



AN OVERVIEW: CLASSIFICATION AND APPLICATION AND METHODS FOR PREPARATION AND CHARACTERIZATION OF LIPOSOMES AS DRUG DELIVERY SYSTEMS

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ABSTRACT

Liposomes as artificially prepared vesicles have become important tools for improving delivery of a large number of drugs: antimicrobial agents, drugs against cancer, antifungal drugs, peptide hormones, enzymes, vaccines and genetic materials. Due to the differences in preparation methods and lipid compositions, liposomes can be classified according to their lamellarity, size, charge and application. The flexibility of their behavior can be exploited for the drug delivery through various routes of administration irrespective of their solubility properties. Encapsulation of drugs in liposomes has provided an opportunity to enhance the therapeutic indices of many drugs mainly through alteration in their biodistribution, targeting the drug to particular tissues. The role of liposomes as drug delivery system is to deliver drug in the controlled manner, reducing undesirable side effects improving its *in vitro* and *in vivo* activity, as well as reducing the toxicity of the drug and enhancing the efficacy of the encapsulated drug. This article provides an overview of methods for preparation of liposomes, as well as analytical methods for control physical, chemical and biological parameters for different types of drugs.

KEY WORDS- Advantage of liposomes, Classification, Method of preparation, Evaluations of liposomes.

1. INTRODUCTION

Paul Ehrlich in 1906 initiated the era of development for targeted delivery. When he predicts a drug delivery mechanism that would target directly to disease cell, what he called as magic bullet,^[1] the objective of any drug delivery system is to control the pharmacokinetics and site of action and greater efficacy and minimize the toxic effects compared to conventional drugs. Many varieties of delivery systems that are used for carrier systems for example microspheres^[2] the name liposome is derived from two Greek words: 'Lipos' meaning fat and 'Soma' meaning body. Liposomes were first described by British haematologist Dr. Alec D Bangham FRS in 1961 (published 1964). 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.^[3] Liposomal vesicles were prepared in the early years (1960's) of their history from various lipid classes identical to those present in most biological membranes.^[4] 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.^[5,6] Has higher protection against the degradation.^[7,8] Liposomes are bilayer structures composed of phospholipids and cholesterol that are used for the administration of nutrients and drugs. For drug delivery applications liposomes are usually unilamellar and range in diameter from about 50 – 150 nm. Larger liposomes are rapidly removed from the blood circulation. Liposomes have lipid bilayer structures, which is present with an aqueous volume wholly enclosed by a membrane, composed of lipid molecules in such a way that both hydrophilic and lipophilic drugs can be successfully entrapped.^[9,10] Liposomes are the drug carrier loaded with great variety of molecules such as small drug molecules, proteins, nucleotides and even plasmids. Liposomes can be formulated and processed to differ in size, composition, charge and lamellarity.^[11] The major structural components of liposomes are phospholipids and cholesterol. The lipid bilayer is composed of phospholipids which have a hydrophilic head group and a hydrophobic tail group. The head is attracted to water, and the tail, which is made of a long hydrocarbon chain, is repelled by water.^[12]

Phospholipids as the main component of the liposomes can easily get combined with the skin lipids improving drug penetration and localization in the skin layers.^[13,14] The cholesterol incorporated in the lipid membrane helps in the increasing the stability of liposomes, as well as it reduces the permeability of the membranes.^[15]

1.2. Liposomes

Paul Ehrlich in 1906 initiated the era of development for targeting delivery when he envisaged a drug delivery mechanism that would target drug directly to diseased cells what he called as magic bullet.^[16] Advances in combinatorial chemistry have led to the discovery of a wide number of new chemical entities (NCE) that have a potential therapeutic action on the biological systems. But most of the NCEs being discovered provide a challenge to the formulation scientist because of their physicochemical properties like poor solubility and permeability. Even though, above problems could be addressed, but most of the molecules fail to show their desired therapeutic action *in vivo*, which leads to lack of *in vitro* – *in vivo* correlation. A liposome is a tiny bubble (vesicle), made out of the same material as a cell membrane. Liposomes can be filled with drugs, and used to deliver drugs for number of diseases.^[16] Liposomes are spherical shaped small vesicles that can be produced from cholesterol, non-toxic surfactants, sphingolipids, glycolipids, long chain fatty acids and even membrane proteins. Phospholipids spontaneously form a closed structure when dissolved in water with internal aqueous environment bounded by phospholipids bilayer membranes, this vesicular system is called as liposome. The name liposome is derived from two Greek words: 'Lipos' meaning fat and 'Soma' meaning body. Structurally, liposomes are concentric bleeder vesicles in which an aqueous Liposomes were first described by British haematologist Dr Alec D Bangham FRS in 1961

