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Liposomes for Targeted 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: Liposomes, phospholipid, lamellarity, stability, entrapped volume.

Contents

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1. 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. 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. Liposomes were first described by British haematologist, Dr Alec D Bangham FRS in 1961 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. 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. The assembly into closed bilayered structures is a spontaneous process. 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. 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• m, the small unilamellar vesicle[SUV] size range is 0.02€0.05 • m, and the large unilamellar vesicle [LUV] size range varies from 0.06 • m 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.

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 blood stream 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 all these parameters determine the fate of liposomes on the shelf and in vivo. **[1, 2, 3,]**

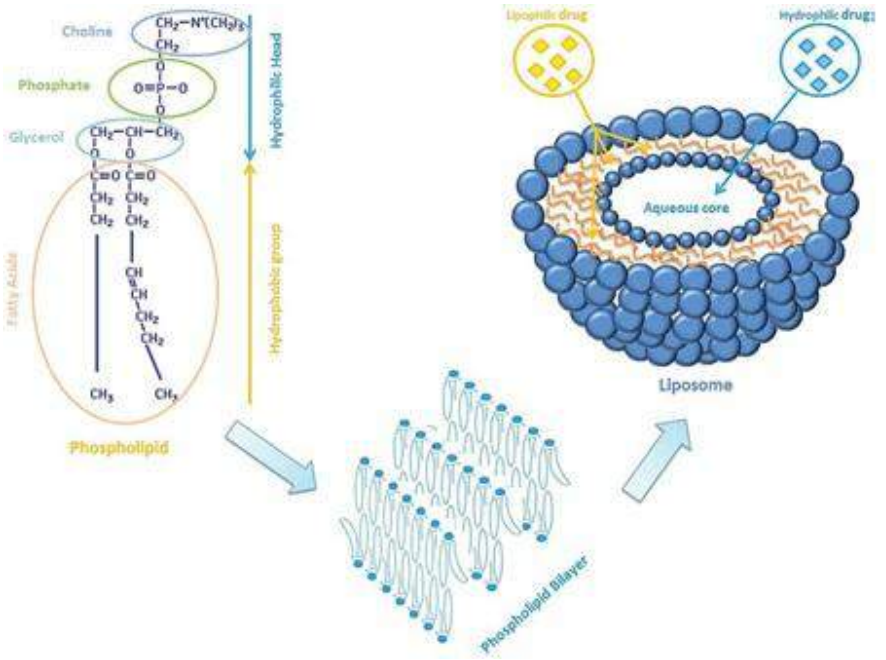

Figure 1: Formation of liposomes

1.1 Advantages[4]

Some of the advantages of liposome are as follows:

- Provides selective passive targeting to tumor tissues (Liposomal doxorubicin).
- Increased efficacy and therapeutic index.
- Increased stability via encapsulation.
- Reduction in toxicity of the encapsulated agents.
- Site avoidance effect.
- Improved pharmacokinetic effects (reduced elimination, increased circulation life times).
- Flexibility to couple with site specific ligands to achieve active targeting.

1.2 Disadvantages[5]

- Production cost is high.
- Leakage and fusion of encapsulated drug /molecules.
- Sometimes phospholipid undergoes oxidation and hydrolysis like reaction.
- Short half-life.
- Low solubility
- Inadequate stability due to uptake by Reticuloendothelial system.

1.3 Reasons to use Liposomes as Drug Carriers [6]

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*f*. 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.

1.4 Mechanism of Liposome Formation [7]

Liposomes are vesicular structures consisting of hydrated bilayers. Liposomes structures used for pharmaceutical purposes consist of a phospholipid backbone. But other classes of molecules can form bilayer based vesicular structures as well. On the other hand not all the hydrated phospholipids form bilayer structures. Other forms of self aggregation such as inverted hexagonal phases or micelles with completely different properties can occur. The common feature that all bilayer forming compounds share is their amphilicity. They have defined polar and nonpolar regions. In water the hydrophobic regions tend to self aggregate and the Polar Regions tend to be in contact with the water phase. Israelachvili and coworkers defined critical packing parameter p by

