



Liposomes-A Novel Drug Delivery System: A Review

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Abstract

Novel drug delivery systems play a major role in providing an optimum dose at a right time and to the right location. Liposomes are the novel drug delivery systems consisting of an aqueous compartment enclosed by means of one or more phospholipid bilayers. Liposomes are the most successful drug delivery systems which uses nanotechnology to potentiate therapeutic efficacy and reduces the toxicities that occurs due to conventional medicines. The ability of liposomes to encapsulate both hydrophilic and lipophilic drugs have allowed these vesicles to become useful drug delivery systems. The present article gives an idea regarding the classification of liposomes, mechanism of vesicle formation, advantages, disadvantages, methods of preparation of liposomes, novel liposomes, applications and marketed products.

Keywords

Liposomes, novel drug delivery system, phospholipids, vesicles.

INTRODUCTION

Liposomes are the microscopic sealed vesicular structures which can encapsulate both hydrophilic and hydrophobic materials and are used as drug carriers in various drug delivery systems ^[1]. Liposomes increases the therapeutic activity of a drug by protecting the drug from the biological environment and by restricting the effect of drug to target cells ^[2]. These are used as vehicles for administration of nutrients and pharmaceutical drugs.

The word liposome is derived from two Greek words lipo and soma. Lipo means fat and soma means body. Liposomes were discovered by Alec Douglas

Bangham and coworkers. Bangham found that closed bilayer structures were formed when he dispersed phosphatidyl choline molecules in water. Weismann named those structures as "Liposomes".

The simplest technique for preparing drug containing liposomes involves drying a solution of lipids in an organic solvent onto the wall of a flask or tube; the lipid is then hydrated and dispersed by adding buffer and vortexing. Drug to be incorporated into the liposomes can be included in the buffer if it is water soluble or included in the organic solvent if it is hydrophobic ^[3]. The structure of liposome is shown in Fig.1.

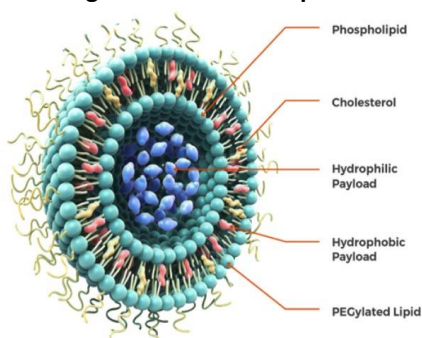
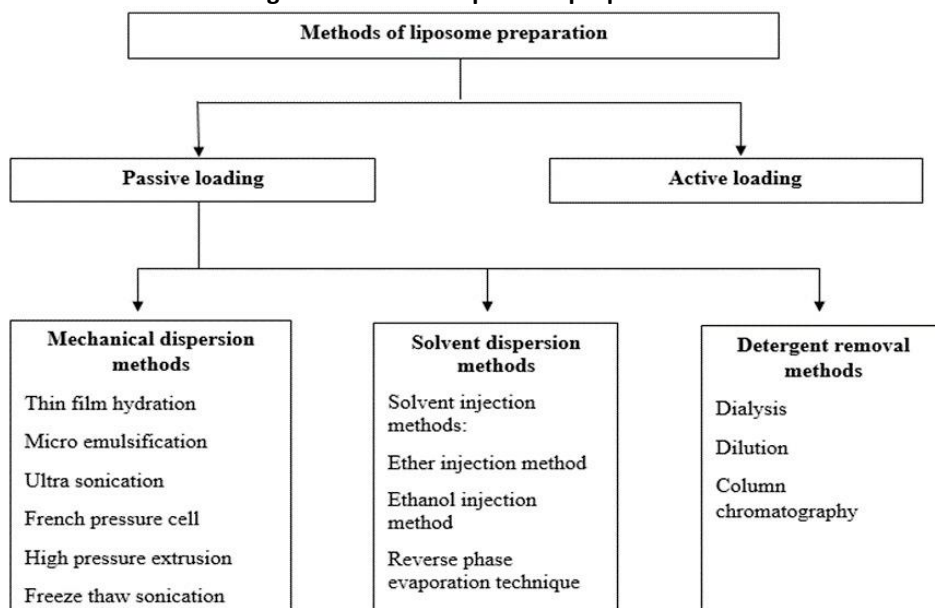
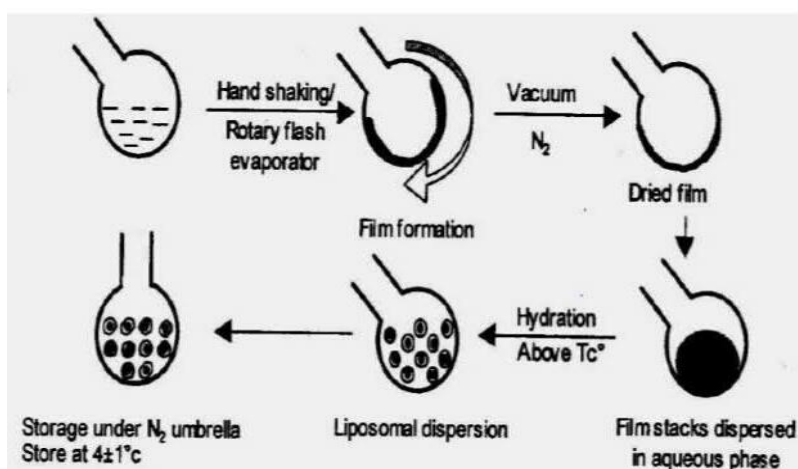
Fig. 1: Structure of Liposome

