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Pharmaceutical Sciences **Review Article……!!!**

LIPOSOMES: NOVEL DRUG DELIVERY SYSTEM

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ABSTRACT

Liposomes are passable and advanced transporters and have capacity to encapsulate hydrophilic and lipophilic drugs as well as maintain them from exterior environmental condition which leads to degradation. Now a days liposomes formulation plays an important role in formulation of potent drug to improve therapeutics index. In consequence of new developments in liposome technology, there are several new methods of liposome preparation based on liposome disposition mechanism and lipid drug interaction including the inhibition of rapid clearance of liposome by controlling particle size, charge and surface hydration. Most clinical applications of liposomal drug delivery are targeting to tissue with or without expression of target recognition molecules on lipid membrane. This review discusses the classification of material used, mechanism of liposome formation, method of preparation, characterization, their application as well as the marketed product with its future prospectus.

1. INTRODUCTION:

When phospholipids are dispersed in water, they spontaneously form closed structure with internal aqueous environment bounded by phospholipid bilayer membranes, this vesicular system is called as liposome [1]. Liposomes are the small vesicle of spherical shape that can be produced from cholesterols, nontoxic surfactants, sphingolipids, glycolipids, long chain fatty acids and even membrane proteins [2]. Liposomes are the drug carrier loaded with great variety of molecules such as small drug molecules, proteins, nucleotides and even plasmids. 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 deliver a wide variety of compounds in its aqueous compartment. Liposome can be formulated and processed to differ in size, composition, charge and lamellarity [2]. Now days liposomal formulations of anti-tumour drugs and antifungal agents have been commercialized. The clinical potential of liposomes as a vehicle for replacement therapy in genetic deficiencies of liposomal enzymes was firstdemonstrated in 1970[3]. Considerable progress was made during 1970s and 1980s in the field of liposome stability leading to long circulation times ofliposomes after intravenous administration resulting in the improvement in bio-distribution of liposomes [4]. 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:

1- Liposome attaches to cellular membrane and appears to fuse with them, releasing their content into the cell.

2- Some times they are taken up by the cell and their phospholipidsare 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.

2. Classification of liposomes[4, 5]:

Table 1. Classification of liposomes depends upon size:

Sr. no	Types	Size
1	Multilamellar large vesicles(MLV)	>0.5 µm
2	Oligolamellar vesicles(OLV)	0.1-1 µm
3	Unilamellar vesicles(UV)	All sizes
4	Small unilamellar vesicles(SUV)	20-100 nm
5	Medium sized unilamellar vesicles(MUV)	-

6	Large unilamellar vesicles (LUV)	>100 nm
7	Giant unilamellar vesicles(GU)	>1 μm
8	Multivesicular vesicles (MVV)	usually >1 μm

Table 2. Classification of liposomes depends upon method of preparation:

Sr. no.	Types	Method of preparation
1	REV(Reverse Evaporation vesicles)	Single or Oligolamellar vesicles made by reverse osmosis
2	MLV-REV	Multilamellar vesicles made by Reverse Phase Evaporation method
3	SPLV	Stable Plurilamellar vesicles
4	FATMLV	Frozen and Thawed MLV
5	VET	Vesicles prepared by Extrusion method
6	DRV	Vesicles prepared by Dehydration-Rehydration method

3. Advantages[6-10]

i. Liposomes are biocompatible, completely biodegradable, non-toxic in nature.

ii. They are suitable for delivery of hydrophobic, amphipathic and hydrophilic drugs.

iii. They protect the encapsulated drug from external environment.

iv. They reduce toxicity and increase stability-Since therapeutic activity of chemotherapeutic agent can be improved through liposome encapsulation. This reduces deleterious effects that are observed at concentration similar to or lower than those required for maximum therapeutic activity.

v. It reduces exposure of sensitive tissue to toxic drugs.

4. Disadvantages[6-8]

i. The production cost is high.

ii. Leakage and fusion of encapsulated drug/molecules can occur.

iii. It has short half-life in reticuloendothelial system, particularly the Kupffer cells in the liver remove liposomes from the circulation.

