Review Article

Liposome Drug Delivery: A Review

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ABSTRACT

Liposomes are result of self assembly of phospholipid in an aqueous media resulting in closed bilayered structures. Liposomes are one of unique drug delivery system which can be use in controlling and targeting drug delivery system. Liposomes are generally classified based upon structure, method of preparation, composition and application, conventional liposome, and specialty liposome. Liposomes are formulated and processed to differ in size, composition, charge and lamellarity, depending upon method of preparation either active loading technique or passive loading technique. The prepared liposomes are characterized for visual appearance, liposomal size distribution, lamillarity, liposome stability, entrapped volume and surface charges. Different marketed formulations are available in market for liposomes. The liposomes have many applications which increase its importance over other formulations.

Keywords: phospholipid, bilayered, targeting drug delivery system.

INTRODUCTION

When phospholipids are dispersed in water, they spontaneously form closed structure with internal aqueous environment bounded by phospholipids bilayer membranes, this vesicular system is called as liposome[1.] Liposomes are the small vesicle of spherical shape that can be produced from cholesterols, non toxic surfactants, sphingolipids, glycolipids, long chain fatty acids and even membrane proteins[2]. Liposomes were first described by British haematologist Dr Alec D Bangham FRS in 1961 (published 1964), at the Babraham Institute, in Cambridge. They were discovered when Bangham and R. W. Horne were testing the institute's new electron microscope by adding negative stain to dry phospholipids. The resemblance to the plasma lemma was obvious, and the microscope pictures served as the first real evidence for the cell membrane being a bilayer lipid structure[3]. Liposomes or lipid based vesicles are microscopic (unilamellar or multilamellar) vesicles that are formed as a result of self-assembly of phospholipids in an aqueous media resulting in closed bilayered structures[4-5]. The assembly into closed bilayered structures is a spontaneous process[6].

Liposome can be formulated and processed to differ in size, composition, charge and lamellarity. Today liposomal formulations of anti-tumor drugs and antifungal agents have been commercialized[7]. Liposomes vary in charge and in size depending on the method of preparation and the lipids used (the multilamellar vesicle [MLV] size range is 0.1–5.0 μm, the small unilamellar vesicle[SUV] size range is0.02–0.05 μm, and the large unilamellar vesicle [LUV] size range varies from 0.06 μm and greater).

Molecules from low molecular weight (glucose) to high molecular weight (peptides and proteins) have been incorporated in liposomes. The water soluble compounds/drugs are present in aqueous compartments while lipid soluble compounds/drugs and amphiphilic compounds/drugs insert themselves in phospholipid bilayers. The liposomes containing drugs can be administrated by many routes (intravenous, oral inhalation, local application, ocular) and these can be used for the treatment of various diseases[8].

