LIPOSOMES: A NOVEL DRUG DELIVERY SYSTEM

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ABSTRACT

Liposomes are formations of vesicles by hydrating mixture of cholesterol and phospholipids. Different novel approaches used for delivering these drugs include liposomes, microspheres, nanotechnology, micro emulsions, antibody-loaded drug delivery, magnetic microcapsules, implantable pumps and noisome. Liposomes and Noisome are equiactive in drug delivery potential and both increase drug efficacy as compared with that of free drug. The application of vesicular (lipid vesicles and non-ionic surfactant vesicles) systems in cosmetics and for therapeutic purpose may offer several advantages. They improve the therapeutic performance of the drug molecules by delayed clearance from the circulation, protecting the drug from biological environment and restricting effects to target cells. Liposomes have been extensively investigated for drug delivery, drug targeting, controlled release and increased solubility. The major rate limiting step in the widespread use of this versatile drug delivery is the stability both physical and chemical. This review article focuses on the advantages, Disadvantages, Design, Classifications, Basic components, Formulations, methods of preparation, characterizations, factors, and applications of Liposomes.

KEYWORDS:- Liposome, Basic components, methods, Evaluation Parameters and applications.

INTRODUCTION

Liposome was discovered about 40 years ago by Bangham and co-workers. This was an accidental discovery, when he dispersed Phosphatidyl choline molecules in water; he found that it was forming a closed bilayer structure containing an aqueous phase entrapped by lipid bilayers. It was defined as microscopic spherical vesicles that form when phospholipids are hydrated or exposed to an aqueous environment. Liposomes are microscopic vesicles composed of a bilayer of phospholipids or any similar amphipathic lipids (Remington et al., 2000). They can encapsulate and effectively deliver both hydrophilic and lipophilic substances, and may be used as a non-toxic vehicle for insoluble drug, because lipids are amphipathic (both hydrophilic and hydrophobic) in aqueous media, their thermodynamic phase properties and self assembling characteristics evoke entropically driven sequestration of their hydrophobic regions into spherical bilayers are referred as lamellar. liposomes vary in charge and size depending on the method of preparation and the lipids used the multi lamellar vesicle [MLV] size range is 0.1-5.0 micrometres. the small unilamellar vesicle [SUV] size range is 0.02-0.05 micrometres, and the large unilamellar vesicle [LUV] size range varies from 0.06 micrometre and greater (Lasic DD et al., 1990).

Definition

Liposomes are defined as structure consisting of one or more concentric spheres of lipid bilayers separated by water or aqueous buffer compartments. (OR) Liposomes are simple microscopic vesicles in which an
aqueous volume is entirely enclosed by a membrane composed of lipid bilayers (Rudy L. Juliano et al., 2009).

This cartoon represents a multilamellar liposome. It contains many layers of phospholipids with water in between the layers. Hydrophobic compounds (green) insert between the phospholipids forming the bilayers of the liposome while water-soluble compounds (pink) are entrapped in the aqueous space between the bilayers.

Advantages
It provides controlled drug delivery.
- It should be biodegradable, biocompatible, and flexible.
- It should be non ionic.
- It can carry both water and lipid soluble drugs.
- The drugs can be stabilized from oxidation.
- It should be improve the protein stabilization.
- It provides controlled hydration.
- It provides sustained release.
- It provides targeted drug delivery or site specific drug delivery.
- Stabilization of entrapped drug from hostile environment.
- Alter pharmacokinetics and pharmacodynamics of drugs.
- It can be administered through various routes.
- It can incorporate micro and macro molecules.
- It acts as reservoir of drugs.
- The therapeutic index of drugs is increased.
- Site avoidance therapy.
- It can modulate the distribution of drug.
- It should be direct interaction of the drug with cell (Alving CR et al., 1998; Gregoriadis G et al., 1972)

Disadvantages: It is less stability
- It is low solubility
- It is short half life
- The phospholipids undergoes oxidation, hydrolysis
- Leakage and fusion
- It is high production cost
- Some time allergic reactions may occur to liposomal constituents

The problem to targeting to various tissue due to their large size (Alving CR et al., 1998; Gregoriadis G et al., 1972; Sharma A et al., 1997)

