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Review Article

Liposomes: As An Important Drug Delivery System

Shachi Pandey*¹, Alka Verma², Arvind Singh³, Aman Kumar Singh⁴

^{1,3,4}Rameshwaram Institute of Technology and Management, Lucknow Uttar Pradesh- 227202, India

²Rameshwaram Pharmacy College, Manwa, Sidhauri Uttar Pradesh- 261303, India

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ABSTRACT

Liposomes are considered as an important drug delivery system with lots of clinical applications. Important discovery in drug delivery system has been begins in 1950's with the new research study of polyclonal antitumor antibodies. Liposomes are developed by Bangham and his colleagues in early 1960's. The term liposomes mean lipid body. These are artificially prepared a simple microscopic vesicle. These vesicles are made up of phospholipid and cholesterol. Several types of drugs can be filled in liposomal formulation according to the needs of patient. It is used to deliver the medicament at the site of action without causing toxicity. Liposomes are used to treat cancer and many other diseases. The normal sizes of the liposome ranges from 25 to 1000 nm. Generally Liposomes are sphingo-lipids, glycol-lipids, long chain fatty acids. It is also known as a vesicular system. Liposome provides an important technology which shows various advantages by delivering active constituents to the site of action. They play an important role for poorly soluble drugs and improve the penetration of these loaded drugs. Liposomes are considered as good carriers for drug delivery which have the ability to incorporate both lipophilic and lipophobic material. This pivotal contribution of liposomal technology is widely observed in the healthcare sector. General introduction classification, liposomal preparation methods, advantages and various applications of the liposomes are discussed by us in this review paper. Liposomes are well known for targeting drug delivery system because it increases the solubility, bioavailability, half life of the poorly soluble drugs. It provides controlled release of the drug at the targeted site in order to reduce the toxicity.

INTRODUCTION

LIPOSOMES:

Paul Ehrlich who is a great German bacteriologist, given the term "magic bullet," in the late 19th century, which is considered as a good carrier for

the transporter of the medicament at the targeted site of action. It shows the property of destroying abnormal cells without affecting normal cells. Many approaches are used to provide targeted drug delivery (1,2) Liposomes are discovered by

*Corresponding Author: Shachi Pandey

Address: Rameshwaram Institute of Technology and Management, Lucknow Uttar Pradesh- 227202, India

Email ✉: shachi5@gmail.com

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A.D Bangham and R.W Thorne in the 1961 from the Braham institute at Cambridge. One of the Bangham's colleagues, Gerald Weismann, proposed the Structure of liposomes is given by Gerald Weismann, who is one of the Bengham's colleagues. The term liposome actually means lipid body. The word liposome is related to the Greek words 'Lipos' which means fat and 'Soma' which means body. It is defined as microscopic vesicles with the diameter ranging from 25 nm to 1000 nm. (3,8,10) Liposome vesicles are generally made up of phospholipids and cholesterol. Where phospholipids act as an important structural component of membrane while cholesterol is used to stabilize the membrane. Phospholipids are considered as amphipathic molecules, which contains lipophobic head and lipophilic tail. Lipophobic head consist of phosphoric acid and lipophilic tail consists of 2 fatty acid chain. The presence of medicament in the vesicles based on the varieties of lipids used in the formulation of liposomes and its physicochemical properties. (4,10, 12) Various types of liposomes are available according to the needs of patient. Name of the liposomes are mainly based on its structural component that is phospholipids and cholesterol while the size of liposomes is not depend on the structural component. Actually Liposomes are considered as artificially prepared formulation which is made up of phopholipid bilayers.(5,8) There are any drugs which are enclosed in liposomes, show the controlled release for targeted drug delivery. The release of the medicament is totally based on the size, surface properties and composition of liposome. Liposomes have the tendency to incapsulate both water soluble as well as also lipid soluble agents. This formulation is suitable for delivery of all type of drug constituents like lipophilic, hydrophilic, and amphipathic in nature.(7,8,11) Liposomes are mostly popular because of its important characteristics which are biocompatible, biodegradable, nontoxic, and non