$$P = v/ a0lc$$

Where v is hte molecular volume of the hydrophobic part, a0 is the optimum surface area per molecule at the hydrocarbon water interface, and lc is the critical half thickness for the hydrocarbon region which must be less than the maximum length of the extended lipid chains. For p < 1/3, spherical micelles are formed. In this category fall single chain lipids with large head group areas.eg lysophosphatidylcholine. For 1/3 < ½ globular or cylindrical micelles are formed. Double chain „fluid state… lipids with large head area (1/2 < p <1) form bilayers and vesicles. This occurs also with double chain „gel state… lipids with small headgroups and p~1. For p>1 inverted structures such as the inverted hexagonal phase can be observed. An addtional condition required for bilayer formation is that the compound can be classified as a nonsoluble swelling ampiphile.

1.4 Type of Liposomes [8]

Depending upon the structure there are two types of liposomes.

a) Unilamellar liposomes: Unilamellar vesicles have a single phospholipid bilayer sphere enclosing aqueous solution.

b) Multilamellar Liposomes: Multilamellar vesicles have onion structure. Typically, several Unilamellar vesicles will form one inside the other in diminishing size, creating a Multilamellar structure of concentric phospholipid spheres separated by layers of water.

1.5 Classification of Liposomes [9]

Liposomes are classified on the basis of:

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

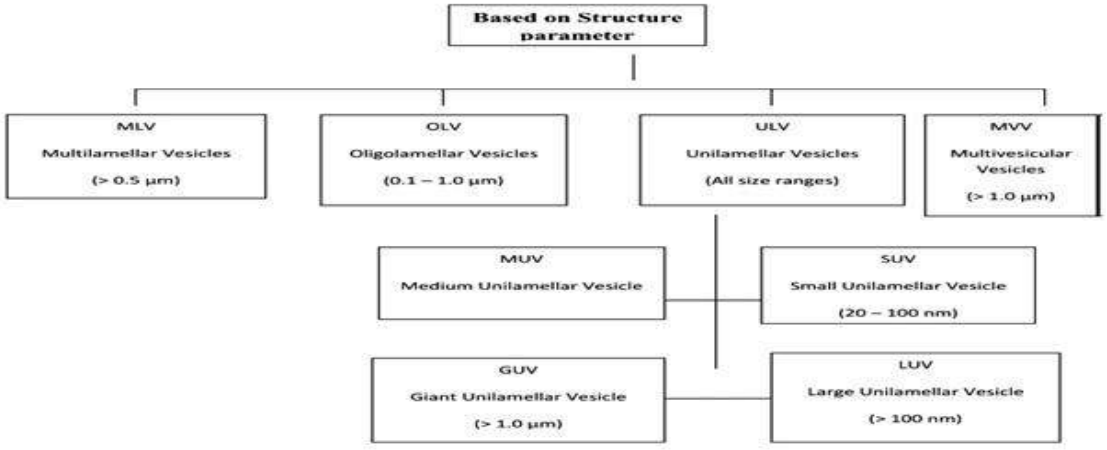

Figure 2

1.5.1 Classification of liposomes based on Structural parameters

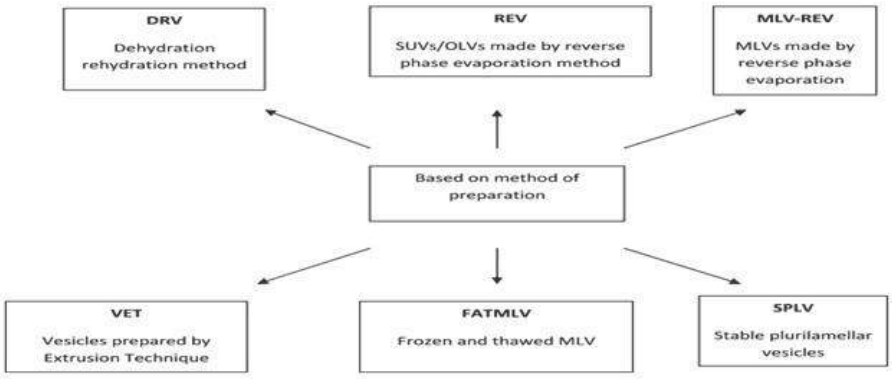

Figure 3

1.5.2Classification of liposomes based on method of preparation

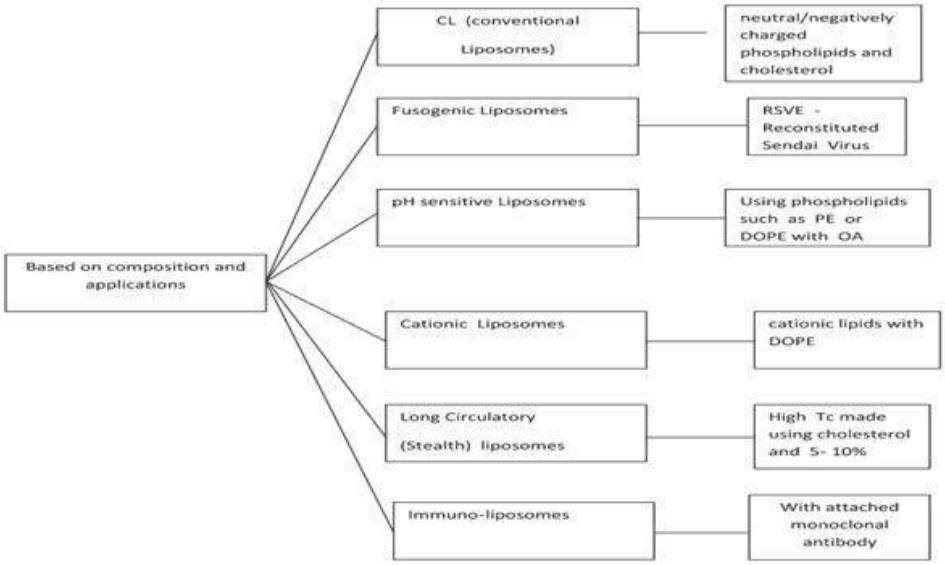

Figure 4

1.5.3 Classification of liposomes based on composition and applications

1.5.4 Based Upon Conventional Liposome

a. Stabilize natural lecithin (PC) mixtures
b. Synthetic identical, chain phospholipids
c. Glycolipids containing liposome

1.5.5 Based Upon Specialty Liposome

a. Bipolar fatty acid
b. Antibody directed liposome.
c. 3. Methyl/ Methylene x- linked liposome.
d. Lipoprotein coated liposome.
e. Carbohydrate coated liposome.
f. Multiple encapsulated liposome.

1.6 Structural Components of Liposomes [10]

Membrane forming components

(a) Phospholipids: Bilayer formers

Phospholipids that are the major components of the biological membranes are the building blocks of the liposomes. The phospholipids have tubular shape owning to the presence of two acyl chains attached to a polar head and on hydration, results into a bilayered membrane. Two types of phospholipids are there i.e. phosphodiglycerides and sphingolipids along with their corresponding hydrolysis products.

Classification of phospholipids

1. Neutral phospholipids e.g. Sphingomyelin, Phosphatidylethanolamine and Phosphatidylcholine.
2. Negatively charged phospholipids e.g. Dipalmitoylphosphatidylcholine, Dipalmitoylphosphatidyl acid [DDPA], Distearoylphosphatidyl choline [DSPC], Dioleoylphosphatidyl choline [DOPC] etc.
3. Positively charged phospholipids e.g. 1, 2-dihexadecyl-N, N-dimethyl€N-trimethyl amine methyl ethanol amine etc.

(b). Membrane Additives [Sterols]

Cholesterol is the most commonly used sterol, which is included in the liposomal membranes. It has been called as the †motar ‡ of bilayers because by virtue of its molecular shape and solubility properties it fills in empty spaces among the phospholipid molecules, anchoring them more strongly into the structure. Cholesterol is an amphipathic molecule and inserts itself into the membrane with its hydroxyl groups oriented towards the aqueous phase and aliphatic chain aligned parallel to acyl chains of the phospholipid molecules. In other words, cholesterol increases the transition temperature of the system by making the membrane more ordered. Cholesterol reduces this type of interaction to a great extent and provides both physical and biological stability.

