https://ijrps.com

ISSN: 0975-7538
Review Article

Liposomes as drug delivery system: A brief review

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ABSTRACT

Liposomes are increasingly popular as drug carriers due to their versatility. These are the most widely investigated carriers among the controlled drug delivery systems used in cancer therapy. Drugs and other pharmaceuticals encapsulated in liposomes show increased efficacy due to their effective protection from external environments as well as sustained and site-specific delivery than conventional formulations. The phamaco-dynamics and pharmacokinetics properties are altered for the liposomal delivery system, which on the whole lead to an increased therapeutic index with decreased toxicity. Various methods are adopted for their production from lab scale to industrial scale. Liposomes are also classified as different types based on their composition, methods of preparation, size and application. The liposomal formulations are assessed for various in vitro characteristics before their in vivo study. In this review, we will primarily discuss about various types of liposomes, composition, properties, different methods of their preparation, important evaluation parameters with an elaborated discussion on their applications in drug delivery research. We believe this concise review will be helpful to gather the basic understanding and some up to date idea on liposomal delivery system.

Keywords: Liposomes; Classification; Drug delivery systems; Application in drug delivery

INTRODUCTION

Liposomes are concentric bilayerd vesicle consisting of lipid bilayers mainly composed of natural or synthetic phospholipids. Liposomes are composed of biocompatible and biodegradable components, which are able to encapsulate both hydrophilic and lipophilic molecules in one platform. The name liposome is derived from two Greek words: 'Lipos' meaning fat and 'Soma' meaning body. A liposome can be formed at a variety of sizes as uni-lamellar or multi-lamellar construction. Drugs can be encapsulated in liposomes, either in the phospholipids bilayer, in the entrapped aqueous volume or at the bilayer interface (Mansoori et al., 2012; Emanuel et al., 1996). Liposomes are biocompatible in nature. This specific property makes them one of the attractive choices for formulation scientists. They can entrap both hydrophilic drug in their internal water compartment and hydrophobic drug into their membrane. Drugs encapsulated in liposomes are protected from the inactivating effect of external environments. Further, they provide sustained release profile and site-specific delivery of pharmaceuticals into cells and also inside individual cellular compartments. By using different methods of preparation and adding new ingredients to the lipid mixture before liposome preparation, it is possible to modify size, charge and surface properties of liposomes. Current research has now more focused on the development of long circulating stealth liposomes and multi functionality liposomes having enhanced in-vivo properties. Further, development of targeted liposomes intended to hit specific antigens or markers for more precise delivery of drugs are now under clinical investigation. In this regard, a comprehensive discussion on the role of liposomes as drug delivery system seems needful. In the present review, we will discuss on the various types of liposomes, their production procedures and characterization parameters along with the applications in pharmaceutical field. Also list of clinically approved liposomal products are given to have an updated idea on the current status.

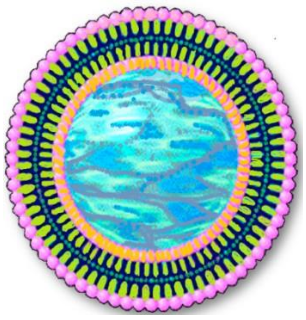

Figure 1: Spherical vesicles with a phospholipid bilayer

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Received on: 01-11-2014
Revised on: 25-12-2014
Accepted on: 27-12-2014

Structural components

a) Phospholipids: Phospholipids are the most commonly used component of liposome formulation. Phospholipids are derived from phosphatidic acid and the responsible part of the molecule is glycerol moiety (Mansori et al., 2012). At C3 position OH group is esterified to phosphoric acid and OH at C1 & C2 are esterified with long chain. One of the remaining OH group of phosphoric acid may be further esterified to organic alcohols including glycerol, choline, ethanolamine, serine and inositol. Thus the parent compound of the series is the phosphoric ester of glycerol (Shailesh S et al., 2009; Kant S et al., 2012).

Examples of phospholipids are –

- Phosphatidyl choline (Lecithin) – PC
- Phosphatidyl inositol (PI)
- Phosphatidyl ethanolamine (cephalin) – PE
- Phosphatidyl serine (PS)
- Phosphatidyl Glycerol (PG) for stable liposomes, saturated fatly acids are used. Unsaturated fatty acids are not used generally.

b) Sphingolipids: Sphingosine is one of the most important parts of sphingolipids. Sphingolipids are obtained from plant and animal cells. Sphingomyelin and glycosphingo lipids are most common sphingolipids. Gangliosides are used as a minor component for liposome production which contain saccharides with one or more salicylic acid residues in their polar head group & thus have one or more negative charge at neutral pH. These are included in liposomes to provide a layer of surface charged group (Mansoori et al., 2012).

c) Sterols

Cholesterol & its derivatives are used in liposomes for decreasing the fluidity or microviscocity of the bilayer. They reduce the permeability of the membrane to water soluble molecules and also stabilize the membrane in the presence of biological fluids such as plasma.