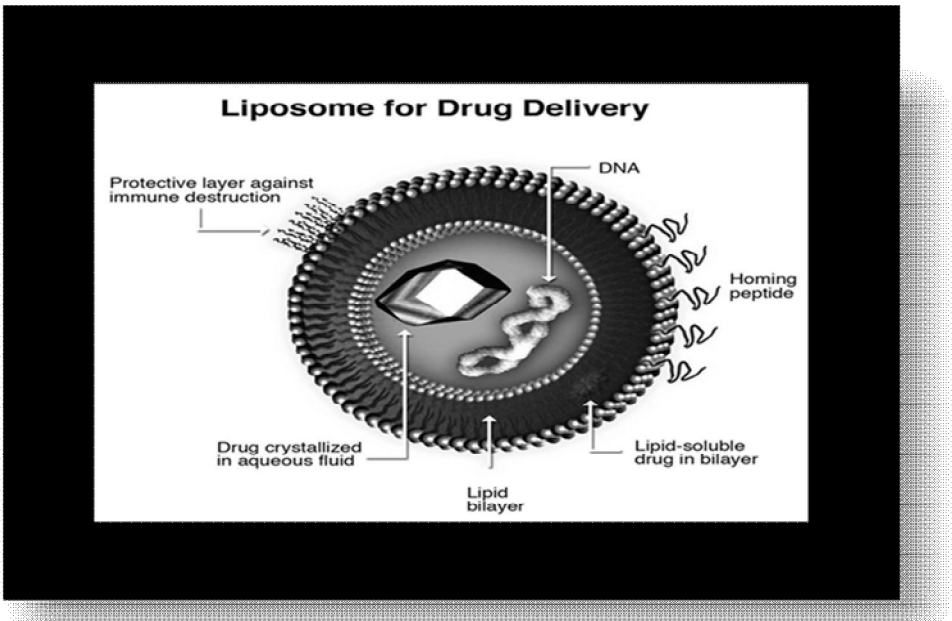


Fig. 1: Liposome for Drug delivery

Liposomes can target a drug to the intended site of action in the body thus enhancing its efficacy. Liposomes can act as a depot from which the entrapped compound is slowly released over time; such a sustained release process can be exploited to maintain therapeutic drug levels in blood stream. Thus liposome surfaces can be readily modified by attaching polyethylene glycol (PEG) units to the bilayer, the circulation time of liposomes in the bloodstream is increased dramatically. Liposome can be formulated and processed to differ in size, composition, charge and lamellarity. To date liposomal formulations of anti-tumor drugs and antifungal agents have been commercialized (fig.1), all these parameters determine the fate of liposomes on the shelf and in vivo.

Reasons to Use Liposomes as Drug Carriers

Solubilisation- Liposomes may solubilise lipophilic drugs that would otherwise be difficult to administer intravenously.

Protection- Liposome-encapsulated drugs are inaccessible to metabolising enzymes; conversely, body components (such as erythrocytes or tissues at the injection site) are not directly exposed to the full dose of the drug.

Duration of action- Liposomes can prolong drug action by slowly releasing the drug in the body.
Directing potential Targeting options change the distribution of the drug through the body.

Internalisation- Liposomes are endocytosed or phagocytosed by cells, opening up opportunities to use 'liposome-dependent drugs'. Lipid based structures (not necessarily liposomes) are also able to bring plasmid material into the cell through the same mechanism (non-viral transfection systems).

Amplification- Liposomes can be used as adjuvant in vaccine formulations.[9]

CLASSIFICATION OF LIPOSOMES

Liposomes are classified on the basis of:

1. Structure.
2. Method of preparation.
3. Composition and application.
4. Conventional liposome.
5. Specialty liposome.

1. Classification Based on Structure (Table 1)

Table 1: Vesicle Types with their Size and Number of Lipid Layers

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

2. Based on Method of Preparation (Table 2)

Table 2: 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
Stable pluri lamellar vesicle	SPLV
Frozen and thawed multi lamellar vesicle	FATMLV
Vesicle prepared by extrusion technique	VET
Dehydration- Rehydration method	DRV

3. Based on Composition and Application (Table 3)

Table 3: Different Liposome with their Compositions

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

4. Based Upon Conventional Liposome

1- Stabilize natural lecithin (PC) mixtures
2- Synthetic identical, chain phospholipids
3- Glycolipids containing liposome

5. Based Upon Specialty Liposome

1- Bipolar fatty acid
2- Antibody directed liposome.
3- Methyl/ Methylene x- linked liposome.
4- Lipoprotein coated liposome.
5- Carbohydrate coated liposome.
6- Multiple encapsulated liposome.[10]