Fig. 2: Methods of liposome preparation

Fig. 3: Thin film hydration technique


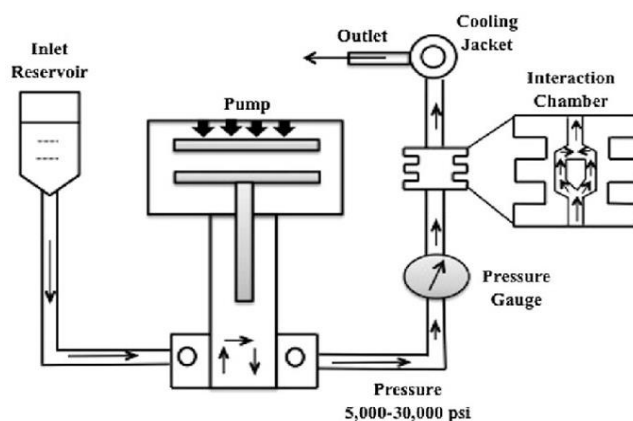
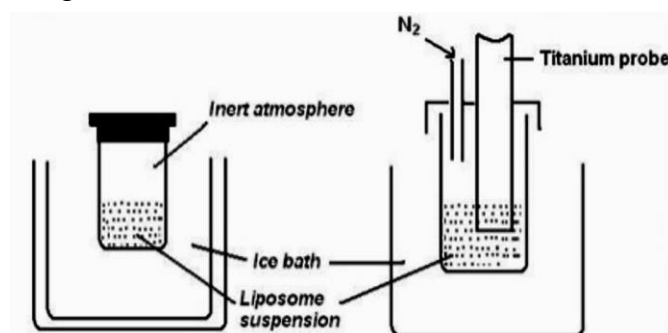
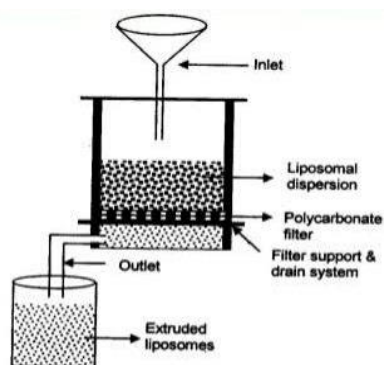
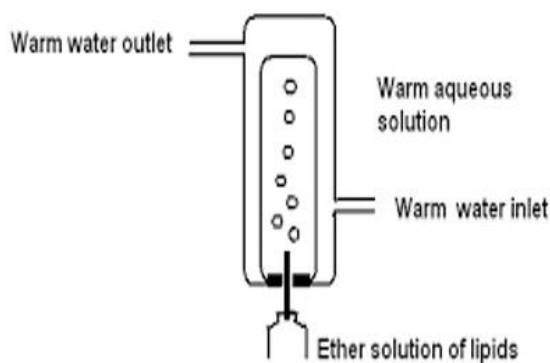
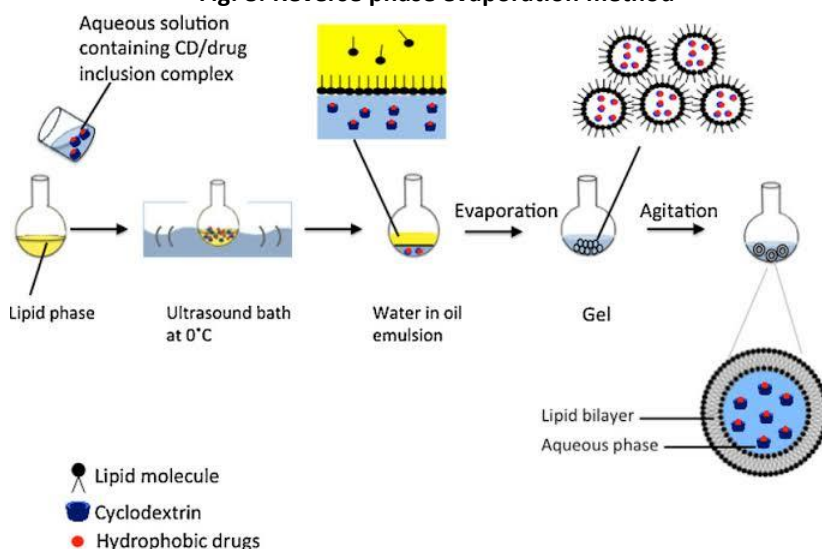
Fig. 4: Microemulsification

