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Review Article

Liposomes: A Targeted Drug Delivery System- A Review

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Abstract

Liposomes are a novel drug delivery system (NDDS), which are vesicular structures consisting of hydrated bilalyers which form spontaneously whenphospholipids are dispersed in water. They are simple microscopic vesicles in which an aqueous volume is entirely enclosed by a membrane composed of lipid bilayers. Novel drug delivery system aims to deliver the drug at a rate directed by the needs of the body during the period of treatment, and channel the active entity to the siteof action. It has been a study interest in the development of a NDDS. Liposomes are colloidal spheres of cholesterol non-toxic surfactants, sphingolipids, glycolipids, long chain fatty acids and even membrane proteins and drug molecules or it is also called vesicular system. It is differ in size, composition and charge. It is a drug carrier loaded with great variety of molecules such as small drug molecules, proteins, nucleotides and even plasmids. Few drugs are also formulated as liposomes to improve their therapeutic index. Consequently a number of vesicular drug delivery systems such as liposomes, niosomes, transfersomes, and pharmacosomes were developed. The focus of this review is to the various method of preparation, characterization of liposomes, advantages and applications etc.

1.Introduction

Liposomes were first described by Bangham in 1965 while studying cell membranes. He found that liposomes are vesicular structures consisting of hydrated bilalyers which form spontaneously when phospholipids are dispersed in water. Since this, further studies into liposomes and their application in various fields such as medicine and research have been explored [1]. Liposomes are defined as structure consisting of one or more concentric pheres of lipid bilayers separated by water or aqueous buffer compartments. Phospholipids are the main component of naturally occurring bilayers. These phospholipids include phosphatidylcholines (PC), phosphatidylethanolamines (PE) and phosphatidylserines (PS).[2] The key common feature that bilayer-forming compounds share is their amphiphilicity i.e., they have defined polar and non polar regions. This is the reason the non-polar regions orientate themselves towards the interior away from the aqueous phase, the polar regions being in contact with it. Liposomes were discovered about 40 years ago by A.D. Bangham which has become the versatile tool in biology, biochemistry and medicine today. In 1960s, liposome has been used as a carrier to transport a wide variety of compounds in its aqueous compartment. 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. [3] The clinical potential of liposomes as a vehicle for replacement therapy in genetic deficiencies of lysosomal enzymes was first established in 1970s.[4,5] Considerable progress was made during 1970s and 1980s in the field of liposome stability leading to long circulation times of liposomes after intravenous administration resulting in the improvement in bio-distribution of liposome. The important anti-tumour drug doxorubicin had been formulated as liposome in 1980s to improve the therapeutic index. There are several mechanisms by which liposomes act within and outside the body which are as follows [6]

1) Liposome attaches to cellular membrane and appears to fuse with them, releasing their content into the cell.
2) Sometimes they are taken up by the cell and their phospholipids are incorporated into the cell membrane by which the drug trapped inside is released.
3) In the case of phagocyte cell, the liposomes are taken up, the phospholipid walls are acted upon by organelles called lysosomes and the active pharmaceutical ingredients are released.

1.1 Attractive biological properties of liposomes:

- Liposomes are biocompatible.
- Liposomes can entrap water-soluble (hydrophilic) pharmaceutical agents in their internal water compartment and water-insoluble (hydrophobic) pharmaceuticals into the membrane.
- Liposome-incorporated pharmaceuticals are protected from the inactivating effect of external conditions; yet do not cause undesirable side reactions.
- Liposomes provide a unique opportunity to deliver pharmaceuticals into cells or even inside individual cellular compartments.
- Size, charge and surface properties of liposomes can be easily changed simply by adding new ingredients to the lipid mixture before liposome preparation and/or by variation of preparation methods

1.2 Advantages of Liposomes:

1) Provide controlled drug delivery
2) Biodegradable, biocompatible, flexible
3) Non ionic
4) Can carry both water and lipid soluble drugs
5) Drugs can be stabilized from oxidation
6) Improve protein stabilization
7) Controlled hydration
8) Provide sustained release
9) Targeted drug delivery or site specific drug delivery
10) Stabilization of entrapped drug from hostile environment
11) Alter pharmacokinetics and pharmacodynamics of drugs
12) Can be administered through various routes
13) Can incorporate micro and macro molecules
14) Act as reservoir of drugs
15) Therapeutic index of drugs is increased
16) Site avoidance therapy
17) Can modulate the distribution of drug