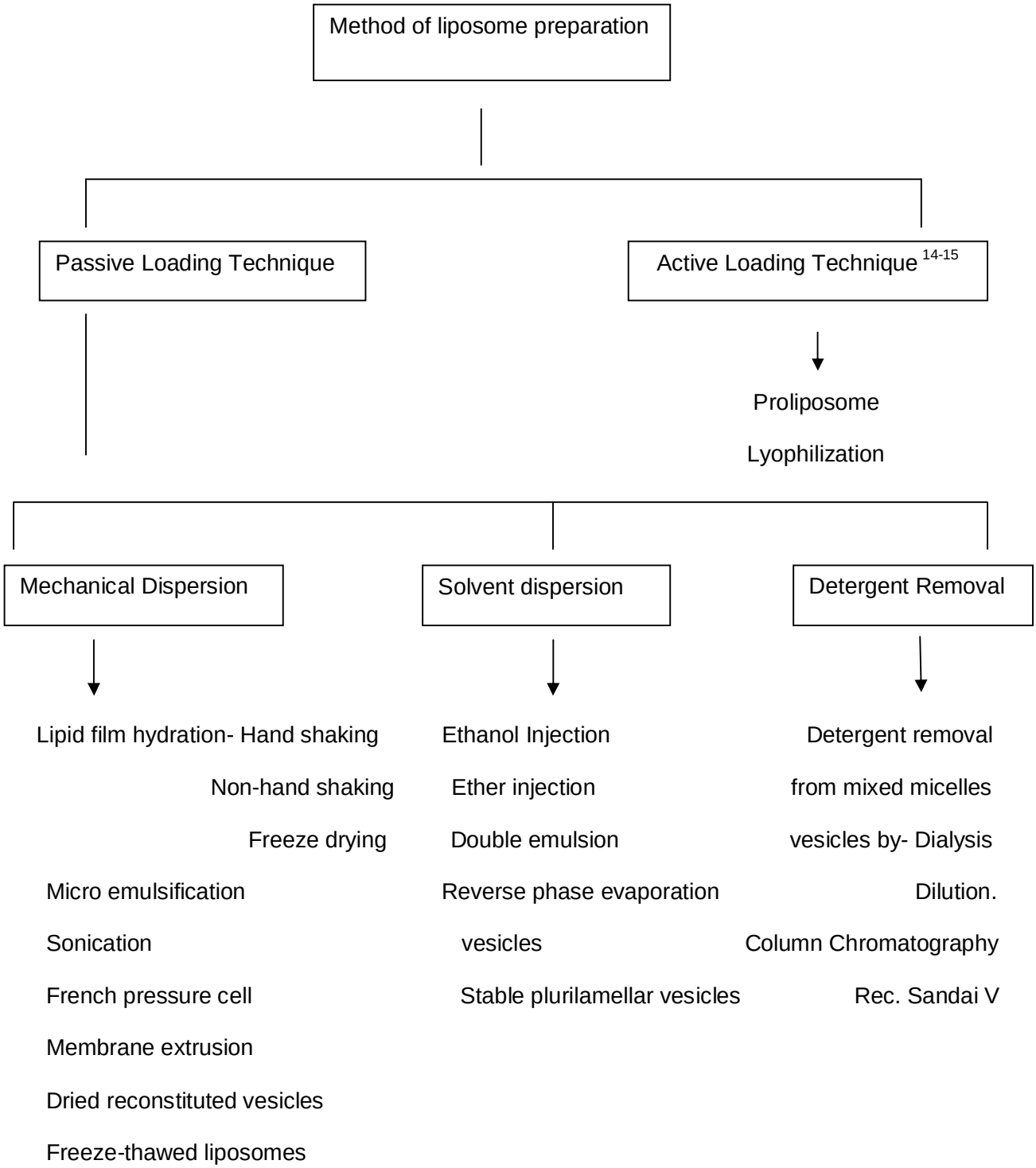
METHOD OF PREPERATION

Preparation of liposomes

The preparation of all types of vesicular systems requires the input of energy[11]. Generally all the methods of liposome preparation involve three basic stages

1. Drying down of mixture of lipids from an organic solvent.
2. Dispersion of lipids in aqueous media.
3. Separation and purification of resultant liposomes.[12]

The various methods of preparations of liposomes are as under[13].



A. Passive loading technique

1. Mechanical dispersion

Lipid Hydration Method

(a) This is the most widely used method for the preparation of MLV. The method involves drying a solution of lipids so that a thin film is formed at the bottom of round bottom flask and then hydrating the film by adding aqueous buffer and vortexing the dispersion for some time. The hydration step is done at a temperature above the gel-liquid crystalline transition temperature Tc of the lipid or above the Tc of the highest melting component in the lipid mixture. The compounds to be encapsulated are added either to aqueous buffer or to organic solvent containing lipids depending upon their solubilities. MLV are simple to prepare by this method and a variety of substances can be encapsulated in these liposomes. The drawbacks of the method are low internal volume, low encapsulation efficiency and the size distribution is heterogeneous [16].

(b) MLVs with high encapsulation efficiency can be prepared by hydrating the lipids in the presence of an immiscible organic solvent (petroleum ether, diethyl ether). The contents are emulsified by vigorous vortexing or sonication. The organic solvent is removed by passing a stream of nitrogen gas over the mixture. MLVs are formed immediately in the aqueous phase after the removal of organic solvent[17-18].

