An Overview on Liposomal Drug Delivery System

Subash Chandran M.P*, Prasobh G.R, Jaghatha T, Aswathy B.S, Remya S.B

Department of Pharmaceutics, SreeKrishna College of Pharmacy and Research Centre, Parassala, Thiruvananthapuram, Kerala, India.

ABSTRACT

The phospholipid bilayered sphere shaped tiny vesicles, liposomes, serves as a better system for delivery of drugs across the cell membrane. The drugs are filled in the vesicles and delivered actively. With the advanced technology, liposomes are able to deliver the drug molecules effectively. Liposomes are classified on the basis of their structure, preparation methods, composition of polymers and applications. The preparation of liposomes involves various methods such as sonication, solvent extraction, detergent removal and cell extrusion. With numerous advantages, liposomes prove their uniqueness in safe drug delivery in various fields of pharmacy and medicine. This overview deals with different classifications, merits, mechanisms of drug delivery, preparation methods and pharmaceutical applications of liposomal drug delivery system.

Key Words: *Liposomal drug delivery system, phospholipids, detergent removal, sonication method, antidiabetic liposomes*

eIJPPR 2019; 9(2):61-68

HOW TO CITE THIS ARTICLE: Subash Chandran M.P, Prasobh G.R, Jaghatha T, Aswathy B.S, Remya S.B (2019). "An Overview on Liposomal Drug Delivery System", International Journal of Pharmaceutical and Phytopharmacological Research, 9(2), pp.61-68.

INTRODUCTION

Liposomes, as the name indicates, they are little spherical shaped artificial vesicles prepared by using various phospholipids [1]. With their hydrophobic, hydrophilic characters and smaller size, they actively deliver the drugs to site of action. The liposomal drug delivery system differs in their properties based on lipids composition, their preparation methods, size and surface charges [2]. The selection of bilayer components determines the charge and fluidity of the vesicles. The permeable less stable bilayers of vesicles can be prepared using unsaturated phosphatidyl-choline species obtained from natural sources. The impermeable rigid bilayer structure is obtained using saturated phospholipids having long acyl chains. The closed structures are prepared by hydrating phospholipids in aqueous solutions (Fig. 1) [3]. Based on the aqueous or lipoidal nature of the drugs, they are transported across cell membrane using one or more phospholipid bilayers. The amphipathic nature of lipids in aqueous media influences tropically focused exclusion of their hydrophobic sections into spherical bilayers with their self -assembling characteristics and thermodynamic phase properties. The particle size of liposomes varies from 30 nanometers to several microns. The self-assembling of polar lipids in different varieties of colloidal particles to self-aggregation of polar lipids forms the bilayer structures which comply with preparation conditions, temperature, molecular shape, and environmental conditions.

The name liposome is derived from two Greek words: 'Lipos' meaning fat and 'Soma' meaning body. The application of liposomal drug delivery system extends to pharmaceutical and cosmetics industries as carriers for number of molecules. To shield their functionality and to entrap unstable compounds, farming and food industries utilize the liposomal encapsulation.

The decomposition of the entrapped combinations can be avoided and the entrapped drug release at site of action was enhanced because of the hydrophobic and hydrophilic characters of the liposomes. With the biocompatibility, low toxicity, biodegradability and aptitude character to enclose both hydrophilic and lipophilic drug materials liposomes have increased rate of

Corresponding author: Subash Chandran M.P
Address: Department of Pharmaceutics, SreeKrishna College of Pharmacy and Research Centre, Parassala, Thiruvananthapuram, Kerala, India.
E-mail: ✉ subashjr @ rediffmail.com
Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest .
Received: 26 September 2018; **Revised:** 28 March2019; **Accepted:** 06 April 2019

use, commercially as a drug delivery system. The recent researches on liposomes mainly focus on specific cell targeting and drug toxicity decreasing.

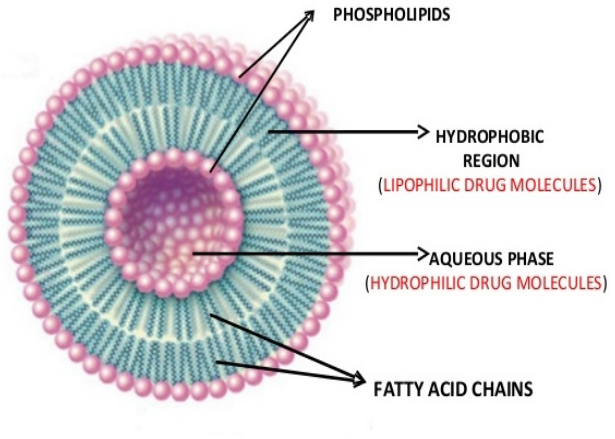

Fig. 1. Structure of a liposome

The liposomal formulations are targeted to deliver the important drug combinations to the body. The sub-microscopic foams generated by encapsulation technology are applied in the formulation of liposomes, which encapsulate numerous materials. These 'liposomes' form a protective barrier around their contents, which is resistant to enzymes in the mouth and stomach, intestinal flora, digestive juices, alkaline solutions and bile salts, that are generated in the human body. The contents of the liposomes are thus prevented from oxidation and degradation. This protective phospholipid barrier remains safe until the liposomal contents are delivered to the exact target gland or system where the contents are to be used [4].