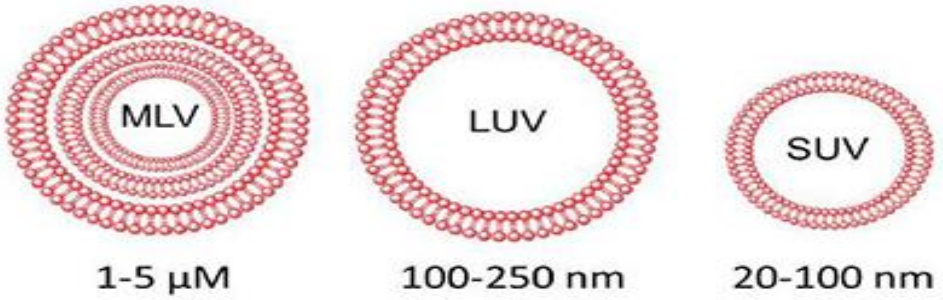

Fig 1. Classification of liposomes based upon size

5. Mechanism of Liposome Formation[11-15]

- Phospholipids being amphipathic (having affinity for both aqueous and polar moieties) molecules, they include a hydrophobic tail and a hydrophilic or polar head.
- The polar domain of fragment is mainly the phosphoric acid bound to a hydrophilic molecule.
- The hydrophilic and hydrophobic end/fragment within the molecular geometry of amphiphilic lipid arrange them self and organize in ordered supra-molecular structure when confronted with solvent.
- In aqueous medium like water and phosphoric acid the molecule in self-assembled structure is oriented to form the structure as a polar portion of molecule remain in contact with the polar environment and simultaneously shield the non-polar part.
- In the excess 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 consist of lamellar, hexagonal or cubic phases dispersed as colloidal Nano construct (artificial membrane) known as liposomes hexasomes or cubosomes respectively.

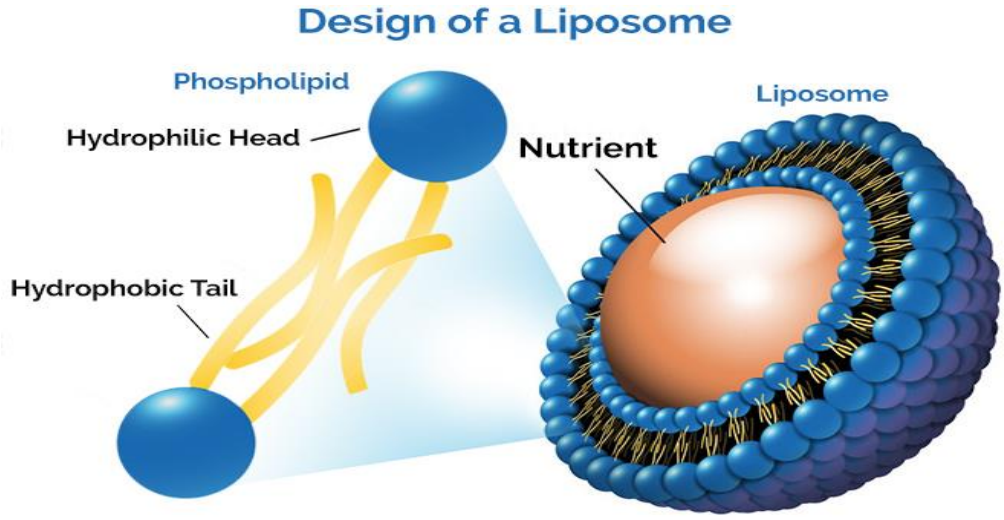

Fig 1. Structure of liposome

6. Materials Used For Preparation of Liposomes [16-18]

6.1. Phospholipids: Glycerol containing phospholipids are most frequently used element of liposome formulation and represent greater than 50% of weight of lipid in biological membranes. These are made up from phosphatidic acid. The back bone of the molecule is glycerol moiety. At C3 position OH group is esterified to phosphoric acid. Fatty acid giving rise to the lipidic nature. One of the remaining OH group of phosphoric acid may be further esterified to a broad range of organic alcohols including glycerol, choline, ethanolamine, serine and inositol. Thus the parent compound of the series is the phosphoric ester of glycerol. Phosphatidylcholine (PC) is routinely used as a neutral bulk phospholipid. As a negatively charged lipid, phosphatidylglycerol (PC) is often selected. Finally, if it is desirable to reduce the permeability of "fluid crystalline state" bilayer, cholesterol is added to bilayer structure. Five groups of phospholipids that can be used for the liposomal preparation can be discerned

1. Phospholipids from natural sources
2. Modified natural phospholipids
3. Semisynthetic phospholipids
4. Fully synthetic phospholipids
5. Phospholipids with non-natural head groups

Examples of phospholipids are

- Phosphatidyl choline (Lecithin) PC
- Phosphatidyl ethanolamine (cephalin) PE
- Phosphatidyl serine (PS)
- Phosphatidyl inositol (PI)
- Phosphatidyl Glycerol (PG)

a) Sphingolipids

Backbone is sphingosine or a related base. These are important constituents of plant and animal cells. Sphingomyelin is capable of hydrogen bonding with adjacent glycerol lipids, thus rising order and stability of vesicle construction.