Synthetic phospholipids

Saturated phospholipids include the following (Kant S. et al., 2012; Kataria S. et al., 2011)

- Dipalmitoyl phosphatidyl choline (DPPC)
- Distearoyl phosphatidyl choline (DSPC)
- Dipalmitoyl phosphatidyl serine (DPPS)
- Dipalmitoyl phosphatidyl ethanolamine (DPPE)
- Dipalmitoyl phosphatidyl glycerol (DPPG)
- Dipalmitoyl phosphatidic acid (DPPA)
- Dioleoyl phosphatidyl choline (DOPC)
- Dioleoyl phosphatidyl glycerol (DOPG)

Mechanism of formation of liposomes

Phospholipids are the backbones for the formulation of liposomes. They are primarily amphipathic molecules having a hydrophobic tail and a hydrophilic or polar head (Lasic D. et al., 1995). The polar end of molecule is mainly the phosphoric acid bound to a water soluble molecule. In aqueous medium, the molecule in self assembled structure is oriented in such a way that the polar portion of molecule remains in contact with the polar environment and at the same time shield thenon-polar part. Among the amphiphiles used in drug delivery, such as soap, detergent, polar lipid, the latter (polar lipid) are often employed to form concentric bilayer structure. The most common natural polar phospholipids are phosphatidylcholine. These are amphipathic molecule in which a glycerol bridge links to a pair of hydrophobic acyl chains with a hydrophilic polar head group, phosphocholine. Thus the amphiphilic nature of the phospholipid and their analogues render them the ability to form closed concentric bilayers in the presence of water. However, in aqueous medium these molecules are able to form various phases, some of them are stable and others remain in the metastable state. At high concentrations of these polar lipids, liquid-crystalline phases are formed that upon dilution with an excess of water can be dispersed into relatively stable colloidal particles. The macroscopic structure most often formed includes lamellar, hexagonal or cubic phases referred to as liposomes, hexasomes or cubosomes respectively.

Liposomes as Drug Carriers

Some special properties of liposomes include

Solubilisation- NLs may solubilise lipophilic drugs that would otherwise be difficult to administer intravenously (Bangham AD. Et al., 1965).

Protection- Liposome-encapsulated drugs are inaccessible to metabolising enzymes;

Conversely, body components are not directly exposed to the full dose of the drug.

Amplification- Liposomes can be used as adjuvant in vaccine formulations (Jayakrishnan et al., 1997).

Internalisation- Liposomes are endocytosed or phagocytosed by cells, opening up opportunities to use 'liposome-dependent drugs'.

Duration of action- Liposomes can prolong drug action by slowly releasing the drug in the body.

Advantage of liposomes

- Provide sustained release.
- Nonionic.
- Targeted drug delivery or site specific drug delivery.

- Stabilization of entrapped drug from hostile environment.
- Can carry both water and lipid soluble drugs.
- Biodegradable drugs can be stabilized from oxidation.
- Improve protein stabilization.
- Controlled hydration.
- Can be administered through various routes
- Alter pharmacokinetics and pharmacodynamics of drugs (Mansoori et al., 2012).

Disadvantages

- Short half-life.
- Less stability.
- Leakage and fusion
- Quick uptake by cells of R.E.S.
- Phospholipids undergoes oxidation, hydrolysis.

CLASSIFICATION OF LIPOSOMES

Liposomes can be classified on the basis of:

1. Method of preparation (Table 1).
2. Structure (Table 2).
3. Composition and application (Table 3).

Methods of liposome preparation

All the methods of preparing the liposomes basically involve four basic stages:

1. Drying down lipids from organic solvent.
2. Dispersing the lipid in aqueous media.
3. Purifying the resultant liposome.
4. Analyzing the final product.

Various methods of liposome preparation (Mayer LD. Et al., 1985)

1. Passive Loading Technique

   a. Mechanical Dispersion

      i. Lipid film hydration (Hand shaking / Non-hand shaking)
      ii. Freeze drying .
      iii. Micro emulsification .
      iv. Sonication
      v. French pressure cell
      vi. Membrane extrusion
      vii. Dried reconstituted vesicles
      viii. Freeze-thawed liposomes

   b. Solvent dispersion

      i. Ethanol Injection
      ii. Ether injection
      iii. Double emulsion
      iv. Reverse phase evaporation

   c. Detergent Removal

      i. Detergent removal from mixed micelles vesicles by- Dialysis Dilution.

2. Active Loading Technique

   a. Proliposome lyophilization

Mechanical dispersion method

Sonication: Sonication is the most extensively used method for the preparation of SUV. MLVs are sonicated with a bath type sonicator or a probe sonicator under a passive atmosphere. The main disadvantages of this method are very low encapsulation efficacy, possible degradation of phospholipids and compounds to be encapsulated.

There are two sonication techniques

a) Probe Sonication: The tip of the sonicator is directly engrossed into the liposome dispersion. In case of probo sonication the energy input into lipid dispersion is very high. The coupling of energy at the tip results in local hotness. So, the vessel must be engrossed into a ice or water bath. Sonication up to 1 h, more than 5% of the lipids can be de-esterified. And also, with this method, titanium may slough off and pollute the solution (Akbarzadeh et al., 2013).

b) Bath Sonication: Now-a-days bath sonicators have largely replaced the probe sonicators. In this method controlling the temperature of the lipid dispersion is usually easier. The material being sonicated can be protected in a sterile vessel or under an inert atmosphere (Kataria S. et al., 2011)

French pressure cell: Extrusion french pressure cell involves the extrusion of MLV through a small orifice and it involves gentle handling of unstable materials. The advantage of the method is that the resulting liposomes are rather larger than sonicated SUVs. But the high temperature is difficult to attain and the working volumes are comparatively smaller (Chauhan T. et al., 2012).

Freeze-thawed liposomes: SUVs are rapidly frozen and thawed slowly. The creation of unilamellar vesicles is as a result of the fusion of SUV throughout the processes of freezing and thawing. Such type of synthesis is strongly inhibited by increasing the phospholipids concentration and also by increasing the ionic strength of the medium (Riaz M. et al., 1996).