(published 1964), at the Babraham Institute, in Cambridge. They were discovered when Bangham and R. W. Horne were testing the institute's new electron microscope by adding negative stain to dry phospholipids. The resemblance to the plasma lemma was obvious, and the microscope pictures served as the first real evidence for the cell membrane being a bilayer lipid structure.^[17] Bangham who was studying phospholipids and blood clotting.^[15] The potential use of liposomes as drug carriers was recognized more than 25 years ago and, since that time, liposomes have been used in a broad range of pharmaceutical applications.^[18] They present many advantages since they can be used as carriers for both hydrophilic and lipophilic molecules, as well as drug delivery systems for controlled drug delivery for different therapeutically purposes. An important aspect of liposomes is the protection that they afford as an encapsulating agent against potentially damaging conditions in external environments. Liposomes are also an important system in their own right in medical, cosmetic, and industrial.^[10] In nature, phospholipids are found in stable membranes composed of two layers (a bilayer). In the presence of water, the heads are attracted to water and line up to form a surface facing the water. The tails are repelled by water, and line up to form a surface away from the water. In a cell, one layer of heads faces outside of the cell, attracted to the water in the environment, and another layer of heads faces inside the cell, attracted by the water inside the cell. The hydrocarbon tails of one layer face the hydrocarbon tails of the other layer, and the combined structure forms bilayer.^[19] the polar character of the liposomal core enables polar drug molecules to be encapsulated. Amphiphilic and lipophilic molecules were solubilised within the phospholipids bilayer according to their affinity towards the phospholipids.^[20]

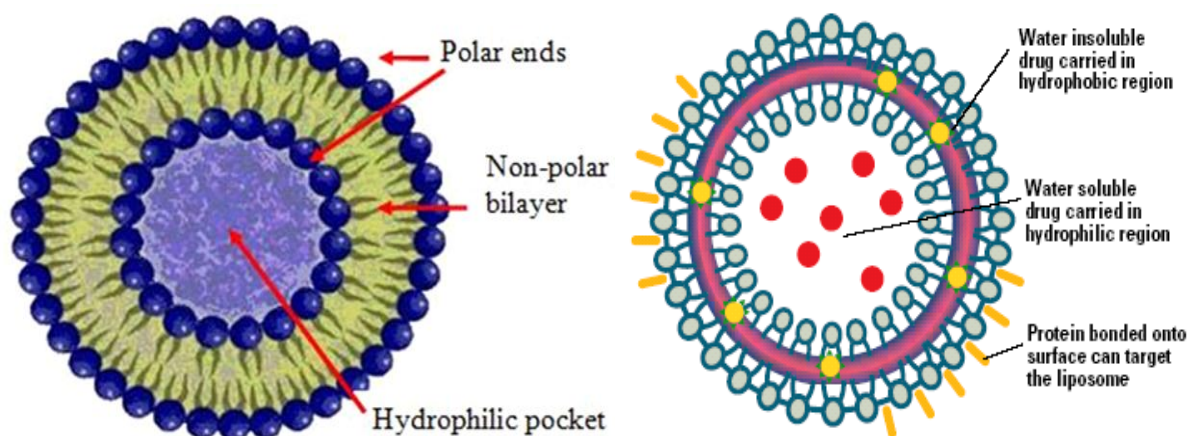


Fig.1 Liposomes

1.3. Classification of liposomes

The liposome system has a major advantage over competing colloidal carrier systems: it allows almost infinite possibilities to alter structural and physicochemical characteristics. This feature of flexibility enables the formulation scientist to modify

liposome behavior *in vivo* and to tailor liposome formulations to specific therapeutic needs. In an attempt to classify the plethora of possible liposome versions, it can be broadly distinguished on the basis of structural parameters and on the basis of composition and application.^[21]