immunogenic. Liposomes provide the protection of encapsulated drug from external environment. Drugs with widely varying lipophilicities can be encapsulated in liposomes, either in phospholipids bilayers in the entrapped aqueous volume or at the bilayer interface.(8,12) Liposome is most commonly used in vesicular research because of their bio-compatibility and structural similarities to biological cells. Liposomes have achieved its acceptance in the vesicular research due to their biocompatibility, similarities to the structure of biological cells. There are various studies which shown that liposomes can be used for safely and effectively administration of therapeutic molecules from various classes such as anti tubercular, anti cancer, anti fungal , anti viral, anti microbial, skin care, vaccines etc.(11,12)

STRUCTURAL COMPONENTS OF LIPOSOMES

Liposomes are generally made up of two components. These components are phospholipids which have a hydrophilic head and two lipophilic tails and cholesterol that is also very important structural components. Cholesterol is mainly used to maintain the stability of membrane. (8, 12)

1. Phospholipids
2. Cholesterol

1. Phospholipids:

Phospholipids are derived from the phosphatic acid. Glycerol is the backbone of this group. . Phospholipids are considered as amphipathic molecules because of having a lipophobic head and two lipophilic tail .The lipophobic head consist of molecules of phosphorous as phosphoric acid group. Lipophilic tail has long hydrocarbon chain groups. Saturated fatty acids are used In order to maintain the stability of liposomes. (1,8)

Common Examples of phospholipids-

- Phosphoglycerides
- Plasmalogen
- Lecithin
- Cephalin



- Phosphatidyl serine (PS)
- Phosphatidyl inositol (PI)
- Phosphatidyl glycerol (PG)

Phosphoglycerides:

It is one of the most commonly used phospholipids which is amphipathic molecule. Phosphoglycerides consist of three hydroxyl groups present in glycerol moiety. Two hydroxyl groups are attached with two fatty acids while one is attached with phosphoric acid. This type of phospholipids should have two nonionic tails of fatty acid where one is saturated and other is unsaturated. phosphoglycerides can be identified because of their attached ionic head alcohol group. (9, 11, 15)

Cephalins:

Cephalin is also known as Phosphatidyl ethanolamine. The structure of cephalins resembles to phosphatidyl choline. Lecithin consists of choline which is replaced by serine in order to form phosphatidyl serine and phosphatidyl ethanoalamine respectively. Cephalin always available in two forms that is α and β . These forms are available due to the presence of two fatty acids. (9)

Plasmalogens:

The structure of plasmalogen is similar to that of lecithins and cephalins but with the change of one fatty acid which is displaced by dehydrated ether. It consists of about only 10% of phospholipids. Structure of plasmalogen contains base of nitrogen. This nitrogenous base is linked via phosphoric acid of phospholipids. This nitrogen base may be cholin, ethanolamine or serine.(7)

Phosphoinositides:

It is also a very important type of phospholipids. Phosphoinositides consist of cyclical structure of hexahydroxy alcohol, It is considered as inositol which is linked via phosphoric acid. When Phosphoinositides undergo the hydrolysis, it results glycerol, inositol and phosphoric acids. (1)

Lecithins: It is also known as Phosphatidyl choline. Lecithin is a very important type of phospholipid which consist of phosphate derived from soya beans and egg yolk. Lecithin consist of anhydrous, non ionic fatty acids. Phosphoric acid and glycerol is linked via choline which is nitrogen base of lecithin.(7,11)

Phosphosphingosides:

The structure of Phosphosphingosides is different from that of other phospholipids because of the presence of nitrogenous sphingosine together with choline. Phosphosphingosides molecules are electrically charged which consist of hydrophilic head phosphocholine. (9,1)

Cholesterol:

Molecular formula of cholesterol is $C_{27}H_{45}OH$. It is optically active and white crystalline solid. Cholesterol is lipid which consists of steroidal ring with attached hydroxyl group. Cholesterol and derivatives are often used in preparation of liposomes for –

- a Maintaining the stability of plasma membrane.
- b Declining the fluidity, microviscosity of the layer(1,11)

ADVANTAGES:

There are various advantages of liposomes.