Fig.5: Bath sonicator
Probe sonicator

Fig. 6: Membrane extrusion method

Fig.7: Ether injection method


Fig. 8: Reverse phase evaporation method



Composition of liposomes:

The major components involved in liposomal preparations are phospholipids and cholesterol.

Phospholipids:

Glycerol containing phospholipids are most commonly used for liposome formulations. They represent more than 50% of the weight of lipid present in the biological membranes. Saturated fatty acids are used in order to produce stable liposomes. Most abundant glycerol phospholipids in plants and animals are phosphatidyl choline (PC) also known as lecithin and phosphatidyl ethanolamine (PE), sometimes referred to as cephalin^[4].

Phospholipids used for liposomal preparation can be of following varieties^[1]

- Natural phospholipids
- Synthetic phospholipids
- Semi synthetic phospholipids
- Modified natural phospholipids
- Phospholipids having non-natural phospholipids

Examples: -

Phosphatidyl choline

Phosphatidyl ethanolamine

Phosphatidyl inositol

Phosphatidyl glycerol

Phosphatidyl serine.

Cholesterol:

Cholesterol is added to improve the characteristics of lipid bilayers. The ratio of cholesterol to phosphatidylcholine can be in the ratio of 1:1 or 1:2. It improves the membrane fluidity, bilayer stability and reduces the permeability of water-soluble molecules through the membrane^[5].

PREPARATION OF LIPOSOMES^[6]

There are two mechanisms by which vesicles formation occurs. They are as follows:

1. The budding theory
2. The bilayer phospholipids theory

Budding theory:

According to this theory, liposomes are formed by the stress induced hydration of phospholipids. Hence, the phospholipids get organized into lamellar arrays which results into budding of lipid bilayers and finally leads to downsizing.

The bilayer phospholipids theory:

This theory involves formation of liposomes by hydration of thin lipid films. During the process of agitation, the hydrated lipid films/ sheets gets detached and self-close to form large, multilamellar vesicles.

CLASSIFICATION OF LIPOSOMES

Based on structural parameters^[1,7]:

MLV	Multilamellar large vesicles
OLV	Oligolamellar vesicles
UV	Unilamellar vesicles
SUV	Small unilamellar vesicles
LUV	Large unilamellar vesicles
GUV	Giant unilamellar vesicles
MVV	Multivesicular vesicles.