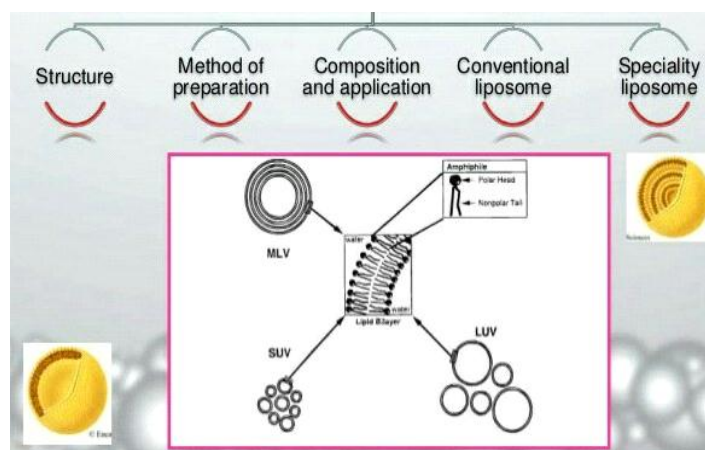


Fig.2. Classification of liposomes

1.3.1. Classification of liposomes based on Structural parameters

1.3.1.1. Multi lamellar vesicles (MLV)

MLV have a size greater than $0.1\ \mu\text{m}$ and consist of two or more bilayers. Their method of preparation is simple, which includes thin film hydration method or hydration of lipids in excess of organic solvent. They are mechanically stable on long storage. Due to the large size, they are cleared rapidly by the reticulo endothelial system (RES) cells and hence can be useful for targeting the organs of RES.^[21] MLV have a moderate trapped volume, i.e., amount of aqueous volume to lipid ratio. The drug entrapment into the vesicles can be enhanced by slower rate of hydration and gentle mixing.^[22] Hydrating thin films of dry lipids can also enhance encapsulation efficiency.^[23] Subsequent lyophilisation and rehydration after mixing with the aqueous phase (containing the drug) can yield MLV with 40% encapsulation efficiency.^[24]

1.3.1.2. Large unilamellar vesicles (LUV)

This class of liposomes consists of single bilayers and has a size greater than $0.1\ \mu\text{m}$. They have higher encapsulation efficiency, since they can hold a large volume of solution in their cavity.^[25] They have high trapped volume and can be useful for encapsulating hydrophilic drugs. Advantage of LUV is that less amount of lipid is required for encapsulating large quantity of drug. Similar to MLV, they are rapidly cleared by RES cells, due to their larger size. LUV can be prepared by various methods like ether injection, detergent dialysis and reverse phase evaporation techniques. Apart from these methods, freeze thawing of liposomes^[26], prepared by the extrusion or sonication of MLV or LUV. All these preparation methods involve the usage of organic solvents or detergents whose presence even in minute quantities can lead to toxicity. In order to avoid this, other methods like polyol dilution^[27], bubble method and heating method^[28] have been developed without using any organic solvents or detergents. Detailed procedures for liposome preparation can be obtained from literature dehydration/ rehydration of SUV and slow swelling of lipids in non-electrolyte solution.^[29]

1.3.1.3. Small unilamellar vesicles (SUV)

SUV are smaller in size (less than $0.1\ \mu\text{m}$) when compared to MLV and LUV, and have a single bilayer. They have a low entrapped aqueous volume to lipid ratio and characterized by having long circulation half life. SUV can be prepared by using solvent injection method (ethanol or ether injection methods)^[30] or alternatively by reducing the size of MLV or LUV using sonication or extrusion process under an inert atmosphere like nitrogen or Argon. The sonication can be performed using either a bath or probe type sonicator. SUV can also be achieved by passing MLV through a narrow orifice under high pressure. These SUV are susceptible to aggregation and fusion at lower or negligible/ no charge.^[31]

1.3.1.4. Oligo lameller vesicles (OLV)

These are made up 2-10 bilayers of lipids surrounding a large internal volume.^[29]

1.3.1.5. Multil vesicular vesicles (MVV)

They have several bilayers. They can compartmentalize the aqueous volume in an infinite numbers of ways. The arrangement can be onion like arrangements of concentrically spherical bilayers of LUV and MLV enclosing a large number of SUV.^[29]

1.3.2. Classification of liposomes on based on method of preparation.

The correct choice of liposome preparation method depends on the following parameters:

- The physicochemical characteristics of the material to be entrapped and those of the liposomal ingredients;
- The nature of the medium in which the lipid vesicles are dispersed; the effective concentration of the entrapped substance and its potential toxicity;
- Additional processes involved during application/ delivery of the vesicles;
- Optimum size, polydispersity and shelf-life of the vesicles for the intended application and batch-to-batch reproducibility and possibility of large-scale production of safe and efficient liposomal products.

1.3.4. Classification of liposomes based on composition

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

Fig.3. Classification of liposomes based on composition

1.4. Advantages of liposomes.^[19,25,30]

Liposomes as drug delivery systems can offer several advantages over conventional dosage forms especially for parenteral (i.e. local or systemic injection or infusion), topical, and pulmonary route of administration. The preceding discussion shows that liposomes exhibit different biodistribution and pharmacokinetics than free drug molecules. In several cases this can be used to improve the therapeutic efficacy of the encapsulated drug molecules. The limitations can be reduced bioavailability of the drug, saturation of the cells of the immune system with lipids and potentially increased toxicity of some drugs due to their increased interactions with particular cells. The benefits of drug loaded liposomes, which can be applied as (colloidal) solution, Aerosol, or in (semi) solid forms, such as creams and gels, can be summarized into seven categories:

➤ Improved solubility of lipophilic and amphiphilic drugs. Examples include Porphyrins, Amphotericin B, Minoxidil, some peptides, and anthracyclines, respectively; furthermore, in some cases hydrophilic drugs, such as anticancer agent Doxorubicin or Acyclovir can be encapsulated in the liposome interior at concentrations several fold above their aqueous solubility. This is possible due to precipitation of the drug or gel formation inside the liposome with appropriate substances encapsulated.