A head group that can vary from simple alcohols such as choline to very complex carbohydrates.

Most common

Sphingolipids – Sphingomyelin, Glycosphingo lipids.

Gangliosides – found on grey matter, used as a minor component for liposome production. This molecule consist of complex saccharides with one or more Salicylic acid residues in their polar head group & therefore have one or more negative charge at neutral pH. These are included in liposomes to provide a layer of surface charged group.

b) Synthetic phospholipids

Saturated phospholipids

1. Dipalmitoyl Phosphatidyl choline (DPPC)
2. Distearoyl Phosphatidyl choline (DSPC)
3. Dipalmitoyl Phosphatidyl ethanolamine(DPPE)
4. Dipalmitoyl Phosphatidyl serine (DPPS)
5. Dipalmitoyl phosphatidic acid (DPPA)
6. Dipalmitoyl Phosphatidyl glycerol (DPPG)

Unsaturated phospholipids

1. Dioleoyl Phosphatidyl choline (DOPC)
2. Dioleoyl Phosphatidyl glycerol (DOPG)

c) Polymeric materials

Synthetic phospholipids including diactylenic group in the hydrocarbon chain polymerizes when exposed to U.V, it will lead to formation of polymerized liposomes which have considerably superior permeability barriers to entrapped aqueous drugs.

E.g. for other Polymerisable lipids are lipids comprising of conjugated diene, methacrylate etc.

6.2. Sterols

Cholesterol & its derivatives are often included in liposomes for

- Cholesterol reduce the fluidity or microviscocity of the outer membrane.
- Cholesterol alters the packing of phospholipid molecule in the structure.
- Cholesterol enhances vesicle resistance capacity to form aggregate.

- Cholesterol decreases the permeability of the bilayer membrane to water soluble (hydrophilic) molecules.
- Cholesterol also helps in the formation of Stable membrane in the presence of biological fluids such as plasma.
- This effect used in formulation of i.v. liposomes

6.3. Other Substances

Varieties of different lipids of surfactants are helps in formation of liposomes. There are various single chain surfactants which can form liposomes by mixing with cholesterol. A different type of polyglycerol and polyethoxylated mono and dialkyl amphiphiles used mainly in cosmetic preparations. Single and double chain lipids having fluoro carbon chains may form very stable liposomes. Ionic surfactant like Steryl amine and dicetyl-phosphate incorporated into liposomes so as to impart either a negative or positive surface charge to these structures. A number of compounds having a single long chain hydrocarbon and an ionic head group which are capable of forming vesicles. These consist of quaternary ammonium salts of dialkyl phosphates.

7. Methods of liposome preparation

The three different strategies for the preparation of liposomesare as follows:

1. Mechanical methods
2. Methods based on replacement of organic solvent
3. Methods based on size transformation or fusion of prepared vesicle

7.1. Mechanical methods

7.1.1. Film Method [19]

The original method is still the simplest procedure for the liposome formation but having some limitation because of its low encapsulation efficiency. In this technique liposome are prepared by hydrating the thin lipid film in an organicsolvent and organic solvent is then removed by film deposition under vacuum. When all the solvent get removed, the solid lipid mixture is hydrated using aqueous buffer. The lipids spontaneously swell and hydrate to form liposome. This method yields a heterogeneous sized population of MLVs over 1 micro meter in diameter.