Designing of Liposomes
Liposomes, with or without entrapped compounds, can be designed to
- Release their contents when they reach a specific temperature.
- Release their contents at a specific pH value.
- Target certain tissues or cell types by changing the types of lipids in the liposome.
- Target tissues, cell types or specific proteins by attaching antibodies to the surface.
- Avoid certain tissues or cells by attaching complex sugars to the surface.
- Evenly distribute fat-soluble (oil-like) compounds such as certain vitamins, antioxidants, antibiotics, flavors, etc. which often can't be mixed in water-based products including most foods.
- Fuse with cells, which is important in delivering DNA to a cell.
- Serve as model cell membranes making it easier to study specific cellular processes and how certain molecules, such as drugs, interact with cells.
- Protect compounds from acidic and enzymatic degradation in the stomach and intestine by using certain molecules to coat the liposome.
- Protect compounds such as vitamins and antioxidants from premature oxidation for increased shelf-life.
- Enhance the intestinal absorption of compounds by coating with certain molecules.
- Carry drugs across the nasal mucosa (nasal drug delivery).
- Deliver drugs directly to lung tissue by inhalation of the liposomes.
- Carry fluorescent dyes or other types of molecules which allow the liposomes to be tracked in the system to which they are added (Alving CR et al., 1998).

CLASSIFICATIONS
Based on composition and mode of drug delivery
1. Conventional liposome or anionic liposome:
Composed of neutral or negatively charged phospholipids and cholesterol. Subject to coated pit endocytosis, contents ultimately delivered to Liposome’s if they do not fuse with the endosomes, useful for E.E.S targeting; rapid and saturable uptake by R.E.S; short circulation half life, dose dependent pharmacokinetics.

2. pH sensitive liposome:
Many formulations of pH-sensitive liposomes have been developed in the past few years. PH-sensitive liposomes are formed from lipids which adopt a lamellar phase at pH around 7.4 (physiological pH). When the pH is decreased to a critical value (around 5.5) the liposome will fuse with the endosomal membrane. Due to the fusion of the liposome with the endosomal membrane the content of the liposome (drugs) are released into the cytosol. The reason for the fusion at the lower pH is the transition from lamellar to hexagonal HII phase. Simultaneously membrane defect occurs which result in the release of the encapsulated substance into the
liposome-surrounding compartment such as endosome. The fusion of liposomes with cell membranes can be monitored by fluorescence resonance energy transfer (FRET).

3. pH-sensitive liposomes:
They are powerful delivery systems for hydrophilic compounds such as proteins, oligonucleotides and proteins. For making pH sensitive liposomes techniques such as reverse-phase evaporation and freeze-thawing followed by extrusion yield high encapsulation value (Alving CR et al., 1998; Ishii F et al., 1992)

4. Encapsulation of Genetic Materials into Liposomal Systems or Cationic Liposome:
Composed of cationic lipids, fuse with cell or endosome membranes; suitable for delivery of negatively charged macromolecules (DNA, RNA); ease of formation, structurally unstable; toxic at high dose, mainly restricted to local administration E.g. DODAC.

5. Long circulating or stealth liposome:
Composed of neutral high transition temperature lipid, cholesterol and 5-10% of PEG-DSPE. Hydrophilic surface coating, low ionization and thus low rate of uptake by RES long circulating half life (40 hrs); Dose independent Pharmacokinetics

6. Immuno liposome:
Conventional or stealth liposomes with attached Antibody or Recognition Sequence. Subject to receptor mediated endocytosis, cell specific binding (targeting); can release contents extra cellular near the target tissue and drugs diffuse through plasma membrane to produce their effects.

7. Magnetic Liposome:
Composed of P.C, cholesterol and small amount of a linear chain aldehyde and colloidal particles of magnetic Iron oxide. These are liposomes that indigenously contain binding sites for attaching other molecules like antibodies on their exterior surface. Can be made use by an external vibrating magnetic field on their deliberate, on site, rapture and immediate release of their components.

8. Temperature (or) heat sensitive liposome:
Composed of Dipalmitoyl P.C. These are vesicles showed maximum release at 41°, the phase transition temperature of Dipalmitoyl P.C. Liposomes release the entrapped content at the target cell surface upon a brief heating to the phase transition temperature of the liposome membrane.