The main aim of any treatment employing drug is not only to increase the therapeutic index of the drug but also to minimize its side effects. The clinical usefulness is restricted either by the incapability to deliver the concentrations of therapeutic drug to the targeting tissues and reducing 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, and organs that are affected by the disease. The vitality of liposomes lies in their composition, which makes them biodegradable and biocompatible [5]. Liposomes are prepared by of natural phospholipids that are biologically inert and feebly immunogenic, and they have low inherent toxicity. Moreover, 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.

Structurally, liposomes are concentric bleeder vesicles in which an aqueous volume is entirely enclosed by a membranous lipid bilayer. Membranes are usually made up 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 [6] (Fig. 2). The bilayer structures are liposomes. The monolayer structures are called micelles.

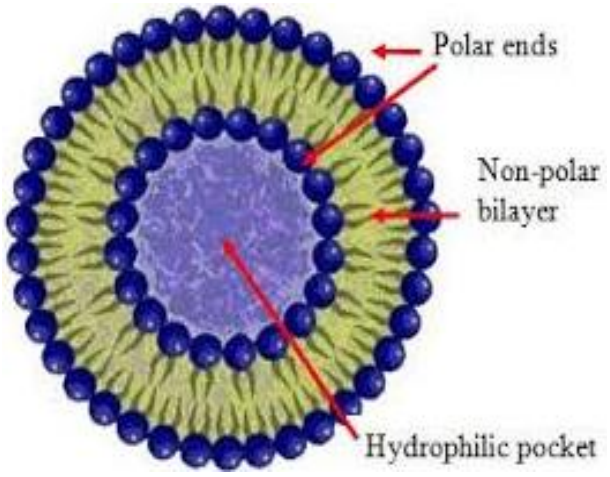

Fig. 2. The nonpolar and the polar heads of liposomes

Phospholipids are amphiphilic with the hydrocarbon tail of the molecule being hydrophobic; its polar head hydrophilic. The lipids in the plasma membrane are chiefly phospholipids like phosphatidyl-ethanolamine and phosphatidyl choline. 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 made up of naturally-derived phospholipids with mixed lipid chains (like egg phosphatidyl-ethanolamine), or of pure surfactant components like dioleoyl phosphatidyl-ethanolamine. Liposomes contain a core of aqueous solution; lipid spheres that contain no aqueous material are called micelles, however, reverse micelles can be accessed to encompass an aqueous environment. The self-aggregation

of polar lipids is not restricted to conventional bilayer structures which rely on molecular shape, and environmental conditions, temperature and preparation conditions, but they may self-assemble into various types of colloidal particles.

As a novel drug delivery system, liposomes and their use have been greatly utilized by pharmaceutical manufacturers in the field of medicine. Liposomes are biocompatible, non-toxic, completely biodegradable, nonimmunogenic and flexible. They have both lipophilic and aqueous environment making them useful for delivering hydrophobic, amphipathic, and hydrophilic medicines. The encapsulation serves to protect sensitive areas from the drug as well. They are extremely versatile in the form which they may be administered. 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 [7]. These forms include suspension, cream, lotion, aerosol, gel, and powder which can then be administered through most common routes of medicinal administration.

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. Liposomes are also flexible in their size, and as is such they can enclose a wide size range of molecules. They possess increased stability via encapsulation. Liposomes provide reduction in toxicity of the encapsulated agents. Liposomes give site avoidance effect [8]. 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.

Liposomes have many advantages when compared with other methods of drug delivery but they also have some limitations [9]. The main limitation of the standard liposome drug delivery system is its fast clearance from circulation due to uptake by the reticuloendothelial system, initially in the liver. The cost of production is high for liposomes when compared to other formulations. The leakage property of liposomes will lead to fusion of the encapsulated drugs. The phospholipid in liposome may undergo oxidation and hydrolysis. Liposomes have a shorter half-life and also have lower solubility and fewer stability problems.

Classification

Depending on application and composition, liposomes are classified as, pH sensitive liposomes, fusogenic liposomes, immuno-liposomes, cationic liposomes and long circulatory or stealth liposomes. Conventional liposomes are neutral or negatively charged phospholipids and cholesterol. Fusogenic liposomes are the reconstituted Sendai virus envelopes. pH sensitive Liposomes includes phospholipids. Cationic liposomes contain cationic lipids with DOPE. LCL have 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 known as pegylation. Immuno-liposomes are liposomes with attached monoclonal antibody or recognition sequence [10].

Structurally liposomes are unilamellar vesicles, oligolamellar vesicles (OLV) and multilamellar vesicles (MLV) (Fig. 3). Unilamellar vesicles are further classified as small unilamellar vesicles (SUV), medium unilamellar vesicles (MUV) and large unilamellar vesicles (LUV). MLV size ranges from 300-5000 nm. LUV size ranges from 100 nm-300 nm. SUV size ranges from 20-100 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 [11].