7.1.2. Ultrasonic method [21]

This method is used for the preparation of SUVs with diameter in the range of 15-25. Ultra sonication of an aqueous dispersion of phospholipids is done by two types of Sonicator i.e. either probe Sonicator or bath Sonicator. The probe Sonicator is used for the small volume which requires high energy while the bath Sonicator is employed for the large volume.

a. Methods based on replacement of organic solvents:

In this method lipids are co-solvated in organic solution, which is then dispersed into aqueous phase containing material to be entrapped within the liposome. This method is of two types:

i. **Reverse Phase Evaporation [22]:** The lipid mixture is added to a round bottom flask and the solvent is removed under reduced pressure by a rotary evaporator. The system ispurged with nitrogen and lipids are re-dissolved in the organic phase which is the phase in which the reverse phase vesicle will form. Diethyl ether and isopropyl ether are the usual solvents of choice. After the lipids are dissolved the emulsion are obtained and then the solvent is removed from an emulsion by evaporation toa semisolid gel under reduced pressure. Non encapsulated material is then removed. The resulting liposomes are called reverse phase evaporation vesicles (REV). This method is used for the preparation of large unilamellar and Oligolamellar vesicles formulations and it has the ability to encapsulate large macromolecules with high efficiency.

ii. **Ether Vaporization Method [23]:**

There are two method according to the solvent used:

i. Ethanol injection method.

ii. Ether injection method.

In ethanol injection method, the lipid is injected rapidly through a fine needle into an excess of saline or other aqueous medium. In ether injection method the lipid is injected very slowly through a fine needle into an excess of saline or other aqueous medium.

b.Methods based on size transformation or fusion of preformed vesicles [25-28]

iii. Freeze Thaw Extrusion Method

The freeze thaw method is an extension of the classical DRV method. Liposomes formed by the film method are vortexes with the solute to be entrapped until the entire film is suspended and the resulted MLVs are frozen in luke warm water and thenvortexes again. After two cycles of freeze thaw and vortexing the sample is extruded three times. This is followed by six freeze thaw cycle and addition eight extrusions. This process ruptures and defuses SUVs during which the solute equilibrates between inside and outside andliposome themselves fuse and increase in size to form large Unilamellar vesicle by extrusion technique (LUVET). For the encapsulation of protein this method is widely used .

iv. **The Dehydration- Rehydration Method**

In this method the empty buffer containing SUVs and rehydrating it with the aqueous fluid containing the material to be entrapped after which they are dried. This leads to a dispersion of solid lipids in finely subdivided form. Freeze drying is often the method of choice. The vesicles are than rehydrated. Liposomes obtained by this method are usually Oligolamellar vesicle.

v. **Characterization Of Liposomes**

The behaviour of liposomes in both physical and biological systems in governed by the factors such as:

- Physical size
- Membrane permeability
- Percent entrapped solutes
- Chemical composition
- Quantity and purity of the starting material

Therefore, the liposomes are characterized for physical attributes:

- Shape size and its distribution
- Percentage drug capture
- Entrapped volume
- Lamellarity
- Percentage drug release
- Chemical compositions:
- Estimation of phospholipids
- Phospholipid oxidation
- Analysis of cholesterol

8.1. Vesicle shape and lamellarity: The shape and lamellarity of liposome 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 liposomes [29].

8.2. Vesicle size and size distribution: The size distribution is normally measured by dynamic light scattering. This method is reliable for liposomes with relatively homogenous 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 [30].

8.3. Surface charge determination: Liposomes are usually prepared using charge imparting constituting lipids and hence it is imperative to study the charge on the vesicle surface. The free flow electrophoresis and zeta potential measurement will be done to determine the charge on the surface of vesicles [31-32].

8.4. Encapsulation efficiency: It describes the percent of drug in aqueous phase and hence percent of water soluble drug ultimately entrapped during preparation of liposomes and is usually

expressed as % entrapment/mg lipid. Encapsulation efficiency is assessed using two techniques including mini column centrifugation method and protamine aggregation method. In mini column centrifugation method, the hydrated gel is filled in a barrel of 1 ml syringe without plunger which is plugged with Whatsmann GF/B filter pad. This barrel is rested in a centrifuge tube. This tube is spun at 2000 rpm for 3 min to remove excess saline solution from gel. After centrifugation the gel column should be dried and the eluted saline is removed from collection tube. Liposome suspension of 0.2 ml is applied drop wise to top gel bed, and the column is spun at 2000 rpm for 3 min to expel the void volume containing the liposomes into centrifugation tube. The elution is then removed and set aside for assay [33].

%Entrapment efficiency = Entrapped drug/Total drug × 100

8.5. Entrapped volume: The entrapped volume of a population of liposomes (in µl/mg phospholipids) can often be deduced from measurements of total quantity of solute entrapped inside liposome assuring that the concentration of solute in the aqueous medium inside liposome is the same after separation from entrapped material[34].