➤ Passive targeting to the cells of the immune system, especially cells of the mononuclear phagocytic system (in older literature reticuloendothelial system). Examples are antimonials, Amphotericin B, porphyrins and also vaccines, immunomodulators or (Immuno) suppressors.

➤ Sustained release system of systemically or locally administered liposomes. Examples are doxorubicin, cytosine arabinose, cortisones, biological proteins or peptides such as vasopressin;

➤ Site-avoidance mechanism: liposomes do not dispose in certain organs, such as heart, kidneys, brain, and nervous system and this reduces cardio-, nephro and neuro-toxicity. Typical examples are reduced

nephrotoxicity of Amphotericin B, and reduced cardio toxicity of Doxorubicin liposomes;

➤ Site specific targeting: in certain cases liposomes with surface attached ligands can bind to target cells ('key and lock' mechanism), or can be delivered into the target tissue by local anatomical conditions such as leaky and badly formed blood vessels, their basal lamina, and capillaries. Examples include anticancer, antiinfection and anti-inflammatory drugs;

➤ Improved transfer of hydrophilic, charged molecules such as chelators, antibiotics, plasmids, and genes into cells; and Improved penetration into tissues, especially in the case of dermally applied liposomal dosage forms. Examples include anesthetics, corticosteroids, and insulin. Among numerous studies which showed improved therapeutic index we shall mention only those which had significant impact and are also in various phases of preclinical and clinical studies in humans. In general, liposome encapsulation is considered when drugs are very potent, toxic and have very short life times in the blood circulation or at the sites of local (subcutaneous, intramuscular or intrapulmonary) administration.

➤ Provides selective passive targeting to tumour tissues (Liposomal doxorubicin).

➤ Increased efficacy and therapeutic index.

➤ Increased stability via encapsulation.

➤ Reduction in toxicity of the encapsulated agent

➤ Site avoidance effect.

➤ Improved pharmacokinetic effects (reduced elimination, increased circulation life times).

➤ Flexibility to couple with site specific ligands to achieve active targeting.

➤ Liposome is used for drug delivery systems due to its unique structural properties.

➤ Liposome herbal therapy acts as a carrier for small cytotoxic molecules and as vehicle for macromolecules as gene.

1.5. Components of Liposome: There are number of the structural and nonstructural components of liposomes, major structural components of liposomes a

1.5.1. Phospholipids: Phospholipids are the major structural components of biological membranes. The most common phospholipids used in liposomal preparation is phosphatidylcholine (PC). Phosphatidylcholine is an amphipathic molecule containing.^[17,32]

- A hydrophilic polar head group, phosphocholine
- A glycerol bridge
- A pair of hydrophobic acyl hydrocarbon chains

Molecules of phosphatidylcholine are not soluble in water. In aqueous media they align themselves closely in planar bilayer sheets in order to minimize the unfavourable action between the bulk aqueous phase and the long hydrocarbon fatty chain. Then the sheets fold on themselves to form closed sealed vesicles. There are several phospholipids that can be used for the liposome preparation such as-

Dilaurylphosphotidyl	choline	(DLPC),
Dimyristoylphosphotidyl	choline	(DMPC),
Dipalmitoylphosphotidyl	choline	(DPPC),
Distearoylphosphotidyl	choline	(DSPC),
Dioleoylphosphotidyl	choline	(DOPC),
Dilaurylphosphotidyl	ethanolamine	(DLPE),
Dimyristoylphosphotidyl	ethanolamine	(DMPE),
Distearoylphosphotidyl	ethanolamine	(DSPE),
Dioleoylphosphotidyl	ethanolamine	(DOPE),
Dilaurylphosphotidyl	glycerol	(DLPG),
Distearoylphosphotidyl	serine	(DSPS)

1.5.2. Soya-L- α -lecithin^[17]

a. Structure

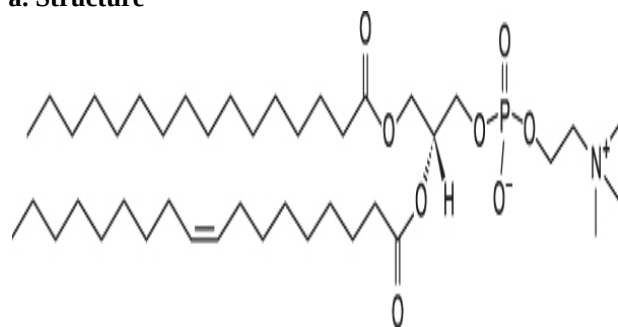


Fig.4. Structure of Soya-L- α -lecithin

b.Synonym-1, 2diacyl glycerol-3-phosphocholine, Phosphatidyl Choline and Lecithin

c. Molecular weight -760.09 g/mol.

