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REVIEW ARTICLE

A Vital Role of Liposome's on Controlled and Novel Drug Delivery

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ABSTRACT

Liposomes, phospholipids' vesicles with a bilayered membrane structure, have been widely used as pharmaceutical carriers for drugs and genes in disease treatment. Liposomes are micro particulate lipoidal vesicles which are under extensive investigation as drug carriers for improving the delivery of therapeutic agents. Due to new developments in liposome technology, several liposome based drug formulations are currently in clinical trial, and recently some of them have been approved for clinical use. Liposome's are the most promising and broadly applicable of all the novel delivery systems. Scientists and physicians are never satisfied only with a favorable drug action against the disease under treatment.. A liposome is an artificially prepared vesicle composed of a lipid bilayer. The liposome can be used as a vehicle for administration of nutrients and pharmaceutical drugs.

Liposomes are composed of natural phospholipids, and may also contain mixed lipid chains with surfactant properties. A liposome design may employ surface ligands for attaching to unhealthy tissue. The major types of liposomes are the multilamellar vesicle (MLV), the small unilamellar vesicle (SUV), and the large unilamellar vesicle (LUV).

Key words: Liposome's, encapsulation, drug delivery, Applications.

INTRODUCTION

Liposome was discovered about 40 years ago by Bangham and co-workers and was defined as microscopic spherical vesicles that form when phospholipids are hydrated or exposed to an aqueous environment.[1] Structurally they are composed of phospholipids which are biodegradable, non toxic and devoid of any antigenic, pyrogenic or allergic reactions, and with careful selection, allow encapsulation of matter that is as small as the lithium ion up to macromolecules as large as genetic material of several hundred thousand Daltons.[2] Liposomes are microscopic vesicles composed of a bilayer of phospholipids or any similar amphipathic lipids. They can encapsulate and effectively deliver both hydrophilic and lipophilic substances, [3] and may be used as a non-toxic vehicle for insoluble drugs. [4] Liposome's are relatively unstable colloidal system manifested by physical and chemical instability. Physical instability is evidenced by vesicle aggregation and fusion, which is associated with changes in vesicle size and loss of entrapped material. [5]

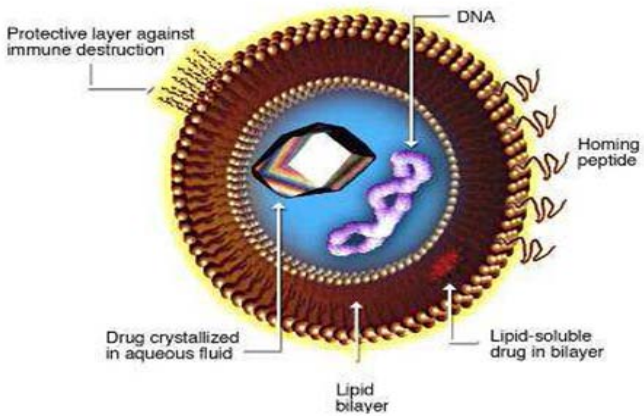

Fig 1: Structure of liposomes

Physical instability is evidenced by vesicle aggregation and fusion, which is associated with changes in vesicle size and loss of entrapped material Chemical stability, is of more importance as it is associated with phospholipids which form the backbone of the bilayer. It is of two types namely hydrolysis of the ester bonds linking the fatty acids to the glycerol backbone and per oxidation of unsaturated acyl chains (if present) which accelerates liposome breakdown and alters drug-release characteristics. These factors

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influence the in vivo performance and storage behavior of liposomes. [6, 7]

Advantages: [8]

It provides controlled drug delivery.

- It should be biodegradable, biocompatible, and flexible
- It should be non ionic.
- It can carry both water and lipid soluble drugs.
- The drugs can be stabilized from oxidation.
- It should be improve the protein stabilization.
- It provides controlled hydration.
- It provides sustained release.
- It provides targeted drug delivery or site specific drug delivery.
- Stabilization of entrapped drug from hostile environment.
- Alter pharmacokinetics and pharmacodynamics of drugs.
- It can be administered through various routes.
- It can incorporate micro and macro molecules.
- It acts as reservoir of drugs.
- The therapeutic index of drugs is increased.
- Site avoidance therapy.
- It can modulate the distribution of drug.
- It should be direct interaction of the drug with cell.

Disadvantages:

It is less stability

- It is low solubility.
- It is short half life.
- The phospholipids undergoes oxidation, hydrolysis.
- Leakage and fusion.
- It is high production cost.
- Some time allergic reactions may occur to liposomal constituents.

TYPES OF LIPOSOMES:

Liposomes are classified based on the size and they are –

- MLV-multi lamellar vesicles-(500 - 10,000 nm)
- SUV-small lamellar vesicles-(<50 nm)
- LUV-large lamellar vesicles-(>50 nm)

The liposomes whose size ranges from (2,000 - 40,000 nm) are called Multi lamellar liposomes. Liposomes of size (10.000 - 10, 00,000 nm) are giant liposomes these can be either unilamellar / multilamellar liposomes6. These are generally preferred carrier system in the current trends as these formulations enhances the therapeutic efficacy at low dose, reduces toxicity and improves pharmacokinetic profile., e.g. liposomal doxorubicin; increased stability of drugs particularly against enzymatic degradation[3][9][10].

FORMULATION OF LIPOSOME:

Liposomes are made from pure lipids or a combination of lipids. The lipids commonly employed in liposome formulations are phospholipids. Liposomes have been prepared from a variety of synthetic and naturally occurring phospholipids; they generally contain cholesterol [11]. The incorporation of cholesterol into the lipid bilayer membrane generally enhances the stability of liposomes in serum, reduces the permeability of the membranes to water soluble molecules and increases the fluidity or microviscosity of the bilayer [12].

The most commonly used phosholipids in liposome preparation are: egg phosphatidylcholine, synthetic dipalmitoyl– DL-α-phosphatidylcholine, brain and synthetic phosphatidylserine, sphingomyelin, phosphatidylinositol and ovolecithin. Usually, a zwitterionic or non-ionic lipid is used as the basic lipid for the preparation of liposomes. The net surface charge of liposome can be modified by the incorporation of positively charged lipids such as stearylamine, or negatively charged lipids such as diacetylphosphate, phosphatidyl glycerol or phosphatidyl serine[13].