8.6. Phase Response and transitional behaviour: Liposome and lipid bilayer exhibit various phase transition that are studied for their role in triggered drug release or stimulus mediated fusion of liposomal constituent with target cell. An understanding of phase transition and fluidity of phospholipids membrane is important both in manufacture and exploitation of liposomes since phase behaviour of liposomal membrane determine such properties such as permeability, fusion, aggregation and protein binding. The phase transition has been evaluated using freeze fracture electron microscopy. They are more comprehensive verified by differential scanning calorimeter analysis [34].

8.7. Stability of liposomes: Liposomal stability includes physical, chemical and biological stability. The physical stability indicates mostly the constancy of the size and the ratio of lipid to active agent. The cationic liposomes can be stable at 4o for 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 phospholipids. These chains are oxidized 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 hydroxyl toluene. Working under nitrogen or argon also minimizes the oxidation of lipids during preparation. The hydrolysis of ester bonds can also be reduced by optimizing pH, temperature, ionic strength, chain length and head group and the

amount of cholesterol incorporated into the bilayer.26 Biological stability of liposomes is limited. Cationic liposomes in plasma are prone to aggregation and exhibit leakage. High density lipoproteins are responsible for destabilization of liposomes prior to interaction of liposomes with circulating phagocytic cells such as monocytes. The destabilization of liposomes is due to the lipid exchange between liposomes and high density lipids [34].

8.8. Drug release: The drug release from the liposomes can be assessed by the use of well calibrated in vitro diffusion cell. The liposome based formulation can be subjected to in vitro assays to predict pharmacokinetics and bio availability of drug before employing costly and time consuming in vivo studies. The dilution induced drug release in buffer and plasma was employed as predictor for pharmacokinetic performance of liposomal formulations and another assay which determined intracellular drug release induced by liposome degradation in presence of mouse-liver lysosome lysate was used to assess the bioavailability of the drug [34].

9. Applications of liposomes[39-42]:

9.1. Cancer chemotherapy: The long term therapy of anticancer drug leads to several toxic side effects. The liposomal therapy to the tumour cell has revolutionized the world of cancer therapy with least side effects. It has been said that the small and stable liposomes are passively targeted to different tumour because they can circulate for longer time. The light sensitive liposomes have been prepared, where light triggers the release of anticancer drugs, like doxorubicin. The light triggered system will reduce the potential toxicity and lead to more effective therapy.

9.2. Gene delivery: Negatively charged or classical liposomes have been used as vehicles for gene transfer into cell in culture. The cationic lipids are able to interact spontaneously with negatively charged DNA to form cluster or aggregated vesicles along the nucleic acid. At a critical liposome density the DNA is condensed and becomes encapsulated with in a lipid bilayer.

9.3. Liposomes for topical applications: Liposomes are proved to be effective in delivering drugs in to the skin. Liposomes increase the permeability of skin for various entrapped drugs. Liposomes can exert different functions after topical application. They can improve drug deposition within the skin at the site of action where the goal is to reduce systemic absorption and thus minimize side effects .They can provide targeted delivery to skin appendages in addition to their potential for transdermal delivery. In the recent studies, it is shown that liposomes penetrate effectively into hair follicles and thus hair follicle penetration can be increased by massaging the skin, which stimulates the in vivo movement of hairs in the hair follicles[43].

9.4. Liposomes for pulmonary delivery: Targeted drug delivery to the lungs, has evolved to be one of the most widely investigated systemic or local drug delivery approaches. The use of drug

delivery system for the treatment of pulmonary diseases is increasing because of their potential for localized topical therapy in the lungs. This route also makes it possible to deposit drugs more site specific at high concentrations within the diseased lung there by reducing the overall amount of drug given to patients, as well as increasing local drug activity while reducing systemic side effects and first pass metabolism.

9.5. Liposome for Nasal administration: For nasally administered products good penetration, is of little use if the formulation is not able to remain in contact with the mucosal surface for a long enough time to enable penetration to occur. Therefore mucoadhesion is a key characteristic of nasally administered formulations**.** The liposomes coated with alginates, chitosan or trimethyl chitosan, which are able to penetrate through the nasal mucosa and offer enhanced penetration over uncoated liposomes when delivered as dry powders. The coating of liposomes may result in some reduction in encapsulation efficiency, still maintained between 60–69% and the structural integrity of the entrapped protein and its release characteristics were maintained.