d. Solubility: Soluble in diethyl ether. Insoluble in cold water, acetone. Soluble in about 12 parts of absolute alcohol. Soluble in chloroform, petroleum ether, mineral oil, fatty acids. Sparingly soluble in benzene. Insoluble in cold vegetable and animal oils.

e. Nutritional Properties

It decreased level of cholesterol and lipids, supplied poly unsaturated fatty acids, protection against fatty, degeneration of the liver/brain, improves memory function, promotes ability to concentrate, and controls lipid metabolic disorders.

f. Pharmacological action

Phosphatidylcholine may have hepatoprotective activity. Phosphatidylcholine is important for normal cellular membrane composition and repair. Phosphatidylcholine is also the major delivery form of the essential nutrient choline. Choline itself is a precursor in the synthesis of the neurotransmitter acetylcholine, the methyl donor betaine and phospholipids, including phosphatidylcholine and spingomyelin among others. Phosphatidylcholine is involved in the hepatic export of very-low-density lipoproteins.^[33] Fatty acids, has a marked fluidizing effect on cellular membranes. Decreased cell-membrane fluidization and breakdown of cell-membrane integrity, as well as impairment of cell-membrane repair mechanisms, are associated with a number of disorders, including liver diseases, neurological diseases, various cancers and cell death.

g. Pharmacokinetics: Phosphatidylcholine is absorbed into the mucosal cells of the small intestine, mainly in the duodenum and upper jejunum, following some digestion by the pancreatic enzyme phospholipase, producing lysophosphatidylcholine (lysolecithin). Reacylation of lysolecithin takes place in intestinal mucosal cells, reforming Phosphatidylcholine This is then transported by the lymphatics in the form of chylomicrons to the blood. Phosphatidylcholine is transported in the blood in various lipoprotein particles, including very-low-density-lipoproteins (VLDL), low-density lipoproteins (LDL) and high-density lipoproteins (HDL); it is then distributed to the various tissues of the body. Some phosphatidylcholines are incorporated into cell membranes. Phosphatidylcholine is also metabolized to choline, fatty acids and glycerol. The fatty acids and glycerol either get oxidized to produce energy or become involved in lipogenesis.^[33]

1.5.2. Cholesterol

The role of cholesterol in formulation of liposomes was given below.^[34]

- Incorporation of sterols in liposome bilayer produces major changes in the preparation of these membranes.
- Cholesterol itself does not form a bilayer structure.
- However, cholesterol acts as a fluidity buffer.

It makes the membrane less ordered and slightly more permeable below the phase transition and makes the membrane more ordered and stable above the phase transition. It can be incorporated into phospholipids membranes in very high concentration up to 1:1 or even 2:1 molar ratios of cholesterol to phospholipids.

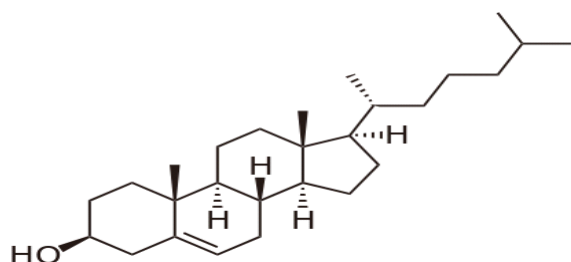


Fig.5.Cholesteroles

1.6. Method of preparation of liposomes^[9,10, 17]

Liposome may be prepared by two techniques

- a) Passive loading technique.
- b) Active loading technique.

1 Passive loading technique

A) Mechanical dispersion method

- Lipid hydration by hand shaking or freeze drying
- Micro emulsification
- Sonication
- French pressure cell

Membrane extrusions

- Dried reconstituted vesicle
- Freeze thawed liposome

B) Solvent dispersion method

- Ethanol injection
- Ether injection
- Double emulsion vesicle
- Reverse phase evaporation vesicle
- Stable plurilamellar vesicle

C) Detergent removal method

- Detergent (cholate, alkylglycoside, Triton x-100) removed from mixed micelles
- Dialysis
- Column chromatography

2) Active loading technique

1) Passive loading technique

Passive loading techniques include three different group of method working on different principles namely mechanical dispersion, solvent dispersion and detergent solubilization.