- Liposomes are non toxic, bio-compatible, bio-degradable.
- Increased therapeutic index and efficacy of drug.
- Increased solubility of drug.
- Increased bioavailability and pharmacokinetics of drug.
- Increased stability of drug via encapsulation.
- Reduced dosing frequency.
- Site avoidance effect.
- Reduced toxicity of drug.(1-5,11)

DISADVANTAGES:

Along with large number of advantages, liposomes have few disadvantages-



- Short shelf life and stability.
- Production cost is high.
- Drug content may undergo leakage.
- Phospholipids may undergo hydrolysis and oxidation.(2,11)

TYPES OF LIPOSOMES:

Classification of liposomes can be done on the basis of the following.(1-3)

Based on composition

On the basis of composition, liposomes can be classified into five types.(1,13,7,)

- Conventional liposome
- Cationic liposome
- Ph sensitive liposome
- Immune liposome
- Long circulating liposome

Based on size and structural parameters

On the basis of size and structures, liposome can be classified into four types.

- ULV- Unilamellar vesicles
- MLV-Multilamellar vesicles
- MVV- Multivesicular vesicles
- OLV-Oligolamellar vesicles

1. Unilamellar Liposomes:

It is one of the most popular type of liposome. Vesicles of unilamellar consist of only one phospholipid bilayer. There are various methods for the preparation of this type of liposome. It is further divided in four types-

1. Large unilamellar vesicles
2. Small unilamellar vesicles
3. Medium sized unilamellar vesicles
4. Giant unilamellar vesicles(2,7)

2. Multilamellar Liposomes:

It consists of various phospholipid bilayer. The structure of multilamellar vesicles is resembles to onion structure. These liposomes are generally considered as traditional liposomes, which consist of numbers of phospholipid bilayers. The size of the multilamellar vesicles is ranges between 0.1 to 20um. Preparation of this type of liposome is very

simple. This type of liposomes is prepared by using a very common thin film hydration method. In this method phospholipid is dissolved in organic solvent with or without using cholesterol within the round bottom clinical flask in order to prepare thin layer of lipid. Organic solvent can be evaporated under vacuum by the use of rotary evaporator. As a result thin film of lipid is formed on the inner wall of the rotary flask. In this way multilamellar vesicles are formed as a result of addition of water by proper shaking in the rotary flask.(7,8,16)

3. Multi Vesicular Liposomes:

When large liposome vesicles resembles to multilamellar vesicles, enclose a group of liposomes, then the subsequent vesicles is known as multivesicular liposomes. Multi vesicular liposomes have advantages over multi lamellar vesicles. Multi vesicular liposomes have high storage stability and easy production.(3)

4. Small Unilamellar Vesicles:

It has a single phospholipid bilayer, which results in a liposome with a size range of 25–1000 nm. SUVs were created by Battsri and Korn (1953) by injecting an ethanolic phospholipid solution with the proper dilution and mixing into the aqueous phase above the phospholipids' phase transition temperature. Typically, probe sonication of MUV dispersion is used to prepare SUVs. SUVs can be produced by probe sonication of big liposomes. By submerging the probe sonicator in liposome dispersion and running it at maximum frequency, this technique breaks apart MUVs into SUVs. (3,5)

5. Large Unilamellar Vesicles:

It is composed of single phospholipid bilayer, which resembles to small unilamellar vesicles. The size of large unilamellar is greater than the size of SUVs, ranging from 0.1um to 1um. This type of liposomes are considered to form high lipophobic entrapment of drug content in compare to multylamellar vesicles.



