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LIPOSOMES AS DRUG DELIVERY - AN OVERVIEW

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ABSTRACT

Liposomes are tiny sphere-shaped vesicles, made out of phospholipid bilayers as a cell membrane. Liposomes can be filled with drugs, and used to deliver drugs for several diseases. Among several new drug delivery systems, liposomes serve an advanced technology to deliver active molecules to the site of action. Based on structure, method of preparation, composition and applications liposomes has been classified. There are several methods by which liposomes are prepared. The drugs are loaded in the liposomes by encapsulation, partitioning or reverse loading techniques. Being unique with several advantages liposomes are applied widely in the field of medicine for safe delivery of drugs. This paper summarizes the various advantages, mechanisms, methods of preparation and applications of liposomal preparations.

KEYWORDS

Liposomes, Phospholipids, Methods of preparation, Pharmaceutical applications and Generations of Liposomes.

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INTRODUCTION

Liposomes are small artificial vesicles of spherical shape that can be made from natural non-toxic phospholipids[1]. Liposomes deliver active molecules to the site of action by their tiny size and hydrophobic and hydrophilic characters. The properties of liposomes differ considerably with composition of lipids, their methods of preparation, surface charges and their size[2]. The rigidity or fluidity and the charge of the bilayer determine the selection of bilayer components. The unsaturated phosphatidyl-choline species from natural sources give much more permeable and less stable bilayers. But the saturated phospholipids with long acyl

chains form a rigid, rather impermeable bilayer structure. When phospholipids are hydrated in aqueous solutions, they form closed structures (Figure No.1)[3]. Such vesicles which have one or more phospholipid bilayer membranes can transport aqueous or lipid drugs, depending on the nature of those drugs. Because lipids are amphipathic (both hydrophobic and hydrophilic) in aqueous media, their thermodynamic phase properties and self - assembling characteristics influence entropically focused confiscation of their hydrophobic sections into spherical bilayers. Liposomes are spherical vesicles with particle sizes ranging from 30 nm to several micrometers. Self-aggregation of polar lipids is not limited to conventional bilayer structures which rely on molecular shape, temperature, and environmental and preparation conditions but may self-assemble into various types of colloidal particles.

Liposomes are extensively used as carriers for numerous molecules in cosmetic and pharmaceutical industries. To grow delivery systems that can entrap unstable compounds and to shield their functionality, food and farming industries uses the liposome encapsulation.

Liposomes can trap both hydrophobic and hydrophilic compounds, avoid decomposition of the entrapped combinations, and release the entrapped at designated targets. As a drug delivery system, liposomes, commercially, have increased rate of use because of their biocompatibility, biodegradability, low toxicity, and aptitude to trap both hydrophilic and lipophilic drugs and simplify site-specific drug delivery to tumor tissues. Many studies have been conducted on liposomes with the aim of decreasing drug toxicity or targeting specific cells.

The proposal of liposomal encapsulation technology targeted the delivery of vital combinations of drugs to the body[4]. Liposomal encapsulation technology is a method of generating sub-microscopic foams called liposomes, which encapsulate numerous materials. These liposomes form a barrier around their contents, which is resistant to enzymes in the mouth and stomach, alkaline solutions, digestive juices, bile salts, and intestinal flora that are generated in the human body, as well as free radicals. The contents of the liposomes are thus protected from oxidation and degradation. This protective phospholipid shield or barrier remains undamaged until the contents of the liposome are delivered to the exact target gland, organ, or system where the contents will be utilized[5].