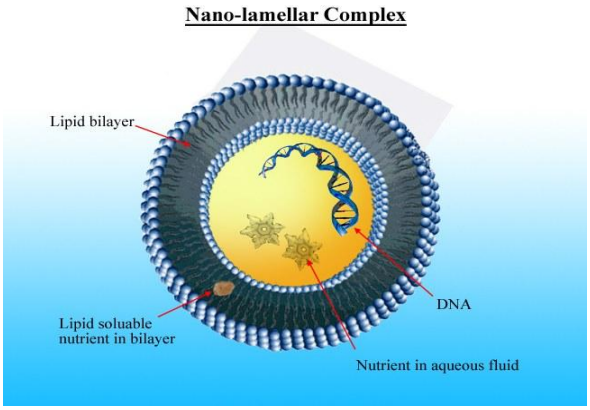

Fig.1; Structure of Liposome (Source: google.com)

2. Classification of Liposomes

2.1 Based on composition and mode of drug delivery

2.1.1. Conventional liposomes:

Composed of neutral or negatively charged phospholipids and cholesterol. Subject to coated pit endocytosis, contents ultimately delivered to Lysosomes 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.

2.1.2. pH sensitive liposomes :

Composed of phospholipids such as phosphatidyl ethanolamine, dioleoyl phosphatidyl ethanolamine. Subjected to coated pit endocytosis at low pH, fuse with cell or endosomes membrane and release their contents in cytoplasm; suitable for intra cellular delivery of weak base and macromolecules. Biodistribution and pharmacokinetics similar to conventional liposomes.

2.1.3. Cationic Liposomes:

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

2.1.4. Long circulating or stealth liposomes:

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

2.1.5. Immuno liposomes:

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

2.1.6. Magnetic Liposomes:

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 likeantibodies 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.

2.1.7. Temperature (or) heat sensitive liposomes:

Composed of Dipalmitoyl P.C. These are vesicles showed maximumrelease at 41o, the phase transition temperature of Dipalmitoyl P.C. Liposomesrelease the entrapped content at the targetcell surface upon a brief heating to thephase transition temperature of theliposome membrane.

2.2Based on Size and Number of Lamellae

2.2.1. Multi lamellar vesicles (M.L.V):

(Size 0.1 - 0.3 micro meter) 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.

a) Oligo lamellar vesicles or Paucilamellar vesicles – Intermediate between L.U.V & MLV.

b) Multi vesicular liposomes – Separate compartments are present in a single M.L.V.

c) Stable Pluri lamellar vesicles – Have unique physical and biological properties due to osmotic compression.

2.2.2. Large Unilamellar Vesicles (L.U.V):

(Size 0.1 - 10 micro meter)Have single bilayer, high aqueous volume tolipid ratio (7: 1 mole lipid), useful forhydrophilic drugs, high capture of macromolecules; rapidly cleared by R.E.S.Prepared by detergent dialysis, etherinjection, reverse phase evaporation oractive loading methods.

2.2.3. 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.

3. Preparation Method Oflipsome[7-44]

A) Multilamellar Liposomes (MLV)

(i) 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.

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. The main drawback of this method is the exposure of the materials to be encapsulated to organic solvent and to sonication.

(ii) Solvent Spherule Method

A method for the preparation of MLVs of homogeneous size distribution was proposed by Kim *et al*. The process involved dispersing in aqueous solution the small spherules of volatile hydrophobic solvent in which lipids had been dissolved. MLVs were formed when controlled evaporation of organic solvent occurred in a water bath.

B) Small Unilamellar Liposomes (SUV)

(i) Sanitation Method

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 along with SUV. Recently, Oezden and Hasirci prepared polymer-coated liposomes by this method.

(ii) 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 (Hamilton and Guo,

1984). 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).

