is achieved using one of three primary mechanisms: encapsulation, partitioning, and reverse loading. Encapsulation. Useful for water-soluble drugs the encapsulation is simple hydration of a lipid with an aqueous solution of drug. The formation of liposomes passively entraps dissolved drug in inter- lamellar spaces, essentially encapsulating a small (captured) volume.

Partitioning

A drug substance that is soluble in organic solvents will go through partitioning. It is dissolved along with phospholipid(s) in a suitable organic solvent. That combination either is dried first or added directly to the aqueous phase, and residual solvent is removed under vacuum. The acyl chains of the phospholipids provide a solubilizing environment for the drug molecule, which will be located in the intrabilayer space.

Reverse Loading

The reverse-loading mechanism uses the fact that certain drugs (such as weak acids) may exist in both charged and uncharged forms depending on the pH of their environment. Such drug molecules can be added to an aqueous phase in the uncharged state to permeate into liposomes through their lipid bilayers. Then the internal pH of the liposomes is adjusted to create a charge on the drug molecule. Once charged, the drug substance no longer is lipophilic enough to pass through the lipid bilayer and return to the external medium. Use of liposomes to carry peptide and protein drugs and DNA vaccines involves simple, easily scaled technology that is capable of high-yield vaccine entrapment. A dehydration–rehydration technique applied for entrapping particulate anti-gens freeze-dries giant vesicles (4–5 m in diameter) in the presence of spores. On rehydration and sucrose-gradient fractionation of the resultant suspension, 30% or more of the spores used will be associated with generated giant liposomes of similar mean size.

Advantages and Their approaches

Liposomes for Gene Delivery [22]

It is important to dissect the overall cell uptake process into individual steps. In fact different studies have indicated that successful gene transfer in vitro involves: 1) the packaging of DNA, 2) the adhesion of packaged DNA to the cell surface, 3)

internalization of DNA, 4) escape of DNA from endosomes if endocytosis is involved, 5) DNA expression in cell nuclei. To perform all of the above steps, liposomes have been explored as a delivery system for DNA as early as in 1979. The encapsulation of plasmid DNA into liposomes and the introduction of poliovirus RNA and SV40 DNA into ells via liposomes were reported between 1979 and 1980.

Liposome for Targeted Delivery [23, 24]

Use of liposome-encapsulated enzymes for delivery into cells was first reported in 1971. About the same time, a specific receptor on hepatocytes was demonstrated to mediate clearance of ß-galactose terminated glycoproteins from circulation. A monoxide specific receptor was recognized on the cell surface of the RES of rats (including the liver sinusoid and macrophages). By grafting different glycosides on the surface of liposomes, it is possible to direct the latter to different cell types of rat liver. Galactosylated liposomes are mainly taken up by liver hepatocytes, whereas mannosylated liposomes are mainly taken up by no parenchymal cells. Grafting specific ligands to the liposome surface facilitates a fusion of the liposome with target cells by endocytosis, thus releasing material to be delivered. In cancer chemotherapy, the toxicity of anticancer drugs is of major concern. Liposomes could be used to deliver such drugs and minimize their toxic effects on healthy cells. Targeted delivery to cancer cells could be achieved by coating monoclonal antibodies (MAbs) raised against tumor-cell specific antigens. In vitro and *in vivo* studies by Ahmad *et al*. of squamous-cell carcinoma in mouse models provided evidence that antibody coated polyethylene glycol liposome's containing doxorubicin were more effective and less toxic than free drugs, drugs in corporate into antibody-free liposome's, and anti- body coated conventional liposomes (1, 4). The major concern in antibody-grafted liposome use is the induction of immune response to the grafted antibodies. Bastton *et al*. suggested a novel approach to overcoming that difficulty they used 125I-labeled antigen to kill the cells responsible for immune induction (the "antigen suicide" technique). Other possible approaches to overcome the immune-system problem include immunosuppressive drugs and humanized antibodies or establishing neutral immune windows for subsequent injection. Liposomes can be designed to release their entrapped contents under certain controlled.

Conditions

pH-sensitive and temperature-dependent liposomal systems. Drug targeting using liposomes as carriers holds much promise, especially in reducing toxicity and targeting delivery to disease sites. The future is bright for liposome research, with a large number of clinical trials ongoing in several countries with liposomal formulations of various anticancer drugs, antisense, cytokines, peptides and proteins. In the near future, several more liposome-based drugs will find their way into the pharmaceutical market.

Clinical Application

Cancer Therapy [25, 26, 27]