Solvent dispersion method

Ethanol injection: A lipid solution of ethanol is rapidly injected to a huge excess of buffer which leads to formation of large size MLVs. The disadvantages of the method are that the population is heterogeneous (30 to 110 nm). The liposomes are very dilute, the removal of all ethanol is difficult and the probability of the various biologically active macromolecules to inactivate in the presence of even low amounts of ethanol is high (Deamer D. et al., 1976; Schieren H. et al., 1978).

Ether injection: A solution of lipids dissolved in ether-methanol mixture or diethyl ether. And the mixture is gradually injected to an aqueous solution of the material to be encapsulated at 55°C to 65°C or under reduced pressure (Mansoori et al., 2012; Batzri S. et al., 1973) Removal of ether from the mixture under vacuum leads to the creation of liposomes. The main drawback of the technique is that the exposure of compounds to be encapsulated to organic solvents at high temperature and population is heterogeneous (70 to 200 nm).

Reverse phase evaporation method: This method provided a progress in liposome technology. Reverse-phase evaporation is based on the creation of inverted micelles these are shaped upon sonication of a mixture of a buffered aqueous phase, which contains the water-soluble molecules to be encapsulated into the liposomes and an organic phase in which the amphiphilic molecules are solubilized. Elimination of the organic solvent leads to the conversion of these inverted micelles into viscous state and gel form. At a critical point in this process, the gel state collapses, and some of the inverted micelles were disturbed and excess of phospholipids in the environment donates to the formation of a complete bilayer around the residual micelles, which results in the creation of liposomes. The main disadvantage of the method is the contact of the materials to be encapsulated to organic solvents and brief periods of sonication which may possibly result in the breakage of DNA strands or the denaturation of some proteins (Akbarzadeh et al., 2013).

Detergent removal method (removal of non-encapsulated material):

Dialysis: The detergents at their critical micelle concentrations (CMC) have been used to solubilize lipid and when the detergent is detached, the micelles become increasingly better-off in phospholipid and lastly combine to form LUVs. Dialysis was used to remove the detergents. A commercial device called Lipo-Prep (Diachema AG, Switzerland) is obtainable for the elimination of detergents.

Detergent removal by adsorbers: Detergent absorption is attained by shaking mixed micelle solution with beaded organic polystyrene adsorbers. The advantage of using detergent adsorbers is that they can eliminate detergents with a very low CMC, which are not entirely depleted.

Organic-beads adsorber – XAD-2 beads (SERVA Electrophoresis GmbH, Heidelberg, Germany).

Bio-beads adsorber – SM2 (Bio-Rad Laboratories, Inc., Hercules, USA).

Gel-permeation chromatography: In this method, the detergent is depleted by size special chromatography. The liposomes do not penetrate into the pores of the beads packed in a column rather; they percolate through the inter-bead spaces at slow flow rates. The separation of liposomes from detergent monomers remains very good. The swollen polysaccharide beads adsorb substantial amounts of amphiphilic lipids; so, pretreatment is necessary. The pre-treatment is done by pre-saturation of the gel filtration column by lipids using empty liposome suspensions.

Dilution: Upon dilution of aqueous mixed micellar solution of detergent and phospholipids with buffer, the micellar size and the polydispersity increase fundamentally, and as the system is diluted beyond the mixed micellar phase boundary, a spontaneous transition from poly-dispersed micelles to vesicles occurs. Sephadex G-50, Sephadex G-l 00 (Sigma-Aldrich, MO, USA) and Sepharose 2B-6B can be used for gel filtration.

Freeze-protectant for liposomes (lyophilization)

Natural products are usually degraded because of oxidation and other chemical reactions. Freeze-drying has been a standard process employed to the production of many pharmaceutical products. There are many pharmaceutical products manufactured that requires freeze-drying from organic co-solvent systems. Freeze-drying involves the removal of water from products in the frozen state at tremendously low pressures. The process is normally used to dry products that are thermo-labile and would be destroyed by heat-drying. The technique has so much potential as a method to solve long-term stability difficulties.

Drug loading in liposomes

Drug loading can be attained either passively (drug is encapsulated during liposome formation) or actively (after liposome formation). Hydrophobic drugs can be directly combined into liposomes during vesicle formation, and the amount of uptake and retention is governed by drug-lipid interactions. Trapping effectiveness is dependent on the solubility of the drug in the liposome membrane. Passive encapsulation of hydrophilic drugs depends on the ability of liposomes to trap aqueous buffer containing a dissolved drug during vesicle formation.

Pharmacokinetics of liposomes

Liposomal drugs can be administered through various routes, but mainly intravenous and topical administration is preferred. After administration, a liposome can interact with the cell by any of the following methods (Anwekar H. et al., 2011).