9. Fluorescent Liposome:
Fluorescent liposome can be made from different fluorescent probes such as:
- Non fixable polar tracers
- Fluorescein derivatives
- Sulforhodamines
- Polysulfonated pyrenes
- Lanthanide Chelates

Fluorescence Lipids: Liposomes can be made from various types of fluorescence lipids. The following is a list of the fluorescence lipids.

Fatty Acid Labeled Lipids
Glycerol Based
- Phosphatidylcholine (NBD)
- Phoshatidic Acid (NBD)
- Phosphatidylethanolamine (NBD)
- Phosphatidylglycerol (NBD)
- Phosphatidylserine (NBD)

Sphingosine Based
- Sphingosine (NBD)
- Sphingosine-1-Phosphate (NBD)
- Ceramide (NBD)
- Sphingomyelin (NBD)
- Phytosphingosine (NBD)
- Galactosyl Cerebroside (NBD)

9. Headgroup Labeled Lipids
Glycerol Based
- Phosphatidylethanolamine (NBD)
- Phosphatidylethanolamine (Lissamine Rhodamine B)
- Dioleoyl Phosphatidylethanolamine (Dansyl, Pyrene, Fluorescein)
- Phosphatidylserine (NBD)

Other Labeled Lipids
25-NBD Cholesterol

10. Surface Modification of Liposomes:
Modification of the liposomal surface is a very important tool for controlling the biological properties of liposomes. Attachment of certain specific ligands such as peptides, proteins (e.g. antibodies), hormones, sugars, metals (magnetic liposome) makes liposomes targeted; coating liposomes with polymers such as PEG (poly ethylene glycol) or poly ethylene Glycolyted liposome give the liposomes the ability to circulate longer in body without being recognized by reticulum endothelial system cells; attachment of contrast agent converts liposome into a powerful diagnostic tool.

Based on Size and Number of Lamellae
a. Multi lamellar vesicles (M.L.V)
- Size : 0.1 - 0.3 micro meters
- Have more than one bilayer; moderate aqueous volume to lipid ratio 4: 1 mole lipid. Greater encapsulation of lipophilic drug, mechanically
stable upon long term storage, rapidly cleared by R.E.S, useful for targeting the cells of R.E.S, simplest to prepare by thin film hydration of lipids in presence of an organic solvent.

1. Oligo lamellar vesicles or Paucilamellar vesicles
   Intermediate between L.U.V & MLV
2. Multi vesicular liposome
   Separate compartments are present in a single M.L.V.
3. Stable Pluri lamellar vesicles
   Have unique physical and biological properties due to osmotic compression.

b. Large Unilamellar Vesicles (L.U.V)
   Size : 0.1 - 10 micro meters
   Have single bilayer, high aqueous volume to lipid ratio (7: 1 mole lipid), useful for hydrophilic drugs, high capture of macro molecules; rapidly cleared by R.E.S. Prepared by detergent dialysis, ether injection, reverse phase evaporation or active loading methods.

c. Small Unilamellar Vesicles (S.U.V)
   Size : 0.1 micro meters
   Single bilayer, homogeneous in size, thermodynamically unstable, susceptible to aggregation and fusion at low or no charge, limited capture of macro molecules, low aqueous volume to lipid ratio (0.2 : 1.5 : 1 mole lipid) prepared by reducing the size of M.L.V or L.U.V using probe sonicator or gas extruder or by active loading or solvent injection technique.

11. Small Molecules Encapsulated into Liposomes:
   E.g.
   1. Clodronate or Dichloromethylene phosphonic acid (DMDP)
   2. Antibiotic Encapsulated Liposomes
   3. Anti-Tumor Drugs Encapsulated Liposomes
   4. Antioxidant Liposomes
   5. NSAIDs encapsulated liposomes
   6. ATP Encapsulated Liposomes
   7. Hemoglobin encapsulated Liposomes (artificial blood)
   8. Vitamin Encapsulated Liposomes

BASIC COMPONENTS OF LIPOSOMES:
A. Phospholipids
   Glycerol containing phospholipids are most common used component of liposome formulation and represent greater than 50% of weight of lipid in biological membranes. These are derived from Phosphatidic acid. The back bone of the molecule is glycerol moiety.

