LIPOSOME AS A DRUG CARRIER - A REVIEW

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ABSTRACT
Liposome is acceptable and superior carrier and has ability to encapsulate hydrophilic and lipophilic drugs and protect them from degradation. Liposomes are vesicles having concentric phospholipids bilayer molecules from low molecular weight to higher molecular weight have been incorporated in liposomes. The water soluble compounds/drugs are present in aqueous compartments while lipid soluble compound/drugs and amphiphilic compounds/drugs insert themselves in phospholipids bilayers. Liposome containing drugs can be administrated by many routes (intravenous, oral, inhalation, local application, ocular) and these can be used for treatment of various diseases. Liposomes are biocompatible, completely biodegradable, non-toxic, and non-immunogenic. Liposomes are prepared by using various methods hydration, ethanol injection method, ether injection method, Sonication method, micro-emulsion method. The different application of liposomes is use for treatment of infection, anti-cancer, vaccination, for human therapy and gene delivery system. After the formulation, the evaluation of liposomes are checked by using physical parameters, chemical parameters, and biologically for the establish the purity and potency of various lipophilic constituents and establish the safety and suitability of formulation for therapeutic application.

Keywords: Liposome, lipophilic drugs, phospholipids, biodegradable.

1.1 INTRODUCTION
Liposomes vesicles were prepared in the early years of their history from various lipid classes identical to those present in most biological membranes. liposomes were discovered in the mid of 1960’s and originally studied as cell membrane model Paul Ehrlich coined the term “magic bullet” in 20” century where carrier system was proposed to simply carry the drug to its of action and releasing its selectively while non – target sits should absolutely be example from drug effect. Liposomes are vesicles having concentric phospholipids bilayer molecules from low molecular weight to high molecular weight have been incorporated in liposomes. The water soluble compounds/drugs are present in aqueous compartments while lipid soluble compound/drugs and amphiphilic compounds/drugs insert themselves in phospholipids bilayer. The liposomes containing drugs can be administrated by many routes (intravenous, oral inhalation, local application, ocular) and these can be used for the treatment of various diseases. Multilamellar liposomes (MLV) usually range from 500 to 10,000 nm. Unilamellar liposomes can be called as small (SUL) and as large (LUV): SUV are usually smaller than 50 nm and LUV are usually large than 50 nm. The liposomes of very large size are called giant liposomes (10,000-10,000 nm). They can be either unilamellar or multilamellar. The liposomes containing encapsulated vesicles are called multi-vesicle liposomes. Their size range from 2,000-40,000 nm. LUVs having asymmetric distribution of phospholipids in the bilayer are called asymmetric liposomes. The delivery of drugs onto the skin is recognized as an effective means of therapy for local dermatologic diseases. Liposome is acceptable and superior carrier and has ability to encapsulate hydrophilic and lipophilic drugs and protect them from degradation. Topical drug administration is a localized drug delivery system anywhere in the body through ophthalmic, rectal, vaginal and skin as topical routes. Skin is one of the most readily accessible organs on human body for topical administration and is main route of topical drug
delivery system. Topical application of liposome vesicles has many advantages over the conventional dosage forms. In general, they are deemed more effective and less toxic than conventional formulations due to the bilayer composition and structure. liposomes are usually applied to the skin as liquids or gels. For topical application of liposomes in gel form, hydrophilic polymers are considered to be suitable thickening agents. Liposome carriers, well known for their potential in topical drug delivery, have been chosen to help transport drug molecules in the skin layers. These vesicles are also expected to provide lipid enriched hydrating conditions to help retain the drug molecules with in the dermal layers, at or near to the site of action. Stability of the liposomes in terms of their drug holding capacity was assessed for a period of 5 weeks, on storage under defined conditions. The delivery of drugs onto the skin is recognized as an effective means of therapy for local dermatologic diseases.