(iii) A new method for the preparation of SUV was given by Lasic *et al*. They deposited egg phosphatidylcholinc mixed with 1.5 %w/v of cetyltetramethylammonium bromide (a detergent) in CHCI3/CH3OH on various supports for example silica gel powder, zeolite X, zeolite ZSM5. After the removal of organic phase, the system was resuspended by shaking or stirring in distilled water or 5 mMNaCl. There was some loss of phospholipid (about 10-20%) due to adsorption on the supports. The loss was 70% and 95% in the case of silica gel and zeolite ZSMS respectively. A homogenous population of vesicle with average diameter of 21.5 nm was obtained when zeolite X (particle size of 0.4 mm) was used as a support.

C) Large Unilamellar Liposomes (LUV)

They have high internal volume/encapsulation efficiency and are now days being used for the encapsulation of drugs and macromolecules.

(i) Solvent Injection Methods

(a) 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.

(b) 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.

(ii) Detergent Removal Methods

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. 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. A commercial device called LIPOPREP (Diachema AG, Switzerland), which is a version of dialysis system, is available for the removal of detergents. Other techniques have been used for the removal of detergents: (a) by using Gel Chromatography involving a column of Sephadex G-25,

(b) By adsorption or binding of Triton X-100 (a detergent) to Bio-Beads SM-2.

(c) By binding of octylglucoside (a detergent) to Amberlite XAD-2 beads.

(iii) Reserves Phase Evaporation Method

First water in oil emulsion is formed by brief sonication of a two-phase system containing phospholipids in organic solvent and aqueous buffer. The organic solvents are removed under reduced pressure, resulting in the formation of a viscous gel. The liposomes are formed when residual solvent is removed by continued rotary evaporation under reduced pressure. With this method high encapsulation efficiency up to 65% can be obtained in a medium of low ionic strength for example 0.01 M NaCl. The method has been used to encapsulate small, large and macromolecules. The main disadvantage of the method is the exposure of the materials to be encapsulated to organic solvents and to brief periods of sonication. These conditions may possibly result in the denaturation of some proteins or breakage of DNA strands. We get a heterogeneous sized dispersion of vesicles by this method. Modified Reverse Phase Evaporation Method was presented by Handa *et al*. and the main advantage of the method is that the liposomes had high encapsulation efficiency (about 80%). The Reverse Phase Evaporation Method of Szoka and Papahadjopoulos have also been modified to entrap plasmids without damaging DNA strands.

(iv) Calcium-Induced Fusion Method

This method is used to prepare LUV from acidic phospholipids. The procedure is based on the observation that calcium addition to SUV induces fusion and results in the formation of multilamellar structures in spiral configuration (Cochleate cylinders). The addition of EDTA to these preparations results in the formation of LUVs. The main advantage of this method is that macromolecules can he encapsulated under gentle conditions. The resulting liposomes are largely unilamellar, although of a heterogeneous size range. The chief disadvantage of this method is that LUVs can only be obtained from acidic phospholipids.

(v) Microfluldization Method

Mayhew *et al*. suggested a technique of microfluidization/microemulsification/ homogenization for the large-scale manufacture of liposomes. The reduction in the size range can be achieved by recycling of the sample. The process is reproducible and yields liposomes with good aqueous phase encapsulation. Riaz and Weiner prepared liposomes consisting of egg yolk, cholesterol and brain phosphatidylserindia sodium salt (57:33:10) by this method. First MLV were prepared by these were passed through a Microluidizer (Microlluidics Corporation, Newton, MA, USA) at 40 psi inlet air pressure. The size range was 150-160 nm after 25 recylces. In the Microluidizer, the interaction of fluid streams takes place at high velocities (pressures) in a precisely defined micro channel, which are present in an interaction chamber. In the chamber pressure reaches up to 10,000 psi this can be cause partial degradation of lipids.

(vi) Extrusion under nitrogen through polycarboriate filters LUV can be prepared by passing MLV under nitrogen through polycarbonate membrane filters. The vesicles produced by this method have narrow size distribution. The extrusion is done under moderate pressures (100-250 psi). A special filter holder is required. Such devices are available commercially under the trade names such as LUVET and EXTRUDER and are equipped with a recirculation mechanism that permits multiple extrusions with little difficulty. Small quantities of liposome preparations (about 10 ml) can be easily prepared by the help of a commercial extruder. Riaz and Weiner prepared liposomes by this technique. The liposomes contained phosphatidylcholine from egg yolk and crude phosphoinositide sodium salt in the ratio of 4:1 and the lipid concentration was 12.5 /mole/ml. MLVs were passed through Extruder Lipex Membrane Inc., Vancouver, Canada) ten times through a stalk of two 100 nm polycarbonate filters (Nudeopore Pleasanton, CA, USA) employing nitrogen pressures upto 250 psi. Freeze fracture electron microscopy and p31-FT NMR revealed that the liposomes were unilamellar. Photon Correlation Spectroscopy revealed that the size range was 99-135 nm.