   Examples of phospholipids are
   o Phosphatidyl choline (Lechitin) – PC
   o Phosphatidyl ethanolamine (cephalic) – PE
   o Phosphatidyl serine (PS)
   o Phosphatidyl inositol (PI)
   o Phosphatidyl Glycerol (PG)

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

B. Sphingolipids
   Backbone is sphingosine or a related base. These are important constituents of plant and animal cells. This contain 3 characteristic building blocks
   o A mol of F.A
   o A mol of sphingosine
   o A head group that can vary from simple alcohols such as choline to very complex carbohydrates.
   o Gangliosides – found on grey matter, used as a minor component for liposome production.

C. Sterols
   Cholesterol & its derivatives are often included in liposomes for
   o decreasing the fluidity or microviscosity of the bilayer
   o reducing the permeability of the membrane to water soluble molecules
   o Stabilizing the membrane in the presence of biological fluids such as plasma.( This effect used in formulation of i.v. liposomes)

   Liposomes without cholesterol are known to interact rapidly with plasma protein such as albumin, transferring, and macroglobulin. These proteins tend to extract bulk phospholipids from liposomes, thereby depleting the outer monolayer of the vesicles leading to physical instability. Cholesterol appears to substantially reduce this type of interaction. Cholesterol has been called the mortar of bilayers, because by virtue of its molecular shape and solubility properties, it fills in empty spaces among the Phospholipids molecules, anchoring them more strongly into the structure. The OH group at 3rd position provides small Polar head group and the hydrocarbon chain at C17 becomes non polar end by these molecules, the cholesterol intercalates in the bilayers (Gonser S et al., 1999).

D. Synthetic phospholipids
   E.g.: for saturated phospholipids are
   o Dipalmitoyl phosphatidyl choline (DPPC)
   o Distearoyl phosphatidyl choline (DSPC)
   o Dipalmitoyl phosphatidyl ethanolamine (DPPE)
   o Dipalmitoyl phosphatidyl serine (DPPS)
   o Dipalmitoyl phosphatidic acid (DPPA)
   o Dipalmitoyl phosphatidyl glycerol (DPPG)
E.g.: for unsaturated phospholipids
1. Dioleoyl phosphatidyl choline (DOPC)
2. Dioleoyl phosphatidyl glycerol (DOPG)

E. Polymeric materials
Synthetic phospholipids with diacytlenic group in the hydrocarbon chain polymerizes when exposed to U.V. leading to formation of polymerized liposomes having significantly higher permeability barriers to entrapped aqueous drugs. E.g.: for other Polymerisable lipids are – lipids containing conjugated diene, Methacrylate etc. Also several Polymerisable surfactants are also synthesized.

F. Polymer bearing lipids
Stability of repulsive interactions with macromolecules is governed mostly by repulsive electrostatic forces. This repulsion can be induced by coating liposome surfaces with charged polymers. Non ionic and water compatible polymers like polyethylene oxide, polyvinyl alcohol, and Poloxazolidines confers higher solubility. But adsorption of such copolymers containing hydrophilic segments with hydrophobic part leads to liposome leakage, so best results can be achieved by covalently attaching polymers to phospholipids

E.g.: Diacyl Phosphatidyl ethanolamine with PEG polymer linked via a carbon at or succinate bond.

The degree of polymerization varies from 15-120 units. Longer polymers give rise to aqueous solubility of polymer lipids and their first removal from membranes in non equilibrium conditions. While shorter polymers do not offer enough repulsive pressure because Vanderwaal's attraction is a long range force.

G. Cationic lipids
E.g.: DODAB/C – Dioctadecyl dimethyl ammonium bromide or chloride
DOTAP – Dioleoyl propyl trimethyl ammonium chloride
– this is an analogue of DOTAP and various others including various analogues of DOTMA and cationic derivatives of cholesterol

H. Other Substances
Variety of other lipids of surfactants are used to form liposomes
➢ Many single chain surfactants can form liposomes on mixing with cholesterol
➢ Non ionic lipids
➢ A variety of Polyglycerol and Polyethoxylated mono and dialkyl amphiphiles used mainly in cosmetic preparations
➢ Single and double chain lipids having fluoro carbon chains can form very stable liposomes
➢ Sterylamine and Dicetyl phosphate

➢ Incorporated into liposomes so as to impart either a negative or positive surface charge to these structures
➢ A number of compounds having a single long chain hydrocarbon and an ionic head group found to be capable of forming vesicles. These include quaternary ammonium salts of dialkyolphosphates.