Based on method of liposome preparation^[1,7]:

REV	Single or oligolamellar vesicles made by reverse-phase evaporation method
MLV-REV	Multilamellar vesicles made by reverse-phase evaporation method
SPLV	Stable plurilamellar vesicles
FATMLV	Frozen and thawed MLV
VET	Vesicles prepared by extrusion techniques
DRV	Dried reconstituted vesicles or Dehydration-rehydration vesicles
FUV	Vesicles prepared by fusion
FPV	Vesicles prepared by French pressure method

BSV Bubblesomes.

Multilamellar vesicles:

The size of MLV ranges from 0.1-0.5 μm . They resemble the structure of onion and can be prepared by thin film hydration technique. The name itself suggests that these are composed of number of concentric lipid bilayers that promotes the encapsulation of more amount of lipophilic drugs [2].

Unilamellar vesicles:

These are of three types: Small unilamellar vesicles, Large unilamellar vesicles, Giant unilamellar vesicles. Small unilamellar vesicles:

They possess single bilayer whose size ranges from 20-100nm. They can be prepared by sonication or extrusion technique or by reducing the size of LUV or MLV.

Large unilamellar vesicles:

These are composed of single bilayer and have high aqueous volume to lipid ratio. The size of LUV is greater than 0.06 μm . They possess abundant space for loading the materials.

Giant unilamellar vesicles:

The size of GUV ranges from 1-200 μm . They can be prepared by Natural swelling, Electroformation, Microfluidics and Gel assisted swelling (Agarose assisted swelling or PVA assisted swelling).

Multivesicular vesicles:

The size of MVV is greater than 1 μm and the vesicles are of various sizes. They possess several non-concentric vesicles which are entrapped within a single bilayer. They have high drug loading capacity [8].

ADVANTAGES

Both hydrophilic and hydrophobic drugs can be entrapped [2].

Reduces the frequency of dosing.

Liposomes prevent the enzymatic degradation of drugs i.e., improves the drug stability [8].

These are biocompatible and biodegradable.

They increase the therapeutic index of drug [8].

Liposomes can complex both with negatively and positively charged materials [9].

Provides greater efficacy.

Liposomes serve as a good carrier i.e., carry large pieces of DNA [9].

Liposomes are used as target selective drug delivery systems [10].

Since the drug does not reach the non-target sites, it reduces the chances of drug toxicity.

DISADVANTAGES

Liposomal drug delivery systems are expensive to produce.

Phospholipids may undergo oxidation and hydrolysis reactions [11].

May undergo leakage during their transit to the site of action.

Difficult in large scale manufacturing and sterilization.

METHODS OF PREPARATION OF LIPOSOMES

Passive loading technique: - Involves loading of entrapped agents before or during the manufacturing process.

Active loading technique: - Involves loading of certain type of compounds into the liposomes after the formation of intact vesicles.

Methods involved in the preparation of liposomes are given in fig.2.

Mechanical dispersion methods:

Thin film hydration technique:

MLVs are produced by this technique. This method involves the dissolution of phospholipids in an organic solvent like Chloroform: Methanol in the ratio of 2:1 v/v in a round bottomed flask. The RBF is then attached to rotary evaporator and is allowed to rotate at a speed of 60rpm. As a result, evaporation of organic solvent occurs. This leads to the formation of thin and homogeneous lipid film on sides of RBF. Nitrogen gas is used to remove the residual solvent. The so formed lipid film is hydrated using an aqueous media. This results in the formation of milky white suspension which is kept aside for 2h at room temperature or above the transition temperature of lipid. Hence, complete swelling of particles occurs which leads to the formation of MLVs [8, 11]. The process is described in Fig.3.