A. Mechanical dispersion method

Sonication-

i. Sonication is perhaps the most extensively used method for the preparation of SUV. Here, MLVs are sonicated either with a bath type sonicator or a probe

sonicator under a passive atmosphere¹¹. The main disadvantages of this method are very low internal volume/encapsulation efficacy, possible degradation of phospholipids and compounds to be

ii. Solvent evaporation method

In this method lipid phase containing soyalecithin and cholesterol were dissolved in organic solvent chloroform. The organic solvent was removed by rotatory evaporation to form a thin film of the lipid mixture in the inner wall of the round bottom flask. The drugs were dissolved in Phosphate buffer (7.4) and ethanol 2:2 was injected to the dried lipid film as rehydration medium. The liposomes were formed after vortexing 15 min.^[35]

iii. Fusion method

Components of lipid phase and propylene glycol was kept at 60°C water bath to form uniform lipid phase. Drug was dissolved in suitable amount of acetone and added to lipid phase. Mixture was kept at 60°C to evaporate acetone subsequently the aqueous phase (phosphate buffer saline) was warm up to 60°C and added to lipid phase. Then vortexing for 15 min. Liposome was not properly formed.^[35]

iv. Probe sonication.

The tip of a sonicator is directly engrossed into the liposome dispersion. The energy input into lipid dispersion is very high in this method. The coupling of energy at the tip results in local hotness; therefore, the vessel must be engrossed into a water/ice bath. Throughout the sonication up to 1 h, more than 5% of the lipids can be de-esterified. Also, with the probe sonicator, titanium will slough off and pollute the solution.^[36]

v. Bath sonication.

The liposome dispersion in a cylinder is placed into a bath sonicator. Controlling the temperature of the lipid dispersion is usually easier in this method, in contrast to sonication by dispersal directly using the tip. The material being sonicated can be protected in a sterile vessel, dissimilar the probe units, or under an inert atmosphere.^[36]

1.6. Characterization and evaluation

Liposome was characterized for visual appearance, turbidity, size distribution, lamellarity, concentration, composition, presence of degradation products, and stability. The behaviour of liposomes in both physical and biological system is governed by these factors; therefore liposomes are characterized for physical attributes and chemical compositions.

1.6.2. Microscopic observation

Examination of macroscopic characteristics is the first approximation of the preparation changes observed in the quality of visual, olfactory and tactile characteristics are indicators of liposomal preparation based on the particle size and composition the appearance of the liposomal

suspension may be varying from translucent to milky. The samples are homogeneous if the turbidity has a bluish shade; the presence of a non-liposomal dispersion is by flat, grey colour and is most likely a disperse inverse hexagonal phase or dispersed micro crystallites.^[24] An optical microscope can detect liposome of size greater than 0.3 µm as well as contamination with larger particles. Microscopic study was done by using binocular microscope.^[36]

1.6.2. Scanning electron microscopy (SEM)

The morphology (shape and surface characteristics) of liposomes was studied scanning electron microscopy.^[37]

1.6.3. Encapsulation efficiency

Entrapment efficiency of liposomes was determined by centrifugation method. Aliquots (1.5 ml) of liposomal dispersion were subjected to centrifugation on a laboratory centrifuge at 3000 rpm at 20°C for a period of 45 min. The clear supernatants (1ml) were diluted with PBS (7.4) and final volume make up to 10 ml then absorbance was measured using by UV-Vis spectroscopy. Entrapment efficiency was calculated by using following formula.^[15]

$$\% EE = \frac{\text{Entrapped CS}}{\text{Total CS}} \times 100$$

1.6.4. In vitro drug release:

In- vitro drug release testing, a measure of release of the active pharmaceutical ingredient (API) from the drug product matrix in controlled laboratory environment, is a key evaluation in drug development and quality control. It involves subjecting the dosage form to a set of conditions that will induce drug release and quantitating the amount of drug released under those conditions. In development, it is an essential test in assessing differences between prototypes, , and modelling *in vivo* behaviour. During this phase, *in vitro* conditions are generally selected to simulate *in vivo* conditions. The release of drug was determined by using the egg membrane mounted on the one end of open tube, containing 2ml of liposomal suspension. The egg membrane was suspended in 100ml beaker containing 50 ml of PBS (pH 7.4).The solution was stirred at 40 rpm with the help of magnetic stirrer at room temperature 37°C. The sample was withdrawn at suitable time interval at (1, 2, and 3,4,5,6, h).The dissolution medium was replaced with same amount of fresh PBS (pH 7.4) solution to maintain the volume 50ml throughout the experiment. The drug content in the withdrawn samples (1ml) making the volume up to 10 ml PBS (pH 7.4) and cumulative percentage of drug released was calculated with by the UV Visible Spectroscopy.^[15]

1.6.5. Zeta potential and Particle Size Determination:

Zeta potentials of the liposomal formulation were measured by the instrument Zetasizer Nano ZS for determination of stability of liposomes.^[38]