FORMULATIONS OF LIPOSOME: (Ishii F et al., 1992)
Liposomes are made from pure lipids or a combination of lipids. The lipids commonly employed in liposome formulations are phospholipids. Liposomes have been prepared from a variety of synthetic and naturally occurring phospholipids, they generally contain cholesterol.

The incorporation of cholesterol into the lipid bilayer membrane generally:
➢ To enhance the stability of liposomes in serum,
➢ To reduces the permeability of the membranes to water soluble molecules and
➢ To increases the fluidity or micro viscosity of the bilayer.

The most commonly used phospholipids in liposome preparation are: eggphosphatidylcholine, synthetic dipalmitoyl-DL-α-phosphatidylcholine, brain and synthetic phosphatidylserine, sphingomyelin, phosphatidylinositol and ovoilecin. Usually, a zwitterionic or non-ionic lipid is used as the basic lipid for the preparation of liposomes.

The net surface charge of liposome can be modified by the incorporation of positively charged lipids such as stearylamine, or negatively charged lipids such as diacetylphosphate, phosphatidyl glycerol or phosphatidyl serine.

The presence of negatively or positively charged lipids:
➢ Used to a greater overall volume for aqueous entrapment and
➢ To reduces the aggregation after preparation of the liposomes.
➢ Detergent (surfactant) for the solubilisation of the lipids. Detergents used include the non-ionic surfactants [e.g., n-octyl-bete-D glucopyranose (octyl glucoside), anionic surfactants (e.g., dodecyl sulphate) and cationic surfactants (e.g., hexadecyltrimethyl ammonium bromide).

METHODS OF PREPARATION OF LIPOSOMES:
(Alving C.R et al., 1998; S.Banker., 2009; Ishii F et al., 1992)
Since the early 1970s many hundreds of drugs, including anti-tumor and antimicrobial agents, chelating agents, peptide hormones, enzymes, other proteins, vaccines
and genetic materials, have been incorporated into the aqueous or lipid phases of liposomes of various sizes, compositions and other characteristics by an ever-increasing number of techniques. Liposomes have evolved from mere experimental tools of research to industrially manufactured products for clinical and veterinary use. This success depends on advanced techniques to obtain efficient drug entrapment and increased stability of the products. The conventional method and the advanced techniques based on this method are discussed as follows:

Physicochemical dispersion methods
a) Non-hand shaking or freeze drying
b) Micro-emulsion
c) Ultrasonication
d) French Pressure cell
e) Membrane Extrusion
f) Freeze-thawing

Solvent injection or dispersion methods
a) Ethanol injection method
b) Ether injection method
c) Double emulsion vesicles method
d) Reverse phase evaporation method
e) Stable plun smeller vesicles method

Detergent removal methods
Detergent can be removed by
a) Dialysis method
b) Column chromatography method
c) Bio-beads method

Miscellaneous methods
a) Hydration of lipids in presence of solvent
b) High pressure extrusion
c) Slow swelling in Non electrolyte solution
d) Removal of Chemotropic ion

ULTRA-SONICATION METHOD:
This method is used in the preparation of SUVs and it involves the subsequent sonication of MLVs prepared by the conventional method, either with a bath type or a probe type sonicator under an inert atmosphere, usually nitrogen or argon. The principle of sonication involves the use of pulsed, high frequency sound waves (sonic energy) to agitate a suspension of the MLVs. Such disruption of the MLVs produces SUVs with diameter in the range of 15–50nm. The purpose of sonication, therefore, is to produce a homogenous dispersion of small vesicles with a potential for greater tissue penetration. The commonly used sonicators are of the bath and probe tip type. A probe tip sonicator delivers high sonic energy to the lipid suspension but has the disadvantage of overheating the lipid suspension to cause degradation. The probe tip also tends to release titanium particles into lipid suspension, which must be removed by centrifugation. For this reason, bath sonication are the more widely used.

By this technique, a test tube containing the suspension is placed in the bath sonicator and sonicating for 5–10 minutes above the transitional temperature of the lipid (i.e., the temperature at which the lipid melts). Comparatively, sonication with a probe results in faster breakdown of the MLVs to smaller structures than can be achieved by a bath sonication. The reduction in size of the liposomes, however, also decreases the amount of interior aqueous space, thereby limiting the amount of water-soluble drugs that can be entrapped. However, degradation of lipids, metal particle shedding from the probe tip (titanium particles) and generation of aerosols from solutions containing radioactive traces, carcinogenic chemicals or infectious agents that have been added to the preparation by probe technique could cause serious biohazards, which are less frequently encountered with bath sonication.