One of the main aims of any treatment employing drug is to increase the therapeutic index of the drug and while minimizing its side effects[6]. The clinical usefulness of most conservative chemotherapeutics is restricted either by the incapability to deliver therapeutic drug concentrations to the target soft tissue and harmful toxic side effects on normal organs and tissues. Different approaches have been made to overcome these difficulties by providing the 'selective' delivery to the target area; the ideal solution would be to target the drug alone to those cells, tissues, organs that are affected by the disease. The importance of liposomes lies in their composition, which makes them biodegradable and biocompatible[7]. Liposomes are composed of natural phospholipids that are biologically inert and feebly immunogenic, and they have low inherent toxicity. Furthermore, drugs with different lipophilicities can be encapsulated into liposomes: strongly lipophilic drugs are entrapped almost totally in the lipid bilayer, intensely hydrophilic drugs are located entirely in the aqueous compartment.

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 volume is entirely enclosed by a membraneous lipid bilayer. Membranes are usually made of phospholipids, which are molecules that have a hydrophilic head group and a hydrophobic tail group. 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 a bilayer. When membrane phospholipids are disrupted, they can reassemble themselves into tiny spheres, smaller than a normal cell, either as bilayers or monolayers[8] (Figure No.2). The bilayer structures are liposomes. The monolayer structures are called micelles.

The lipids in the plasma membrane are chiefly phospholipids like phosphatidyl-ethanolamine and phosphatidylcholine. Phospholipids are amphiphilic with the hydrocarbon tail of the molecule being hydrophobic; its polar head hydrophilic. As the plasma membrane faces watery solutions on both sides, its phospholipids accommodate this by forming a phospholipid bilayer with the hydrophobic tails facing each other.

Liposomes can be composed of naturally-derived phospholipids with mixed lipid chains (like egg phosphatidyl-ethanolamine), or of pure surfactant components like dioleoyl phosphatidyl-ethanolamine (DOPE). Liposomes, usually but not by definition, contain a core of aqueous solution; lipid spheres that contain no aqueous material are called micelles, however, reverse micelles can be made to encompass an aqueous environment. The self-aggregation of polar lipids is not limited to conventional bilayer structures which rely on molecular shape, temperature, and environmental and preparation conditions but may self-assemble into various types of colloidal particles[9].

As drug delivery, liposomes and their use have been greatly explored by pharmaceutical companies in the field of medicine. Liposomes are biocompatible, completely biodegradable, non-toxic, flexible, and nonimmunogenic. They have both lipophilic and aqueous environment making them useful for delivering hydrophobic, amphipathic, and hydrophilic medicines. Liposomes with their layers encapsulate the drug and serves as a protection of the drug from the environment as well as acting as a sustained release mechanism[10]. This encapsulation also serves to protect sensitive areas from the drug as well. They are extremely versatile in the form which they may be administered. These forms include suspension, aerosol, gel, cream, lotion, and powder which can then by administered through most common routes of medicinal administration.

Liposomes are also flexible in their size, and as is such they can enclose a wide size range of molecules. A special quality of liposome is that they enable water soluble and water insoluble materials to be used together in a formulation without the use of surfactants and emulsifiers. The liposome wall holds fat soluble materials such as oils[11]. They possess increased stability via encapsulation. Liposomes provide reduction in toxicity of the encapsulated agents. Liposomes give site avoidance effect[12]. Liposomes can aid with active targeting as it has flexibility in coupling with site-specific ligands. They are biocompatible, completely biodegradable, non-toxic, flexible and nonimmunogenic for systemic and non-systemic administrations. Liposomes could provide increased efficacy and therapeutic index[13].

Though Liposomes have many advantages, when compared with other methods of drug delivery they also have some limitations[14]. The main disadvantage of the standard liposome formulation is its rapid clearance from circulation due to uptake, by the reticulo endothelial system, primarily in the liver. High production cost is required for liposomes encapsulated drugs. Liposomes may have leakage property which leads to fusion of encapsulated drugs. The liposome phospholipid may undergo oxidation and hydrolysis. Liposomes have a shorter half-life. Liposomes have lower solubility fewer stability problems.

