LIPOSOMES-CONTROLLED AND TARGETED DRUG DELIVERY SYSTEM

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Abstract

Liposomes are spherical-enclosed membrane vesicles mainly constructed with lipids. Lipid nanoparticles are loaded with therapeutics and may not contain an enclosed bilayer. The majority of those clinically approved have diameters of 50–300 nm. The growing interest in Nanomedicine has fueledlipid–drug and lipid–protein studies, which provide a foundation for developing lipid particles that improve drug potency and reduce off-target effects. At present, about 600 clinical trials involve lipid particle drug delivery systems.

Most clinical applications of liposomal drug delivery are targeting to tissue with or without expression of target recognition molecules on lipid. The liposomes are characterized with respect to physical, chemical and biological parameters. Due to new development of liposome technology, several liposomes -based drug formulations are currently in clinical trials and recently some of them have been approved for clinical use.

Keywords

Liposome, sonication, Zeta potential, Nanoparticles.etc

Introduction

Liposomes mainly made up of phospholipid. Liposomes are surrounded by single or multiple layers of phospholipid. In liposomes hydrophobic as well as hydrophilic drugs are incorporated.

Liposomes are simple microscopic vesicles in which an aqueous volume is entirely enclosed by a membrane composed of lipid molecule.

Structurally, liposomes are concentric bilayer vesicles in which aqueous volume is entirely enclosed by membranous lipid bilayers mainly composed of natural or synthetic phospholipids.

Liposomes is Greek words means ‘Lipo’ mean ‘Fat’ and ‘Somes’ mean ‘Body’.

Liposomes are made of molecules with hydrophilic and hydrophobic ends that form hollow spheres which can encapsulate water-soluble ingredients (drugs)in their inner water space and oil-soluble ingredients(drugs)in their phospholipid membranes that are made up of one or more concentric lipid bilayers, and range in size from 50 nanometers to several micrometers in diameter.

The unique property of liposomes, namely their versatile, biodegradable, hypoallergenic nature, along with their similarity to biological membranes are the important factors in the continued efforts to develop liposomal drug delivery forms.

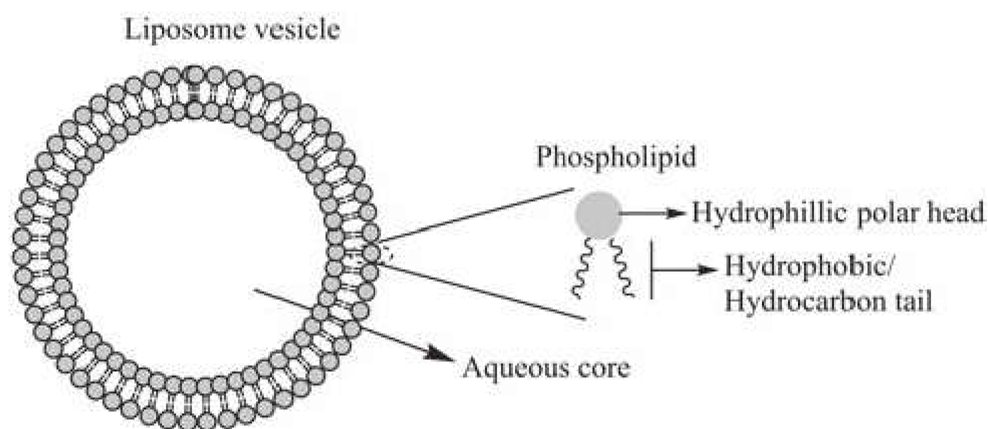


Figure 1 Diagramatic representation of liposomes

Advantages-

1.Drug directly delivery in a body cell or individual cellular compartment.

2.These are targeted drug delivery system.

3.Liposomes are non-toxic, non-pyrogenic.

4.Drug released at target site that`s because other tissues and cells are protected.

5.Used as carriers for controlled and sustained drug delivery.

6.Increased efficacy and therapeutic index (Actinomycin D).

7.Increase stability via encapsulation.

8.Improve pharmacokinetic effect.

9.Liposomes help to reduced exposure of sensitive tissue to toxic drugs.

10.Provides selective passive targeting to tumor tissues.

Disadvantages-

1.They are quickly eliminated by cells of reticuloendothelial system from blood after IV injection.