9.6. Liposomes in parasitic diseases: The conventional liposomes are digested by phagocytic cells in the body after intravenous management; they are ideal vehicles for the targeting drug molecules into these macrophages. Leishmaniasis is a parasitic infection of macrophages which affects over 100 million people in tropical regions and is often deadly. The effectual dose of drugs, mostly different antimonies, is not much lower than the toxic one. Liposomes accumulate in the very same cell population which is infected. The best results reported so far in human therapy are probably liposomes as carriers for Amphotericin B in antifungal therapies. This is the drug of choice in dispersed fungal infections[44].

9.7. Ophthalmic delivery of drugs: Liposomes has been investigated for ophthalmic drug delivery since it offers advantages as a carrier system. It is biodegradable and biocompatible Nano carrier. It can enhance the permeation of poorly absorbed drug molecules by binding to the corneal surface and improving residence time. To reduce the drug loss and side effects associated with conventional eye drops, a novel approach was introduced, where the liposomes made up of dimyristoylphosphatidylcholine are dispersed in contact lens hydrogels made up of poly-2-hydroxyethyl methacrylate. The contact lens loaded with hydrogels is transparent in nature and deliver drugs at therapeutic level for few days.

9.8. Liposomes for Brain targeting: The liposomal technology is quite advanced to design with better site specific action. The basic reason for the acceptance of liposomal carrier is due to their controlled profile or drug release nature as well as due to their selected targeting mechanism. The surface modified liposomes can be used to directly encapsulate drug molecules to diseased tissues

or organs. The brain distribution of liposomes can be modulated by conjugation of appropriate targeting vectors, like monoclonal antibody. The mechanism involved in the concentration of liposomes in brain by crossing blood brain barrier–coupling of liposomes with brain drug transport vector through absorptive mediated transcystosis or by receptor mediated transcystosis.

Table no.3: List of some marketed products of liposomes

Marketed product	Drug used	Target disease	Company
DaunoXomeTM	Daunsolid, tumoursorubicin	Kaposi's sarcoma Breast and lung cancer	NeXstar, USA
AmphotecTM	Amphotericin B	Fungal infections	SEQUUS, USA
ALECTM	Dry protein free powder of DPPC-PG	Lung disease in babies	Britannia pharm, UK
VENTUSTM	Prostaglandin-E1	Systemic	The liposome
Novasome	Smallpox vaccine	Smallpox	Novavax, USA
Depocyt	Cytarabine	Cancer therapy	Skye pharm, USA
Topex-Br	Terbutaline sulphate	Asthma	Ozone, USA
Fungizone	AmphotericinB	Fungal infections	SEQUUS, USA
Avian retrovirus	Killed Avian	Chickenpox	Vineland lab, USA
DoxilTM	Doxorubicin	Kaposi's sarcoma	SEQUUS, USA

Table no. 4: List of some liposomal cosmetics formulation in the market

Product	Manufacturer	Liposomes and key ingredient
Nactosomes Lancome	Lancome	Vitamins
Niosomes Lancome	L'Oreal	Glyceropolyether with moisturizers
Future perfect skin gel	Estee Lauder	TMF vitamins E, A Palmitate
Formulae liposome	Payot	Thyroxin, hyaluronic acid

gel		
Efect du soleil	L'Oreal	Tanning agent in liposome
Inovita	Pharm Apotheke	Thymus extract, hyaluronic
Eye perfector	Avon soothing	Niosomes. Creams to reduce eye
Natipide II	Natter Mann PL	Liposomal gel for do it yourself
Flaw less finish	Elizabeth Arden	Liquid makeup
Symphatic 2000	Biopharm, GmbH	Thymus extract of Vitamin A Palmitate

Conclusion:

Almost from the time of their discovery in 1960's and the demonstration of their entrapment potential, liposome vesicle have drawn attention of researchers as potential carriers of various bioactive molecules that could be used for therapeutic applications in human and animals. Many factors contribute to their success as drug delivery vehicles. Liposomes solubilise lipophilic drug candidates that would otherwise be difficult to administer intravenously. The encapsulated drug is inaccessible to metabolizing enzyme; conversely, body component such as erythrocyte and tissue injection site are not directly exposed to full dose of the drug. Liposomes can cross the BBB because of the lipophilic nature of the phospholipids, so even the hydrophilic drugs (which otherwise cannot easily cross the BBB) might be formulated as liposomes. Liposome can prolong the drug action by slowly releasing the drug in the body. The new developments in the liposome are the specific binding properties of a drug-carrying liposome to a target cell (tumour cell and specific molecules), stealth liposomes for targeting hydrophilic (water soluble) anticancer drugs like doxorubicin, mitoxantrone which leads to decrease in side effects becausethe drug is mostly concentrated at the site of action. Several commercialliposomes have already been discovered, registered and introduced with great success in pharmaceutical market.