The presence of negatively or positively charged lipids leads to a greater overall volume for aqueous entrapment and reduces the likelihood of aggregation after preparation of the liposomes [14].

Cationic liposomes display some disadvantages such as cytolytic and cytotoxic activities. Yoshihara and Nakae

[15] have demonstrated that cationic liposomes containing stearylamine showed an in vivo toxicity in rabbits. This effect was attributed to haemolysis of the erythrocytes and was directly related to the amount of stearylamine present in the liposome composition.

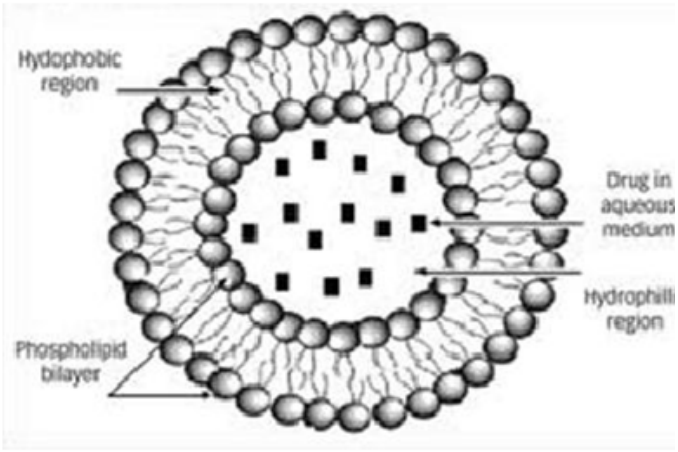

Fig 2: Structure of liposomes

Formulation factors affecting the degree of drug entrapment:

The extents of drug entrapment and retention as well as factors influencing them are important considerations in the design of liposome-mediated drug delivery systems. Drugs may be entrapped in the aqueous or lipid phase of the liposome.

(a) Aqueous entrapment :

This relates to the aqueous volume in the liposome. The larger the aqueous volume the greater the amounts of polar drugs that can be encapsulated [16].

Multiple compartment liposomes encapsulate higher percentages of aqueous soluble drugs than single compartment vesicles, because of the larger volume of encapsulated aqueous space in the former. Formulations that promote formation of MLVs are thus associated with higher aqueous entrapment. Osmotic swelling and/or incorporation of charged lipids, e.g., phosphatidylserine into bilayers are measures for increasing the aqueous volume in liposomes [17].

Aqueous solubility of the drug is another factor, hence, the extent of drug entrapment in liposomes (MLVs) can vary markedly as seen in the following examples: 2.2–8.4% for penicillin, 2.3–11.6% for actinomycin D, 18% for methothrexate and up to 60% for bleomycin. Leakage of entrapped solute is another formulation problem. Cholesterol modifies the fluidity of lipid membranes, thereby influencing the degree of retention of drugs by vesicles as well as stabilizing the system against enzymatic degradation[17]. Large molecules (e.g., peptides and proteins) are better retained than smaller molecules, which can diffuse slowly through the lipid layers.

(b) Lipid entrapment:

Lipid soluble drugs are entrapped in the lipid layers of liposome. Here, the entrapment efficiency can be as high as 100%, irrespective of liposomal type and composition. An example of a drug that is hydrophobic in nature is camptothecin [18].

The retention of such hydrophobic drugs is also high when the liposomes are placed in aqueous biological environment because of their high lipid-water partition coefficients.

Formulation factors affecting stability of liposomes:

The stability of liposomes refers to their ability to retain entrapped solutes, chemical stability of both the entrapped solutes and the lipid membranes. Solute leakage depends on Membrane permeability and on the interaction with components of biological fluids. Membrane fluidity can be controlled to reduce leakage by supplementing the lipid bilayer with cholesterol [19] or by manipulating the hydrophobic/lipophobic character of the bilayers, for example, with the use of fluorinated lipid [20][21].

The rate of solute leakage also depends on the lamellar structure of liposomes, for instance, MLVs are less prone to leakage than ULVs. In order to minimize leakages, liposomes are stored in the form of freeze-dried powders.

The lipid vesicles can undergo chemical degradation. For instance, phospholipids can be hydrolysed to lysophospholipids, which are also subject to further hydrolysis [22].

The 2- lysophospholipids are the main initial products of hydrolysis [23].

Hydrolytic degradation of either the lipid or entrapped drug may be pH related but can be prevented or minimized by freeze-drying of liposome suspension to dry powders. When unsaturated phospholipids are used to prepare liposomes, special precautions must be taken to minimize oxidation. These include the use of light resistant containers, use of antioxidants

Such as α-tocophenol, de-aeration with argon or nitrogen to minimize exposure to oxygen, and removal of heavy metals from the preparation.

METHODS FOR PREPARATION OF LIPOSOMES:

The main goal of an ideal method of liposome formulation is to obtain efficient drug entrapment, narrow particles size distribution and long term stability of liposome products. The general procedure for all methods of liposomes preparation involves hydrating of the lipid, followed by sizing of the particles and removing of the non encapsulated drug. There are two types used for the preparation of liposomes: passive loading mechanical dispersion methods and active loading methods. The most common used methods in the preparation for liposomes are: thin-film hydration method, micro emulsification, sonication, membrane extrusion, freeze thawed method, ether injection method, ethanol injection method, reverse phase evaporation method, dehydration-rehydration, and calcium-induced fusion method [24-26].

In the passive loading method the drug is encapsulated by introducing an aqueous phase of a water-soluble drug or an organic phase of a lipid-soluble drug before or at some stage during the preparation of the liposomes. The high drug encapsulation efficiency can be achieved by using

Passive loading method for lipid-soluble drugs with a high affinity to the lipid membrane. In the active loading method, the drugs can be loaded by creating diffusion gradients for the ions or drugs across the external and internal aqueous phases. The classification of liposomes based on methods for their preparation depends on using the organic solvent, obtaining liposomes with different lamellarity, transforming the size and applications of the liposomes. The phospholipids play important role in the preparation of liposomes, as well as in their stability [26].