2. There is chances of oxidation of phospholipid.
3. Liposomes are less stable.
4. Drug released in slow manner.
5. Leakage of encapsulated drug during storage.
6. Production cost is high.
7. Short half-life.
8. Possibility of dumping, due to faulty administration.
9. Difficult in large scale production manufacturing and sterilization.
10.Batch to batch variation.

Classification of liposomes-

On the basis of structural parameters

1. Unilamellar vesicles (UV)
2.Oligolamellar vesicles (OLV)
3. Multilamellar vesicles (MLV)

On the basis of liposome preparation

1.Vesicles prepared by reverse phase evaporation method (REV)
2. Multilamellar vesicles by REV.
3.Stable plurilamellar vesicles (SPLV)
4. Vesicles prepared by extrusion techniques (VET)
5. Frozen and thawed MLV
6. Dried reconstituted vesicles (DRV)

On the basis of composition and applications-

1. Conventional liposomes
2. Fusogenic liposomes
3. Cationic liposomes
4. PH sensitive liposomes
5. Immuno- liposomes
6. Long circulatory liposomes

On the basis of structural parameters-

1. Unilamellar vesicles- They are divided into three subregions.

a) Small unilamellar vesicles- size range 20-40nm.
b) Medium unilamellar vesicles- size range 40-80nm.
c) Large unilamellar vesicles- size range 100-1000nm.

2.Oligolamellar vesicles (OLV)- The vesicles made up of 2-10 bilayers of lipids.

3. Multilamellar vesicles (MLV)- They have several bilayers of phospholipid.

They are differing according to way by which they are prepared.

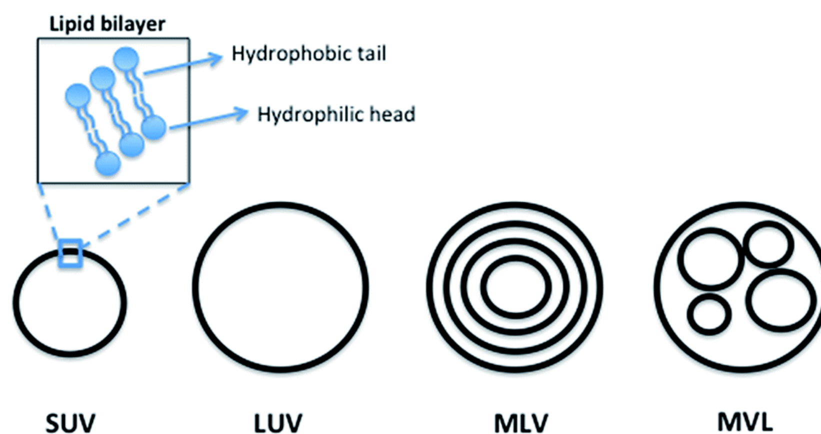


Figure 2 Different types of liposomes

Methods of preparation of liposomes-

1. Hand shaking method
2. Sonication method
3. Zeta potential method
4. Reverse phase evaporation method
5. Solvent dispersion method
6. Freeze dried rehydration method

1. Hand shaking method-

i) Preparation of film for hydration-

This method developed by Banghametal for the preparation of multilamellar vesicles. In this method phospholipid are dissolve in organic solvent such as chloroform and methanol. Lipid solution are prepared at 10-20mg lipid/ml of organic solvents.

Once the lipids are mixed in organic solvents, the solvent is removed to yield a lipid film. For small volume solvent removal(<1m) and solvent may evaporate by using dry Nitrogen gas and Argon stream. For larger volume, solvent remove by Rotary Evaporation to form thin lipid film. Lipid film is a dried to removed organic solvent by placing in vacuum overnight. Lipid film can also be prepared by freezing in dry ice/dry ice-acetone/alcohol bath. The lipid cake is placed in vacuum pump and lyophilized until dry (1-3 days). The thickness of lipid cake should not be more than

the diameter of the container being used for lyophilization. Dry lipid film removed from vacuum, container closed tightly, taped and store frozen.

ii) Hydration of lipid film-

Hydration of lipid film is method of adding an aqueous media to the container containing dry lipid and agitating and over night stand.

Temperature of hydrating medium should be above transition temperature of lipid.

Hydration media are used in lipid film are buffer solution, saline 5% dextrose, 10 % sucrose.

The particles can be separated once a stable, hydrated LMV (Large multilamellar vesicles) suspension has been created.