1. Endocytosis by phagocytotic cells of the reticulo endothelial system (RES).

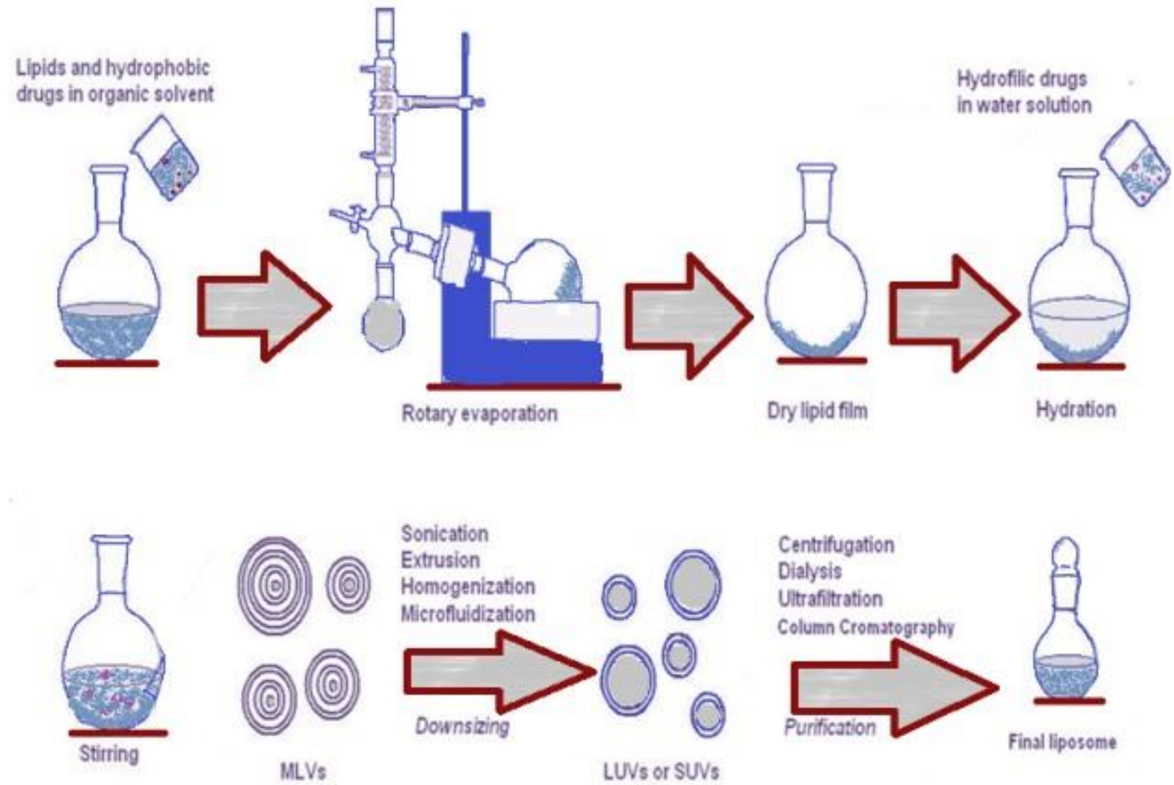

Figure 1: Representation of liposome production by lipid hydration followed by vortex

Table 1: Different preparation methods and the vesicles formed by these methods

Method of Preparation	Vesicle Type
Single or oligo lamellar vesicle made by reverse phase evaporation method	REVS
Multi lamellar vesicle made by reverse phase evaporation method	MLV-REV
Vesicle prepared by extrusion technique	VET
Dehydration- Rehydration method	DRV
Stable pluri lamellar vesicle	SPLV
Frozen and thawed multi lamellar vesicle	FATMLV

Table 2: Vesicle Types with their Size and Number of Lipid Layers

Vesicle type	Abbreviation	No.of lipid bilayer	Diameter size
Small Unilamellar	SUV	One	20-100 nm
Unilamellar	UV	One	All size ranges
Medium Unilamellar	MUV	One	More than 100 nm
Large Unilamellar	LUV	One	More than 100 nm
Giant Unilamellar	GUV	One	More than 1 micrometer
Oligolamellar	OLV	5	0.1 – 1 micrometer
Multilamellar	MLV	5-25	More than 0.5 micrometer
Multi vesicular	MV	Multi compartmental structure	More than 1 micrometer

2. Adsorption to the cell surface.
3. Fusion with the plasma cell membrane.
4. Transfer of liposomal lipids to cellular or sub cellular membrane.

Pharmocodynamics of liposome encapsulated drugs

To get the action of drugs to a particular site in the body, the general approach is to deposit drug bearing liposome directly into the site where therapy is desired. The liposomes slowly release drug into the target site and otherwise the drug loaded liposomes might interact directly with cells in the target site, without producing release. The goal of this approach is to maximize the amount of effective drug at the target site and decreasing systemic toxicity (Tatsuhiro I. et al., 2002).

Stability of Liposomes

Stability of liposomes depends on number of chemical and physical destabilization processes. Therefore liposomes stability is an important part while studying liposomes. These aspects of liposomes stability have two aspects physical and chemical stability (Kulkarni PR. et al., 2010).

Physical stability

Physical processes that affect shelf life, loss of liposome associated drug, changes in size, aggregation and fusion are the critical factors. Aggregation is the formation of larger units of liposomal material. Aggregation is initially induced by applying mild shears forces, or by changing the temperature or by binding metal ions. Fusion indicates that new colloidal structure was formed and it is an irreversible process, the original liposomes can never be retrieved. Drug molecules may be leaked from liposomes and it depends on the bilayer composition and the physiochemical nature of the drug. Liquid state bilayers are more prone to drug loss and are less stable during storage.

Chemical stability

a) Hydrolysis of the ester bonds: Phosphatidylcholine have four ester bonds. The glycerophosphate and phosphocholine ester bonds are more stable. The two acyl ester bonds are most liable to hydrolysis. The 1-acyl-lysophosphatidylcholine (LPC) and 2- acyl LPC are both formed at similar rates.

b) Lipid peroxidatin of phospholipids: The polyunsaturated acyl chains of phospholipids are sensitive to oxidation. Hydroperoxides, alkanes, cyclic peroxides and malonilaldehyde are the major degradation products. Absence of heavy metals, Low oxygen pressure, complexing agents, addition of anti-oxidants and quenchers (beta-carotene) of the photo-oxidation reactions improve resistance against lipid peroxidation.