1) Thin-Film Hydration Method:

The thin-film hydration procedure is the most common and simple method for preparation of MLV by dissolving the phospholipids in the organic solvents: dichloromethane [27], chloroform [28][29][30], ethanol and chloroform-methanol mixture (2:1 v/v; 9:1 v/v; 3:1 v/v)[32,33].

A thin and homogeneous lipid film is formed when solvent is evaporated under vacuum at the temperature: 45-60 ºC. Nitrogen gas is involved in order to completely remove the residual solvent [31, 34].

A solution of distilled water [35][36], phosphate buffer, phosphate saline buffer[33] at pH 7.4 and normal saline buffer are used in hydration step. The time for the hydration process varied from 1 hr to 2 hr at the temperature 60-70 ºC. In order to obtain full lipid hydration, the liposomal suspension is left overnight at 4 ºC [36]. The thin-film hydration method can be used for all different kinds of lipid mixtures. The main drawbacks of the method are related to low

Encapsulation, difficulty of scaling up and the size distribution is heterogeneous [26,27].

2) Injection Methods :

1. Ether Injection Method:

In ether injection method a solution of lipids is dissolved in ether or diethyl ether/methanol mixture which is slowly injected to an aqueous solution of the material to be capsulated. The subsequent removal of the organic solvent under reduced pressure leads to the formation of liposomes [26, 37].

The main disadvantage of the method is heterogeneous population and the exposure of compounds to be encapsulated to organic solvents or high temperature [38].

2. Ethanol Injection Method:

In ethanol injection method the ethanolic lipid solution is rapidly injected to a vast excess of preheated distilled water [26] or TRIS-HCl buffer. The incorporation of the drug in liposomal vesicle depends on its hydrophilic/hydrophobic character. Nimesulide as lipid soluble component incorporates better in liposomes than 5-fluorouracil which migrates to external aqueous phase [39]. The main advantage of ethanol injection method is including of non harmful solvent as ethanol, as well as easy scale up of the method. The possibility of formation of azeotrope with water reduces its applicability [26, 37].

3. Sonication Method:

The sonication method is based on size transformation and involves the subsequent sonication of MLVs prepared by thin-film hydration method, using sonic energy usually under an inert atmosphere including nitrogen or argon. The sonication method enables homogenous dispersion of small vesicles using bath type or probe type sonicator with a potential for greater tissue penetration. The

probe tip sonicator delivers high energy to the lipid suspension. The possibility of overheating of the lipid suspension causes degradation [34,35,40].

Sonication tips tend to release titanium particles into the lipid suspension which must be removed by centrifugation prior to use. The bath sonicators are the most widely used instrumentation for preparation of SUV [30,36,39][41-43].

They are used for large volume of dilute lipids. The oxidation of unsaturated bonds in the fatty acid chains of phospholipids and hydrolysis to lysophospholipids and free fatty acids, as well as denaturation of thermo labile substances and very low encapsulation efficiency of internal volume are the main drawbacks of the method [26,30].

4. High-Pressure Extrusion Method:

MLVs prepared by thin-film hydration method are repeatedly passed through filters polycarbonate membranes reducing the liposome size in high-pressure extrusion method [38, 44].

The liposomes are prepared using thin-film hydration method subsequently using an extruder for ten cycles to obtain extruded liposomes with uniform diameters [44].

5. Reverse-Phase Evaporation Method:

The reverse-phase evaporation method is used with the organic solvents such as diethyl ether/isopropyl ether or mixture of diethyl ether and chloroform (1:1 v/v) [39] and a mixture of chloroform methanol (2:1 v/v) [42] containing phospholipids. The organic phase should be immiscible with aqueous phase, thus an oil/water emulsion is created. Phosphate buffer saline [42,45] or citric-Na_2HPO_4 buffer is added to aqueous phase with aim to improve the efficiency of liposome formulations. The formation of liposomes is allowed by continued rotary evaporation of the organic solvents under vacuum [39,46,42,45]. The main advantage of the method is a very high encapsulation rate [42][47].

The main drawback of the method is the possibility of remaining the solvent in the formulation and it has difficulties to scale up [39, 42].

6. Calcium-Induced Fusion Method:

The calcium-induced method is based on adding of calcium to SUV. The formation of multilamellar vesicles is as result of fusion. The addition of ethylene diamine tetra acetic acid (EDTA) to the preparations results in the formation of LUV liposomes [48].

The preparation of LUV liposomes can be obtained only from acidic phospholipids [38, 48].

7. Dehydration-Rehydration Method:

The method of dehydration-rehydration is used as method for the preparation of liposomes, also [49, 50]. The small unilamellar vesicles which are composed of phosphatidylcholine, 1,2-dioleoyl-3- (tri methyl ammonium) propane, cholesterol and plasmid DNA are prepared by sonication method[50].

The obtained formulation is frozen and left freeze-dried overnight. The formation of multilamellar dehydration-rehydration vesicles containing DNA in their structure due to the bound of the cationic charges of the inner bilayers is as a result of a controlled rehydration of the dry powders [50].

8. Freeze-Thaw Method:

The method of freezing and thawing is introduced for increasing the trapped volume of liposomal preparations. The freeze-thaw method is dependent on the ionic strength of the medium and the phospholipids concentration [51].

It influences to a physical disruption of lamellar structure leading to formation of unilamellar vesicles.

The unilamellar vesicles are rapidly frozen followed by slow thawing [51, 52], while the freeze and thawing cycles are repeated. The preparation of MLV propranolol liposomes by freeze-thaw method is described in the literature [53]. The liposomal propranolol formulation is prepared by using distearoyl phosphatidylcholine and dimyristoyl phosphatidylcholine as phospholipids in phosphate buffered saline buffer, followed by six freeze-thaw cycles [53].

9. Micro fluidization:

A method based on micro fluidization i.e. micro emulsification is used for the large

scale manufacture of liposomes. The preparation of antibiotic liposomes by thin-layer hydration method followed by sonication with a bath-type sonicator and micro fluidization in order to achieve partial homogenization was described by Boltič *et al*., [54].

The process of micro fluidization is reproducible and yield liposomes with good aqueous phase encapsulation [51, 55].