(c). Charge inducers and steric stabilizers

Stearylamine, dicetylphosphate, solulan C-24 and diacylglycerol are commonly used to impart either a negative or a positive surface charge. Since it is a well-known fact that negatively charged and positively charged liposomes are more rapidly up taken by the reticulo-endothelial system as compared to neutral liposomes, charge inducers are used to overcome this problem. Also they proved to be useful in reducing aggregation as neutral liposomes show higher tendency to undergo aggregation.

(d). Other substances:

In case, the drug is very prone to oxidation, antioxidants e.g. tocopherol, butylatedhydroxy toluene and stabilizers are used. The use of preservatives is very common to increase the shelf-life of liposomal formulations.

2. Preparation Method

2.1 Classical technique [11]

There are four classical methods of liposome manufacture. The difference between the various methods is the way in which lipids are drying down from organic solvents and then redispersed in aqueous media. These steps are performed individually or are mostly combined.

2.1.1 Hydration of a thin lipid film: bangham method

This is the original method which was initially used for liposomes production. A mixture of phospholipid and cholesterol were dispersed in organic solvent. Then, the organic solvent was removed by means of evaporation (using a Rotary Evaporator at reduced pressure). Finally, the dry lipidic film deposited on the flask wall was hydrated by adding an aqueous buffer solution under agitation at temperature above the lipid transition temperature. This method is widespread and easy to handle, however, dispersed-phospholipids in aqueous buffer yields a population of multilamellar liposomes (MLVs) heterogeneous both in size and shape (1€5 €m diameter). Thus, liposome size reduction techniques, such as sonication for SUVs formation or extrusion through polycarbonate filters forming LUVs were useful to produce smaller and more uniformly sized population of vesicles.

2.1.2 Reverse-phase evaporation (rev) technique

A lipidic film is prepared by evaporating organic solvent under reduced pressure. The system is purged with nitrogen and the lipids are re-dissolved in a second organic phase which is usually constituted by diethyl ether and/or isopropyl ether. Large unilamellar and oligo lamellar vesicles are formed when an aqueous buffer is introduced into this mixture. The organic solvent is subsequently removed and the system is maintained under continuous nitrogen. These vesicles have aqueous volume to lipid ratios that are 30 times higher than sonicated preparations and 4 times higher than multilamellar vesicles. Most importantly, a substantial fraction of the aqueous phase (up to 62% at low salt concentrations) is entrapped within the vesicles, encapsulating even large macromolecular assemblies with high efficiency.

2.1.3 Solvent (ether/ethanol) injection technique

The solvent injection methods involve the dissolution of the lipid into an organic phase (ethanol or ether), followed by the injection of the lipid solution into aqueous media, forming liposomes. The ethanol injection method was first described in 1973. The main relevance of the ethanol injection method resides in the observation that a narrow distribution of small liposomes (under 100 nm) can be obtained by simply injecting an ethanolic lipid solution in water, in one step, without extrusion or sonication.

The ether injection method differs from the ethanol injection method since the ether is immiscible with the aqueous phase, which is also heated so that the solvent is removed from the liposomal product. The method involves injection of ether-lipid solutions into warmed aqueous phases above the boiling point of the ether. The ether vaporizes upon contacting the aqueous phase, and the dispersed lipid forms primarily unilamellar liposomes. An advantage of the ether injection method compared to the ethanol injection method is the removal of the solvent from

the product, enabling the process to be run for extended periods forming a concentrated liposomal product with high entrapment efficiencies.

2.1.4 Detergent dialysis

Liposomes, in the size range of 40€180 nm, are formed when lipids are solubilized with detergent, yielding defined mixed micelles. As the detergent is subsequently removed by controlled dialysis, phospholipids form homogeneous unilamellar vesicles with usefully large encapsulated volume. Other methods have been already used for liposomes preparation such as: calcium induced fusion, nanoprecipitation, and emulsion techniques. However, these classical techniques require large amounts of organic solvent, which are harmful both to the environment and to human health, requiring complete removal of residual organic solvent. Furthermore, conventional methods consist of many steps for size homogenization and consume a large amount of energy which is unsuitable for the mass production of liposomes.