Microemulsification method:

Microemulsification method yields small MLVs. This method involves the usage of Microfluidizer, wherein the lipids are introduced into it either as large MLVs or as slurry of unhydrated lipids in an organic medium. Microfluidizer pumps the fluid through an orifice (5 μm) at a pressure of 10,000 psi. Due to this high pressure, the fluid passes through the microchannels which directs the two streams of fluid to collide with each other at right angles with higher velocity. The so formed fluid is collected and recycled through the pump and interaction chamber until the spherical vesicles are produced. After a single pass, the size of vesicles gets reduced to a diameter of 0.1 and 0.2 μm [11, 12] which is shown in fig.4.

Ultrasonication method:

This method involves preparation of SUVs from MLVs. Bath type or probe type sonicators are generally used under an inert atmosphere of nitrogen or argon.

Principle: - Pulsed, high frequency sound waves are used to agitate MLVs suspension.

Probe type sonicator:

This method involves the usage of titanium probe which delivers high energy to the liposomal suspension.

Disadvantages:

Thermo labile materials like proteins or DNA gets denatured or inactivated.

Degradation of liposomal suspension may occur due to the release of titanium particles into it ^[2, 8].

Bath type sonicator:

To overcome the drawbacks associated with the probe type sonicator; bath type sonicators are majorly preferred.

In this method, test tube containing MLV dispersion is placed in a bath type sonicator. MLV dispersion is subjected to sonication for about 5-10 min at a temperature above the transition temperature of the lipid. Due to this, slightly hazy transparent solution is obtained which is then subjected to centrifugation in order to produce SUV dispersion. Upon centrifugation, MLVs and titanium particles forms a sediment. Later, the tube is removed from the rotar and by using the Pasteur pipette, top clear liquid layer is decanted leaving behind the central layer having MLVs and pellet. The top layer constitutes the pure SUVs dispersion ^[12].

Both bath and probe type sonicators are shown in fig.5.

French pressure cell:

French pressure cell is made up of stainless steel and is capable of withstanding pressures of 20,000 – 40,000psi. The body of cell contains pressure chamber, pressure relief valve, piston, bottom seal and valve closure. Both piston and bottom seal have rubber O-ring each. This method is expensive, and cleaning of cell is difficult.

This method involves addition of liposomal suspension to the pressure chamber and the piston is pushed into the body. Then the cell is turned at an angle of 180°. After filling, the bottom seal is pressed down and the pressure chamber is closed. Later, the cell is brought back to upright position and inserted in a hydraulic press due to which pressure is developed. Finally, the valve is opened slowly and the product (liposomes) is allowed to exit in a drop-wise manner. ULVs or OLVs having a size of 30-80nm are obtained ^[11].

High pressure extrusion method:

This method offers advantages like large capacity, simple and rapid. It is used to produce SUVs and LUVs. The mechanism of action appears like peeling of an onion. This involves the usage of polycarbonate filter membranes. MLV suspension is passed through the filter at a pressure of 250psi. During this process, the successive layers gets peeled off, thereby leaving only a single layer. Hence, uniform sized liposomes

are obtained. Liposomes produced by this technique are known as LUVETs ^[2, 8]. High pressure extrusion technique is shown in fig.6.

Freeze thaw sonication:

This technique involves series of steps like freezing, thawing and sonication. This method involves freezing of unilamellar dispersion (SUV). Then the frozen dispersion is allowed for thawing at room temperature for about 15min. Later, sonication is done. Hence, aggregation of SUVs occurs leading to the formation of LUVs ^[11, 12].

Solvent dispersion methods:

Ether injection method:

This method involves dissolution of lipids in diethyl ether or ether/methanol. This lipid mixture is then injected into an aqueous solution containing material to be encapsulated. This is performed at a temperature of 55-65°C or under reduced pressure. Evaporation of organic solvent is brought about by vacuum application. Finally, liposomes are obtained ^[8, 13] which is shown in fig 7.

Ethanol injection method:

Ethanol solution containing lipids is injected into excess of saline or aqueous solution through fine needle. Then mixing is done to produce SUVs ^[12, 13].