Bath sonication is a closed system allowing for temperature control to minimize thermo degradation of the lipid and entrapped substance. The position of the tube and water level in the bath is also regulated for maximum efficiency. The major drawbacks in the preparation of liposomes by sonication include oxidation of unsaturated bonds in the fatty acid chains of phospholipids and hydrolysis to lysophospholipids and free fatty acids. Another drawback is the denaturation or inactivation of some thermolabile substances (e.g., DNA, certain proteins, etc) to be entrapped.

HIGH-PRESSURE EXTRUSION METHOD:
This is another method for converting MLV to SUV suspensions. By this method, suspensions of MLVs prepared by the conventional method are repeatedly passed through filters polycarbonate membranes with very small pore diameter (0.8–1.0...m) under high pressure up to 250psi. By choosing filters with appropriate pore sizes, liposomes of desirable diameters can be produced. The mechanism of action of the high pressure extrusion method appears to be much like peeling an onion. As the MLVs are forced through the small pores, successive layers are peeled off until only one remains. Besides reducing the liposome size, the extrusion method produces liposomes of homogeneous size distributions. A variety of different lipids can be used to form stable liposomes by this method Extrusion at low pressures <1Mpa is possible when lipid concentration is low, 30 but the most commonly used pressures are about 5Mpa. The method is amenable to small scale production only. In order to overcome
this problem, Schneider and co-workers designed and tested a new continuous extrusion apparatus, which operates at high pressures of up to 10.5Mpa with the advantage of a high output.

SOLUBULISATION AND DETERGENT REMOVAL METHOD:

This method is used in the preparation of LUVs and it involves the use of detergent (surfactant) for the solubilisation of the lipids. Detergents include the non-ionic surfactants [e.g., n-octyl-bet-D glucopyranose (octyl gluaside), anionic surfactants (e.g., dodecyl sulphate) and cationic surfactants (e.g., hexadecyltrimethyl ammonium bromide)]. The procedure involves the solubilisation of the lipids in an aqueous solution of the detergent and the protein(s) to be encapsulated. The detergent should have a high critical micelle concentration (CMC), so that it is easily removed. The detergent is subsequently removed by dialysis or column chromatography. During detergent removal, LUVs of diameter 0.08–0.2m are produced. This detergent removal method has been found suitable for the encapsulation of proteins of biomedical importance.

REVERSE PHASE EVAPORATION TECHNIQUE:

Szoka and Papahadjopoulos pioneered the preparation of lipid vesicles by reversed phase evaporation technique. It consists of a rapid injection of aqueous solution of the drug into an organic solvent, which contains the lipid dissolved with simultaneous bath sonication of the mixture leading to the formation of water droplets in the organic solvent (i.e., a “water-in-oil” emulsion). The resulting emulsion is dried down to a semi solid gel in a rotary evaporator. The next step is to subject the gel to vigorous mechanical agitation to induce a phase reversal from water-in-oil to oil-in-water dispersion (i.e., an aqueous suspension of the vesicles). During the agitation, some of the water droplets collapse to form the external phase while the remaining portion forms the entrapped aqueous volume. Large unilamellar vesicles (diameter 0.1–1 m) are formed in the process. This method has been used to encapsulate both small and macromolecules such as RNA and various enzymes without loss of activity. The expected limitation of this method is the exposure of the material to be encapsulated to organic solvents and mechanical agitation, which can lead to the denaturation of some proteins or breakage of DNA strands.

FACTORS AFFECTING CLEARANCE AND DISTRIBUTION: (Aulton, 2002; Banker, 2009)

A. Particle size and charge

Large liposomes are cleared more rapidly than small ones and negatively charged vesicles are cleared more for rapidly than neutral or positive ones.

B. Chemical composition

If cholesterol is not present, liposomes can bind to plasma proteins. If membrane stabilizers against serum lipoproteins are added then decreases clearance.

C. Dose or load of liposome administered

The particle clearance rate of RES is inversely proportional to the load of liposomes i.e., rate of clearance for larger dose is smaller than for smaller dose.

D. Structure of capillary endothelium

E. Phagocytotic capabilities of RES

F. Fluidity of liposomal membrane

G. Stability

There are many ways to increase the chemical and physical stability of liposomes.