2.2 New large-scale liposome technique

Since industrial scale production of liposomes has become reality, the range of liposome preparation methods has been extended by a number of techniques such as Heating Method, Spray drying, Freeze Drying, Super Critical Reverse Phase Evaporation (SCRPE), and several modified ethanol injection techniques which are increasingly attractive.

2.2.1 Heating method

A new method for fast production of liposomes without the use of any hazardous chemical or process has been described. This method involves the hydration of liposome components in an aqueous medium followed by the heating of these components, in the presence of glycerol (3% v/v), up to 120^0C. Glycerol is a water-soluble and physiologically acceptable chemical with the ability to increase the stability of lipid vesicles and does not need to be removed from the final Liposomal product. Temperature and mechanical stirring provide adequate energy for the formation of stable liposomes. Reza Mozafari et al. confirmed by TLC that no degradation of the used lipids occurred at the above mentioned temperatures. The particle size can be controlled by the phospholipid nature and charge, the speed of the stirring and the shape of the reaction vessel. Otherwise, employment of heat abolishes the need to carry out any further sterilisation procedure reducing the time and cost of liposome production.

2.2.2 Spray-drying

Since spray-drying is a very simple and industrially applicable method, the direct spray-drying of a mixture of lipid and drug was applied in the preparation of liposomes. The spray-drying process is considered to be a fast single-step procedure applied in the nanoparticles formulation. Hence, liposomes were prepared by suspending lecithin and mannitol in chloroform. The mixture was sonicated for 8 min (bath sonicator) and subjected to spray-drying on a Buchi 190 M Mini Spray Dryer. The spray-drying conditions were as follows: inlet and outlet temperatures were 120^0C and 80^0C, respectively; airflow rate was 700 NI/hr; and the flow rate was 1000 ml/hr. The dried product was hydrated with different volumes of phosphate buffered saline (PBS; pH 7.4) by stirring for 45 min. The main factor influencing the liposomal size was the volume of aqueous medium used for hydration of the spray-dried product. However, mannitol plays an important role in increasing the surface area of the lipid mixture, enabling successful hydration of the spray-dried product.

2.3. Freeze drying

This new method was described for the preparation of sterile and pyrogen-free submicron narrow sized liposomes. It is based on the formation of a homogenous dispersion of lipids in water-soluble carrier materials. Liposome-forming lipids and water-soluble carrier materials such as sucrose were dissolved in tert-butyl alcohol/water cosolvent systems in appropriate ratios to form a clear isotropic monophase solution. Then the monophase solution was sterilized by filtration and filled into freeze-drying vials. In recent study, a laboratory freeze drier was used and freeze-drying process was as follows: freezing at ˆ 40^0C for 8 h; primary drying at ˆ 40^0C for 48 h and secondary drying at 25 ^{0}C for 10h.The chamber pressure was maintained at 20 Pascal during the drying process. On addition of water, the lyophilized product spontaneously forms homogenous liposome preparation. After investigation of the various parameters associated with this method it is found that the lipid/carrier ratio is the key factor affecting the size and the polydispersity of the liposome preparation. Therefore, TBA/water cosolvent system was used for economy concerns.

2.4. Super critical reverse phase evaporation (scrpe)

The SCRPE is a one-step new method that has been developed for liposomes preparation using supercritical carbon dioxide. This method allowed aqueous dispersions of liposomes to be obtained through emulsion formation by introducing a given amount of water into a homogeneous mixture of supercritical carbon dioxide/LR-Dipalmitoyl phosphatidylcholine /ethanol under sufficient stirring and subsequent pressure reduction. Transmission electron microscopy observations revealed that vesicles are large unilamellar with diameters of 0.1€1.2•m. The trapping efficiency of these liposomes indicated more than 5 times higher values for the water-soluble solute compared to multilamellar vesicles prepared by the Bangham method. The trapping efficiency for an oil soluble substance, the cholesterol, was about 63%. Results showed that the SCRPE is an excellent technique that permits one-step preparation of large unilamellar liposome exhibiting a high trapping efficiency for both water-soluble and oil-soluble compounds.