Liposomes are formed open hydration of lipid molecules normally lipids are hydrated from a dry state(thin or thick lipid film, spray dried powder),and stacks of crystalline bilayer become fluid and swell myelin-long, thin cylinders grow and upon agitation detach self close in to large, multilamellar liposomes because this eliminates, unfavorable interactions at the edges. Once the large particles are formed they can be either broken by mechanical treatment in to smaller bilayered fragments, which close into smaller liposomes .The size of liposomes in the budding off mechanism is very difficult to calculate, in the self closing bilayer mechanism the liposomes size depends, the bending elasticity of the bilayer and the edges interaction of open fragments. These factors determine the size of the vesicle size. Liposomes have been reported to be effective drug carriers. Local anesthetics encapsulated into liposomes show longer duration of action, reduction in circulating plasma levels, reduced central nervous system toxicity, and reduced cardiovascular toxicity. Liposomes are single or multilayered vesicles that completely enclose an aqueous phase within one or several phospholipids bilayer membrane. An important aspect of liposomes is the protection that they afford as an encapsulating agent against potentially damaging conditions in external environments. Liposomes are also a system in their own right in medical, cosmetic, and industrial application.

However major limitation of using liposomes topically is the liquid nature of preparation. They can be overcome by their incorporation in adequate vehicles where original structure of vesicles is preserved. It has already been shown that liposomes are fairly compatible with gels made from polymers derived from cross linked polyacrylic acid, such as carbopol resins.

1.2 Classification of liposomes

Liposome vesicles were prepared in the early years of their history from various lipid classes identical to those present in most biological membranes. Basic studies on liposomes vesicles resulted in numerous methods of their preparation and characterization. Liposomes are broadly defined as lipid bilayer surrounding an aqueous space. Multilamellar vesicles (MLV) consist of several (up to 14) lipid layers (in an onion-like arrangement) separated nanometers in diameter. Small unilamellar vesicles (SUV) are surrounded by a single lipid layer and are 25-50nm.

- Based on structural parameters
  - Small unilamellar vesicles (SUV): Size range from 20-40nm.
  - Medium unilamellar vesicles (MUV): Size range from 40-80nm.
  - Large unilamellar vesicles (LUV): Size range from 100-1000nm.
  - Oligolamellar vesicles (OLV)
    These are made up of 2-10 bilayers of lipids surrounding a large internal volume.

- Multilamellar vesicles (MLU)
  They have several bilayers. They can compartmentalize the aqueous volume in an infinite numbers of ways. They differ according to way by which they are prepared. The arrangements can be onion like arrangements of concentric spherical bilayer of LUV/MLV enclosing a large number of SUVs.

- Based on Method of liposome preparation
  - REV: single or oligolamellar vesicles made 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 repaired by extrusion technique.
  - DRV: Dehydration-rehydration Method.

- Based upon composition and Application:
Conventional liposomes (CL): Neutral or negatively charged phospholipids and Chol.

Fig. 1:

1.3 Advantages of liposomes
- Liposomes are biocompatible, completely biodegradable, non-toxic and non-immunogenic.
- Liposomes are suitable for delivery of hydrophobic, amphiphatic and hydrophilic drugs.
- Liposomes are protecting the encapsulated drug from the external environment.
- Liposomes are reduced the toxicity and increased the therapeutical effect of the drugs.
- They are increase the activity of chemotherapeutic drugs and can improved through liposome encapsulation this reduce deleterious effect that are observed at concentration similar to or lower than those required for maximum therapeutic activity.
- They reduce exposure of sensitive tissues to toxic drugs.

1.4 Disadvantages of liposomes
- Production cost is high.
- Leakage and fusion of encapsulated drug/molecules.
- Short half-life.
- Stability problem.

2.1 Methods of Preparation of liposomes
- Hydration method.
- Ethanol injection method.
- Ether injection method.
- Sonication method.
- Micro-emulsification method.

2.2 Hydration method
This is simplest and widely used method. The Lipid mixture and charged components are dissolved in chloroform. Methanol mixture and then this mixture is introduced in to a 250 ml Round bottomed flask. The flask is attached to rotary evaporator connected with vacuum pump and rotated at 60 rpm. The organic solvents are evaporated at about 30°. A dry lipid residue is formed at the walls of the flask and rotation is continued for 15 minutes after dry lipid residue appeared. The evaporator is detached from vacuum pump and nitrogen is introduced into it. The flask is then removed from evaporator and fixed onto lyophilized to remove residual solvent. Then the flask is again flushed with nitrogen and 5 ml of phosphate buffer is added. The flask is attached to evaporator again and rotated at 60 rpm speed for 30 minutes or until all lipid has been removed from the wall of the flask. A milky white suspension is formed finally. The suspension is allowed to stand for 2 hours in order to complete swelling process to give MLVs.