CLASSIFICATION

Based upon composition and application **l**iposomes are classified as conventional liposomes (CL), fusogenic liposomes, pH sensitive liposomes,

cationic liposomes, long circulatory (stealth) liposomes (LCL) and immuno-liposome. CL is neutral or negatively charged phospholipids and cholesterol. Fusogenic liposomes are Reconstituted Sendai virus envelopes. pH sensitive Liposomes includes Phospholipids such as PE or DOPE with either CHEMS or OA. Cationic liposomes are cationic lipids with DOPE. LCL has polyethylene glycol (PEG) derivatives attached to their surface to decrease their detection by phagocyte system. The attachment of PEG to liposomes decreases the clearance from blood stream and extends circulation time of liposomes in the body. The attachment of PEG is also known as pegylation. Immuno-Liposomes are CL or LCL with attached monoclonal antibody or recognition sequence[15].

Based on structural parameters liposomes are classified as unilamellar vesicles, oligolamellar vesicles (OLV) and multilamellar vesicles (MLV) (Figure No.3). Unilamellar vesicles are further classified as small unilamellar vesicles (SUV), medium unilamellar vesicles (MUV) and large unilamellar vesicles (LUV). SUV size ranges from 20-40 nm. MUV size ranges from 40-80 nm. LUV size ranges from 100 nm-1,000 nm. OLV are made up of 2-10 bilayers of lipids surrounding a large internal volume. MLV have several bilayers. They can compartmentalize the aqueous volume in an infinite numbers of ways. They differ according to way by which they are prepared. The arrangements can be onion like arrangements of concentric spherical bilayers of LUV/MLV enclosing a large number of SUV etc[16].

Based on method of preparation liposomes are classified as OLV made by Reverse-Phase Evaporation method, MLV made by Reverse-Phase Evaporation method, stable plurilamellar vesicles, frozen and thawed MLV, vesicles prepared by extrusion technique and vesicles prepared by dehydration-rehydration method[17].

METHODS OF PREPARATION

The parameters to be considered while selecting liposome preparation method are 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, batch-to-batch reproducibility and possibility of large-scale production of safe and efficient liposomal products[18].

Liposomes are prepared by using various procedures in which the water soluble (hydrophilic) materials are entrapped by using aqueous solution of these materials as hydrating fluid. The lipid soluble (lipophilic) materials are solubilized in the organic solution of the constitutive lipid and then evaporated to a dry drug containing lipid film followed by its hydration[19].

The general method of preparing the liposomes involves four basic stages namely, drying down lipids from organic solvent, dispersing the lipid in aqueous media, purifying the resultant liposome and analyzing the final product[20]. Liposomes are prepared using two techniques namely passive loading techniques and active loading technique. Passive loading techniques include three different methods like mechanical dispersion method, solvent dispersion method and detergent removal method. The different types of mechanical dispersion methods are sonication, French pressure cell: extrusion, Freeze-thawed liposomes, lipid film hydration by hand shaking, non-hand shaking or freeze drying, micro-emulsification, membrane extrusion and dried reconstituted vesicles[21].

Sonication method is used for the preparation of SUV and MLV. Here, MLVs are sonicated either with a bath type sonicator or a probe sonicator under a passive atmosphere. The main disadvantages of this method are very low internal volume/encapsulation efficacy, possible degradation of phospholipids and compounds to be encapsulated, elimination of large molecules, metal pollution from probe tip, and presence of MLV along with SUV. The two sonication techniques are probe sonication and bath sonication[22].

The French pressure cell extrusion method involves the extrusion of MLV through a small orifice. It involves gentle handling of unstable materials. The main advantage of this method is the resulting liposomes are rather larger than sonicated SUVs. The drawbacks of the method are that the high temperature is difficult to attain, and the working volumes are comparatively small[23].

In Freeze-thawed liposome method the unilamellar vesicles are prepared by the fusion of SUV throughout the processes of freezing and thawing (Figure No.4). This type of synthesis is strongly inhibited by increasing the phospholipid concentration and by increasing the ionic strength of the medium. The encapsulation efficacies from 20% to 30% were obtained[24].