2.5. Modified ethanol injection method

Novel approaches based on the principle of the ethanol injection technique such as the micro fluidic channel method, the cross flow-injection technique, and the membrane contactor method were recently reported for liposome production.

2.5.1. The Cross flow Injection Technique

The concept of continuous cross flow injection is a promising approach as a novel scalable liposome preparation technique for pharmaceutical application. Wagner et al. used a cross flow injection module made of two tubes welded together forming a cross. At the connecting point, the modules were adapted with an injection hole. The influencing parameters such as the lipid concentration, the injection hole diameter, the injection pressure, the buffer flow rate, and system performance were investigated. A minimum of buffer flow rate is required to affect batch homogeneity and strongly influencing parameters are lipid concentration in combination with increasing injection pressures. After exceeding the upper pressure limit of the linear range, where injection velocities remain constant, the vesicle batches are narrowly distributed, also when injecting higher lipid concentrations. Reproducibility and scalability data show similar results with respect to vesicle size and size distribution and demonstrate the stability and robustness of the novel continuous liposome preparation technique.

2.5.2. Microfluidization

By using a micro fluidic hydrodynamic focusing (MHF) platform, Jahn et al. generated liposomes by injecting the lipid phase and the water phase into a micro channel. Microfluidic flow is generally laminar due to the small channel dimensions and relatively low flow rates. Well-defined mixing is then obtained by interfacial diffusion when multiple flow streams are injected in a microchannel. The size of the liposomes was mainly controlled by changing the flow rate.

2.5.3. Membrane contactor

Recently, Jaafar- Maalej et al. applied the ethanol injection technique while using a membrane contactor for large scale liposomes production. In this method, a lipid phase (ethanol, phospholipid and cholesterol) was pressed through the membrane with a specified pore size. Nitrogen gas at pressure below 5 bar was sufficient for passing the organic phase through the membrane. At the same time, the aqueous phase flew tangentially to the membrane surface and swept away the formed liposomes within the membrane device. The new process advantages are the design simplicity, the control of the liposome size by tuning the process parameters and the scaling-up abilities. As a result, these techniques lead from the conventional batch process to potential large scale continuous procedures.

3. Stability of Liposomes

Liposomes face a number of chemical and physical destabilization processes. So liposomes stability is an important consideration while studying liposomes. This aspect of liposomes stability has two aspects physical and chemical stability.

3.1 Physical stability[12,13]

Physical processes that effect shelf life includes loss of liposome associated drug and changes in size, aggregation and fusion. Aggregation is the formation of larger units of liposome material, these units are still composed of individual liposomes. In principle, this process is reversible, e.g., by applying mild shears forces, or by changing the temperature or by binding metal ions that initially induced aggregation. Fusion indicates that new colloidal structures were formed. As fusion is an irreversible process, the original liposomes can never be retrieved. Drug molecules can leak from liposomes. The leakage rate strongly depends on the bilayer composition and the physiochemical nature of the drug. Bilayers in the gel state or those containing substantial (molar) fractions of cholesterol tend to lose associated drug only slowly; liquid state bilayers are more prone to drug loss and are less stable during storage. Bilayer permeability is not necessarily a constant parameter. Chang in bilayer permeability can occur as a result of chemical degradation processes, such as the formation of lypo-PC and FA.

3.2 Chemical stability

a) Hydrolysis of the ester bonds

Phosphatidylcholine possesses for ester bonds. The two acyl ester bonds are most liable to hydrolysis. The glycerophosphate and phosphocholine ester bonds are more stable. The 1-acyl-lysophosphatidylcholine (LPC) and 2- acyl LPC are both formed at comparable rates.

b) Lipid peroxidatin of phospholipids

The polyunsaturated acyl chains of phospholipids are sensitive to oxidation via free radical reactions. Cyclic peroxides, hydroperoxides, malonilaldehyde, alkanes are the major degradation products. Low oxygen pressure, absence of heavy metals, addition of anti-oxidants, complexing agents (EDTA, etc), quenchers (beta-carotene) of the photo-oxidation reactions improve resistance against lipid peroxidation.