❖ EVALUATION OF LIPOSOMES:

In vitro and in vivo formulations are carried out after the formulations of liposomes. As the liposomes are produced from different techniques they may differ from physicochemical characteristics. These differences may have impact on their behavior in both in vitro and *in vivo*. The characterization parameter is classified as the physical, chemical and biological parameters.

Physical characters include purity and potency of various liposomal constituents. Physical and chemical characterization is very important for the comparison of different liposomes prepared from different batches. Biological parameters are helpful in the establishing the safety and suitability of formulation for *in vivo* or the therapeutic applications [17, 56, 57].

1. Visual Appearance:

Liposome suspension can range from translucent to milky, depending on the composition and particle size. If the turbidity has a bluish shade this means that particles in the sample are homogeneous; a flat, gray color indicates that presence of a non liposomal dispersion and is most likely a disperse inverse hexagonal phase or dispersed microcrystallines. An optical microscope (phase contrast) can detect liposome> 0.3 μm and contamination with larger particles [67].

2. Particle size and particle size distribution:

The Particle size analyzer (sympate HELOS, Germany H1004) measures the particle size based the laser diffraction theory. The apparatus consists of a He-Ne laser beam of 632.8 nm focused with a minimum power of 5 MW using a Fourier lens (R-5) to a point at the centre of multi element detector and a sample holding unit.

The liposomes were diluted for about 10 times in the de ionized water before measurement and are added to the sample dispersion unit and kept for stirring at high speed to reduce the inter particle aggregation and laser beam was focused [58].

3. Drug entrapment efficiency:

The entrapment efficiency was calculated by using the formula:

% Entrapment efficiency = Entrapped drug / total drug added X100

To find out the drug content (drug entrapment) the liposomal suspension was ultra-centrifuged at 5000 rpm for 15 min at 4^{o}C temperatures by using semi cooling centrifuge to separate the free drug.

The free drug was formed at wall of the centrifuge tube and suspended containing liposomes were in suspended stage. The supernatant liquid was collected and centrifuged by maintaining same rpm and temperature for about 30 min which forms a clear solution of supernatant and pellets of liposomes.

The unentrapped drug were soaked for about 10 min by using methanol and sonicated for about 10min, which causes breakdown of the vesicles to release the drug and the drug content was estimated by UV spectrophotometrically [42, 59,60].

4. Microscopy:

The morphology of the liposomes was studied under microscope. The liposomes were viewed under microscope called as LECIA DMIL which is an inverted fluorescence microscope to study their shape and lamellarity. The liposomes are diluted and viewed under 45x [32, 42, 61].

5. Differential scanning calorimetry (DSC):

In this method drug loaded multilamellar liposomes were submitted to DSC analyzer (modelTA-60, shimadzu). The DSC was performed on the 5 mg sealed in standard aluminum pans. Thermo grams were obtained at a scanning rate of 20 oC / min. Each sample was scanned between 0 ^{o}C to 200 ^{o}C. This is used to determine phase transition temperature of phospholipids sample in order to characterize the state of drug [42, 62].

6. *In vitro* drug release study: [63-66]

The *in vitro* diffusion studies were carried out by using the Franz diffusion cell which has a diameter of 25 mm and a diffusion area of 4.90 cm^2. It contains the reservoir compartment of 22 mL, which was filled with a buffer which contains 20% v / v methanol to maintain sink condition. There is regenerated cellulose acetate membrane which is placed in between the lower cell reservoir and the glass cell top containing the sample and is clinched with a clamp [63, 64].

The whole system was maintained at the temperature of 37 ^oC ± 5 ^oC by using magnetic heater resulting in the membrane surface temperature of 32 ^oC [65].

The sample was placed on the surface of the donor compartment and then for the assay process the 2mL of the samples were received from receptor fluid and replaced with 2mL of fresh solution. The time period to carry out the process depends on the half-life of the drug selected and further dilutions are carried out and measured spectophotometrically [66].

7. Quasi-elastic light scattering (QELS):

The hydrodynamic mean diameters (MD) of the vesicles and mixed aggregates were determined by quasi elastic light scattering (QELS) using a N4 Coultronics apparatus. Calculations were made according to the Stokes– Einstein equation assuming the particles to be independent and spherical. The MD values correspond to the average of three measurements with a standard deviation lower than 5% [68].

8. Entrapped Volume:

The entrapped volume of a population of liposome (in μL/ mg phospholipid) can often be deduced from measurements of the total quantity of solute entrapped inside liposome assuring that the concentration of solute in the aqueous medium inside liposomes is the same after separation from unentrapped material. For example, in two phase method of preparation, water can be lost from the internal compartment during the drying down step to remove organic solvent [28].

9. Surface Charge:

Liposomes are usually prepared using charge imparting constituting lipids and hence it is imparting to study the charge on the vesicle surface. In general two methods are used to assess the charge, namely free flow electrophoresis and zeta potential measurement. From the mobility of the liposomal dispersion in a suitable buffer, the surface charge on the vesicles [69].

10. High-Performance Thin-Layer Chromatography (HPTLC):

A HPTLC method for the determination of ketorolac tromethamine is introduced by Nava *et al*., 69. The content of the drug is determined by spotting samples on TLC plates, Alugram® Sil G/UV_{254} and developing in ethyl acetate:chloroform:acetic acid (8:3:0.1)[69].

❖ **Stability of Liposomes:** [70]

In general successful formulation of stable liposomal drug product requires the following precautions

i. Processing with fresh, purified lipids and solvents.
ii. Avoidance of high temperature and excessive shear forces.
iii. Maintenance of low oxygen potential (Nitrogen purging).
iv. Use of antioxidant or metal chelators.
v. Formulating at neutral pH.
vi. Use of lyo-protectant when freeze drying.

Classification of Stability of Liposomes:

Liposome stability can be subdivided into physical, chemical and biological stabilities, which are all inter-related. Generally, the shelf-life stability of liposomes is determined by the physical and chemical stability (Uniformity of size distribution and encapsulation efficiency, and minimal degradation of all compounds, respectively). By optimizing the size distribution, pH and ionic strength, as well as the addition of antioxidants and chelating agents, liquid liposome formulations can be stable [70, 71].