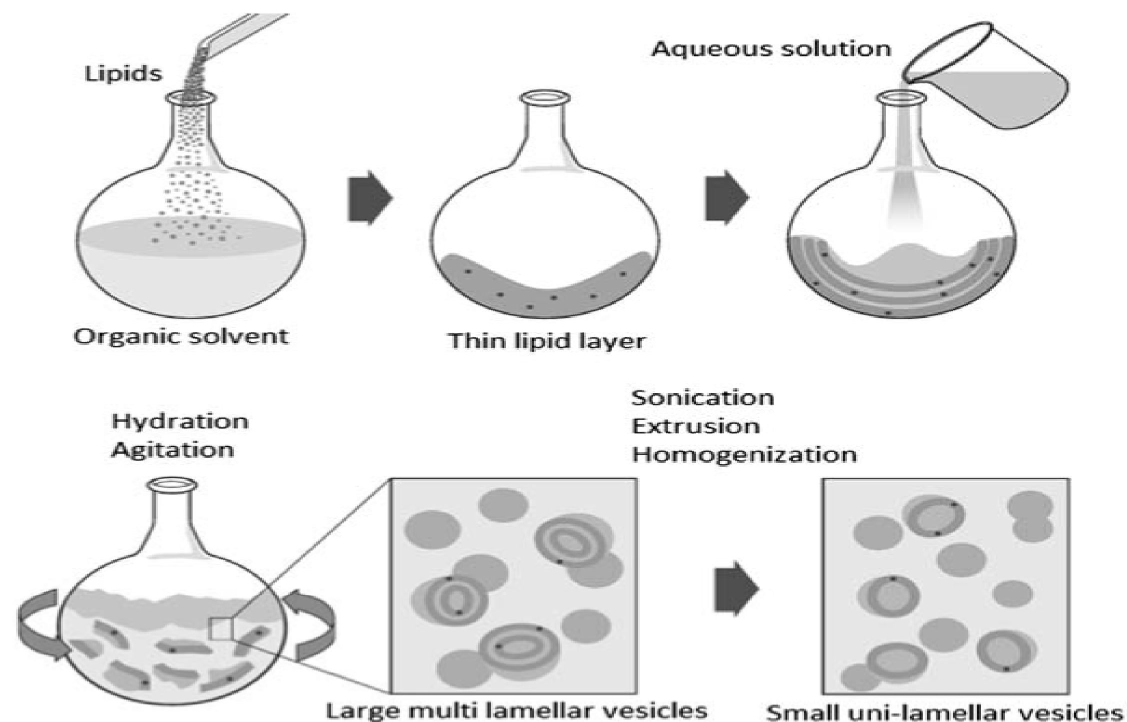


Figure 3. Hand shaking method

2.Sonication method-

Most commonly used for preparation of small unilamellar vesicles liposomes range between (20-100nm). Sonication done by bath type sonicator and probe sonicator under passive atmosphere.

i) Probe sonication-

In this procedure, the sonicator tip is directly immersed in the liposome dispersion is quite high. Because energy dissipation at the tip causes local overheating. The vessel must be insulated into an ice bath throughout sonication upto 2 hours more than 5% of lipids can be de-esterify.

Also, with probe sonicator, titanium will slough off and pollute the solution.

ii) Bath sonication- In a bath sonicator, the liposomes dispersion is placed in a cylinder. The temperature of the lipid dispersion is normally controlled is easier in this method as compared as sonication by dispersion using the tip.

The material are used for sonication can be protected in a sterile vessel, comparable Probe units or in an inert atmosphere.