4. Characterization of Liposomes

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.

4.1 Visual Appearance [14]

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 anon liposomal dispersion and is most likely a disperse inverse hexagonal phase or dispersed microcrystallites. An optical microscope (phase contrast) can detect liposome> 0.3 •m and contamination with larger particles.

4.2 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 -4Band -2B columns can separate SUV from micelles.

4.3 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.

4.4 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 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 tocopherol and butyl hydroxy 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 the pH, temperature, ionic strength, chain length and head group and the amount of cholesterol incorporated into the bilayer. There are many aspects to physical instability. Stabilization may be achieved by careful selection of the bilayer components, for example cholesterol is added to permeable bilayers to decrease leakage rates. Physical stability study is performed to investigate the leak out of the drug from liposome during storage.

4.5 Entrapped Volume

The entrapped volume of a population of liposome (in •l/ 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.

%Entrapment efficiency = Entrapped drug (mg) /Total Drug Added (mg) ×100

4.6 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 met-hods 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.

5. Applications

I. Liposome as drug/protein delivery vehicles[15,16, 17, 18]

- Controlled and sustained drug release
- Enhanced drug solubilization
- Altered pharmacokinetics and bio-distribution
- Enzyme replacement therapy andbio-distribution
- Enzyme replacement therapy and lysosomal storage disorders

II. Liposome in anti-microbial, antifungal and antiviral therapy

- Liposomal drugs
- Liposomal biological response modifiers

III. Liposome in tumor therapy

- Carrier of small cytotoxic molecules

- Vehicle for macromolecules as cytokines orgenes

IV. Liposome in gene delivery

- Gene and antisense therapy
- Genetic [DNA] vaccination

V. Liposome in immunology

- Immunoadjuvant
- Immunomodulator
- Immunodiagnosis

VI. Liposome as artificial blood surrogates

VII. Liposome as radiopharmaceutical and radio diagnostic carriers

VIII. Liposome in cosmetics and dermatology

IX. Liposome in enzyme immobilization and bioreactor technology

X. Miscellaneous Applications

- PEGylated Liposomal doxorubicin in the treatment of ovarian cancer.
- Liposomal daunorubicin in the treatment of leukemia.
- Liposomal neomycin and penicillin are widely used in antimicrobial therapy.
- Liposomal rifabutin is active against M.avium infection.
- Liposomes are used for gene therapy such as Allovectin-7 TM for treatment of metastatic
- Melanoma and colorectal carcinoma.
- Leuprolide acetate liposomes are widely used in the intravenous administration of peptide drugs.
- Liposomal antioxidants are widely used for the treatment of oxidative stress such as quercetin, Astaxanthin, Resveratrol
- Liposomal verteporfin is used for the treatment of ocular histoplasmosis and subfoveal Choroidal neovascularization [CNV].
- Silica coated liposomes showed enhanced insulin delivery and very active in reducing the Glucose levels.
- Stealth liposomes are used in vaccines, diagnostic imaging, inflammatory diseases, Gene transfection and targeted drug delivery.
- Transferosomes are widely used for the treatment of skin diseases by employing corticosteroids.

6. List of Marketed Liposomal Products [19]

Table 1: List of liposomal products available in market

S.No	Product name	Drug	Manufacturer [country]
1	Abelcet	Amphotericin B	The liposome company [USA]
2	Allovecti-711	HLB-B7 plasmid	Vical incorporation[USA]
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]
7	L-AMP-LRC-1	Amphotericin B	Seth G.S. medical college and K.E.M. hospital[India]
8	MiKasomeTM	Amikacin	NeXatar pharmaceuticals[USA]

7. 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.