Microemulsification[19]

This method is provided for preparing small lipid vesicles in commercial quantities by microemulsifying lipid compositions using very high shear forces generated in a homogenizing apparatus operated at high pressures at a selected temperature. At least 20 circulations (approximately 10 minutes) but not greater than 200 circulations (100 minutes) are sufficient to produce a micro emulsion of small vesicles suitable for biological application.

Sonication

Here MLVs are sonicated either with a bath type sonicator or a probe sonicator under an inert atmosphere. The main drawbacks of this method are very low internal volume/encapsulation efficiency, possibly degradation of phospholipids and compounds to be encapsulated, exclusion of large molecules, metal contamination from probe tip and presence of MLV alongwith SUV. Recently, Oezden and Hasirci (1991) prepared a polymer coated liposomes by this method.

French Pressure Cell Method

The method involves the extrusion of MLV at 20,000 psi at 4°C through a small orifice. The method has several advantages over sonication method. The method is simple rapid, reproducible and involves gentle handling of unstable materials[20]. The resulting liposomes are somewhat larger than sonicated SUVs. The drawbacks of the method are that the temperature is difficult to achieve and the working volumes are relatively small (about 50 mL maximum).

Membrane extrusion[21]

A suspension of heterogeneous size liposomes is passed through a polymer filter having a web-like construction providing a network of interconnected, tortuous-path capillary pore, and a membrane thickness of at least about 100 microns. The processed liposomes have selected average size less than about 0.4 microns, and a narrow size distribution.

Dried reconstituted vesicles[22]

In Dried reconstituted vesicles method preformed liposomes are added to an aqueous solution containing an active agent or are mixed with a lyophilized protein, followed by dehydration of mixture.

Freeze-Thaw Method

SUVs are rapidly frozen and followed by slow thawing. The brief sonication disperses aggregated materials to LUV. The formation of unilamellar vesicles is due to the fusion of SUV during the processes of freezing and

or thawing[23-25]. This type of fusion is strongly inhibited by increasing the ionic strength of the medium and by increasing the phospholipid concentration. The encapsulation efficiencies from 20 to 30% were obtained[23].

2. Solvent dispersion

Ethanol Injection Method

A lipid solution of ethanol is rapidly injected to a vast excess of buffer. The MLVs are immediately formed. The drawbacks of the method are that the population is heterogeneous (30-110 nm), liposomes are very dilute, it is difficult to remove all ethanol because it forms azeotrope with water and the possibility of various biologically active macromolecules to inactivation in the presence of even low amounts of ethanol[26].

Ether Infusion Method

A solution of lipids dissolved in diethyl ether or ether/methanol mixture is slowly injected to an aqueous solution of the material to be encapsulated at 55-65°C or under reduced pressure. The subsequent removal of ether under vacuum leads to the formation of liposomes. The main drawbacks of the method are that the population is heterogeneous (70-190 nm) and the exposure of compounds to be encapsulated to organic solvents or high temperature[27-28].

Double emulsification[29-31]

In this process, an active ingredient is first dissolved in an aqueous phase (w1) which is then emulsified in an organic solvent of a polymer to make a primary w1/o emulsion. This primary emulsion is further mixed in an emulsifier-containing aqueous solution (w2) to make a w1/o/w2 double emulsion. The removal of the solvent leaves microspheres in the aqueous continuous phase, making it possible to collect them by filtering or centrifuging.

Reverse-phase evaporation

In this method, several phospholipids (pure/mixed with cholesterol) can be used. The lipid mixture is added to a round bottom flask and the solvent is removed under reduced pressure by a rotary evaporator. The system is purged with nitrogen and the lipids are re-dissolved in the organic phase. This is the phase that the reverse phase vesicles will form. Diethyl ether and isopropyl ether are the usual solvents of choice.

After the lipids are re-dissolved in this phase the aqueous phase (contains compound to be encapsulated) is added. The system is kept under continuous nitrogen and the two-phase system is sonicated until the mixture becomes a clear one-phase dispersion. The mixture is then placed on the rotary evaporator and the organic solvent removed until a gel is formed. Non-encapsulated material is removed. The resulting liposomes are called reverse-phase evaporation vesicles (REV). The large unilamellar and oligolamellar vesicles formed have the ability to encapsulate large macromolecular vesicles with high efficiency[32].