Characterization of liposomes

The most important parameters of liposome characterization include visual appearance, size distribution, turbidity, lamellarity, concentration, composition, presence of degradation products, and stability (Kulkarni PR. et al., 2010). liposomes are characterized for physical attributes and chemical compositions and biological system (Table 4).

1. Visual Appearance

Visually, liposome suspension appears semi-transparent milky white, depending on the composition and particle size. If the turbidity has a bluish shade this means that particles in the sample are homogeneous. A flat, gray color indicates that presence of nonliposomal dispersion and is most likely a disperse inverse hexagonal phase or dispersed micro crystallites.

2. Determination of Liposomal Size Distribution

Size distribution is normally measured by dynamic light scattering (DLS) and it is reliable for liposomes with relatively homogeneous size distribution. A simple but powerful method is gel exclusion chromatography. By this method hydrodynamic radius can be detected. Sephacryl-S100 can separate liposome in size range of 30-300nm and Sepharose -4B and -2B columns can separate SUV from micelles.

3. Determination of Lamillarity

The lamellarity of liposomes is measured by spectroscopic techniques or by electron microscopy. Encapsulation efficiency is measured by encapsulating a hydrophilic marker. The nuclear magnetic resonance spectrum of liposome is recorded with and without the addition of a paramagnetic agent that shifts or bleaches the signal of the observed nuclei on the outer surface of liposome.

4. Drug entrapment efficiency

To find out the drug entrapment (drug content) the liposomal suspension was ultra-centrifuged at 5000 rpm for 15 min at 4°C to separate the free drug. The free drug was formed at wall of the centrifuge tube and liposomes were in suspended stage. The clear supernatant liquid was collected. The unentrapped drug were soaked for about 10 min by using methanol and sonicated for about 10min, which causes breakdown of the vesicles to release the drug and the drug content was estimated by UV spectrophotometer (Rewar S . et al., 2014).

$$\%Entrapment\ efficiency = \frac{Entrapped\ drug\ (mg)}{Total\ Drug\ Added\ (mg)} \times 100$$

5. Surface Charge

It is important to study the charge on the vesicle surface. Zeta potential measurement and free flow electrophoresis are used to assess the charge of liposomes. From the mobility of the liposomal dispersion in a suitable buffer, the surface charge on the vesicles can be measured (Rewar S. et al., 2014).

6. Differential scanning calorimetry (DSC)

DSC is the most common thermal analysis technique to assess any possible type of chemical interaction between the materials and now is a useful tool in many analytical, process control, quality assurance, and R&D laboratories.

DSC measurement of liposome was performed with an instrument for measurement of thermotropic transition of the experimental materials (Mettler TA4000, Toledo, OH). Empty aluminium pans were used as reference and samples were carefully placed in another aluminium pan. The measurement was done in an inert atmosphere within the temperature range of 30°C to 200°C, at 5°C per min (Rudra A . et al., 2010).

8. In vitro release study

In vitro release of liposomes is conducted by various methods, among which dialysis method is the most widely used method. In this method, a weighed amount of freshly prepared lyophilized liposomes is reconstituted in release medium and taken inside a dialysis chamber. Then the whole system is kept on a

Table 3: Different liposomes with their compositions

Type of liposome	Abbreviation	Composition
Conventional liposome	CL	Neutral of negatively charge phospholipids and cholesterol
Cationic liposome	--	Cationic lipid with DOPE
Long circulatory liposome	LCL	Neutral high temp, cholesterol and 5--10% PEG, DSP
Fusogenic liposome	RSVE	Reconstituted sendai virus enveops
pH sensitive liposomes	--	Phospholipids such as PER or DOPE with either CHEMS or OA
Immune liposome	IL	CL or LCL with attached monoclonal antibody or recognition sequences

Table 4: Liposome characterization

Characterization parameters	Analysis for	Analytical methods/instrumentation
Physical Characterization		
Vesicle(Size & Surface morphology, Size distribution)	-----	TEM, Freeze fracture electron microscopy DLS, Zetasizer, TEM, PCR, gel permeation, Exclusion
Electric surface potential &pH	-----	Zeta potential measurement, pH Probes
Lamellarity	-----	SAXS, NMR, Freeze fracture EM
Phase behavior	-----	Freeze fracture EM, DSC
% Entrapment Efficiency	-----	Mini--column centrifugation, gel exclusion, ion exchange, prota---mine aggregation, radiolabelling
Drug release	-----	Diffusion
Chemical characterization		
Concentration	Phospholipid Cholestrol Drug	Barlett/Stewart assay, HPLC Cholestrol oxidase assay, HPLC Method as in individual monograph
Phospholipid	Peroxidation Hydrolysis	UV absorbance, TBA, iodometric, GLC HPLC, TLC, Fatty Acid Conc.
Cholestrol auto--oxidation	-----	HPLC, TLC
Anti--oxidant degradation	-----	HPLC, TLC
pH	-----	pH meter
Osmolarity	-----	Osmometer
Biological characterization		
Sterility	-----	Aerobic or anaerobic cultures
Pyrogenicity	-----	LAL test
Animal toxicity	-----	Monitoring survival rates, Histopathology

magnetic stirrer and maintained at 37°C. Sampling is done by withdrawing 1 ml from the released medium along with addition of 1 ml of fresh buffer simultane---ously. Samples are measured spectrophotometrically.