(vii) Lasic *et al*. reported a method for the instant formation of a rather homogeneous preparation of LUV by a simple technique. The formation of multilammelar liposomes is prevented by inducing a surface charge (+ve) on the bilayer while the size of the vesicles is controlled by the topography of the wafer support surface on which phospholipid film was formed. They deposited 0.5-1.0 mg egg yolk lecithin doped with 3 ml of CHCl3/CH3OH on a specially etched 2-inch silicon wafer. This wafer was put in place of the original bottom of an Erlenmeyer flask that is bottom of the flask is replaced by wafer. After having dried overnight at 102 tore (about 1 Pa), the film was resuspended by gentle shaking in 1-2 ml water. Liposomes were formed instantly. The contamination of liposomes with large structures such as MLVs, giant vesicles and phospholipids particles was ruled out by video enhanced phase contrast microscopy.

(viii) A method for the extemporaneous preparation of LUVs has been described by Liautard and Phillippot. The method was recommended for immediate clinical use of liposomes.

(ix) 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. 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.

(D) Giant Liposomes

(i) The procedure for the formation of giant liposomes involves the dialysis, of a methanol solution of phosphatidylcholine in the presence of methyl glucoside detergent against an aqueous solution containing up to 1 M NaCl. The liposomes range in diameter from 10 to 100 mm.

(ii) A method for the formation of giant single lamellar liposomes with size in the range of 10 to 20 prn by the removal of sodium trichloroacetate by dialysis was presented by Oku and MacDonald.

(E) Multivesicular Liposomes

(i) The formation of multivesicular liposomes has been reported by Kim *et al*.. The water in oil emulsion was converted to organic solvent spherules by the addition of the emulsion to across solution. The evaporation of organic solvent resulted in the formation of multivesicular vesicles. The diameter of liposomes ranges from 5.6 to 29 pm. The materials which can be encapsulated include glucose, EDTA, human DNA. These liposomes have very high encapsulation efficiency (up to 89%).

(ii) Cullis *et al*. (1987) found that when MLV preparations were subjected to five cycles of freeze on liquid nitrogen)-thaw and followed by thawing in warm water, the liposomes of high encapsulation efficiency (up to 88%) could be obtained. Freeze fracture electron micrographs revealed vesicles within vesicles.

(F) Assymetric Liposomes

It has been shown that the phospholipid distribution in natural membranes is asymmetric. For example phosphatidytcholine and sphingomyelin concentrate at the outer half of lipid bilayer whereas phosphatidyl ethanolamine, phosphatidyl inositoland phosphatidylserine are mainly localized in the inner half of bilayer. Due to this, attempts have been made to prepare LUVs in which phospholipid distribution in both halves of bilayer is different. It appears that as model membranes the asymmetric liposomes are nearer to natural membranes than the conventional unilamellar liposomes. In the latter the phospholipids distribution is symmetrical in bilayer.

(i) Cestaro *et al*. (1982) described a procedure for the preparation of asymmetric liposomes which contain cerebroside sulfate only at the outer leaflet of phospholipids bilayer. Cerebroside sulfate was adsorbed on to a filter paper (cellulose) support and then the support was incubated with small or large fused unilamellar liposomes. After six hours sulfatide contents reached about 6 mole percentage of the total quantity of phospholipid, corresponding to about 10 mole % of phospholipid present in the outer layer. The sulfatide could not be removed by washing with 1M NaCl or 1M urea.

(ii) Pagano *et al*. (1981) reported the formation of asymmetric phospholipid vesicles, which contained fluorescent lipid analogue in either the outer or inner leaflet of the liposome bilayer. The procedure is based on the observation that the lipid analogues undergo rapid exchange (transfer) between the vesicles populations.