8. References

1. Chauhan Tikshdeep, Arora Sonia, Parashar Bharat and Chandel Abhishek. Liposome Drug Delivery: A Review, International Journal Of Pharmaceutical And Chemical Sciences Jul-Sep **2012**, Vol. 1 (3); 754-61.
2. Bangham AD, Standish MM and Watkens JC. Diffusion of univalent ions across the lamellae of swollen phospholipids, J Mol Biol, **1965**, Vol.13(1): 238.

3. Bangham AD and Horne RW. Negative staining of phospholipids and their structural modification by surface-active agents as observed in the electron microscope, J Mol Bio, **1964**, Vol.8 (2): 660-668.
4. Prabhakar Vishvakrama and Saurabh Sharma, Liposomes: An Overview, International Journal of Research in Pharmaceutical and Biomedical Sciences, **2012**,Vol. 3(3): 1074-76
5. Kant Shashi, kumar Satinder, Prashar bharat, A Complete review on liposomes, international research journal of pharmacy, **2013**, 3(7): 10.
6. Storm G and Crommelin DJA. Liposomes: quo vadis, Pharmaceutical Science & Technology Today. **1998**, 1(1): 19€31.
7. Priyanka R Kulkarni, Jaydeep D Yadav, Kumar A Vaidya, Liposomes: A Novel Drug Delivery System, International journal of current pharmaceutical research, **2011**, 3(2): 11-12.
8. Lalita K. Lende, N. D. Grampurohit,D.D, Gaikwad, M. V. Gadhave, Jadhav S.L, World Journal Of Pharmacy And Pharmaceutical Sciences, **2012**, 1(4): 1210-11.
9. J.S. Dua, A. C. Rana, A. K. Bhandari, Liposome: Methods of Preparation and Applications, International Journal of Pharmaceutical Studies and Research, **2012**: 3(2): 14-16.
10. V.N.L.Sirisha, I.Bhavani Harika, B.Sruthi, M.Namrata, P.Kirankumar, Y.Kiran Kumar Rao, K.Pranavi, S.Sindhura, N.Vamsi Krishna, O.UmaMaheshwaraRao, IOSR Journal of Pharmacy, **2012**, 2(5): 27,35.
11. A. Laouini1, C. Jaafar-Maalej1, I. Limayem-Blouza2, S. Sfar2, C. Charcosset1, and H. Fessi1, Preparation, Characterization and Applications of Liposomes: State of the Art, Journal of Colloid Science and Biotechnology, **2012**, 1: 151-53.
12. Senior J., Fate and behaviour of liposomes in vivo: A review of controlling factors. CRC Crit. Reviews Therapeut., Drug Carrier Systems, **1987**, 3(3): 123.
13. Varde Neha M, Joshi Darshana P, C. Sini Srendran, Shah Viral H, Upadhyay U.M, Formulation Optimization and Evaluation of Liposomal Gel of Prednisolone by Applying Statistical Design, International Journal of Chemistry and Pharmaceutical Sciences, **2013**, 1(2): 147-164
14. Prasanth V.V, Maharshi S, Sam T. Mathew, Liposomes: An Overview, IJPI*f*s Journal of Pharmaceutics and Cosmetology, **2012**, 2(8): 70-72
15. Hsin-I Chang and Ming-Kung Yeh, Clinical development of liposome-based drugs: formulation, characterization, and therapeutic efficacy, Int J Nanomedicine. **2012**, 7(1): 49€60.
16. Vladimir P. Torchilin, Recent Advances with liposomes as pharmaceutical carriers, nature reviews, Drug Discovery. **2005**, 4(2): 148.
17. Sipai Altaf Bhai.M, Vandana Yadav, Mamatha.Y, prasanth V.V, liposomes a review, Journal of Pharmaceutical and scientific innovation, **2012**, (4): 13-21.
18. Navneet Kumar, Verma, Anubha Gupta, Harendra Prasad, A Brief Study on Liposomes-A Review, Asian Journal of Chemical and Pharmaceutical Research, **2014,** 2(1): 112-123
19. ParveenGoyal, KumudGoyal, SengodanGurusamy, Vijaya Kumar, Ajit Singh, Om Prakash Katare, DinaNath Mishra, Liposomal drug delivery systems € Clinica Applications,Acta Pharm, **2005**, 55(3): 1€25.