The different types of solvent dispersion methods are ether injection, ethanol injection and reverse phase evaporation method. In ether injection (solvent vaporization) method a lipid solution dissolved in diethyl ether or ether-methanol mixture is gradually injected to an aqueous solution of the material to be encapsulated at 55°C to 65°C or under reduced pressure[25]. The liposomes are obtained by removal of ether under vacuum. The main disadvantages of the technique are that the population is heterogeneous (70 to 200 nm) and the exposure of compounds to be encapsulated to organic solvents at high temperature. In ethanol injection method the MLVs are prepared by injecting a lipid solution of ethanol to a huge excess of buffer[26]. The disadvantages of the method are that the liposomes are very dilute, the removal all ethanol is difficult because it forms into azeotrope with water, and the probability of the various biologically active macromolecules to inactivate in the presence of even low amounts of ethanol is high.

In reverse phase evaporation method the liposomes are prepared with a high aqueous space-to-lipid ratio and have the capability to entrap a large percentage of the aqueous material. The inverted micelles formed are shaped upon sonication of a mixture of a buffered aqueous phase, which contains the water-soluble molecules to be encapsulated into the liposomes and an organic phase in which the amphiphilic molecules are solubilized. The method has been used to encapsulate small, large, and macromolecules. The main drawback of the technique is the contact of the materials to be encapsulated to organic solvents[27].

In Detergent removal method **t**he detergents at their critical micelle concentrations (CMC) have been used to solubilize lipids (Figure No.5)[28]. As the detergent is detached, the micelles become increasingly better-off in phospholipid and lastly combine to form LUVs. The detergents were removed by dialysis. A commercial device is obtainable for the elimination of detergents. The dialysis can be performed in dialysis bags engrossed in large detergent free buffers (equilibrium dialysis). Detergent absorption is attained by shaking mixed micelle solution with beaded organic polystyrene adsorbers such as beads and Bio-beads[29]. The great benefit of using detergent adsorbers is that they can eliminate detergents with a very low CMC, which are not entirely depleted.

In Gel-permeation chromatography method, the detergent is depleted by size special chromatography. Sephadex G-50, Sephadex G-l 00 can be used for gel filtration. The liposomes do not penetrate into the pores of the beads packed in a column. They percolate through the inter-bead spaces. At slow flow rates, the separation of liposomes from detergent monomers is very good. The swollen polysaccharide beads adsorb substantial amounts of amphiphilic lipids; therefore, pre-treatment is necessary. The pre-treatment is done by pre-saturation of the gel filtration column by lipids using empty liposome suspensions[30]. Upon dilution of aqueous mixed micellar solution of detergent and phospholipids with buffer, the micellar size and the polydispersity increase fundamentally, and as the system are diluted beyond the mixed micellar phase boundary, a spontaneous transition from polydispersed micelles to vesicles occurs[31].

A stealth liposome is a sphere-shaped vesicle with a membrane composed of phospholipid bilayer used to deliver drugs or genetic material into a cell[32]. A liposome can be composed of naturally derived phospholipids with mixed lipid chains coated or

steadied by polymers of PEG and colloidal in nature. Stealth liposomes are attained and grown in new drug delivery and in controlled release. This stealth principle has been used to develop the successful doxorubicin-loaded liposome product that is presently marketed as Doxil for the treatment of solid tumors. Pharmacological action of vasopressin is formulated in long circulating liposome.

DRUG LOADING IN LIPOSOMES

The mechanism of introducing drugs into liposomes is achieved by three primary mechanisms: encapsulation, partitioning and reverse loading. For encapsulation the physicochemical properties of the drug itself, especially solubility and partition coefficient, are important determinant of the extent of its incorporation in liposomes (Figure No.6)[33]. It is useful for water-soluble drugs (doxorubicin, penicillin G); the encapsulation is simple hydration of a lipid with an aqueous solution of drug. The formation of liposomes passively entraps dissolved drug in the interlamellar spaces, essentially encapsulating a small volume.