References:

1. Vyas SP, Khar RK. Targeted & Controlled drug delivery-Carrier Concept in drug delivery. New Delhi, CBS Publishers. 2002: 38-80.
2. Loveleenpreet Kaur. Liposome As A Drug Carrier – A Review. IJRPC.2013; 3(1): 121-128.
3. Mansoori and Agarwal. A Review On Liposome. IJARPB. 2012; 2 (4):453-464.
4. Deepthi V and Kavitha A NLiposomal Drug Delivery System-A ReviewJournal of Pharmaceutical Sciences, 2014;4(2).

5. Samad A, Sultana Y, Aqil M. Liposomal Drug Delivery Systems: An Update Review, *Current Drug Delivery*, 2007, *4*, 297-305 297.
6. Nagasamy Venkatesh Dhandapani. Liposome As Novel Drug Delivery System: A Comprehensive Review. International Journal of Research and Pharmaceutical Science. 4(2): 187-193.
7. Vladimir P.Torchilin. Recent Advances With Liposomes As Pharmaceutical Carriers:Nature Reviews. Drug Discovery. 2005; 4(5): 145-160.
8. K. Egbaria and N. Weiner. Liposomes as a Topical Drug Delivery system. Advanced Drug Delivery Reviews. 1990; 5(1): 287-300.
9. Akbarzadeh et al. Liposome: Classification, preparation, and application.A springer open journalof Nano scale Research. 2013; 8(102): 1088-1107.
10. Patidar et al. Liposome: new strategy in drug delivery. IJPLCP. 2015; 6(7): 4615-4619.
11. Sonia Tyagi, Pramod Kumar Sharma and Rishabha Malviya. Advancement and Patents on Liposomal Drug Delivery. Global Journal of Pharmacology. 2015; 9(2): 166-173.
12. Hemanth Kumar et al. Liposomal Encapsulation Technology: A Novel Drug Delivery System Designed For Ayurvedic Drug Preparation. IRJP. 2011; 2(10):4- 6.
13. Olga Popovska et al.An Overview: Methods for Preparation and Characterization of Liposomes as Drug Delivery Systems. International journal of Pharmacy and Phytopharmacol research. 2013; 3(2):2249-6084.
14. Zhang et al. Preparation, Characterization, and Pharmacodynamics of Thermo sensitive Liposomes Containing Docetaxel. Research Journal of Pharmaceutical Sciences. 2014; 1(3):2177–2183.
15. Rakesh et al. Ethosomes for Transdermal And Topical Drug Delivery. International journal of Pharmacyand Pharmaceutical Science. 4(3):17-24.
16. R.G.S. Maheshwari et al. Ethosomes and ultra-deformable liposomes for transdermal delivery of Clotrimazole. A comparative assessment Saudi Pharmaceutical Journal. 2012; 2(1):161-170.
17. Devi R et al. Formulation, characterization and evaluation of fluconazole liposomes. Pelagia Research Library Der Pharmacia Sinica. 2015;6(5):61-66.
18. S. Rathod et al. Design and Evaluation of Liposomal Formulation of Pilocarpine Nitrate. Indian Journal of Pharmaceutical Science: 72(2): Mar-Apr 2010.
19. Prem Kumar et al. Formulation and Evaluation of Econazole Niosomes. Sch. Acad. J. Pharm: 2(4):315-318, 2013.
20. Arvind. Formulation and Characterization of Amphotericin B Liposomes Prepared By Thin Film Hydration Method. Int J Pharm: 3(3): 540-547, 2013.

21. K L Senthilkumar, Preparation and Characterization of Nabumetone Liposomes Issn 2250-3137 www.ijlbpr.comVol.1, Issue. 1, January 2012 © 2012 IJLBPR.

22. Sakthivel, Formulation And In Vitro Evaluation Of Niosomes Containing Oxcarbazepine Int J Pharm Sci, Vol 4, Issue 3, ISSN- 0975-1491 563-567.