Reverse phase evaporation technique:

This method is generally used to encapsulate RNA and various enzymes. This technique involves injection of aqueous solution of drug into an organic solvent containing lipid followed by sonication of the biphasic mixture. This leads to the formation of water-in-oil type of emulsion. Later, the emulsion is dried in a rotary evaporator to obtain semisolid gel. The gel is then agitated mechanically due to which phase inversion occurs i.e., water-in-oil turns to oil-in-water type of emulsion. During the process of agitation, some of the water droplets collapses to form the external phase, while remaining portion forms the entrapped aqueous volume ^[2]. This method is described in fig.8.

Solubilization and detergent removal method:

This technique is used for the encapsulation of proteins and other biological molecules. LUVs are obtained by this method.

Detergents are used in order to solubilize the lipids. Commonly used detergents are non-ionic surfactants [e.g. n-octyl-beta-d-glucopyranose], anionic surfactants (e.g. dodecyl sulphate) and cationic surfactants (e.g. Hexadecyltrimethyl ammonium bromide). This method involves the solubilisation of lipids in an aqueous solution of detergent and proteins to be encapsulated. Later, the detergents are removed by column chromatography or dialysis method. During the process of detergent removal, LUVs are obtained ^[2].

Disadvantages of detergent removal method:

Time consuming.

Liposomal concentration in solution is low.

Traces of detergent may remain in the formulation.

Entrapment of hydrophobic compounds is low.

Removal of hydrophilic compounds may occur during detergent removal [8].

Examples:

Mechanical dispersion method:

Incorporation of vegetable oils in ketoconazole liposomes using thin film hydration technique:

Ketoconazole is an antifungal drug that shows action against variety of fungi and yeast. Using thin film hydration technique, MLVs of ketoconazole can be formulated wherein olive oil and sunflower oil are used as solvents. A variety of materials included in the preparation are ketoconazole, cholesterol, hydration media (water or 0.9% NaCl), solvents (olive and sunflower oil), phospholipid (lecithin from egg yolk).

Ketoconazole, cholesterol and lecithin of suitable quantities must be taken in a round bottomed flask. To this 5ml of solvents must be added. Then the mixture is homogenized using magnetic stirrer (1000rpm) at a temperature of 40° for about 10min. Later, 5ml of hydration media must be added at a temperature of 80°C. The so formed mixture must be stirred on the rotary evaporator at 65° for 1h at a rotational speed of 90rpm to produce liposomes. The untrapped drug (ketoconazole) can be removed from the liposomal dispersion by the centrifugation process at 4000rpm or 5000rpm for about 15 min [14]. Encapsulation of ketoconazole in liposomes promotes prolonged drug delivery by enhancing half-life and also reduces the side effects [15].

Solvent dispersion method:

Novel camptothecin analogue (Gimatecan) containing liposomes prepared by Ethanol injection method:

Gimatecan is an analogue of camptothecin that shows an action against tumours [16]. This method involves dissolution of lipids in an alcoholic solvent followed by dissolving gimatecan in an alcoholic lipid mixture. The so formed mixture must be injected into water or saline solution using syringe to yield liposomes. Gimatecan liposomal formulation shows better antitumoral activity upon intravenous administration [17].

Detergent removal method:

Preparation of pH- sensitive, large unilamellar liposomes by detergent dialysis method:

LUVs can be prepared using detergent dialysis method. 10μmol of lipid should be dried using a stream of nitrogen gas. The dried lipid was subjected to vacuum for about 30min and later it is suspended

into 900μl of PBS buffer (phosphate buffered saline)/ 50Mm Calcein/ 0.55Mm EGTA. Cholestanyl ether is also incorporated into lipid mixture in order to monitor the lipids. Then the lipid suspension is subjected to bath sonication and pH 8.0 is maintained. Later, 10μmol of octyl glucoside is added. The so formed mixture is vortexed and dialyzed at room temperature against 100ml of buffer having 1g of SM-2 beads. The liposomes were then separated from free calcein by Bio-gel A 1.5m column chromatography [18].

NOVEL LIPOSOMES

Archaeosomes:

These are the novel generation liposomes in which lipids are obtained from *archaeobacteria*. *Archaeobacteria* is a domain of prokaryotes. The method used for obtaining lipids from *archaeobacteria* is solvent extraction method. The membranes of *archaeobacteria* contains diether/ tetraether linkages which promotes the generation of lipid layers of archaeosomes. The major advantage is that they show greater stability even in harsh conditions [19, 20]. Archaeosomes shows good biocompatibility compared to liposomes.