1. Chemical stability:

As phospholipids usually form the backbone of the bilayer their chemical stability is important. Two types of chemical degradation reactions can affect the performance of phospholipid bilayers: hydrolysis of the ester bonds linking the fatty acids to the glycerol backbone and peroxidation of unsaturated acyl chains (If present). The oxidation and hydrolysis of lipids may lead to the appearance of short-chain lipids and then soluble derivatives will form in the

membrane, resulting in the decrease of the quality of liposome products [70, 72].

2. Physical stability:

Physical processes such as aggregation/flocculation and fusion/coalescence that affect the shelf life of liposomes can result in loss of liposome associated drug and changes in size. Aggregation is the formation of larger units of liposomal material; these units are still composed of individual liposomes.

In principle, this process is reversible e.g. by applying mild shears forces, by changing the temperature or by binding metal ions that initially induced aggregation. However, the presence of aggregation can accelerate the process of coalescence of liposomes, which indicates that new colloidal structures are formed. As coalescence is an irreversible process; the original liposomes cannot be retrieved.

A colloidal dispersion is often thermodynamically unstable. Spontaneous processes occur in the direction of decreasing Gibbs free energy; therefore the separation of a two-phase dispersed system to form two distinct layers is a change in the direction of decreasing Gibbs free energy. There is more surface energy in a liposome suspension when the dispersed phase is in a highly subdivided state than when it is in a coarser state of subdivision. The central feature of coalescence is the fact that the total surface area is reduced in the coarsening process of thermodynamically unstable liposome dispersion, while there is no reduction of surface in aggregation, although certain surface sites may be blocked at the points at which the smaller particles touch13. After small particles coalescence, only the new larger particle remains. With aggregation, however, the small particles retain their identity and the aggregation moves as a single unit [70].

3. Biological stability:

Biological stability of liposomes, however, depends on the presence of agents such as proteins that interact with liposomes upon application to the subject and depends on the administration route. Strategies used to enhance biological stability of liposomes will improve liposome-mediated drug delivery in vivo and increase circulation time in the blood stream. It was observed that aerosols of interleukin 2 liposomes were biologically stable and retained in the lung after nebulization15. Incorporating steric stability, e.g. the incorporation of N-acyl-phosphatidyl ethanolamine into liposomes has shown to increase the liposomal biological stability towards plasma components [70].

Storage of liposomes:

Freeze drying is preferred for the storage of liposomes as they favour hydrolytic degradation and leakage [73].

The converted powder can be re constituted to the suspension form before use up on rehydration. Fusion and leakage can be prevented by adding carbohydrate during storage [74].

❖ **APPLICATIONS OF LIPOSOMES:**

Pharmaceutical Application:[75-77]

I. The liposome is applicable in various fields:[75]

a) Cell -liposome interaction.
b) Localized drug effect.
c) Enhanced drug uptake.
d) Molecules with wide range of solubility and molecular weight can be accommodated
e) Flexibility in structural characteristics.

II. Liposomes are used to Target drugs to the tumors:[75][76]

a) The liposomal Ara -C inhibit DNA synthesis in the lungs.
b) For targeted drug delivery for blood born Neoplasm's.
c) By active targeting using monoclonal antibodies, by magnetosomes or by temperature sensitive liposomes.
d) By passive targeting to liver, spleen, R.E.S cancers.

III. Liposomes are used to Reduction of Toxicity:[75]

This is usually due to targeted or site specific delivery.

E.g. Hydrophobic drugs including alkylating agents, antimitoticagents, anthracyclines.

IV. Liposome's are used to treatment of localized drug effect:

V. Liposomes are used to treatment of Gene therapy:[76]

E.g. Liposomes can be used to deliver DNA into the cell.

VI. Liposomes can be used as carriers for vaccines:[75-77]

E.g. Diphtheria toxoid vaccine

VII. Liposomes can be used as carrier of drug in oral treatment:[75]

a. Arthritis

Treated with steroids using MLVs prepared by DPPC and P.A.

E.g. Drugs are Ibuprofen, cortisol palmitate

b. Diabetes

Alternation in blood glucose level in diabetic animals was obtained by oral

Administration of liposome encapsulated insulin (PC: CH liposomes).

VIII. Liposomes used for topical application[76, 77]

E.g.Triamcinolone ointments, Hydrocortisone ointment, Diclofenac gel etc.

IX. Liposomes used for pulmonary delivery system:[75, 77]

E.g. Cytosine arabinoside, Pentamidine, Sodium cromoglycate, Metepreterenol.

X. Liposomes used for ophthalmic delivery of drugs:[76, 77]

In order to maintain optimal drug concentration at the site of action liposomes are used a carriers or vehicles.

E.g. Treatment of Keratitis by Idoxuridine Also increases (2 times) the Trans corneal flux of penicillin G, indoxol and carbachol.

Other Applications:

1. **Treatment of Diseases:** [78]

   Drug containing liposomes have been shown to be effective against diseases in test animals and in some cases in human beings.

2. **As Vaccine Carriers:**[78]

   Liposomes carrying antigens (derived from Infectious organisms) particularly viral antigens, malaria antigens and bacterial toxins have been successfully used to produce humoral or cellular immunity in test animals. This indicates that liposomes have potential as vaccine carriers.

   E.g. Rabies glycoprotein's (Antigens as Liposomal Preparation) - Interleukin-2 Enhancement (Applications)

3. **In Cell Physiology:**

   As Diagnostic Agents: The intravenous administration of liposomes containing contrast agents such as 99m Tc have been used to visualize certain malignant tissues such as cancerous tissues of breast, rheumatoid factor, etc.

4. **In Cosmetics:**[81]

   To investigate the use of liposomes in cosmetics, studies have been made to study interaction between skin and liposomes. Liposomes entrap a variety of active molecules like anti-oxidants, vitamins, natural or synthetic drugs, and can therefore be utilized for skin creams, anti-aging creams, after shave lotions, lipsticks, sun-screen and make-up creams.

5. **In Textiles:**

   A new method of wool chlorination at pH < 7 using liposomes as vehicles for oxidizing agents is suitable for inhibiting or modulating the formation of cystic acid in wool fibers.

6. **Liposome for Respiratory Drug Delivery System:** [79]

   Liposome is widely used in several types of respiratory disorders.