23. Patel et al, Formulation and Evaluation of Liposomes of Ketoconazole IJDDT July-September 2009, Vol 1, Issue 1(16-23).

24. S. S. Patel, Development and Evaluation of Liposomes for Topical Delivery of Tacrolimus (Fk-506) J. Sci. Res. 2(3) 585-596 (2010).

25. Patel H, Development Of Stabilized Calcium Phosphate Coated Liposome Ijprbs, 2014; Volume 3(3) ISSN: 2277-8713 : 559-580.

26. Alok Kumar Srivastav , To Study the Formulation of Niosome of Ofloxacin and Its Evaluation for Efficacy of Anti-Microbial Activity IJIRSET Vol. 3, Issue 12, December 2014 ISSN: 2319-8753 17958- 17965.

27. Eskandar moghimi pour, Trimethylchitosan coated insulin liposomes for oral delivery, preparation and characterization Int. J. Curr. Res. Chem. Pharma. Sci. 2(10): (2015): (p-ISSN: 2348-5213) 58–66.

28. Tanmay N. Patel, Preparation and Evaluation of Imiquimod Loaded Liposomal Dispersion: Part-I Journal of Biomedical and Pharmaceutical Research 2 (2) 2013, ISSN: 2279 - 0594 56-62.

29. Nam-Joon Cho, Comparison of Extruded and Sonicated Vesicles for Planar Bilayer Self-Assembly Materials 2013, 6, 3294-3308.

30. Raheem saba, Abdul ridha, Effect of method of physical properties of ciprofloxacin hcl elastic liposomes intended to be utilized in treatment of acne vulgaris , INT. J. RES. Ayurveda pharm. 4(5), sep-oct 2013 , 742-746.

31. R. Silva, Effect of ultrasound parameters for unilamellar liposome preparation Ultrasonic sonochemistry 17(2010)628-632.

32. Ryan caddell, Ultrasound-enhanced Microfluidic Synthesis of Liposomes Anticancer Research 30: 463-466 (2010).

33. P. Roy Vagelos, Procedure for preparation of liposomes with large internal aqueous space and high capture by reverse-phase evaporation Proc. Natl. Acad. Sci. USA Vol. 75, No. 9, pp. 4194-4198.

34. Machado, Application of sonication and mixing for Nano encapsulation of the cyanobacterium Spirulina platensis in liposomes, IFRJ 21(6) (2014): 2201-2206.

35. Rawal Gaurav, Liposomal Drug Delivery System: An Overview, International Journal of Pharmaceutical &Biological Archives 2011; 2(6) ISSN 0976 – 3333 :1575-1580.

36. Robert L. Hamilton, Unilamellar Liposomes Made With The French Pressure Cell: A Simple Preparative And Semiq Uant Itat Ive , Technique Journal of Lipid Research Volume 2 1, 1980 , 981-992.

37. D. Lichtenberg, A New Method For Preparation Of Phospholipid Vesicles (Liposomes) French Press, Biomedical Press Volume 99, number 1 March 1979 , 210-214.

38. Peter I. Lelkes, Interaction Of French-Pressed Liposomes With Isolated Bovine Adrenal Chromaffin Cells the journal of biological chemistry Vol. 260, No. 3, Issue of February 10, pp. 1796-1803,1985.

39. Chiraz Jaafar-Maalej , Ethanol Injection Method For Hydrophilic And Lipophilic Drug-Loaded Liposome Preparation, Informa Health Care Journal Of Liposome Research, 2010; 20(3): 228–243.

40. Musavad Sunny, Liposomes – A Unique Transdermal Drug Delivery System IJPCS Vol. 1 (3) 500, ISSN: 2277 Jul-Sep 2012.

41. Navneet Kumar Verma, A Brief Study on Liposomes-A Review AJCPR 2014 Vol 2 , (1) ISSN: 2347-8322 : 112-12.

42. Sayali V.Tarkunde, Liposome – A Novel Drug Delivery Int. J. Pure App. Biosci. 2 (6): 92-102 (2014) ISSN: 2320 – 7051.

43. Anshita saini, A platform for liposomal drug delivery; Int.J.Pharm Drug Anal Vol: 3 Issue:1 ,JAN 2015 ISSN: 2348-8948 Page:6-11.

44. Leslie A. Morton, Jonel Saludes, Constant Pressure-controlled Extrusion Method for the Preparation of Nanosized Lipid Vesicles, J Vis Exp. 2012; (64): 4151.