1.6.6. Rheological study: It is used for the determination of the setting behaviour and arrangement the vehicle of particle structure for the purpose of composition of liposomes. Rheological analysis of the experimental liposome was performed using rheometer. Liposomal viscosity was measured according to the applied shear rate. Experimental conditions were kept constant during the progress of the measurements. Experiments were conducted by changing the rotational speed, at different rpm 0.3, 0.6, 1.5,6.0, 12,15,40,60, respectively.^[39]

1.6.7. Refractive index: It is used for determining how much light is bent or refracted when entering the material it also determines the amount of light that is reflected when reaching the interface. The refractive index analysis of liposomal preparation was performed by using abbe's Refractometer in that a drop of sample was sandwiched in to a thin layer between an Calumniating prism and a refracting prism and subsequently the observation was done by a detector placed on the backside of the refracting prism would show a light and dark region and at that point reading is recorded [39].

1.6.8. Determination of pH: pH determination of the experimental liposomal preparation was performed using pH meter using glass electrode.^[40]

1.6.9. Stability: The stability of a pharmaceutical delivery system may be defined as the capability of a particular formulation, in a specific container to remain within its Physical, chemical, microbiological, therapeutic and toxicological specification.^[9,10]

CONCLUSION

Liposomes are one of the exclusive drug delivery system, which can be of potential use in controlling and targeting drug delivery. Liposomes are administrated orally, parenteral and topically as well as used in cosmetic and hair technologies, sustained release formulations, diagnostic purpose and as good carriers in gene delivery various drugs with liposomal delivery systems have been approved. Nowadays liposomes are used as versatile carriers for targeted delivery of drug. These review discusses the classification of liposomes and Advantage, Method of preparation, and evaluation of liposomes was studied.

REFERENCE

1. Alving CR. Macrophages as targeting for delivery of liposome encapsulated antimicrobial agents advance drug delivery. 1998; 12(5): 90-98.
2. Gabions A. Stealth liposomes and tumor targeting: one step further in the quest for the magic bullet. Adv drug deliv rev. 2001; 76(7): 2223-2225.
3. Bangham AD, Standish MM, Watkins JC. Diffusion of univalent ions across the lamellae of swollen phospholipids, J. Mol. Biol. 1965; 20(12): 238-252.
4. Fielding, M.R. Liposomal drug delivery advantages and limitations from a clinical pharmacokinetics and