   Liposomal aerosol has several advantages over ordinary aerosol which are as follows:

   1. Sustained release
   2. Prevention of local irritation
   3. Reduced toxicity and
   4. Improved stability in the large aqueous core.

7. **Food industry:** [80]

   In food industry, natural antioxidants like vitamin C and E when used as a liposome formulation improves the activity of the compounds and reduce the use of artificial preservatives which cause unwanted side effects.

8. **Agro bio-ecological systems:**

   Even agro-food industry employs liposomes in application to herbicides and pesticides by adopting the sustained release from the liposomes. This enables the prolonged contact time of the toxins to the pests thus ensuring the improved efficacy of the Herbicides and pesticides[82].

   Liposomes have found to be applicable in maintaining the ecological health to clear the oil leaks in the sea by liposomal trapped floating blooms [83].

9. **Enhancement of antimicrobial efficacy / safety:**[84]

   Most of the antimicrobial agents (antibiotics) favour the conditions for the enzymatic degradation and most of the antibiotics such as the penicillin and cephalosporins are very sensitive to the β-lactamase degrading action and these also require high doses to show their effective therapeutic action. To overcome these problems the antimicrobial agent are encapsulated in the liposomes, such that they show their therapeutic efficacy in relative

lower doses and reduces dose related toxicity (e.g. Amphotercin B) .

CONCLUSION

From the above article it is concluded that the considering the advantages of this Novel drug delivery system. Liposome vesicles 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. Several methods of preparing liposomes were identified, which could influence the particle structure, degree of drug entrapment and leakage of the liposomes. It was also identified that there are improved pharmacokinetic properties with liposomal drugs compared to the free drugs.

Liposomes are tools for drug targeting in certain biomedical situations (e.g., cancer) and for reducing the incidence of dose-related drug toxicity. Instability of the preparations (particularly leakage) is a problem, which is yet to be overcome before full commercialization of the process can be realized. The development of 'pharmaceutical' liposomes is currently a growth area. The increasing variety of suggested applications, and encouraging results from early clinical applications and clinical trials of different liposomal drugs.

REFERENCES

1. Bangham AD, Standish MM and Watkins JC. Diffusion of univalent ions across the lamellae of swollen phospholipids. J Mol Biol 1965; 13: 238–252.
2. Jain SK, Jain NK. Liposomes as Drug Carriers. In: N K Jain (ed.), Controlled and Novel Drug Delivery, CBS, New Delhi, 2008, pp. 304-345.
3. Fielding MR. Liposomal drug delivery: advantages and limitations from a clinical pharmacokinetics and therapeutic perpective. Clin Pharmacokinet 1991; 21: 155–164.
4. Akbarieh M, Besner JG, Galal A and Tawashi R. Liposomal delivery system for the targeting and controlled release of praziquantel. Drug Dev Ind Pharm 1992; 18: 303–317.
5. Lidgate DM, Felgner PL, Fleitman JS, Whatley J and Fu RC. Invitro and invivo studies evaluating a liposome system for drug solubilisation. Pharm Res 1988; 5: 759–764.
6. Taylor KMG, Elhissi AMA. Preparation of Liposomes for Pulmonary Delivery Using Medical Nebulizers. In: Gregory Gregoriadis (ed.), Liposome Technology Liposome Preparation and Related Techniques, 3rd Edn, Vol I, Informa Healthcare, New York, 2007, pp. 67-84.
7. Stark B, Pabst G, Prassl R, Long-term stability of sterically stabilized liposomes by freezing and freeze-drying: Effects of cryoprotectants on structure. Euro. J. Pharm. Sci, 41: 546–555, (2010).
8. Alving CR *et al*. Macrophages, as targets for delivery of liposome encapsulated antimicrobial agents. Adv Drug Delivery Rev. 1998; 2: 107–128.
9. Gregoriadis G. Overview of liposomes. J Antimicrob Chemother 1991; 28(supp. B):39–48.
10. Xian-rong Q, Yoshie M and Tsuneji N. Effect of soybean-derived sterols on the invitro stability and the blood circulation of liposomes in mice. Int J Pharm 1995; 114: 33–41.
11. Rogers JA and Aderson KE. The potential of liposomes in oral drug delivery. Crit Rev Ther Drug Carrier System 1998; 13(5): 421–480.
12. Senior J and Gregoriadis G. Stability of small unilamellar liposomes in serum and clearance from the circulation: the effect of the phospholipid and cholesterol com-ponents. Life Sci 1982; 30: 2123–2136.
13. Frezard F. Liposomes: from biophysics to the design of peptide vaccines. Braz J Biol Res 1999; 32(2): 181–189.
14. Goldbach P, Herve B, Pascal W and Andre S. Sterile filtration of liposomes: retention of encapsulated carboxyfluorescein. Int J Pharm 1995; 117: 225–230.
15. Yoshihara E and Nakae T. Cytolytic activity of liposomes containing stearylamine. Biochim Biophys Acta 1986; 854: 530–546.
16. Fendler JH. Optimizing drug entrapment in liposomes: chemical and biophysical consideration in liposomes in biological systems. In: G Gregoriadis and AC Allison (Eds.). New York: John Wiley and Sons Ltd, 1980; 87–100.
17. Weiner N, Martin F and Riox M. Liposomes as drug delivery system. Drug Dev Ind Pharm 1989; 15(10): 1523–1554.