Liposome for targeted delivery

Targeted drug delivery is a method of deliver---ing medication to a patient in a manner that increases the concentration of the medication in some parts of the body relative to others. The goal of liposome drug delivery system is to prolong, localize, target and have a protected drug interaction with the diseased tissue. The advantages to the targeted release system is the reduction of dosing frequency, having a more uniform effect of the drug, reduction of drug side--effects, and reduced fluctuation in circulating drug levels. Delivery of agents to the reticulo endothelial system (RES) is easily achieved, since most conventional liposomes are trapped by the RES. For the purpose of delivery of agents to target organs, long--circulating liposomes have been developed by modifying the liposomal sur---face. In cancer chemotherapy, the toxicity of anti--cancer 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 antibod---ies (MAbs) raised against tumor---cell specific antigens. Liposomes can be designed to release their entrapped contents under certain controlled conditions: pH--sensitive and temperature--dependent liposomal systems. The future is bright for liposome research, with liposomal formulations of various anticancer drugs, antisense, cytokines, peptides and proteins are already under clinical trial (Muller R. et al., 2004).

Table 5: List of clinically- approved liposomal drugs

Name	Trade name	Company	Indication
Liposomal Amphotericin B	Abelcet	Enzon	Fungal infections
Liposomal Amphotericin B	Ambisome	Gilead Sciences	Fungal and protozoal Infections
Liposomal cytarabine	Depocyt	Pacira (formerlySkyePharma)	Malignant lymphomatous meningitis
Liposomal Daunorubicin	DaunoXome	Gilead Sciences	HIV-related Kaposi's Sarcoma
Liposoma Doxorubicin	Myocet	Zeneus	Combinationtherapywith cyclophosphamide in metastatic breast cancer
Micellular estradiol	Estrasorb	Novavax	Menopausal therapy
Vincristine	Onco TCS	---	Non-Hodgkin's lymphoma
Lurtotecan	NX211	---	Ovarian cancer
Nystatin	Nyotran	---	Topical antifungal agent
Liposome-PEG Doxoru-bicin	Doxil/Caelyx	Ortho Biotech, Schering-Plough	HIV-relatedKaposi's sarcoma, metastatic breastcancer, metastatic ovarian cancer
Liposomal Vaccine	Epaxal	Berna Biotech	Hepatitis A
Liposomal Vaccine	Inflexal V	Berna Biotech	Influenza
Liposomal morphine	DepoDur	SkyePharma, Endo	Postsurgical analgesia
Liposomal Verteporfion	Visudyne	QLT, Novartis	Age-related macular degeneration, pathologic myopia, ocular histoplasmosis
All-trans retinoic acid	Altragen	---	Acute promyelocytic leukaemia; non-Hodgkin's lymphoma; renalcell carcinoma; Kaposi's sarcoma
Platinum compounds	Platar	---	Solid tumours
Annamycin	---	---	Doxorubicin-resistant tumours
E1A gene	---	---	Various tumours
DNA plasmid encoding HLA-B7 and α2 microglobulin	Allovectin-7	---	Metastatic melanoma

New ligands for targeting liposomes

Antibody-mediated liposome targeting

Various types of monoclonal antibodies have been shown to deliver liposomes to many targets, the optimization of properties of immunoliposomes is an important concern. The majority of research in this area relates to cancer targeting, which utilizes a variety of antibodies (Vladimir P. et al., 2005). Nucleosome-specific antibodies capable of recognizing various tumor cells. This method of liposome targeting has several potential advantages. A single liposome, targeted with MoAb[3] (immunoliposome), can potentially deliver hundreds to thousands of drug molecules into an individual target cell, whereas only a few molecules can be directly coupled to each MAb molecule (Berinstein et al., 1987; Huang. A. et al., 1982). A combination of immunoliposome and endosome with disruptive peptide improves the cytosolic delivery of the liposomal drug, increases cytotoxicity and opens up new avenues for constructing targeted liposomal systems and this was shown with the diphtheria toxin A chain, which was combined with pH-dependent fusogenic peptide diINF-7 into integrated liposomes specifically targeted to ovarian carcinoma (Huang et al., 1983).

Folate-mediated liposome targeting

Folate receptors (FR) are frequently overexpressed in a range of tumor cells, therefore targeting tumors with folate-modified liposomes represents a popular approach. Folate-targeted liposomes have been proposed as delivery vehicles for targeting tumors with happens for tumor immunotherapy and boron neutron capture therapy. Folate-targeted liposomes have been used for both gene targeting to tumour cells and for targeting tumors with antisense oligonucleotides (Lu et al., 2002; Gabizon et al., 2004). Liposomal daunorubicin as well as doxorubicin have been delivered into various tumor cells through FR and demonstrated increased cytotoxicity.

The drug DOX was selected for determining the targeting efficiency of folate liposomes, because it can be efficiently loaded into liposomes via a pH gradient, easily quantifiable due to its fluorescence properties, and an FDA approved non-targeted liposomal-DOX formulation (DOXIL) is currently used clinically (E .M. Bolotin et al., 1994).

Immunoliposomes

To increase liposomal drug accumulation in the desired tissues and organs, targeted liposomes with surface-attached ligands are used, which are capable of recognizing and binding to cells. Target cell recognition by immunoliposomes is influenced by two factors, the type of the antibody molecule and the chemistry of conjugation. It has been extensively shown that whole antibodies coupled to liposomes are highly immunogenic. These liposomes are rapidly eliminated through Fc-mediated phagocytosis by macrophages of the liver and spleen, and also by tumor localized macrophages. Immunoglobulins (Ig) of the IgG class and their fragments are the most widely used targeting moieties for liposomes, which can be attached to liposomes. Therapeutic efficacy of immunoliposomes similar to regular liposomes is also dependent on the rate of release of drug and the lipid composition of the liposomes (Vladimir P., 2005).