The major drawbacks of this method are following-

- a The population of liposome is not homogeneous (70 -190).
- b The disclosure of encapsulated drug to high temperature or organic solvents.(3,18)

TRANSPOTATION MECHANISM:

There are various mechanisms which are used for the transportation of drug content from liposome toward the cell membrane. Generally it is very difficult to determine which mechanism is used, because more than one mechanism is used even at the single time.(8, 12,18)

- Endocytosis
- Adsorption
- Fusion
- Transfer

Endocytosis:

It is a cellular process which is done by the phagocytic cells. These cells are present in reticuloendothelial system .In this Process external material is moved toward the cell.

Adsorption:

Adsorption of the drug content can be done on the surface of the cell either by the interaction of cellular components or by weak lipophilic forces.

Fusion:

Liposomes can be fused with the plasma lipid membrane by addition of lipid bilayer of the liposome into the plasma membrane.

Transfer:

Liposomal phospholipids can be transferred to the plasma membrane without any cooperation of liposome content.

METHOD OF LIPOSOME PREPARATION:

There are various preparation methods of liposome.(1,2,7,9)

Mechanical dispersion method:

- Lipid film hydration by hand shaking method
- Non-hand shaking method
- Micro-emulsification method
- Membrane extrusion

- French-pressure cell
- Dried reconstituted vesicles
- Sonication

Solvent dispersion method:

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

Detergent removal methods:

- Column chromatography
- Dialysis
- Dilution

PHYSICAL DISPERSION METHOD:

Generally Liposome can be prepared by using this method. In this method soya lecithin is kept in different ratio while cholesterol remains constant. Here in this method firstly cholesterol and soya lecithin is taken in the different ratio and mix in 5 ml of chloroform. A flat bottom conical flask is required to carry out this method. Resulting mixture is spread over flat bottom conical flask. Now this solution containing flask is kept for overnight to evaporate at normal room temperature in order to prepare the thin film without disturbing the solution. Now 10 mg desired drug is dissolved in phosphate buffer PH 6.8 for the preparation of aqueous solution. This resulting solution is properly mixed with the lipid film for the purpose of hydration. For hydration conical flask is dawn to one side and then aqueous medium is added to the dawn side of the flask.After that flask is gradually returned toward the upright orientation. Now flat bottom conical flask is placed on water bath at temperature $37\pm 2^{\circ}\text{C}$ for two hrs in order to complete the hydration. The lipid layer is removed from side of conical flask by the gentle shaking of the conical flask and resulting liposome suspension. This liposome suspension is stored at 4°C for one day for the purpose of maturation. Now the prepared liposome suspension is

centrifuged at 15000 rpm around 20 minutes. After that the precipitate is collected and diluted with distilled water for more study. (1,7,)

ETHER INJECTION METHOD:

Different ratio of soya lecithin can be used in this method for the preparation of the liposomes but cholesterol is kept as constant. Here firstly cholesterol and soya lecithin is taken in the different ratio and dissolved in 10 ml of ether and ethanol. After that measured quantity of drug is dissolved in phosphate buffer pH6.8. This solution act as aqueous medium. This solution is placed in water bath at 60°C, Now Ether –lipid solution is injected drop by drop into the above prepared solution. The ether is evaporated when come into contact with aqueous phase, unilamellar liposomes will be formed by dispersed lipid. The final product will be collected. The maturation of the liposome is completed by the storage at 4°C. After that resulting liposomal suspension is centrifuged at 15,000 rpm for 20 minutes. The final precipitate is diluted with distilled water for evaluation studies. (5,9,11)

ETHANOL INJECTION:

Here in this method, liposomes are prepared by the rapid injection of lipid solution of ethanol into the buffer solution. The resulting liposomes are generally considered as multilamellar vesicles. (5,11)

Disadvantages:

There are following disadvantages of this method.

- a Heterogenous population.
- b Removal of ethanol is difficult.

SONICATION: The Sonication is most commonly used method in order to prepare small unilamellar vesicles. (8, 9)

Disadvantages:

There are some disadvantages of the sonication method.