18. Saetern AM, Flaten GE and Brandl MA. A method to determine the incorporation capacity of camptothecin in liposomes. AASP Pharm Sci Tech 2004; 5(3): article 40.
19. Senior J and Gregoriadis G. Stability of small unilamellar liposomes in serum and clearance from the circulation: the effect of the phospholipid and cholesterol components. Life Sci 1982; 30: 2123–2136.
20. Frezard F, Santaella C, Montisci MJ, Vierling P and Riess JG. Florinate phospholipidbase liposomes: H+/Na+ permeability, active doxorubicin encapsulation and stability in human serum. Biochim Biophys Acta 1994; 1194: 61–68.
21. Frezard F, Santaella C, Vierling P and Riess JG. Permeability and stability in buffer and in human serum of fluorinated phospholipid- based liposomes. Biochim Biophys Acta 1994; 1192: 61–70.
22. Grit M, Smidt J, Struijke A and Crommelin DJA. Hydrolysis of phosphatidycholine in aqueous liposome dispersions. Int J Pharm 1989; 50: 1–6.
23. Kemps JMA and Crommelin DJA. Chemische stabiliteit van fosfolipiden in farmaceutische preparaten. I. Hydrolyse van fosfolipiden in waterig milieu. Pharm Weekbl 1988; 123: 355–363.
24. Anwekar H, Patel S, *et al* "Liposome-as drug carriers" Int J of Pharm & Life Sci, 2011, 2 (7): 945-951.
25. Sirisha VNL, BhavaniHarika I, *et al* "Liposomes-the potential drug carriers" J Pharm, 2012, 2 (5): 26-38.
26. Sipai ABM, Vandana Y, *et al* "Liposomes: an overview" JPSI, 2012, 1 (1): 13-21.
27. Chanda H, Das P, *et al* "Development and evaluation of liposomes of fluconazole" JPBMS, 2011, 5 (27): 1-9.
28. Aukunuru J, Joginapally S, *et al* "Preparation, characterization and evaluation of hepatoprotective activity of an intravenous liposomal formulation of bis-demethoxy curcumin analogue (BDMCA)" Int J Drug Dev & Res, 2009, 1 (1): 37-46.
29. Devi M, Kumar MS, *et al* "Amphotericin-B loaded vesicular systems for the treatment of topical fungal infection" IJRAPR, 2011, 4: 37-46.
30. Jadhav MP, Nagarsenker MS, *et al* "Formulation and evaluation of long circulating liposomal amphotericin B: a scinti-kinetic study using 99mTc in BALB/C mice" Indian J Pharm Sci, 2011, 73 (1): 57-64.
31. Achim M, Precup C, *et al* "Thermo sensitive liposomes containing doxorubicin. Preparation and in vitro evaluation" Farmacia, 2009, 57 (6): 703-710.
32. Bhatia A "Tamoxifen in topical liposomes: development, characterization and in vitro evaluation" J Pharm Pharmaceut Sci, 2004, 7 (2): 252-259.
33. Ghanbarzadeh S, Valizadeh H, *et al* "Application of response surface methodology in development of sirolimus liposomes prepared by thin film hydration technique" BioImpacts, 2013, 3 (2): 75-81.
34. Lopes LB, Scarpa MV, *et al* "Interaction of sodium diclofenac with freeze-dried soya phosphatidylcholine and unilamellar liposomes" Braz J Pharm Sci, 2006, 42 (4): 497-504.
35. Hwang TL, Lee WR, *et al* "Cisplatin encapsulated in phosphatidylethanolamine liposomes enhances the in vitro cytotoxicity and in vivo intratumor drug accumulation against melanomas" J Dermatol Sci, 2007, 46: 11-20.
36. Channarong S, Chaicumpa W, *et al* "Development and evaluation of chitosan-coated liposomes for oral DNA vaccine: the improvement of Peyer's patch targeting using a polyplex-loaded liposomes" AAPS PharmSciTech, 2011, 12 (1): 192-200.
37. Kumar A, Badde S, *et al* "Development and characterization of liposomal drug delivery system for nimesulide" Int J Pharm Pharm Sci, 2010, 2 (Suppl 4): 87-89.
38. Nidhal KM, Athmar DH "Preparation and evaluation of salbutamol liposomal suspension using chloroform film method" Mustansiriya Medical Journal, 2012, 11 (2): 39-44.
39. Da Costa CAM, Moraes AM "Encapsulation of 5-fluorouracil in liposomes for topical administration" Maringá, 2003, 25 (1): 53-61.
40. Prabhu P, Kumar N, *et al* "Preparation and evaluation of liposomes of brimonidine

tartrate as an ocular drug delivery system" Int J Res Pharm Sci, 2010, 1(4): 502-508.
41. Makhmalzadeh BS, Azh Z, *et al* "Preparation and evaluation of mafenide acetate liposomal formulation as eschar delivery system" Int J Drug Dev & Res, 2011, 3 (4): 129-140.
42. Hathout RM, Mansour S, *et al* "Liposomes as an ocular delivery system for acetazolamide: in vitro and in vivo studies" AAPS PharmSciTech, 2007, 8 (1): E1-E12.
43. Rathod S, Deshpande SG "Design and evaluation of liposomal formulation of pilocarpine nitrate" Indian J Pharm Sci, 2010, 72 (2): 155-160.
44. Ramana LN, Sethuraman S, *et al* "Development of a liposomal nanodelivery system for nevirapine" J Biomed Sci, 2010, 17: 1-9.
45. Mishra N, Gupta PN, *et al* "Liposomes as adjuvant for combination vaccines" Indian J Exp Biol, 2007, 45: 237-241.
46. Niu M, Lu Y, *et al* "Liposomes containing glycocholate as potential oral insulin delivery systems: preparation, in vitro characterization, and improved protection against enzymatic degradation" Int J Nano medicine, 2011, 6: 1115-1166.
47. Mirzaee M, Owlia P, *et al* "Comparison of the bactericidal activity of amikacin in free and liposomal formulation against gram-negative and gram-positive bacteria" JJNPP, 2009, 4 (1): 1-7.
48. Mulye C, Mishra R, *et al* "Formulation and evaluation of liposome mediated drug delivery" UJP, 2013, 2 (2): 156-160.
49. Anwekar H, Patel S, *et al* "Liposome-as drug carriers" Int J of Pharm & Life Sci, 2011, 2 (7): 945-951.
50. Perrie Y, Frederik PM *et al* "Liposome-mediated DNA vaccination: the effect of vesicle composition" Vaccine, 2001, 19: 3301-3310.
51. Gaurav R, Tejal S "Liposomal drug delivery system: an overview" IJPBA, 2011, 2 (6): 1575-1580.
52. Traïkia M, Warschawski DE, *et al* "Formation of unilamellar vesicles by repetitive freeze-thaw cycles: characterization by electron microscopy and 31P-nuclear magnetic resonance" Eur Biophys J, 2000, 29: 184-195.
53. Shazly GA "Propranolol liposomes: formulation, characterization, and in vitro release" J Optoelectron Biomed Mater, 2013, 5 (1): 17-25.
54. Boltič Z, Petkovska M, *et al* "In vitro evaluation of the controlled release of antibiotics from liposomes" Chem Ind, 2003, 57 (12): 589-595.
55. Dua JS, Rana AC, *et al* "Liposome: methods of preparation and applications" IJPSR, 2012, 3 (2): 14-20.
56. Ostro, M.J. (1987). In: Liposomes: From biophysics to therapeutics, Marcel Dekker, Newyork, 231-233.
57. New, R.R.C. (1989). In: Liposomes: A practical approach, OIRL Press, Oxford, Londan, 145-150.
58. Solanki, A., Parikh, J., & Parikh, R. (2008). Preparation, Characterization, Optimization, and Stability Studies of Aceclofenac Proniosomes. Iranian Journal of Pharmaceutical Research. 7 (4), 237-246.
59. Sandhya, K.V., Devi, G.S., & Mathew, S.T. (2007). Liposomal Formulations of Serratiopeptidase: In Vitro Studies Using PAMPA and Caco-2 Models. Molecular Pharmaceutics, 5(1), 92–97.
60. Liang, M.T., Davies, N.M., & Toth, I. (2005). Encapsulation of lipopeptides with in liposomes: Effect of number of lipid chains, chain length and method of liposome preparation. International Journal of Pharmaceutics 301, 247–254.
61. Saetern, A.M., Flaten, G.E., & Brandl, M. (2004). A Method to Determine the Incorporation Capacity of Camptothecin in Liposomes. AAPS PharmSciTech. 5(3), 1 -8.
62. Sharma, S.K., Kumar, M., & Ahuja, M. (2008). Hepatoprotective Study of Curcumin-Soya Lecithin Complex. Sci. Pharm., 76: 761–774.
63. Liu, H., Li, S., Wang, Y., Han, F., & Dong, Y. (2006). Bicontinuous water – AOT /Tween 85-Isopropyl myristate micro emulsion: a new vehicle for transdermal delivery of cyclosporine A. Drug Dev. and Ind. Pharmacy., 32(5), 549-557.
64. Siewert, M., Dresssmen, J., Brown, C. K., & Shah, V.P. (2003). FIP/AAPS Guideline to dissolution/in-vitro release testing of novel/special dosage form. AAPS PharmSci Tech., 4 (1), 1-10.