2.2 Ethanol injection method
This is simple method. In this method an ethanol solution of the lipids is directly inject rapidly to an excess of saline or other aqueous medium through a fine needle. The ethanol is diluted in water and phospholipids molecules are dispersed evenly through the medium. The procedure yields a high proportion of SUVs.
2.3 Ether injection
The method is similar to above one. It involves injecting the immiscible organic solution very slowly into an aqueous phase through a narrow needle at temperature of vaporizing of organic solvent. In this method the lipids are carefully treated and there is very less risk of oxidative degradation.

2.4 Sonication
This method reduces the size of the vesicles and impart energy to lipid suspension. This can be achieved by exposing the MLV to ultrasonic irradiation. There are two methods of Sonication.

(a) Using bath sonicator.
(b) Using probe sonicator.

![Fig. 3:](image)

The probe Sonication is used for suspension which requires high energy in small volume. The bath sonicator is used for large volume of dilute lipids. The disadvantage of probe sonicator is contamination of preparation with metal from tip of probe. By this method small unilamellar vesicles are formed and they are purified by ultracentrifugation.

2.5 Micro-emulsification method
Equipment called micro-fluidizer is used to prepare small vesicles from concentrated lipid suspension. The lipids can be introduced in to the fluidizer as a suspension of large MLVs. The equipment pumps the fluid at very high pressure through 5 micrometer screen. Then it is forced long micro channels, which dried two streams of fluids collide together at right angles at very high velocity. The fluid collected can be recycled through the pump and interaction chamber until vesicles of spherical dimensions are obtained.

3.1 Application of liposomes
Liposomes were developed as an advanced drug delivery vehicle. They are generally considered non-toxic, biodegradable and non-immunogenic. Associating a drug with liposomes markedly changes its pharmacokinetics and lowers systemic toxicity; furthermore, the drug is prevented from early degradation and/or inactivation after introduction to the target organism.

Liposomes in infection treatment
Liposomes are useful in the treatment of parasitic infections of the MPS, such as leishmaniasis. Encapsulating the amphotericin B into liposomes reduces the renal and general toxicity, and the therapeutic efficiency is improved. The encapsulation of the anti-tuberculosis drug Rifampicin or Isoniazid in liposomes targeted to lung improves the efficacy of the drug.

Liposomes as vaccine system
Liposomes can be used as enhancers of the immunological response by incorporation of antigens 61. For this purpose the liposomes are administered intramuscularly, a location where the encapsulated antigen is released slowly and accumulate passively within regional lymph nodes. To control the antigen release and improve the antibody response, the liposomes encapsulating antigens are subsequently encapsulated into alginate lysine microcapsules. Hepatitis A virus incorporated into liposomes proved to be a suitable formulation in term of rapid seroconversion high level of mean antibody content and low reactogenicity. Also, there is in clinical trial vaccines against influenza, Hepatitis B, diphtheria, tetanus, E.coli infection.

Liposomes in human therapy
Despite of the good and encouraging results obtained using liposomes as vehicles for drugs in numerous diseased animal models, in human therapy; the use of liposomes is restricted to systemic fungal infections and cancer therapy, only. However, liposomes based vaccines show great promise and vaccine against hepatitis A is already on the market.
Liposomes in anticancer therapy
Based on the early studies that showed that encapsulation of a drug inside of liposomes reduces its toxic side effects, the liposomes were considered as attractive candidates for the delivery of anticancer agents. Intravenously administered stealth liposomes were passive targeted to solid tumors due to their extravasations in leaky blood vessels supporting the tumor. The good result obtained with Liposomal encapsulation Doxorubicin and Daunorubicin have lead to two products licensed for use in the treatment of Kaposi’s sarcoma, namely Doxil and Daunoxome.

Liposomes in Gene delivery
Gene therapy is the process which DNA delivers sequences encoding specific altered genes to cells with the goal of treating or curing genetic diseases. Thus, instead of treating the symptoms of the diseases as in conventional medicines, gene therapy has the potential to correct the underlying cause of genetic diseases. While the idea of gene therapy is a simple concept, the delivery of genes to the diseased areas turned out to be a difficult task. The problems associated with the use of viral vectors for gene therapy, lead to the search for less-hazardous, non-viral delivery systems. As an alternative to viral vectors, cationic liposomes have been developed for gene transfer since they have no limit for the size of the gene to be delivered and exhibit low immunogenicity.