A drug substance that is soluble in organic solvents (cyclosporine) will go through partitioning. It is dissolve along with phospholipid in a suitable organic solvent. This combination is dried first after than added directly to the aqueous phase and solvent residues remove under vacuum[34]. The acyl chains of the phospholipids provide a solubilizing environment for the drug molecule. The drug substance is specifically distributed to the bottom phase of a poly (ethylene glycol)/ dextran two-phase system through interactions with which the affinity ligand was produced. This will be located in the intrabilayer space. The affinity of two-phase partitioning is based on an immunoaffinity sandwich approach for the rapid and selective purification of membranes.

The reverse-loading mechanism uses for certain drugs (5-fluorouracil, mercapto-purine) may exist in both charged and uncharged forms depending on the ph of the environment[35]. This type of drug can be added to an aqueous phases in the uncharged state to permeate into liposomes through their lipid bilayers. Then the internal pH of the liposome is adjusted to create a charge on the drug molecules. Once, charged the drug molecules no longer is lipophilic enough to pass through the lipid bilayer and return to the external medium.

MECHANISM OF TRANSPORTATION THROUGH LIPOSOME

A liposome encapsulates a region of aqueous solution inside a hydrophobic membrane; dissolved hydrophilic solutes cannot readily pass through the lipids[36]. Hydrophobic chemicals can be dissolved into the membrane, and in this way liposome can carry both hydrophobic molecules and hydrophilic molecules. To deliver the molecules to sites of action, the lipid bilayer can fuse with other bilayers such as the cell membrane, thus delivering the liposome contents. By making liposomes in a solution of DNA or drugs they can be delivered past the lipid bilayer. A liposome does not necessarily have lipophobic contents, such as water, although it usually does.

Liposomes are used as models for artificial cells. Liposomes can also be designed to deliver drugs in other ways. Liposomes that contain low pH can be constructed such that dissolved aqueous drugs will be charged in solution. As the pH naturally neutralizes within the liposome, the drug will also be neutralized, allowing it to freely pass through a membrane. These liposomes work to deliver drug by diffusion rather than by direct cell fusion. Liposomes can be made in a particular size range that makes them viable targets for natural macrophage phagocytosis. These liposomes may be digested while in the macrophage's phagosome, thus releasing its drug. Liposomes can also be decorated with opsonins and ligands to activate endocytosis in other cell types.

The use of liposomes for transformation or transfection of DNA into a host cell is known as lipofection. It generally uses a positively charged lipid to form an aggregate with the negatively charged genetic material. A net positive charge on this aggregrate has been assumed to increase the effectiveness of transfection through the negatively

charged phospholipid bilayer. Numerous drugs has been tried as liposomes (Table No.1) many trials are going on for the development of liposomes as drug delivery.

GENERATIONS OF LIPOSOMES

The development of liposomes has undergone several generations from the time of their discovery[52]. The first-generation liposomes are conventional liposomes comprised a liposome-containing drugs[53]. They are difficult to formulate and manufacture. Eg: amphotericin B, Ambi-some, Myocet, doxorubicin. The second-generation liposomes are long-circulating liposomes with "pure lipid approach"[54]. They are easy to formulate and difficult to manufacture. Eg: Daunoxome. The third-generation liposomes were surface-modified liposomes with gangliosides or sialic acid, which can evade the immune system responsible for removing liposomes from circulation[55]. The fourth-generation lipo-somes were called "stealth liposomes" because of their ability to evade interception by the immune system, in the same way as the stealth bomber was able to evade radar. Eg: pegylated liposomal doxorubicin[56]. The new generation liposomes are comprised of natural soy phospholipids and contains generally recognized as safe (GRAS) excipients. Eg: Paclitaxel, docetaxel, cabazitaxel[57].