1. One way is to avoid using unsaturated phospholipids. Unsaturated phospholipids are subject to peroxidation. However this might not always be doable and the use of unsaturated lipids might be necessary for some liposomes formulations. If unsaturated phospholipids are used then it is important to do the following:

   * Maintain an oxygen free environment during the manufacturing process and storage
   * Add tocopherols or other membrane active antioxidants
   * Limit the light exposure during the manufacturing process and storage

2. Store the liposomes in 4°C.

3. Never freeze the liposomes.

4. Buffer and maintain the pH of liposomes at 6-6.5 during the manufacturing and storage to avoid both acid- and base- catalyzed hydrolysis.

5. Most buffers enhance hydrolysis; ensure minimal concentration of buffer sufficient to maintain pH.

6. Add ethylene diamine tetra acetic acid (EDTA) to ensure the absence of multivalent cations. The presence of multivalent cations may cause aggregation and bilayer destabilization.

7. Control the temperature or select lipids so that lipid phase transitions do not occur during storage.

8. Monitor the aggregation state of the liposomes and adjust the aqueous phase or lipid composition accordingly.

9. Avoid using lipid compositions which spontaneously fuse (i.e. DSPC, SUV) or increase fusion activity upon accumulation of hydrolysis products.

10. Incorporation of cholesterol into liposomes can reduce destabilizing effects of hydrolysis products.
EVALUATION PARAMETERS OF LIPOSOMES:
(Aulton, 2002; Banker, 2009; Ishii F et al., 1992)
APPLICATIONS OF LIPOSOMES: (Lasic DD et al., 1998; Gregoriadis G et al., 1992; Su.D et al., 2009)

1. The liposome is applicable in various fields;
   a. Cell -liposome interaction
   b. Localized drug effect
   c. Enhanced drug uptake
   d. Molecules with wide range of solubility and molecular weight can be accommodated
   e. Flexibility in structural characteristics.

2. Liposomes are used to Target drugs to the tumors.
   a. the liposomal Ara -C inhibit DNA synthesis in the lungs
   b. For targeted drug delivery for blood born Neoplasms
   c. By active targeting using monoclonal antibodies, by magnetosomes or by temperature sensitive liposomes
   d. By passive targeting to liver, spleen, R.E.S cancers.

3. Liposomes are used to Reduction of Toxicity
   This is usually due to targeted or site specific delivery.
   E.g. Hydrophobic drugs including alkylating agents, antimitoticagents, anthracyclines

4. Liposome’s are used to treatment of localized drug effect

5. Liposomes are used to treatment of Gene therapy
   E.g. Liposomes can be used to deliver DNA into the cell

6. Liposomes can be used as carriers for vaccines
   E.g. Diphtheria toxoid vaccine

7. Liposomes can be used as carrier of drug in oral treatment
   a. Arthritis
      Treated with steroids using MLVs prepared by DPPC and P.A.
      E.g. Drugs are Ibuprofen, cortisol palmitate
   b. Diabetes
      Alternation in blood glucose level in diabetic animals was obtained by oral administration of liposome encapsulated insulin (PC: CH liposomes).

8. Liposomes used for topical application
   E.g. Triamcinolone ointments, Hydrocortisone ointment, Diclofenac gel etc.

9. Liposomes used for pulmonary delivery system
   E.g. Cytosine arabinoside, Pentamidine, Sodium cromoglycate, Metepreterenol.

10. Liposomes used for ophthalmic delivery of drugs
    In order to maintain optimal drug concentration at the site of action liposomes are used as carriers or vehicles.
    E.g. Treatment of Keratitis by Idoxuridine Also increases (2 times) the Trans corneal flux of penicillin G, indoxol and carbachol.