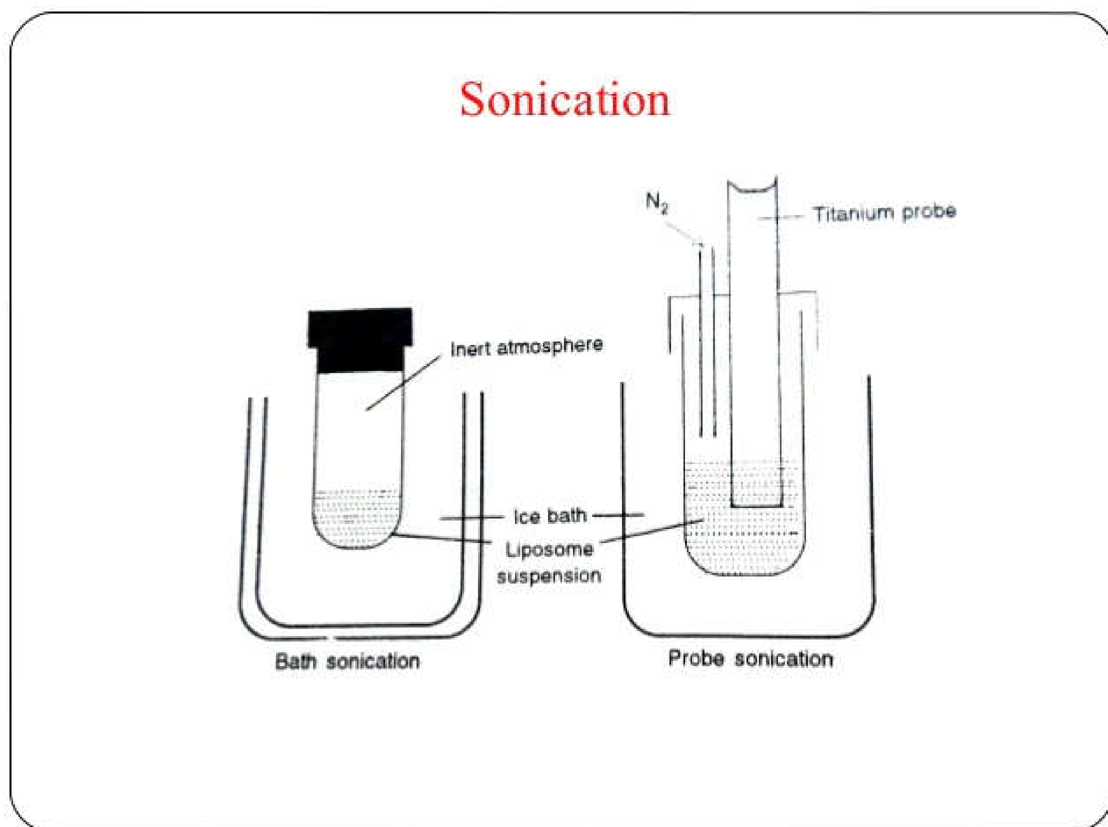


Figure 4. sonication

3. Zeta potential-

Zeta potential is term used to describe the potential of a molecule. Each liposomes has a charge either positive, negative or neutral, depending on the makeup and associated ligand. By using zeta potential method, stability of liposomes can be estimated.

Liposomes with low surface area or uncharged tend to aggregate over time, whereas liposomes with higher negative or positive charge will have repulsive force in medium which causes agglomeration. Incident light illuminates in a sample cell, and the zeta potential is determined by changes in the scattered light when the liposomes move due to the application of force. The movement of liposomes is proportional to the associated charge. A doppler shift in frequency the detected laser light is generated by the movement of liposomes.

4.Reverse phase evaporation method-

The lipid is added to a round bottom flask, and the solvent is evaporated using a Rotary evaporator under reduce pressure. The system is flushed with nitrogen, and the lipids are redissolved in the organic phase which is the phase in which reverse phase vesicle will form. The most common solvents are Diethyl ether and Isopropyl ether. The emulsion is obtained after the lipids have been redissolved. Evaporation is used to remove the solvent from an emulsion under reduced pressure. Semisolid gel material that hasn't been encased is then taken away. Non encapsulated material is then removed. The liposomes that result is known as reverse phase liposomes. The method has been used to encapsulate small and large macromolecules and it has ability to encapsulate large macromolecules with high efficiency. The main disadvantages of the method are the exposure of materials to be encapsulated to organic solvents.

5. Solvent dispersion method-

i) Ether injection-

Ether injection method is also known as solvent vaporization method. A lipid solution is dissolved in diethyl ether or mixture of ether and methanol is gradually injected into an aqueous solution of the material to be encapsulate at 55 ^{o}C to 65 ^{o}C or under reduced pressure.

The consequent removal of ether under vacuum leads to the creation of liposomes.

The main drawbacks of this technique is population of heterogenous (wavelength range70 to 200nm) and exposure of number of chemicals to be encapsulated in an organic solvents at high temperature.

ii) Ethanol injection-

A lipid solution of ethanol is injected rapidly into a range amount of buffer. The MLV's are generated at same time.

The drawbacks of this method there is wide range of population in wavelength 30 to 110nm, because liposomes available in very dilute form and removal of ethanol is

difficult because it forms azeotrope with water and there is chance that are physiologically active macromolecules will inactivate in the presence of even a small amount of ethanol. Ethanol is present in little concentration, and thus it has high level of toxicity.