APPLICATIONS

Liposomes have many applications as a method of drug delivery. They are used for drug delivery to sites of action. They are used as models for artificial cells[58]. The use of liposomes for transformation or transfection of DNA into a host cell is known as lipofection[59]. In addition to gene and drug delivery applications, liposomes can be used as carriers for the delivery of dyes to textiles, pesticides to plants, enzymes and nutritional supplements to foods and cosmetics to the skin. The use of liposomes in nano cosmetology also has many benefits, including improved penetration and diffusion of active ingredients, selective transport of active ingredients; longer release time, greater stability of active ingredients, reduction of unwanted side effects and high biocompatibility[60].

Liposomes are used in treatment of cancer because of their natural ability to target cancer. They are used to protect the entrapped drug against enzymatic degradation during circulation. Liposomes, tagged on the lipid vesicles, can be applied in drug targeting. Since liposomes increase the permeability of skin for various entrapped drugs they can be used in topical drug delivery. Enhanced antimicrobial efficacy/ safety antimicrobial agents have been encapsulated in liposomes.

FUTURE OF LIPOSOMES

For solubilizing new generation of small molecules liposomes are unique systems. They can be produced synthetically and in large quantities. Since well-characterized lipids are available surge of activities can be performed in developing a pharmaceutically-acceptable liposomal product. Numerous clinical trials are ongoing in the designing and development of liposomes as drug delivery systems.

Table No.1: List of Some Drugs Tried as Liposome Drug Delivery

S.No	DRUG	APPLICATIONS
1	Acetazolamide	In lowering intraocular pressure[37]
2	Amphotericin-B	Broad-spectrum antifungal agent[38]
3	Anthracyclins	Cardiac-sparing effect in induced cardiotoxicity[39]
4	Artemisinin	For the treatment of malaria[40]
5	Bevacizumab	In ophthalmic drug delivery[41]
6	Copper palmitate	Blocks porphyrin induced photosensitivity in rats [42]
7	Cytarabine	For the intrathecal treatment of lymphomatous meningitis[43]
8	Doxophylline	In the treatment of asthma[44]
9	Doxyrubicin	In tumor cell implantation[45]
10	Fluconazole	In the treatment of candidal keratitis in rabbits[46]
11	Ganciclovir	Treatment against herpes simplex virus type 1[47]
12	Minoxidil	In hair loss treatment[48]
13	Muramyl-tripeptide-phosphatidyl ethanolamine, and glycoprotein D	In the treatment of herpes simplex virus infections[49]
14	Prednisolone	Inhibits growth of tumors in mice[50]
15	Rhodamine-conjugated liposomes	In the treatment of uveitis[51]