(iii)Denkins and Schroit (1986) prepared asymmetric liposomes by the enzymatic conversion of the fluorescent lipid-analogue of phosphatidylserine (NBD-PS) in the outer leaflet of LUV to NBD-phosphatidylethanolamine (NBD-PE).

(iv) Low and Zilversmit (1980) reported that lipid exchange proteins could be effectively being used so remove phosphatidylinositol at the outer leaflet of unilamellar liposomes. Therefore, it appears that these proteins may be used for the preparation of asymmetric liposomes.

(v) Collis *et al*. (1987) found that in SUV, distribution of lipid was not symmetrical and ratio of lipid in the outer monolayer to lipid in the inner monolayer could be as large as 2:1. Therefore, small unilamellar liposomes can be also be called as asymmetric to some extent.

4. Characterization

4.1. Particle Size and Surface Charge

The droplet size and zeta potential of the liposomes was determined by a Laser Scattering Particle Size Distribution Analyzer and Zeta Potential Analyzer at room temperature. One ml of the liposome suspensions was diluted with 14 ml and 2 ml deionized water, respectively.

4.2. Transmission Electron Microscopy

Transmission Electron Microscopy (TEM) was used to visualize the liposomal vesicles. The vesicles were dried on a copper grid and adsorbed with filter paper. After drying, the sample was viewed under the microscope at 10–100 k magnification at an accelerating voltage of 100 kV.

4.3. Entrapment Efficiency (%EE) and Loading Efficiency

The concentration of MX in the formulation was determined by HPLC analysis after disruption of the vesicles (liposomes) with Triton X-100 (0.1% w/v) at a 1:1 volume ratio and appropriate dilution with PBS (pH 7.4). The vesicle/Triton X-100 solutions was centrifuged at 10,000 rpm at C for 10 min. The supernatant was filtered with a 0.45 m nylon syringe filter. The entrapment efficiencies and the loading efficiencies of the MX-loaded formulation were calculated by (1) and (2), respectively. (1) Where is the concentration of MX loaded in the formulation as described in the above methods, and is the initial concentration of MX added into the formulation (2) where is the total amount of MX in the formulation and is the total amount of PC added into the formulation.

4.4. Stability Evaluation of Liposomes

Liposomes were stored at C and C (room temperature, RT) for 30 days. Both the physical and the chemical stability of MX were evaluated. The physical stability was assessed by visual observation for sedimentation and particle size determination. The chemical stability was determined by measuring the MX content by HPLC on days 0, 1, 7, 14, and 30.

5. Advancements in Liposomes

There are various types of liposomes classified on the basis of the targeted site, technology used, and use. The drug release from the liposomal action occurs by different mechanisms and the targeting of the liposomes to a specific site is achieved in various ways. All the types, mechanisms of action and targeting strategies of the liposomes have been reviewed [45]. To overcome the drawbacks of the liposomes and to further enhance the drug delivery some advancement were made to conventional liposomes. This brought up the development of newer concepts like *Plarosomes*, featuring the approaches to target the Anthracycline drugs at the site of action, *Pulsatory liposomes* for the well-controlled delivery of biotechnological products, *Thermosensitive liposomes* in combination with localized hyperthermia, to improve the targeted drug delivery for more effective management of melanoma, *Gaint liposomes* for stability and the simulation of the cells models, *Dry liposomes* using sugars which stabilize the membranes by lowering lipid phase transition temperature and prevent liposome aggretation and fusion, thus achieving a stable liposomal formulation. This stabilization mechanism was explained that the sugar molecules form hydrogen bonds with the polar heads of the phospholipids on the liposomal membranes, *Sterically stabilized liposomes*, challenging the stability problem. Detailed information on the preparation and evaluation of every type of liposomes stated above can be studied from the citations quoted against each type [46-49].