Cytotoxic drugs can distribute non-specifically throughout the body, lead to death of normal as well as malignant cells, thereby giving rise to a variety of toxic side effects. Entrapment of these drugs into liposomes resulted in increased circulation lifetime, enhanced deposition in the infected tissues, and protection from the drug metabolic degradation, altered tissue distribution of the drug, with its enhanced uptake in organs rich in mononuclear phagocytic cells (liver, spleen and bone marrow) and decreased uptake in the kidney, myocardium and brain. To target tumors, liposomes must be capable of leaving the blood and accessing the tumor. However, because of their size liposomes cannot normally undergo trans capillary passage. In spite of this, various studies have demonstrated accumulation of liposomes in certain tumors in a higher concentration than found in normal tissues. Many research efforts' have been directed towards improving the safety profile of the anthracycline cytotoxic, doxorubicin (DXR) and daunorubicin (DNR), along with vincristine (VCR), which are associated with severe cardio toxic side effects, although acute gastrointestinal effects and other toxicities may also occur. Liposomal entrapment of these drugs showed reduced cardio toxicity, dermal toxicity and better survival of ex-perimental animals compared to the controls receiving free drugs. Such beneficial effects of liposomal anthracyclines have been observed with a variety of liposomal formulations regardless of their lipid composition provided that lipids used high cholesterol (Cho) concentration or phospholipids with high phase transition temperature (Tc) are conducive to drug retention by the vesicles in the systemic circulation. DXR entrapped in liposomes shows reduced non-specific toxicity and maintains or enhances anticancer effect. DXR hydrochloride constitutes the first liposomal product (Doxil TM) to be licensed in the United States. Surface grafted methoxypolyethylene glycol (MPEG) provides the hydrophilic stealth coating, which allows the Doxil TM liposomes to circulate in the blood stream for prolonged periods. The lipid matrix and an internal buffer system combine to keep virtually all the DXR encapsulated during liposome residence in the circulation. This means that the drug is not free to exert its toxic effects. Liposome association alters the drug pharmaco- kinetics and thus the liposome has a half-life of approximately 55 hours in humans, whereas the free drug distributes to the tissues within a few minutes and is entirely cleared from circulation within 24 hours. Liposomal formulation showed decreased toxic effects of DXR; a dose higher than the LD50 could be administered without acute toxicity, which suggests that these liposomes extravasate from the endothelium of tumor tissues and reside around tumor cells where they release the drug into the interstitial fluid. Preclinical and clinical investigations have demonstrated significantly increased efficacy and decreased toxicity of liposomes containing DNR (Dauno Xome TM) in comparison with free DNR in the treatment of acute leukemia. However, in the treatment of hepatocellular carcinoma and liver cirrhosis liposomal DNR showed mild hematological toxicities and significant hepatic toxicity, which warns against further assessment of these liposome's in patients with hepato cellular carcinoma and liver cirrhosis. However, liposomal DNR showed encouraging results in the treatment of advanced cutaneous T-cell lymphoma. Furthermore, liposomal DNR and carboplatin plus etoposide, used to treat children with

recurrent high-grade glioma after surgery and with progressive teratoid/rhabdoid tumor, showed encouraging results with only little and transient hematological toxicity. Liposomal encapsulation of VCR resulted in increased and prolonged plasma concentration, which is associated with increased antitumor activity (murine P388 ascetic tumor) but not increased drug toxicities comparedto the encapsulated drug. Guthlein *et al*. found that VCR entrapped into a vesicular phospholipids gel consisting of densely packed liposomes was an effective delivery system with superior antitumor activity compared to conventional VCR against human small cell lung carcinoma LXFS 650 and the human mammary carcinoma MX1. Sustained release and passive tumor targeting can explain the enhanced efficacy.

2. Antimicrobial Therapy [28, 29]

Incorporation of rifabutin in liposomes resulted in a significant enhancement of activity against Mycobacterium avium infection compared to free rifabutin. Moreover, the anti- tubercular activity of rifampin was considerably increased when encapsulated in egg phosphatidylcholine liposome. A further increase in the activity was observed when the macrophage activator tetra peptide tuftsin was grafted on the surface of drug-loaded liposomes. Rifampin delivered twice weekly for two weeks in tuftsin- bearing liposomes was at least 2,000 times more effective than the free drug in lowering the load of lung bacilli in infected animals. Liposome encapsulated clarithromycin may be more effective than the free form against Mycobacterium avium intracellular (MAI) infections *in vivo*, and the use of a combination therapy with ethambutol could further enhance the efficacy. Furthermore, when the activity of TLC G- 65 (liposomal gentamicin preparation), alone and in combination with rifapentine, clarithromycin, clofazimine and ethambutol, was evaluated in the beige mouse model of disseminated Mycobacterium avium infection showed that the combination of rifapentine and TLC G- 65 was more active than either agent alone. The activity of clarithromycin in combination with TLC G- 65 was similar to that of either agent alone. Clofazimine improved the activity of TLC G- 65 with respect to the spleen, while ethambutol improved the activity with respect to the liver. Entrapment of ciprofloxacin in liposomes increases the circulation half- life of the drug when given by intravenous route in mice, which is associated with enhanced delivery of the drug to the liver, spleen, kidneys, and lungs. Furthermore, liposomal entrapment was associated with increased therapeutic efficacy against the Salmonella typhimuriusm infection model in mice. Stevenson and coworkers showed enhanced activity of streptomycin and chloramphenicol against Escherichia coli in the cells of the J774 murine macrophage line mediated by liposome delivery.

Result and Discussion

We have study the liposome technique for drug delivery and it shows versatile tool for drug targeting into specific organ or tissue and cell. Now days most of drugs are incorporated by the liposomal technique and it help to reduce the toxicity and side effect of drug.

Marketed liposomal and lipid-based product and their use

Conclusion

Almost from the time of their discovery in 1960's and the demonstration of their entrapment potential, liposome vesicle have drawn attention of researchers as potential carriers of various bioactive molecules that could be used for therapeutic application in humans and animal. liposome solubilise lipophillic drug candidates that would otherwise be difficult to administer i.v. the encapsulated drug is inaccessible to metabolizing enzyme; conversely, body compartment such as erythrocyte and tissue injection site are not directly exposed to full dose of the drug.

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