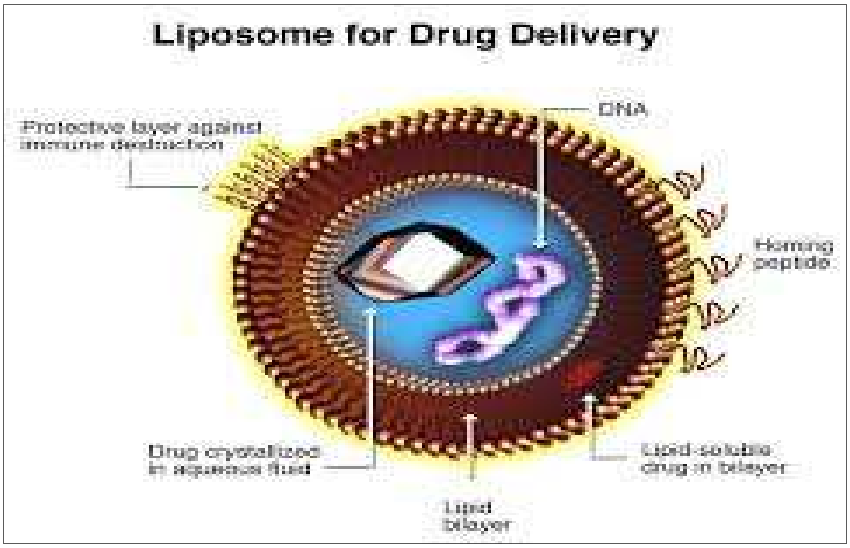

Figure No.1: Scheme of a liposome formed by phospholipids in an aqueous solution

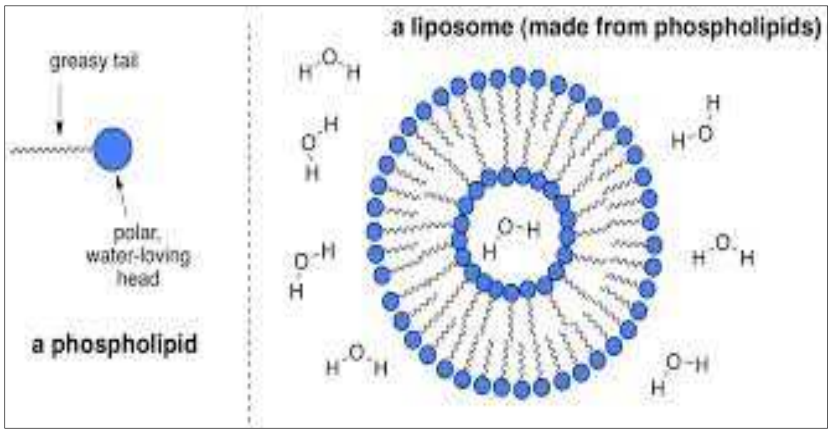

Figure No.2: Nonpolar and polar, water-loving heads outside or inside the sphere

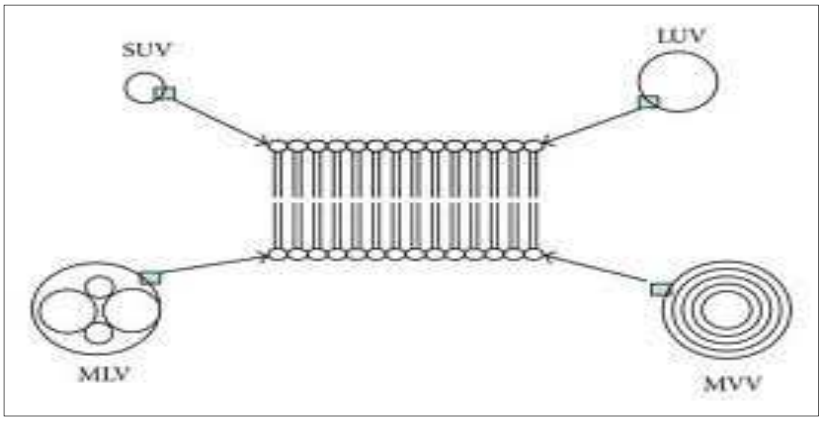

Figure No.3: Classification of liposomes. SUV, LUV AND MLV

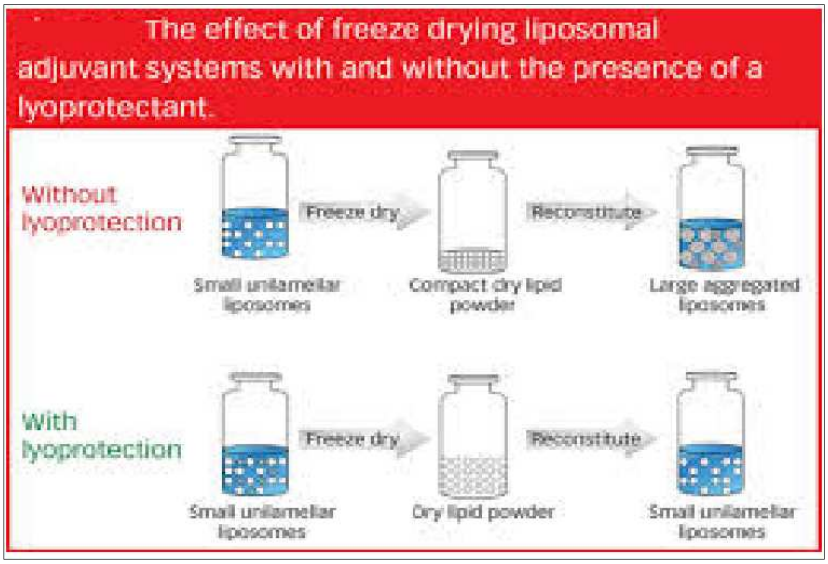

Figure No.4: Development of a freeze-dried adjuvant vaccine product

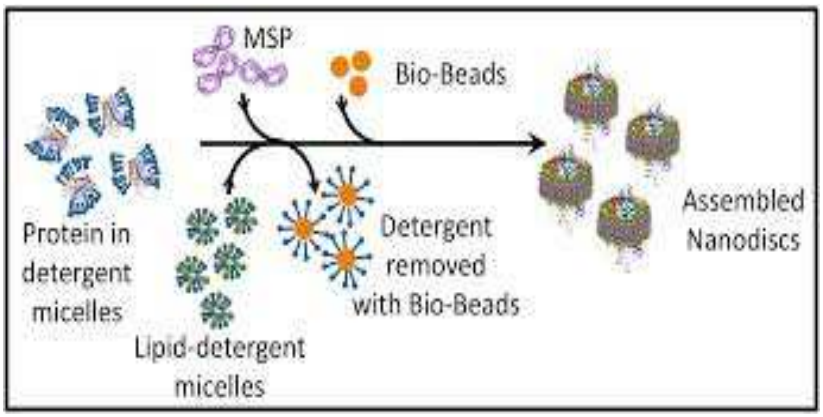

Figure No.5: Detergent removal method

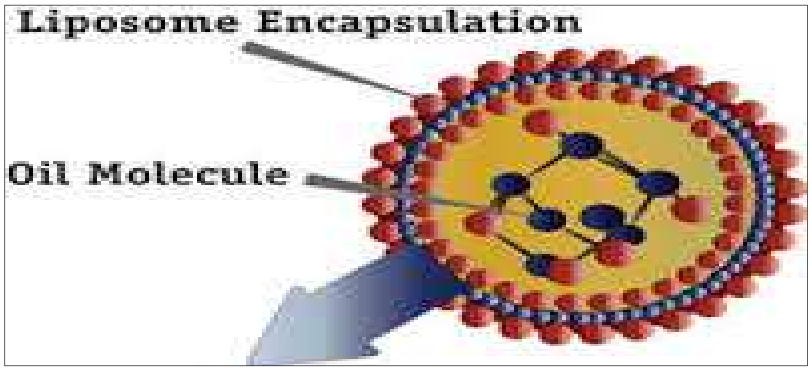

Figure No.6: Encapsulated liposome

CONCLUSION

Liposome vesicles are potential carriers of various drugs that could be used for therapeutic applications. There are many factors which contribute to their success as drug delivery vehicles. The drugs which are difficult to administer intravenously are solubilised as liposomes. The hydrophilic drugs can be formulated as liposomes, since liposomes can cross the Blood brain barrier. By slowly releasing the drug in the body, liposome can prolong the drug action. The specific binding properties of a drug-carrying liposome to a target cell, stealth liposomes for targeting hydrophilic anticancer drugs which leads to decrease in side effects, the most concentration of drug at the site of action are some of the newer developments. Though many commercial liposomes have already been discovered, registered and introduced in pharmaceutical and cosmetical market, there is greater promise in future for marketing of highly stabilized and more sophisticated liposomal formulations. The future of liposomal drug delivery system will be revolutionized with wide application especially in the treatment of tumour and various disorders.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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