Table 1: Application of Liposomes

Drug	Method of Preparation	Composition	Achievement	Ref
Ketaconazole	Thin film hydration	Soya lecithin, cholesterol tocopheryl acetate	Improve therapeutic response and reduce adverse effects	[50]
Acetazolamide	Reverse phase evaporation and thin film hydration	Egg phosphotidyl choline, cholesterol (steryl amine and diacetyl phosphate as + or – charge inducers)	Increasing the stability and reducing the intra ocular pressure	[51]
N-Methyl-*N*-D-fructosyl amphotericin B methyl ester (MFAME)	chloroform film method	dimyristoyl phosphatidylcholine cholesterol or ergosterol	Reduction In toxicity of amphotericin	[52]
Cyproterone acetate	solvent evaporation and thin film formation technique	phosphatidylcholine (PC): cholesterol	better penetration	[53]
Doxorubicin	Thin Film hydration method	Cholesterol Phosphatidylserhe, phosphatidylglycerol or cardiolipin Saturated or unsaturated Phospholipid acyl chains	Reduction I cardiotoxicity and enhanced antitumor activity	[54]
Ferrous sulphate	thin-film hydration, thin-film sonication, reverse-phase evaporation and freeze-thawing,	egg lecithin, 10% (mol/mol) cholesterol and 10% (mol/mol) Tween 80. Ascorbic acid	Increased electrostatic and steric stability	[55]
Hydroxyzine	Ethanol injection method and lipid film hydration method	L-α-phosphatidylcholine 95%(PC), cholesterol	Increase in drug concentration in skin and enhanced efficacy	[56]
TopotecanHCl	chloroform film method	soyabean phosphatidylcholine or hydrogenated soybean phosphatidylcholine and cholesterol (PEG Ligated)	Improved stability and enhanced efficacy by accumulation in tumor cells	[57]
Paclitaxel	thin-film hydration method	Soyabean phosphatidyl choline (S100PC) and 1,2- distearoyl-*sn*-glycero-3-phosphoethanolamine [methoxy (polyethyleneglycol)-2000]Cholesterol (CH)4 .C for further experiments.	Increased aqueous solubility	[58]

Table 2: List of Marketed Liposomal Products [59]

S.No	Product name	Drug	Manufacturer
1	Abelcet	Amphotericin B	The liposome company [USA]
2	Allovecti-711	HLB-B7 plasmid	Vical incorporation[USA]
3	3 AmBisome	Amphotericin B	NeXatar pharmaceuticals[USA]
4	Amphocil	Amphotericin B	SEQUUS Pharmaceuticals[USA]
5	Doxilt	Doxorubicin	SEQUUS Pharmaceuticals[USA]
6	Doxosome	Doxorubicin	Indian Institute of chemical biology[India]

6. Conclusion

In the development of novel drug delivery system (NDDS), Liposomes are highly useful for cancer therapy and vaccination. DOXIL, SPI077, Lipoplatin, S-CKD-602 have been approved or in advance trial of PEGylated liposomal formulations. PEG-derivatized liposomes with increased stability can easily be modified using a wide array of targeting moieties (MAb, ligands) to deliver the drug specifically to the target tissues with increasing accuracy. The development of liposome delivery to particular subcellular compartments is a field of great interest in different fields, such as gene therapy and vaccination. The interaction of stealth liposomes with cell membranes, and release of the drug in the neighborhood of target tissues are still under investigation, but some recent studies indicate that the use of detachable PEG may facilitate cell penetration and/or intracellular delivery of vesicles. PEG-coated liposomes are becoming increasingly important, giving technological and biological stability to liposomal systems. Application of Liposomes is also reviewed in this article.

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References

[1] Bangham, A.D. Liposomes in Biological Systems, John Wiley and Sons: Chichester, 1980.
[2] Chapman D. Liposomal technology. 1983; 1:1-18.
[3] Jayakrishnan, A.; Latha, M.S. Controlled and Novel Drug Delivery, B.S. Pub: New Delhi, 1997.
[4] Sessa, G.; Weismann, G. Journal of Journal of *Biological Chemistry*, 1970;, 245: 3295.
[5] Gregoriadis, G. *Methods in Enzymology*, 1976; 44: 278.
[6] Dunnick, J. K.; Rooke, J D.; Aragon, S.; Kriss, J. P. *Cancer. Res.,* 1976; 36: 2385-2389.
[7] Bangham A.D., Standish M.M. and Watlins J.C. *J. Mol. Biol*. 1965; 13: 238.
[8] Bangham A.D., Hill M.W. and Miller N.G.A. n: Methods in Membrane Biology (Korn N.D., ed.) Plenum. N.Y., 1974; Vol.1, p.l.
[9] Papahadjopoulos D. and Watkins J.C. *Biochimicaet Biophysica*, 1978; 135: 639.
[10] Gruner S.M., Leak R.P., Jan off S. and Ostro M.J. *Biochem.* 1985; 24: 2833.
[11] Kim S., Jacobs R.E. and White S.H. *Biochimicaet Biophysica Acta*, 1985; 812: 793.
[12] Oezden M.Y. and Hasirci V.N. *Biochimicaet Biophysica* Acta. 1991; 1075: 102.
[13] Hamilton R.L. and Guo L.S.S. In: Liposome Technology (Gregoriadis G. ed.) CRC Press, Florida, 1984; Vol.1, Chapter 4, p.37.