6. Freeze dried rehydration method-

Freeze dried liposomes are formed from performed liposomes. Dehydration causes the lipid bilayers as well as material to be encapsulated in liposomes are brought in into close proximity. Upon reswelling the chances for encapsulation of the adhered molecules are much higher.

The rehydration is crucial step in the recovery process and should be carried out with caution. The watery phase should be added with a micropipette in very small amounts to the dried materials. The tube should be vortexed after each addition.

The total volume used for rehydration must be smaller than the starting volume of the liposome dispersion.

Liposomal Amphotericin B (AmBisome)-

Amphotericin B is the treatment choice for the systemic fungal infection such as candidosis and aspergillosis. Amphotericin B liposomal formulation in which the medication is tightly bound to the bilayer structure of tiny unilamellar liposome.

AmBisome is composed of hydrogenated soy and phosphatidylcholine, disteroyl phosphatidylcholine and cholesterol. It is very stable, over a 72 hours incubation period in human plasma.

This resistance to drug loss is a critical feature in AmBisome is capacity to significantly minimize the well-known acute and chronic toxicities associated with AmBisome B therapy. AmBisome therapeutic efficacy for a wide spectrum of fungal infection has been demonstrated in numerous animal and clinical investigations.

AmBisome has a physiochemical property and a pharmacokinetic profile that are significantly different from those of currently available lipid complexed. This formulations with much higher area under the plasma concentration-time curve and much lower clearance at equivalent doses, due to its size and in vivo stability.

At fungal infection sites AmBisome liposomes can be detected accumulating. After attachment to the fungal cell wall, AmBisome liposomes are disrupted, causing amphotericin B to bind to the ergosterol in the fungal cell membrane resulting in cell lysis.

AmBisome has been demonstrated to permeate both extracellular and intracellular foetal cell walls.

Pharmacokinetics of liposomal amphotericin B in critically ill patients-

The parent drug's acute and chronic side effects are considerably by the liposomal formulation of amphotericin B. The current study compares the pharmacokinetics of AmBisome, which was given to 10 patients at a dose of 2.8 to 3.0mg/kg of body weight, to the pharmacokinetics pf amphotericin B deoxycholate, which was given to 6 patients at a typical dose of 1.0mg/kg.

Patients treated with AmBisome had a 8 to 10 fold more interpatient variability of amphotericin B peak concentration (Cmax) and areas under the concentration time curves (AUC) than patients treated with amphotericin B deoxycholate.

Cmaxes were 8.4 times higher at the three-fold higher dose of AmBisome, and median AUCs were g times higher than those seen with amphotericin B deoxycholate. This was explained in part by the fact AmBisome, on the other hand, had a twofold shorter apparent half-life of elimination (p=0.03).

In one case, neither hemodialysis nor hemofiltration had a substantial impact on AmBisome pharmacokinetics.

Finally, the liposomal amphotericin B formulation considerably (p=0.001) lower the volume of drug distribution.

Characterization of amphotericin B liposome formulations-

Liposomes composed of hydrogenated soya/ phosphatidylcholine/cholesterol/charged lipids [diacetylphosphate (-) or stearylamine (+) were developed.

The hydrogenated /soya/phosphatidylcholine/cholesterol/charged lipids were synthesized using a chloroform film process with: sonication in molar ratios of 1:1:0, 7:2:0, 7:2:1(-), 7:2:1(+) with or without entrapped amphotericin B (0.05 mg AmB/mg lipid).

A zeta meter used to determine the charges of liposomes. The surface charge density of negative liposomes with or without encapsulated AmB was higher than that of other formulations.

The AmB contains in liposomes were determined by high performance liquid chromatography with ultraviolet detection at 382nm.

Mechanism of action-

Amphotericin B binds to ergosterol in the fungal cell membrane, which leads to the formation of pores, ion exchange and ultimately fungal cell death.