Long-circulating liposomes

Long-circulating liposomes are now being investigated in detail and are widely used in biomedical *in vitro* and *in vivo* studies. Different methods have been suggested to achieve long circulation of liposomes in vivo, including coating the liposome surface with biocompatible polymers, such as PEG, which form a protective layer over the liposome surface. The development of long-circulating liposomes has significant benefits in terms of drug delivery. When long-circulating liposomes administered intravenously, it remain in the blood circulation longer, allowing a greater percentage of the liposomes to come in contact with and become trapped in the intended targeted tissue of the body. An important role of protective polymers is their flexibility, which allows a relatively small number of surface-grafted polymer molecules to create an impermeable layer over the liposome surface. Long-circulating liposomes demonstrate dose-independent, non-saturable, log-linear kinetics and increased bioavailability (Vladimir P., 2005).

pH-sensitive liposomes

To achieve the pH-sensitive release of liposome content, liposomes are constructed from pH-sensitive components and after being endocytosed in the intact form. These fuse with the endovacuolar membrane as a result of the lower pH inside the endosome and release their contents into the cytoplasm. Antisense oligonucleotides can be delivered into cells by anionic pH-sensitive phosphatidylethanolamine (PE)-containing liposomes and long-circulating PEGylated pH-sensitive liposomes having low pH sensitivity that can effectively deliver their contents into the cytoplasm (Vladimir P., 2005).

Magnetic liposomes

Liposomes are composed of lamellar phase lipid bilayer membranes, they can contain both aqueous and lipophilic drugs, and the surfaces of liposomes can also be modified by the addition of a specific antibody, ligands and polymers. Target-selective drug delivery systems for delivering drugs to target organs, tissues and cells are expected to greatly reduce side effects in normal cells. Magnetic liposomes contain magnetic ion oxide. These liposomes can be directed by a vibrating magnetic field to deliver at intended sites. In the osteosarcoma model in which the magnet was implanted into the tumor, magnetic liposomes loaded with adriamycin demonstrated better accumulation in tumour vasculature and resulted in enhanced tumor-growth inhibition (Kubo et al., 2010). Magnetic materials that also have biological properties will be useful for improving DDSs, which should lead to reduction in side effects in the near future (Shashi K. et al., 2012).

Cytoskeleton-specific immunoliposomes

Cytoskeleton-specific immunoliposomes can combine with the damaged cells and so they were used as carriers for successful gene delivery in to hypoxic cells (Khaw et al., 2001). Anticardiac myosin monoclonal antibodies have an outstanding capacity to identify and bind hypoxic cells with damaged plasma membranes (Gabizon et al., 2004). This quality of the antimyosin antibody has been successfully used for the delivery of antibody-bearing liposomes in the field of myocardial infarction. Immunoliposomes specifically targeting ischaemically damaged cell membrane and decrease the level of cell death both *in vitro* and in the isolated rat heart model (Khudairi et al., 2004). Cytoskeletal antigen (myosin) specific immunoliposomes (CSIL) were shown to seal membrane lesions in hypoxis cardiocytes by anchoring CSIL to the exposed cytoskeletal antigen.

Liposomal haemoglobin

Liposomal encapsulation of haemoglobin is capable to deliver high concentration of haemoglobin and to provide sufficient oxygen in conditions of significant blood loss (Yadav VR. et al., 2014). Active research continues in the area of liposomal haemoglobin as a blood substitute. PEGylated liposomal haemoglobin was found to be stable at storage for 1 year at normal room temperature. Many experimental studies have confirmed that liposomal haemoglobin reduce the toxicity causes by free haemoglobin in plasma (Sakai et al., 2000). The use of saturated lipids is preferable because they successfully escaped from lipid peroxidation along with good microvascular perfusion (Sakai et al., 1999).

Applications of liposomes in drug delivery

Liposome formulations of some drugs have shown a significant increase in therapeutic efficacy in preclinical models and in humans, compared to the non-liposomal formulations.

It is now possible to produce a wide range of liposome of varying sizes, varying compositions and surface morphology suitable for wide range of applications (Mayer et al., 1998). The therapeutic applications of liposomes generally fall into several categories briefly described below (A et al., 1997).

Formulation aid

Hydrophobic drugs are usually formulated in surfactants and organic co-solvents for systemic administration which usually cause toxicity. Liposomes are made up of phospholipids which are biocompatible and biodegradable molecules, relatively non-toxic, non-immunogenic and can encapsulate a broad range of water-insoluble (lipophilic) drugs. Currently, phospholipid mixtures are being used as excipients for preparing better-tolerated preclinical and clinical formulations of several lipophilic, slightly water soluble drugs such as amphotericin B (A et al., 1997). Liposomes have been evaluated as a vehicle for the delivery of paclitaxel and its analogs as an alternative to the ethanol / cremophor vehicle.

Intracellular drug delivery

Drugs with intracellular receptors are required to cross the plasma membrane to show the pharmacological activity. Liposomal delivery of drugs which normally enter the cells by pinocytosis can be very effective because liposomes can contain greater concentrations of drug compared to the extracellular fluid. The endocytosis process by which negatively charged liposomes are predominantly taken up by the cells, is more efficient than pinocytosis.

Liposomes can be used to increase cytosolic delivery of certain drugs which is normally poorly taken up into cells (V et al., 2011).