- therapeutic perspective. *Clin Pharmacokinetics*, 1991; 9(2): 155-164.
5. Reddy KH, Reddy MM. In-Vitro formulation and evaluation of cefezime liposome formulation, *Research article pharmaceutical science* 2012; 1(6): 198-207.
 6. Akbarieh M, Besner JG, Galal A, Tawashi R. Liposomal delivery system for the targeting and controlled release of praziquantel. *Drug Dev Ind Pharm*. 1998; 18(2): 303-317.
 7. Verma S, Singh SK, Syan N, Mathur P, Valecha V. *Journal of Chemical and Pharmaceutical research*. 2010; 2(2): 496-509.
 8. Mohammad Zishan, Zeeshan Ahmad, Faisal Saeed, Mohd Wasim Hussain, Suhail Ahmad, Mohammad Saidurrahman, Sahar Idris, Najma Khan, Vesiclar drug delivery system for liver diseases, *World journal of Pharmaceutical sciences* 2017; 5(4): 28-35.
 9. Vyas SP, Khar RK. Targeted and controlled drug delivery, *Novel carrier systems*. CBS publishers. 2002; 173-248.
 10. Jain NK Controlled and novel drug delivery. CBS publishers and distributors, New Delhi. 2007; 304-352.
 11. Singh A, Vengurlekar P, Rathod S. Design, development and characterization of liposomal neem gel, *International Journal of Pharma Sciences and Research*, 2014; 18(7): 140-148.
 12. Popovska O, Simonovska J, Kavrovski J, Rafajlovska. An Overview: Methods for Preparation and Characterization of Liposomes as Drug Delivery Systems, *International Journal of Pharmaceutical and Phytopharmacological Research*, 2013; 3(2): 14-20.
 13. Achim M, Precup, C. "Thermo sensitive liposomes containing doxorubicin. Preparation and in vitro evaluation *Farmacia*, 2009; 57(6): 703-710.
 14. Bhatia A. Tamoxifen in topical liposomes: development, characterization and in vitro evaluation" *J Pharm Pharmaceut Sci.*, 2004; 7(2): 252-259.
 15. Devi R, Prasad CM, Rengnathan A, Kasthuri S, Saundharajan R, Deepa N. Formulation characterization and evaluation of fluconazole liposomes. *Pelagia Research library*. 2015; 6(5): 61-66.
 16. Shashi K, Satinder K, Bharat P. A complete review on liposomes, *International research journal of pharmacy*. 2012; 4(8): 3-7.
 17. Bangham AD, Standish MM, Watkins JC Diffusion of univalent ions across the lamellae of swollen phospholipids, *J. Mol. Biol.* 1965; 20(12): 238-252
 18. Argan SL, Harikumar, Nirmala. topical liposomal gel: a novel drug delivery system. *wjop*. 2011; 25(6): 36-43.
 19. Dua JS, Rana AC, Bhandari. Liposome: methods of preparation and applications. *International Journal of Pharmaceutical Studies and Research*. 2012; 17(10): 14-20.
 20. Perri Y, Mohammed AR. Vangala, McNeil SE. Environment scanning electron microscopy offers real-time morphological analysis of liposomes and niosomes, *J. of liposome res.* 2007; 17(1): 27-37.
 21. Sharma M. Applications of nanotechnology based dosage forms for delivery of herbal drugs, research and reviews, *Journal of pharmaceuticals and nanotechnology*. 2014; 11(8): 23-30.
 22. Ohsawa T, Miura H, Harada K. A novel method for preparing liposome with a high capacity to encapsulate proteinous drugs: freeze-drying method, *Chem Pharm Bull*. 1984; 32(12): 2442-2445.
 23. Olson F, Hunt T, Szoka F, Vail WJ, Papahadjopoulos D. Preparation of liposomes of defined size distribution by extrusion through polycarbonate membranes. *Biochim Biophys Acta*. 1979; 557: 9-23.
 24. Ortan A, Parvn DC. Rheological study of a liposomal hydrogel based on carbopol. *Romanian biotechnological letters*. 2011; 16(9): 47-54.
 25. Vemuri S, Rhodes CT. Preparation and characterization of liposomes as therapeutic delivery systems: a review. *Pharmaceutica Acta Helvetiae*. 1995; 70(5): 95-111.
 26. Pick UI. Liposomes with a large trapping capacity prepared by freezing and thawing of sonicated phospholipid mixtures. *Arch Biochem Biophys*. 1981; 212(12): 186-194.
 27. Kikuchi H, Yamauchi H, Hirota S. A polyol dilution method for mass production of liposomes. *J Liposome Res*. 1994; 26(4): 71-91.
 28. Talsma H, Steenbergen MJ, Borchert JCH, Crommelin DJA. A novel technique for the one-step preparation of liposomes and nonionic surfactant vesicles without the use of organic solvents. Liposome formation in a continuous gas stream: The bubble method. *J Pharm Sci*. 1994; 83(8): 276-280.
 29. Shew RL, Deamer DA novel method for encapsulation of macromolecules in liposomes. *Biochim Biophys Acta*. 1985; 816(5): 1-8.
 30. Hamilton RL, Goerke J, Guo L. Unilamellar liposomes made with the French pressure cell: A simple preparative and semi-quantitative technique. *J Lipid Res*. 1980; 18(21): 981-992.
 31. Gregoriadis G. Liposome technology: Liposome preparation and related techniques. *Informa Health care*, New York. 2007; 3(1): 21-24.
 32. McIntosh TJ. The effect of cholesterol on the structure of phosphatidylcholine bilayers, *Biochim. Biophys. Acta*. 1978; 513(5): 43-58.
 33. Suzuki S, Yamamoto H, Sakai M. Oral Administration of Soybean Lecithin Transphosphatidylated Phosphatidylserine Improves Memory Impairment in Aged Rats. *Journal of nutrition*. 2001; 13(11): 2951-2956.
 34. Yeagle PL. Modulation of membrane function by cholesterol. *Biochimie*. 1991; 73(10): 1303-10.
 35. Asili J, Mosallaei N, Shaterzadeh A, Nikouei B. Preparation and characterization of liposomes containing methanol extract of aerial parts of

- Platyclus orientalis franco, Avicenna journal of phytomedicine. 2012; 65(10): 17-24.
36. Khaw B, Torchilin VP, Berdichevski VR, Barsukov AA, Klibanov AL, Smirnov VN. Enhancing specificity and stability of targeted liposomes by co incorporation of sialoglycoprotein and antibody on liposomes. Exp biol med. 1983; 95(12): 776-781.
 37. Umalkar, D.G., Rajesh, K.S. Formulation evaluation of liposomal gel for treatment of psoriasis, Int J Pharm Bio Sci, 2013; 39(8): 90-98.
 38. Laouini, Jaafar J, Sfar S, Charcosset C, Fessi H. Preparation, Classification and Application of liposome, State of art. J. Colloid Sci. Biotechnol. 2012; 109(1): 147-168.
 39. Malana AM, Zohra R. Rheological characteristic of novel physically crosslinked polymeric hydrogels at different temperatures. Biochem biophys acta, 2012; 48(24): 155-162.
 40. Brandi M. Liposome as drug carriers: a technological approach. Biotechnol. annu. rev. 2009; (7): 59-85