CURRENT LIPOSOMAL DRUG PREPARATIONS
(Alving CR et al., 1998)
**Figure 3:** Liposomes contain hydrophilic and hydrophobic layer

**Figure 4:** Genetic materials encapsulated into the liposome

**Figure 5:** PEGylated liposome

**Figure 6:** Immunoliposome

**Figure 7:** A liposome made from a labelled lipid

**Figure 8:** Proteo liposome

**Figure 9:** Multilamellar liposome

**Figure 10:** Unilamellar liposome
Figure 11: Drug encapsulated liposome

Figure 12: Formulation of Liposome's

Figure 13: General method of preparation

Figure 14: Liposome's by Ultra Sonication Method

Figure 15: Liposome's by Reverse Phase Evaporation Method
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### II. CHEMICAL STABILITY

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<td>5</td>
<td>Anti oxidant degradation</td>
<td>HPLC/TLC/FA concentration</td>
</tr>
</tbody>
</table>

### III. PHYSICAL STABILITY

<table>
<thead>
<tr>
<th>S.No</th>
<th>Assay/Characterisation</th>
<th>Methodology</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vesicle size distribution DLS</td>
<td>Vesicle size distribution DLS</td>
</tr>
<tr>
<td>2</td>
<td>Sub micron range</td>
<td>Coulter counter, light microscopy</td>
</tr>
<tr>
<td>3</td>
<td>Micron range</td>
<td>Laser diffraction, GEC</td>
</tr>
</tbody>
</table>

### IV. ELECTRICAL SURFACE POTENTIAL OR ZETA POTENTIAL

<table>
<thead>
<tr>
<th>S.No</th>
<th>Assay/Characterisation</th>
<th>Methodology</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Number of bilayers</td>
<td>SAXS, NMR</td>
</tr>
<tr>
<td>2</td>
<td>Percentage of free drug</td>
<td>GEC, IEC</td>
</tr>
<tr>
<td>3</td>
<td>Dilution dependent drug release</td>
<td>Retention loss of dilution</td>
</tr>
<tr>
<td>4</td>
<td>Relevant body fluid induced leakage</td>
<td>GEC/IEC, protamine precipitation</td>
</tr>
</tbody>
</table>

### V. BIOLOGICAL CHARACTERISATION

<table>
<thead>
<tr>
<th>S.No</th>
<th>Assay/Characterisation</th>
<th>Methodology</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sterility</td>
<td>Aerobic or anaerobic cultures</td>
</tr>
<tr>
<td>2</td>
<td>Pyrogenicity</td>
<td>Rabbit or LAL test</td>
</tr>
<tr>
<td>3</td>
<td>Animal toxicity</td>
<td>Monitor survival, histology, pathology</td>
</tr>
</tbody>
</table>

### S.No | Type of Agents | Examples |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Anticancer Drugs</td>
<td>Duanorubicin, Doxorubicin*, Epirubicin, Methotrexate, Cisplatin*, Cytarabin</td>
</tr>
<tr>
<td>2.</td>
<td>Antibacterial Drugs</td>
<td>Triclosan, Clindamycin hydrochloride, Ampicillin, Peperacillin, Rifamicin</td>
</tr>
<tr>
<td>3.</td>
<td>Antiviral Drugs</td>
<td>AZT</td>
</tr>
<tr>
<td>4.</td>
<td>DNA Material</td>
<td>cDNA - CFTR*</td>
</tr>
<tr>
<td>5.</td>
<td>Enzymes</td>
<td>Hexosaminidase A , Glucocerebrosidase, Peroxidase</td>
</tr>
<tr>
<td>6.</td>
<td>Radionuclie</td>
<td>In-111*, Tc-99m</td>
</tr>
<tr>
<td>7.</td>
<td>Fungicides</td>
<td>Amphotericin B*</td>
</tr>
<tr>
<td>8.</td>
<td>Vaccines</td>
<td>Malaria merozoite, Malaria sporozoite, Hepatitis B antigen, Rabies virus glycoprotein</td>
</tr>
</tbody>
</table>

### CONCLUSION

From the above article it is concluded that the considering the advantages of this Novel drug delivery system. A novel technology has been developed by which water-soluble substances can be solubilized in the absence of water into oils. The formation of anhydrous reverse micelles might play an important role in the solubilization and the resulting oil solutions are physically and chemically stable. Two types of formulations are developed. On exposure to water, a substantial amount of drug can be released from type 1 formulations in the liposome-entrapped form; therefore, type 1 formulations can be used as lipid depot drug delivery systems. In contrast, type 2 formulations are self-emulsifying drug delivery systems and might find application in oral delivery of peptides/proteins. Liposomes also its modifications or upgraded versions like Enzymosomes, Hemosomes, Virosomes, Erythrosomes, Virosomes, etc.

### ACKNOWLEDGMENT

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REFERENCES