Sustained release drug delivery

Sustained release systems are required to achieve and then to maintain the concentration of drug administered within the therapeutically effective range needed for medication, it is often necessary to take this type of drug delivery systems several times in a day. This results in a fluctuated drug level and consequently undesirable toxicity and poor efficiency (Yie W Chien et al., 1992; Yie W Chien. Et al., 1988). To minimize this fluctuation, novel drug delivery systems have been developed, which include niosomes, liposomes.

Gene therapy

Liposomes have been used widely in the analytical sciences as well as for drug and gene delivery.

A number of systemic diseases are caused by lack of enzymes or factors which are due to missing or defective genes. In recent years, several attempts have been made to restore gene expression by delivery of the relevant exogenous DNA or genes to cells. Because of the poly anionic nature of DNA, cationic (and neutral) lipids are typically used for gene delivery, while the use of anionic liposomes has been fairly restricted to the delivery of other therapeutic macromolecules (Yie W Chien. et al., 1992). Some of the widely used cationic liposome formulations are: lipofectin, cytofectin, lipofectamine, transfectace, DC-cholesterol and transfectam (dioctadecyldimethyl ammonium Chloride) (Lu et al., 2002).

Site-avoidance delivery

The biodistribution pattern of liposomes may lead to a relative reduction of drug concentration in tissues specifically sensitive to the delivered drug. This may have implications with regard to the theraputic window of various cytotoxic drugs, such as the cardiotoxic anthacyclines, provided that anti-tumor efficacy is not negatively affected. Drugs having narrow therapeutic index (TI) which used in the treatment of diseases like cancer that can be highly toxic to normal tissues. The toxicity of these drugs may be minimized by decreasing delivery to critical normal organs (Lu et al., 2002). Liposomes are taken up poorly by tissues such as heart, kidney, and GI tract, which are major sites for toxic side-effects of a variety of antineoplastic drugs and liposome formulation may improve the TI by altering the bio distribution of drug away from drug sensitive normal tissues**.**

Cancer Therapy: Cytotoxic drugs can distribute non-specifically throughout the body, leading 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 results in increased circulation lifetime, enhanced deposition in the infected tissues, protection from metabolic degradation, altered tissue distribution of the drug etc. Researches have been shown to improve the safety profile of the anthracy--- cline cytotoxics, doxorubicin and daunorubicin, along with vincristine, which are associated with severe car--- diotoxic side effects. Liposomal entrapment of these drugs showed a significant reduction cardiotoxicity, dermal toxicity and better survival of experimental animals compared to the controls receiving free drugs (G et al., 1971).

Occular Application: Ocular drug delivery is challenging in terms of achieving optimum drug concentration due to special protective mechanisms of the eye. Development of a drug delivery system for attaining therapeutic concentration at the target site requires a comprehensive understanding of static and dynamic barriers of the eye. The usual routes of drug administration for the treatment of eye disorders are topical, systemic, periocular, and intravitreal. Topical administration is the most preferred route because of highest patient compliance and least invasive nature. Drugs encapsu--- lated in liposomes showed improved efficacy than con--- ventional formulations. Enhanced efficacy of liposome encapsulated idoxuridine in herpes simplex infected corneal lesions in rabbits was first reported in 1981 (Smolin G. et al., 1981). Lee in 1985 concluded that ocular delivery of drugs could be either promoted by the use of liposome carriers, depending on the physio--- chemical properties of the drugs and lipid mixture em- ployed. Ganglioside-containing liposomes and wheat germ agglutinin that has a high binding affinity for both cornea and ganglioside, were tested for corneal adhe--- sion (Shaeffer H. et al., 1982). Corneal binding as well as accumulation and transcorneal flux of carbachol was enhanced 2.5 to 3 fold over 90 min exposure times (Davies N. et al., 1992) proposed the use of mucoad--- hesive polymers, carbopol 934P and carbopol 1342 to retain liposomes at the cornea. While precorneal re--- tention time was significantly enhanced under appro--- priate conditions, liposomes even in the presence of the mucoadhesive had migrated toward the conjuctival sac with very little activity remaining at the corneal surface.

Antimicrobial efficacy

Many Antimicrobial agents are unstable to processing and react to many environmental stimuli. Encapsula--- tion has shown to be beneficial by increasing their sta--- bility, activity and by protecting them from the envi-- ronment. First, they protect the entrapped drug against enzymatic degradation. For example, cephalo--- sporin, penicillin etc. Secondly, the lipid nature of the vesicles promotes enhanced cellular uptake of the an--- tibiotics into the microorganisms, thus reducing the effective dose and the incidence of toxicity, for exam- ple liposomal formulation of amphotericin B (J et al., 2012).

CONCLUSION

Liposomes have been recognized as extremely useful carrier systems for targeted drug delivery. They are also showing particular promise as intracellular deliv--- ery systems for anti-sense molecules, ribosomes, pro-- teins/peptides, and DNA. Liposomes with enhanced drug delivery to disease locations along with long circu--- lation residence times, are now achieving clinical ac--- ceptance. Also, liposomes promote targeting of thera--- peutics to particular diseased cells within the disease site which lead to reduce toxicities and to enhance efficacy compared with free drug. Considering the ad--- vantages of this drug delivery system, its modifications or upgraded versions like Enzymosomes, Hemosomes, Virosomes, Erythrosomes, Virosomes, etc. are now being investigated as new modes for targeted drug delivery. Many liposomal products are already in the market and many are under the clinical trials to get the approval. Surely, the liposomal delivery system has the potential to revolutionize the traditional therapy for treatment of various life threatening diseases including cancer.

ACKNOWLEDGEMENT

The authors are highly thankful to the department of pharmaceutical sciences, Utkal University, Bhubanes--- war, Odisha for their kind support.

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