- a Very low internal volume
- b Low encapsulation efficacy

Types of Sonication methods:-

1. Probe sonication

2. Bath sonication:

DIALYSIS:

The detergents are most commonly used in order to dissolve the lipids at their critical micelle concentration. When the detergent is removed, the micelles become increasingly better off in phospholipids. The micelles are combined to form LUVs. When the liposomes are formed, the detergents are removed by dialysis.(5,8,9)

STORAGE STABILITY OF LIPOSOMES:

The purpose of the liposome formulation is to reduced drug toxicity, dosing frequency and increase the bioavailability of drug at the targeted site. Generally liposomes are unstable as liquid dispersion. The liability of the phospholipids to degrade by oxidation or hydrolysis can cause liposome aggregation followed by leakage of entrapped material. Oxidation of the phospholipids can be reduced by the use of antioxidants. It can be also reduced by decrease in the storage temperature to 4°C. Freeze drying, spray drying, dispersion methods are the most commonly used techniques to maintain the stability of liposomes.(20,22)

APPLICATIONS OF LIPOSOMES:

There are various applications of liposomes. Some are given -

Systemic Application:

Liposomes are considered as a very good drug delivery vehicles. Liposomes are also known as nanoscale drug delivery system which can be used in order to treat systemic fungal infection. (2,28,35)

Topical Application:

There are various skin disorders which can be easily treated by using liposomal formulation. In the beginning of the liposomes, they are used for moisturizing and restoring effect. Generally liposomes are prepared by using phospholipid and cholesterol which can easily deliver the drug content to the dermatological field. (3,29,40)



Cosmetic Applications:

Liposomes are also used in order to deliver various important constituents in cosmetics. These are most commonly used in cosmetics because lipid are hydrated and decrease the roughness, wrinkles of skin. These are the basic reasons of ageing. In 1989, Christian Dior formulated an antiageing cream which is known as first liposomal cosmetic product. Treatment of hair loss can be also done by using liposomal formulation. There are various liposomal formulation which can be used to decrease the transdermal loss of water .It is also used to treat the dry skin. (2,35)

Gene Therapy:

Liposomes are used to deliver genetic material to the cells. Rationality is the ability of liposome to increase the accumulation in cells and provide the transfer of large and heavily charged molecules across the cellular membrane.(5, 8, 31)

Cancer Treatment:

There are many drugs which are used to treat the cancer but these drugs may be toxic in its free form, In order to decrease the toxicity of drugs, various liposomal formulations are prepared. It is good choice to deliver the drug at the targeted site without causing toxicity. Drug toxicity can be decreased about 50 % by the liposome formulation by decreasing the distribution of drug molecule toward the targeted organ. Liposomal encapsulation of anticancer drug provide sustained release and better efficacy of drug, mainly in case of systemic lymphoma.(17,8,38)

Fungal Infection:

liposome act as a carrier for various antifungal agents in antifungal therapies. Fungal infections can be easily treated by using a very important antifungal drug that is Amphotericin B. It is considered as the very important drug in order to treat the fungal infections. Instead of having numbers of advantages this drug is toxic in nature. In order to eliminate the toxicity of drug, liposomal formulation of amphotericin B is widely

used which prevent the storage of drug at the targeted site.(8,19,42)

Eye Disorders:

liposomes are widely used to medicate eye disorders. There are various diseases of eye like – Keratitis, which is noted as swelling of the cornea. Uveitis is considered as an eye disorder which is denoted by the swelling of second layer of the eye ball which is known as uvea. It is characterized by redness, pain of the eye and also loss of vision. Endophthalmitis, which is identified by swelling of inner layer of the eye. It is mainly caused by infection.(1,13,43,)

CONCLUSION:

Liposomes are considered as a very important transporter system in order to target the delivery of drug. They have various chemical and pharmaceutical applications. We shortly explain various advantages of liposome with special emphasis on formulation techniques, classification and recent advancement in the field of application. Liposomes have many advantages over the traditional approach as a drug carrier. They are available in variable concerning size and surface properties. They provide the sustained release. Liposomes having enhanced targeted drug delivery which increased the residence time in targeted organ. Various formulations of liposome are available in the market according to the needs of patient but many other formulations are under the process of clinical trial in order to get the final approval.(2,8,46)

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