[14] Lasic D.D., Kidric J. and Zagorc S. *Biochimicaet Biophysica* Acta. 1987; 896: 117.
[15] Deamer D. and Bangham A.D. *Biochimicaet Biophysica Acta*. 1976; 443: 629.
[16] Schieren H., Rudolph S., Findelstein M., Coleman P. and Weissmann G. *Biochimicaet Biophysica Acta*. 1978; 542: 137.
[17] Batzri S. and Korn E.D. *Biochimicaet Biophysica*. 1973; 298: 1015.
[18] Kagawa Y. and Racker E. *Journal of Biological Chemistry*. 1974; 246: 5477.
[19] Milsmann M.H.W., Schwendener R.A. and Wader H. *Biochimicaet Biophysica Acta*. 1978; 512: 147.
[20] Alpes H., Allmann K., Plattner H., Reichert J., Rick R. and Schulz S. *Biochimicaet Biophysica Acta*. 1986; 862: 294.
[21] Enoch H.G. and Strittmatter P. Proceedings of the National Academy of Sciences, USA. 1979;76: 145.
[22] Gerritson W.J., Verkley A.J., Zwaal R.F.A. and van Deenan L.L.M. *European Journal of Biochemistry*, 1978; 85: 255.
[23] Philippot J.R., Mutafschicv S. and Liautard J.P. *Biochimicaet Biophysica Acta*. 1985; 821: 79.
[24] Szoka F. Jr. and Papahadjopoulos D. Proceedings of the National Academy of Sciences, USA. 1978; 75: 4194.
[25] Handa T., Takeuchi H., Phokubo Y. and Kawashima Y. *Chemical&* pharmaceutical bulletin. 1987; 35: 748.
[26] Haga N. and Yogi K. *Journal of Clinical Biochemistry* and Nutrition. 1989; 7: 175.
[27] Papahadjopoulos D. and Vail W.J. *Annals of the New York Academy of Sciences*. 1978; 308: 259.
[28] Mayhew E., Lazo R., Vail WJ, King J. and Green A.M. *Biochimicaet Biophysica Acta*. 1984; 775: 169.
[29] Riaz M. and Weiner N. *Pakistan Journal of Pharmaceutical Sciences*. 1995;
[30] Jousma H., Talsma H., Spies F., Joosten J.G.H., Junginger H.E. and Crommelin D.J.A. *International Journal of Pharmaceutics*. 1987; 35: 263.
[31] Riaz M. and Weiner N. *Pakistan Journal of Pharmaceutical Sciences*. 1994; 7: 61.
[32] Lasic D.D., Belie A. and Valentincic T. *J. Ant. Client. Soc.* 1988; 110: 970.
[33] Liautard J.P. and Phillippot J.R. *Journal of Liposome Research.* 1993; 63.
[34] Pick U. *Arch. Biochem. Biophys*. 1981; 212: 186.
[35] Ohsawa T., Miura H. and Harada K. *Chemical&* pharmaceutical bulletin. 1985; 33: 3945.
[36] Liu L. and Yonetaini T. Journal of *Microencapsulation.* 1994; 11: 409.
[37] Oku N., Scheerer J.F. and McDonald R.C. *Biochimicaet Biophysic acta*. 1982; 692: 384.
[38] Oku N. and MacDonald R.C. *Biochimicaet Biophysica Acta*. 1983; 734: 54.
[39] Kim S., Turker M.S., Chi E.Y., Scla S. and Martin G.M. *Biochimicaet Biophysica Acta*. 1983; 728: 339.
[40] Cullis R.P., Hope M.J., Bally M.B., Madden T.D. and Jan off AS. In: Liposomes from Biophysics to Therapeutics (Ostro M. J., Ed.) Marcel Dekker, N.Y., Chapter 2, 1987; p.39.39
[41] Op den Kamp J.A.F. *Ann. Rev. Biochem.* 1979; 48: 47.
[42] Cestaro B., Pistolesi E., Hershkowitz N. and Galt S. *Biochimicaet Biophysica Acta*. 1982; 685:13.
[43] Pagano R.E., Martin O.C., Schroit A.J. and Struck D.K. *Biochemistry.* 1981; 20: 4920.