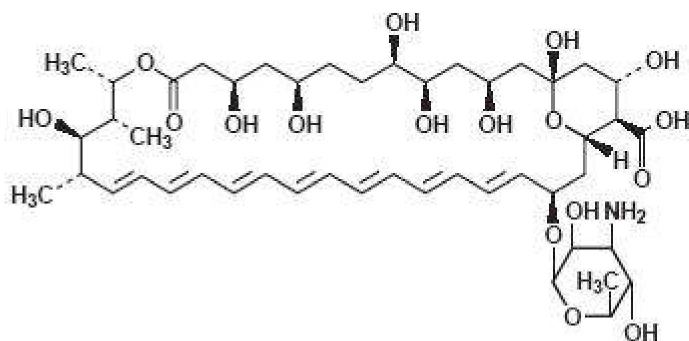
The binding of liposome to the cell wall of pathogenic yeasts and moulds has been demonstrated in vitro using and in vivo using fluorescently labeled liposomes and gold-labeled liposomes. Liposomes without AmB bind to the cell wall, but both the ‘empty’ liposomes and fungal cell remain intact.

In contrast binding of amphotericin B containing liposomes results in fungal cell death suggesting that binding results in liposomal disruption and released of amphotericin B, which is then free to exert its fungicidal activity by binding to ergosterol in the fungal cell membrane.

The precise mechanism by which amphotericin B transferred from the liposome through the fungal cell wall to the fungal membrane was not known. It is likely that the process is facilitated by the higher binding affinity of amphotericin B for ergosterol the sterol present in fungal cell membranes) compared with cholesterol, which is the principle lipid component of the liposome.

Temperature also appears to be important in the transfer of amphotericin B between the liposome and the fungus and occurs most efficiently at body temperature.

Structure of amphotericin B-



Pharmacodynamics-

LAmB is less potent on a mg-per-mg basis compared with amphotericin B deoxycholate. Difference in exposure-response relationships is most obvious from an in vitro model of the human alveolus, where the effective dose for 50% effect (ED50) was 1.03 and 0.12 mg/L for LAmB and DAmB, respectively. Similar findings are apparent in other animal models of invasive fungal infection including a murine aspergillosis model and a rabbit aspergillosis study. These results suggest that much of the active compound is ‘locked’ in the liposome and is in effect biologically inert.

LAmB has antifungal activity in the central nervous system of a rabbit model of candida meningoencephalitis and leads to complete sterilization within the cerebrum. LAmB is also effective in a murine model of CNS aspergilllosis.

In a preclinical study of disseminated candidiasis, blastomycosis, mucormycosis histoplasmosis and cryptococcosis dose-dependent antifungal activity is consistently observed.

Side effects-

Common side effects of AmBisome include:

- Fever,
- Shaking,
- Chills,
- Flushing (warmth, redness, or tingly feeling),
- Loss of appetite,
- Dizziness,
- Nausea,
- Vomiting,
- Stomach pain,
- Diarrhea,
- Headache,
- Shortness of breath,
- Fast breathing 1 to 2 hours after the infusion is started,
- Sleep problems (insomnia), or
- Skin rash.

Tell your doctor if you have serious side effects of AmBisome including:

- Swelling or pain at injection site,
- Muscle or joint pain,
- Weakness,
- Changes in the amount of urine,
- Painful urination,
- Vision changes,
- Numbness or tingling of arms or legs,
- Hearing changes (e.g. ringing of ears)

- Dark urine,
- Fast/slow/irregular heartbeat,
- Yellowing eyes or skin,
- Blue lips,
- Mental/ mood changes,
- Easy bruising or bleeding.

Dosage for AmBisome-

AmBisome is administered by intravenous infusion, using a controlled infusion device, over a period of approximately 2 hours. Dose is based on patient's weight.

What Drugs or supplements interact with AmBisome-

AmBisome may interact with flucytosine, digoxin, pentamidine, tacrolimus, muscle relaxers, steroids, antifungal antibiotics, or cancer medicines.

AmBisime during Pregnancy and Breastfeeding-

During pregnancy, AmBisome should be used only when prescribed. It is unknown if this drug passes into breast milk. Consult your doctor before breastfeeding.

Signs of an allergic reaction-

Hives, swelling of face, lips, tongue or throat.

Therapeutic application of liposomal amphotericin B-

A liposomal amphotericin B formulation appropriate for use in human patients has been developed and successfully tested. A phase I clinical trial to assess its safety has been completed, and phase II study to assess its efficacy in patients with systemic fungal infection is currently underway, with promising results.

It can be used safely in immunocompromised patients, those with impaired renal function and those who are sick.

One of the primary goals of the treatment, as proposed by Gregoriadis and Ryman1, is to transport medications efficiently and particularly to the site of sickness, or to use liposome as a carrier to treat lysosomal enzyme disorder with low toxicity.