3. Detergent removal

The detergents at their critical micelles concentrations have been used to solubilize lipids. As the detergent is removed the micelles become progressively richer in phospholipid and finally combine to form LUVs. The detergents were removed by dialysis[33-35]. The advantages of detergent dialysis method are excellent reproducibility and production of liposome populations which are homogenous in size. The main drawback of the method is the retention of traces of detergent(s) within the liposomes.

B. Active loading technique

Proliposome

In Proliposome, lipid and drug are coated onto a soluble carrier to form free-flowing granular material which on hydration forms an isotonic liposomal suspension. The proliposome approach may provide an opportunity for cost-effective large scale manufacture of liposomes containing particularly lipophilic drugs.

Lyophilization

Freeze-drying (lyophilization) involves the removal of water from products in the frozen state at extremely low pressures. The process is generally used to dry products that Riaz 75 are thermo labile and would be destroyed by heat-drying. The technique has a great potential as a method to solve long term stability problems with respect to liposomal stability. It is exposed that leakage of entrapped materials may take place during the process of freeze- drying and on reconstitution.

CHARACTERIZATION OF LIPOSOMES[36-38]

Liposome prepared by one of the preceding method must be characterized. The most important parameters of liposome characterization include visual appearance, turbidity, size distribution, lamellarity, concentration, composition, presence of degradation products, and stability. The behavior of liposomes in both physical and biological system is governed by these factors; therefore liposomes are characterized for physical attributes and chemical compositions.

Liposome characterization[39]

Characterization parameters	Analysis for	Analytical methods/ instrumentation
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
Physical Characterization		
Vesicle(Size & Surface morphology, Size distribution)		TEM, Freeze fracture electron microscopy DLS,Zetasizer,TEM,PCR,gel permeation, exclusion
Surface charge		Free flow electrophoresis
Electric surface potential &pH		Zeta potential measurement, pH probes
Lamellarity		SAXS,[31] NMR, Freeze fracture EM
Phase behavior		Freeze fracture EM,DSC
% Entrapment Efficiency		Minicolumn centrifugation, gel exclusion, ion exachange,protamine aggregation,radiolabelling
Drug release		Diffusion
Biological characterization		
Sterility		Aerobic or anaerobic cultures
Pyrogenicity		LAL test
Animal toxicity		Monitoring survival rates, Histopathology

1. Visual Appearance

Liposome suspension can range from translucent to milky, 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 a nonliposomal dispersion and is most likely a disperse inverse hexagonal phase or dispersed microcrystallites. An optical microscope (phase contrast) can detect liposome> 0.3 µm and contamination with larger particles.

2. Determination of Liposomal Size Distribution

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

3. Determination of Lamillarity

The lamellarity of liposomes is measured by electron microscopy or by spectroscopic techniques. Most frequently 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. Encapsulation efficiency is measured by encapsulating a hydrophilic marker

4. Liposome Stability

Liposome stability is a complex issue, and consists of physical, chemical, and biological stability. In the pharmaceutical industry and in drug delivery, shelf life stability is also important. Physical stability indicates mostly the constancy of the size and the ratio of lipid to active agent. The cationic liposomes can be stable at 4°C for a long period of time, if properly sterilized. The chemical instability mainly concerns two degradation pathways, oxidative and hydrolytic. Oxidation of phospholipids in liposomes mainly takes place in unsaturated fatty acyl chain-carrying phosphlipids. These chains are oxidised via a free radical chain mechanism in the absence of particular oxidants. Storage at low temperatures and protection from light and oxygen will reduce the chance of oxidation. Further protection could be enhanced with the addition of antioxidants such as α-tocopherol and butyl hydroxy toluene. Working under nitrogen or argon also minimises the oxidation of lipids during preparation. The hydrolysis of ester bonds can also be reduced by optimising the pH, temperature, ionic strength, chain length and head group and the amount of cholesterol incorporated into the bilayer[40]

There are many aspects to physical instability. Stabilisation may be achieved by careful selection of the bilayer components, for example cholesterol is added to permeable bilayers to decrease leakage rates [41.] Physical stability study is performed to investigate the leak out of the drug from liposome during storage.