[44] Low M.G. and Zilversmit D.D. *Biochimicaet Biophysica Acta*. 1980; 596: 223.
[45] Christopher Kirby, Jacqui Clarke, Gregory Gregoriadis. Effect of the Cholesterol Content of Small Unilamellar. Liposomes on their Stability in vivo and in vitro. *Biochem. J*., 1980; 186: 591-598.
[46] Omar Moh'd Atrouse. The Effect of liposome Composition and Temperature on the Stability of Liposomes and the Interaction of liposomes with Human Neutrophils. *Pakisthan Journal of Biological Sciences*, 2002; 5(9): 948-951.
[47] Arkadiusz Kozubek, Jerzy Gubernator, Ewa Przeworska, Maria Stasiuk Liposomal drug delivery, a novel approach: PLA Rosomes. *Acta Biochimica Polonica*, 2000; 47(3): 639–649.
[48] Popescu D, Popescu AG, Amuzescu B Pulsatory. Liposomes – A Possible Biotechnological Device for Controlled Drug Delivery. I. The Liposome Swelling. *Romanian J. Biophys*., 2010; 20(1): 37–46.
[49] Tiwari Sandip B, Udupa N, Rao BSS, Uma Devi P. Thermosensitive Liposomes And Localised Hyperthermia – An Effective Bimodality Approach For Tumour Management. *Indian Journal of Pharmacology*, 2000; 32: 214-220.
[50] Rakesh P Patel, Hardik Patel, Ashok H Baria. Formulation and Evaluation of Liposomes of Ketoconazole. *International Journal of Drug Delivery Technology*, 2009; 1(1): 16-23.
[51] Rania M Hathout, Samar Mansour, Nahed D Mortada, Ahmed S Guinedi. Liposomes as an Ocular Delivery System for Acetazolamide. In Vitro and In Vivo Studies. *AAPS Pharm SciTech*., 2007; 8(1): E1-12.
[52] Barbara Cybulska, Karolina Kupczyk, Joanna Szlinder-Richert, Edward Borowski. Comparative *in vitro* studies on liposomal formulations of amphotericin B and its derivative, N-methyl-N-D-fructosyl amphotericin B methyl ester (MFAME). *Acta Biochimica Polonica,* 2002; 49(1): 67-75.
[53] Soleiman Mohammadi-Samani, Hashem Montaseri, Minoo Jamshidnejad. Preparation and Evaluation of Cyproterone Acetate Liposome for Topical Drug Delivery. I*ranian Journal of Pharmaceutical Sciences Autumn*, 2009; 5(4): 199-204.
[54] Paul G Tardi, Nancy L Boman, Pieter R Cullis. Liposomal Doxorubicin. *J. drug targeting.,* 1996; 4(3): 129-140.
[55] Shuqin Xia, Shiying Xu. Ferrous sulfate liposomes: preparation, stability and application in fluid milk. Food Research International, 2005; 38: 289–296.
[56] Abeer AW Elzainy *et al*; Hydroxyzine from Topical Phospholipid Liposomal Formulations: Evaluation of Peripheral Antihistaminic Activity and Systemic Absorption in a Rabbit Model. *AAPS Pharm Sci*., 2003; 5(4): 41-48.
[57] Yan-Li Hao *et al*; *In Vitro* and *In Vivo* Studies of Different Liposomes Containing Topotecan. *Arch Pharm Res*., 2005; 28(5): 626-635.
[58] Tao Yang *et al*; Enhanced solubility and stability of PEGylated liposomal paclitaxel: In vitro and in vivo evaluation. *International Journal of Pharmaceutics,* 2007; 338: 317–326.
[59] Parveen Goyal *et al*; Liposomal drug delivery systems *Clinica Applications, Acta Pharm*, 2005; 55(3): 1-25.