These lead to the development of the liposomes, which come in a variety of composition, sizes, and surfaces. However, progress in the design of selective drugs has been slow.

Targeted site to improve drug therapy by

i) drug delivery was conceptualized as early as the controlled drug delivery.

ii) targeted drug delivery and beginning of this century by microbiologist, paul.

iii) site avoidance drug delivery.

The treatment of fungal diseases and leishmaniasis using amphotericin B liposome formulation has proven to be quite effective.

However, the increased cost prevents it from being widely used in developing countries. As a result, eve device, a modified ethanol injection method for producing AmB liposomes.

Applications of liposomes-

1. liposomes as drug delivery vehicle-

- Liposomes enhance solubilization of drugs (Amphotericin B, Paclitaxel, Cyclosporine, Minoxidil).
- Provide protection to sensitive drug molecules (Cystone, arabinose, DNA, RNA, Ribozymes).
- Enhance intra cellular uptake (anti-cancer, anti-viral and anti-microbial agents).
- Alter pharmacokinetics and distribution of drugs.

2. liposome as vaccine carrier-

- Liposomes potentiate both cell medicated and humoral immunity.
- Liposomal vaccines based on immunopotentiating reconstituted influenza virosomes (IRIV)
  are prepared.
- For immunopotentiation, immunomodulating agents like muramyl dipeptide, lipopolysaccharide, and lipid can be incorporated into liposome.
  Advantages-
  1. Non-toxic, bio-compatible and biodegradable.
  2. Incorporate adjuvants to provide strong immune system.
  3. Convert loaded non-immunogenic substance to immunogenic (Proliposome).
  4. Minimize and eliminate toxicity of toxic antigens and allergic reaction.

3. liposome in tumor therapy-

- Liposomes as a drug carrier can be administered I.V route.
- If liposomes are modified more hydrophilic, with lipids their circulation time in blood stream increases.

- These are called stealth liposomes, used as carriers for hydrophilic anti-cancer drugs (Doxorubicin, Mitoxantrone)
- In this form they can extravasate the tumor vascular endothelium.

4. liposome in gene therapy-
- The non-viral vector system, are especially engineered liposome such as PH sensitive liposomes, cationic liposomes, fusogenic liposomes, genosomes, lipoplex, and lipopliyplex have been extensively investigated for their gene delivery potential.
- Cationic liposomes deliver the content through membrane fusion, thereby avoiding lysosomal and nucleolus degradation of DNA.
- pH sensitive liposomes use endosomal acidification for fusion with endosomal membrane.
- Genosomes are complex formulations of DNA with various cationic liposomes.
- Lipoplex aggregates with DNA to form large and heterogenous particles.
- Lipopolyplex is composed of liposome + polycation +DNA.

5. Liposome as artificial blood surrogates-
- Liposome encapsulated hemoglobin products can be used as artificial RBC.
- Sterically stabilized liposome bearing hemoglobin as better oxygen carriers.
- These have low toxicity, less platelet activation and aggregation and less haemostatic generation.

6. liposome as radio-pharmaceutical and radio- diagnostic carrier-
- Liposomal radio- diagnostic applications include imaging of liver, brain, lymphatics, tumor, bold pool, cardiovascular pathogenesis, visualization of inflammation, infection sites, bone marrow, eye vasculature.
- Liposome imaging agents are used for magnetic resonance, computed tomography and ultra sound imaging of tumors.

7. Enzyme immobilization-
- Liposomes can deliver enzymes to lysosomal system and other sites.

8. Liposomes in cosmetics and dermatology-
- Liposomes with essential oils provide an effective nourishing treatment that penetrates deeply in to the skin.

- Liposomes based on anti- aging formulations (e.g. creams, lotions, gels, and hydrogels) have been formulated and launched in the cosmetic market by L' oreal in 1986.
- Liposomal preparation reduces the roughness because of its interaction with corneocytes, the intracellular lipid resulting in skin softening and smoothing.
- Various liposome-based products for facial and body care, make-up, mascara and foundation, haircare, sunscreen products and perfumes are in market.

Conclusion-

The liposomes have great potency in drug delivery system. Drug of both (hydrophilic/lipophilic) easily embedded in the liposomes. The drug was delivered in the body in the controlled manner or wants to be site specific. In many hard diseases (cancers, tumors, HIV) the drug was easily and effective delivered by the means of liposomes.

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