Transferosomes:

The name transferosomes implies a “carrying body”. Transferosomes penetrates through the skin by getting squeezed through the lipids present in the cells of stratum corneum. These are generally composed of amphipathic molecule like phosphatidyl choline which acts as a vesicle forming agent and also contains bilayer softening agent like surfactant which is responsible for flexibility of the transferosome [21]. Transferosomes offers higher entrapment efficiency and also provides protection to the drugs from metabolic degradation. They have ability to penetrate through small pores. These are biodegradable and biocompatible.

Ethosomes:

Ethosomes are the vesicles comprising of phospholipids along with high concentration of alcohol. The alcohols may be either ethanol or isopropyl alcohol. The phospholipids may be phosphatidylcholine, phosphatidic acid, phosphatidylethanolamine etc. Ethosomes enables the drugs to permeate through the stratum corneum. These are considered to be safe drug delivery system which is approved for cosmetic and pharmaceutical use. These are more stable than conventional liposomes. They enhance the delivery of active agents.

Stealth liposomes:

These are the spherical vesicles with a membrane composed of phospholipid bilayer, polymers and are

used for delivering drugs or genetic materials into the cells. In this, the liposomes are coated with hydrophilic polymer (polyethylene glycol) [22]. They serve as reservoir for extended drug release.

Pharmacosomes:

Pharmacosomes are the molecules having positive and negative charges and possess hydrophilic and hydrophobic properties. They improve the permeation rate and possess broader stability profile. Pharmacosomes are widely used because of their greater shelf life.

Immunoliposomes:

These are the liposomal drug delivery systems in which antibody molecules are conjugated to the surface of liposomes. They play a major role in treatment of cancer cells [23].

Virosomes:

These are the reconstituted viral envelopes which serves as vaccines and as vehicles for cellular delivery of various types of macromolecules. These are non-toxic, biocompatible and biodegradable. These are used to encapsulate various pharmacologically active substances. *Influenza virus* is most commonly used to prepare virosomes [24].

APPLICATIONS

In cancer chemotherapy.

In ophthalmic drug delivery.

In oral drug delivery.

Topical application.

Liposomes can be used as immunological adjuvants.

Helps in the treatment of leishmaniasis [1, 25].

Gene therapy: The genetic material can be placed in the liposomes in order to increase the DNA uptake in tissue culture.

Used in food industry.

Used in various cosmetic preparations.

MARKETED LIPOSOMES

Some of the commercially available marketed liposomal products are as follows: -

Ambisome®: - It is a lipid associated formulation containing the drug Amphotericin B and is used in treatment of systemic fungal infections [26].

Doxil®: - It is a long- acting PEGylated liposomal product containing Doxorubicin used for the treatment of Kaposi's sarcoma and multiple myeloma [27].

Depocyt®: - It is a pyrogen- free liposomal parenteral suspension of Cytarabine developed for the treatment of neoplastic meningitis [28].

Efudex®: - It is a liposomal formulation composed of N₃-O-toluy- fluorouracil (an intermediate metabolite of Atofluding) used for the inhibition of various types of tumours [29].

DaunoXome®: - It is a liposomal preparation composed of Daunorubicin citrate used in the treatment of AIDS associated Kaposi's sarcoma [30].

DepoDur™: - It is a morphine encapsulated liposomal preparation used as post-surgical pain reliever [31].

Lipusu®: -It is a liposomal formulation containing Paclitaxel and is used in the treatment of various types of cancers like ovarian cancer, prostate cancer and lung cancer [32].

CONCLUSION

From the review of Liposomes - A novel drug delivery system, it was concluded that liposomes serve as a promising carrier for improving site targeted delivery of number of drugs. These are considered to be the significant candidates in improving the drug delivery. Liposomes help in reducing the frequency of administration and lower the toxicity. Liposomes provide unique opportunity of delivering the drugs into the cells or even inside the individual cellular components.

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