65. Suwanpidokkul, N., Thongnopnua, P., & Umprayn, K., (2004). Transdermal delivery of zidovudine (AZT): the effects of vehicles, enhancers, and polymer membranes on permeation across cadaver pig skin. AAPS PharmSciTech. 5, 48.
66. Jantharaprapap, R., & Stagni, G. (2007). Effects of penetration enhancers on in-vitro permeability of meloxicam gels. International Journal of Pharmaceutics. 343, 26–33.
67. Wiener, N. Lieb, L. "Medical Applications Of Liposome." Elsevier: Oxford, 1998.
68. Karine Andrieux, Laura Forte, Sylviane Lesieur, Maité Paternostre,Michel Ollivon, Cécile Grabielle-Madelmont. "Solubilisation Of Dipalmitoylphosphatidylcholine Bilayers By Sodium Taurocholate:A Model To Study The Stability Of Liposomes In The Gastrointestinal Tractand Their Mechanism Of Interaction With A Model Bile Salt." European Journal of Pharmaceutics and Biopharmaceutics, (2009): 346–355.
69. Nava G, Piñón E, *et al* "Formulation and in vitro, ex vivo and in vivo evaluation of elastic liposomes for transdermal delivery of ketorolac tromethamine" Pharmaceutics, 2011, 3: 954-970.
70. Yadav A.V, Murthy M.S, Shete A. S and Sfurti Sakhare. "Stability Aspects of Liposomes." Indian Journal Of Pharmaceutical Education And Research, Oct-Dec 2011: 402-413.
71. Lasic, D.D.,. "Novel Application of Liposomes" Tibitech 16, 1998: 307-321.
72. Chen, C.M. And D. Alli. J. Pharm. Sci, 1987: 419.
73. Kirby C and Gregoriadis G. Dehydration-rehydration vesicles: a simple method for highyield drug entrapment in liposomes. Bio-technology 1984; 2: 979–984.
74. Crowe LM, Womersley C, Crowe JH, Reid D, Appel L and Rudolph A. Prevention of fusion and leakage in freeze-dried liposomes by carbohydrates. Biochim. Biophys.Acta. 1986; 861: 131–140.
75. Gregoriadis G et al. Liposome Technology, CRC Press, Boca Raton, 1992. Vol- I, II and III, 2nd Edition.
76. Su D *et al*. The role of Macrophages in the immune adjacent action of liposomes, Immune response against intravenously injected liposome associated albumin antigen. Immunology. 2009.
77. Lasic DD et al. Liposome a controlled drug delivery system. 1990; 172; 33-70.
78. Riaz, Mohammad. "Stability And Uses Of Liposomes." Pakistan Journal Of Pharmaceutical Sciences, July 1995: 69-79.
79. Abdus Samad, Y.Sultana, M.Aqil. "Liposomal Drug Delivery System Ana Update Review." Current Drug Delivery, 2007: 297-305.
80. Kirby C Delivery systems for enzymes. Chem. Br., 1990, 847–851.
81. Lasic DD Applications of Liposomes. In: Lipowsky R, Sackmann E, editors. Handbook of Biological Physics Liposome Technology. California, USA: Elsevier Science B. V, 1995, 491-519.
82. Tahibi A, Sakurai JD, Mathur R, Wallach DFH. Novasome vesicles in extended pesticide formulation. Proc. Symp. Contr. Rel. Bioact. Mat., 18, 1991, 231–232.
83. Gatt S, Bercovier JH, Barenholz Y. Use of liposomes to combat oil spills and their potential application to bioreclamation. In: Hinchee RE, Olfenbuttel RF, editors. On Site Bioreclamation. Stoneham, Butterworth, 1991, 293–312.
84. Gorin NC, Prentice HG, Meunier F, Ringden O and Tura S. Amphotericin B-liposome for confirmed opportunistic deep mycoses in patients with leukemia. 23rd Congress of the International Society of Hematoloy, and the 32nd annual Meeting of the American Society of Haematology, November 28th to December 4th 1990.