4.1 Evaluation of liposomes
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 3 broad categories which include physical, chemical, and biological parameters.

- Physical characterization evaluates various parameters including size, shape, surface features, lamellarity, phase behaviors and drug release profile.
- Ma et al. Evaluated structural integrity of Liposomal phospholipids membrane by a new technique of gamma-ray perturb angular correlation (PAC) spectroscopy. In this 111In label diethylene triamine penta acetic acid (DTPA) Derivative dipalmityl phosphatidyl ethanoamine (DPPE) lipid were incorporated in the SUVs. This helped in the continuous non-invasive monitoring of the microenvironment of the lipid bilayer.
- Chemical Characterization includes those studies which establish the purity and potency of various lipophilic constituents.
- Biological Characterization parameters are helpful in establishing the safety and suitability of formulation for therapeutic application.

4.2 Vesicle shape and Lamellarity
Vesicle shape can be assessed using Electron Microscopic Techniques. Lamellarity of vesicles i.e. number of bilayer present in liposomes is determined using Freeze-Fracture Electron Microscopy and P-31 Nuclear Magnetic Resonance Analysis.

4.3 Vesicle size and size distribution
Various techniques are described in literature for determination of size and size distribution. These include Light Microscopy, Electron Microscopy (especially Transmission Electron Microscopy), Laser light scattering Photon correlation Spectroscopy, Field Flow Fractionation, Gel permeation and Gel Exclusion. The most precise method of determine size of liposome is Electron Microscopy Since it permit one to view each individual liposome and obtain exact information about profile of liposome population over the whole range of sizes. Unfortunately, it is very time consuming and require equipments that may not always be immediately to hand. In contrast, laser light scattering method is very simple and rapid to perform but having disadvantage of measuring an
average property of bulk of liposomes. Another more recently developed microscopic technique known as atomic force microscopy has been utilized to study liposome morphology, size, and stability.

Most of methods used in size, shape and distribution analysis can be grouped into various categories namely microscopic, diffraction, Scattering, and hydrodynamic techniques.

### Biological Characterization

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<th>Instrument for Analysis</th>
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<td>Aerobic/Aerobic Culture</td>
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### Physical Characterization

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### Chemical Characterization

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<td>HPLC/Barlet assay</td>
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### 4.4 Stabilization of liposome

The stability of liposome should meet the same standard as conventional pharmaceutical formulation. The stability of any pharmaceutical product is the capabilities of the delivery system in the prescribed formulation to remain within defined or pre-established limits for predetermined period of time.

- **Chemical Stability** involves prevention of both the hydrolysis of ester bonds in the phospholipids bilayer and the oxidation of unsaturated sites in the lipid chain.

- **Chemical instability** leads to physical instability or leakage of encapsulated drug from the bilayers and fusion and finally aggregation of vesicles.

- Chen et al. Introduced the pro-liposome concept of liposome preparation to avoid physicochemical instability encountered in liposome suspension such as aggregation, fusion, hydrolysis and / or oxidation.

- Approaches that can be taken to increase Liposomal stability involve efficient formulation and lyophilization. Formulation involves the selection of the appropriate lipid composition, concentration of bilayers, aqueous phase ingredients such as buffers, antioxidant, metal chelators and cryo protectants. Charge inducing lipids such as phosphotidyl glycerol can be incorporated into liposome bilayers to decrease permability and leakage of encapsulated drugs. Buffers at neutral pH can decrease hydrolysis, addition of antioxidant such as sodium ascorbate can decrease oxidation.
- Oxygen potential is kept to minimum during processing by nitrogen purging solution.
- In general successful formulation of stable Liposomal drug product requires the following precautions.
- Processing with fresh, purified lipid and solvents.
- Avoidance of high temperature and excessive shear forces.
- Maintenance of low oxygen potential (Nitrogen purging).
- Use of antioxidant or metal chelators.
- Formulating at natural pH.
- Use of lyo-protectant when freeze drying.

CONCLUSION
Twenty five years of Research into the use of liposome in drug delivery. Liposomes are one of the unique drug delivery system, They can be use in controlling and targeting drug delivery. Now, in days the Liposomal topical formulations are more effectively and give the safe therapeutic efficacy. These are also used in the cosmetic and hair Technologies, diagnostic purpose and good carrier in gene delivery. Liposomes are giving a good and encouraging result in the anticaner therapy and human therapy.

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