5. Entrapped Volume

The entrapped volume of a population of liposome (in µL/ mg phospholipid) can often be deduced from measurements of the total quantity of solute entrapped inside liposome assuring that the concentration of solute in the aqueous medium inside liposomes is the same after separation from unentrapped material. For example, in two phase method of preparation, water can be lost from the internal compartment during the drying down step to remove organic solvent.

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

6. Surface Charge

Liposomes are usually prepared using charge imparting constituting lipids and hence it is imparting to study the charge on the vesicle surface. In general two methods are used to assess

the charge, namely free flow electrophoresis and zeta potential measurement. From the mobility of the liposomal dispersion in a suitable buffer, the surface charge on the vesicles.

MARKETED FORMULATIONS

Various Marketed Formulations of Liposomes Product	Drug	Company
Ambisome™	Amphotericin B	NeXstar Pharmaceuticals, Inc., CO
Abelcet™	Amphotericin B	The Liposome Company, NJ
Amphocil™	Amphotericin B	Sequus Pharmaceuticals, Inc., C.A.
Doxil™	Doxorubicin	Sequus Pharmaceuticals, Inc., C.A.
DaunoXome™	Daunorubicin	NeXstar Pharmaceuticals, Inc., CO
MiKasome™	Amikacin	NeXstar Pharmaceuticals, Inc., CO
DC99™	Doxorubicin	Liposome Co., NJ, USA
Epaxel™	Hepatitis A Vaccine	Swiss Serum Institute, Switzerland
ELA-Max™	Lidocaine	Biozone Labs, CA, USA

APPLICATIONS

- Liposomes are used as a model, tool, or reagent in the basic studies of cell interactions, recognition processes, and of the mode of action of certain substances[42]
- Improved solubility of lipophilic and amphiphilic drugs. This is possible due to precipitation of the drug or gel formation inside the liposome with appropriate substances encapsulated.[43]
- Passive targeting to the cells of the immune system, especially cells of the mononuclear phagocytic system (in older literature reticuloendothelial system). Examples are antimonials, Amphotericin B, porphyrins and also vaccines, immunomodulators or (immuno) suppressor.
- Sustained release system of systemically or locally administered liposomes.
  Site-avoidance mechanism: liposomes do not dispose in certain organs, such as heart, kidneys, brain, and nervous system and this reduces cardio-, nephro-, and neuro-toxicity.
- Site specific targeting: in certain cases liposomes with surface attached ligands can bind to target cells (‘key and lock’ mechanism).
- Improved penetration into tissues, especially in the case of dermally applied liposomal dosage forms.
- liposome encapsulation prevents accumulation of drug in organs and drastically reduces toxicity[44]
- Natural toxins induce strong macrophage response which results in macrophage activation. This can be duplicated and improved by the use of liposomes because small molecules with immunogenic properties (haptens) cannot induce immune response without being attached to a larger particle. Activation of macrophages was proven useful in the treatment of viral, bacterial, and fungal infections as well. Activating factors such as cytokines and lymphokines, including interferon are attached[45]
- Small liposomes composed of lipids with long and saturated hydrocarbon chains in mixtures with cholesterol were shown to accumulate at the sites of inflammations. Such liposomes were used for diagnostic purposes[46]
- Long circulation times significantly, i.e. 200-fold, increased the area under curve of drug concentration vs. time and accumulation in various tumours was proportional to the liposome circulation time[47]
- After preliminary studies in which liposome systems were optimized the cheese ripening times can be shortened by 30–50%[48]

- Due to the surfactant action liposomes also improve the coagulation and sinking of oil spread on the water surface or its cleaning up with floating booms[49]

CONCLUSION

After many years of search liposomes are considered as a good drug delivery vehicle with general applications. Liposomes are considered as better targeter of the drug at appropriate tissue destination by incorporating the drug into liposomes, which is widely accepted by researchers because liposomes are unique drug delivery system which can be administrated orally, parenterally and topically and thus can be used in controlling and targeting drug delivery. Liposomes are used in sustain release, diagnostic purpose, intracellular delivery systems for proteins/peptides, antisense molecules, ribozymes and DNA.

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