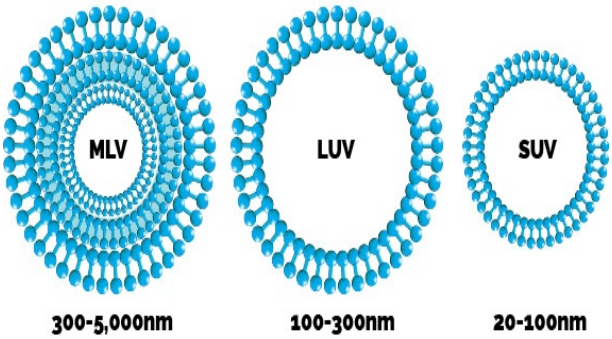

Fig. 3. Classification of liposomes – MLV, LUV and SUV

On preparation methods liposomes are classified as OLV made by reverse-phase evaporation method, stable plurilamellar vesicles, MLV made by reverse-phase evaporation method, frozen and thawed MLV, vesicles prepared by dehydration-rehydration method and vesicles prepared by extrusion technique [12].

PREPARATION METHODS

The physicochemical characteristics of the material to be entrapped should be considered while selecting liposome preparation method. The physicochemical characteristics of the liposomal ingredients, the nature of the medium in which the lipid vesicles are dispersed, additional

processes involved during application/ delivery of the vesicles, the effective concentration of the entrapped substance and its potential toxicity, polydispersity, optimum size 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.

Liposomes are prepared by using various methods in which the entrapment of water soluble (hydrophilic) materials in aqueous solution of these materials as hydrating fluid. The organic solution of the constitutive lipid is used to solubilize the lipid soluble (lipophilic) materials and by hydration, they are evaporated to a dry lipid film with drug.

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. Two techniques namely passive loading techniques and active loading techniques are used to prepare liposomes. Passive loading techniques include three different methods like solvent dispersion method, mechanical dispersion method and detergent removal method. The different types of mechanical dispersion methods are French pressure cell extrusion, sonication, Freeze-thawed liposomes, non-hand shaking or freeze drying, micro-emulsification, lipid film hydration by hand shaking, membrane extrusion and dried reconstituted vesicles [13].

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.

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 [14].

In Freeze-thawed liposome preparation method the unilamellar vesicles are prepared by the fusion of SUV throughout the processes of freezing and thawing. 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 [15].

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. 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 [16]. The disadvantages of the method are that the liposomes are very dilute, the removal of all ethanol is difficult because it forms 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.

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. 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 is diluted beyond the mixed micellar phase boundary, a spontaneous transition from polydispersed micelles to vesicles occurs [17].

In Detergent removal method the detergents at their critical micelle concentrations (CMC) have been used to solubilize lipids. 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. The great benefit of using detergent adsorbers is that they can eliminate detergents with a very low CMC, which are not entirely depleted [18].

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 [19]. 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 for the treatment of solid tumors. Pharmacological action of vasopressin is formulated in long circulating liposome.

Loading of Drug in Liposomal Drug Delivery System

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. 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 will go through partitioning. It is dissolve along with phospholipid in a suitable organic solvent. This combination is dried first after added directly to the aqueous phase and solvent residues remove under vacuum [20]. 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 immune-affinity sandwich approach for the rapid and selective purification of membranes.

The reverse-loading mechanism uses for certain drugs may exist in both charged and uncharged forms depending on the pH of the environment. This type of drug can be added to an aqueous phase 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.

Transportation Mechanism through Liposomes

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.

A liposome encapsulates a region of aqueous solution inside a hydrophobic membrane; dissolved hydrophilic solutes cannot readily pass through the lipids [21]. 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.

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 aggregate has been assumed to increase the effectiveness of transfection through the negatively charged phospholipid bilayer.

Numerous drugs have been tried as liposomes (Table 1). Many trials are going on for the development of liposomes as drug delivery.

Table 1: List of some drugs tried to deliver by liposome drug delivery system

Sl.No	Drug	Applications
1	Amphotericin-B	Broad-spectrum antifungal agent [22]
2	Artemisinin	For the treatment of malaria [23]
3	Copper palmitate	Blocks porphyrin-induced photosensitivity in rats [24]
4	Doxophylline	In the treatment of asthma [25]
5	Gliclazide	Antidiabetic agent [26]
6	Glimepiride	Antidiabetic agent [27]
7	Muramyl-tripeptide-phosphatidyl	In the treatment of herpes simplex virus infections [28]

	ethanolamine, and glycoprotein D	
8	Rhodamine-conjugated liposomes	In the treatment of uveitis [29]

Applications

Liposomes have many applications as a method of drug delivery. Glimepiride when formulated showed sustained release property which could be successfully developed as liposomes [30]. The liposomes have been evidenced to play a vital role in treatment of disorders of both anterior and posterior segment of eye. Dry eyes, keratitis, corneal transplant rejection, uveitis, ondopthelmitis and proliferative vitro retinopathy are the examples of eye disorders against which liposomes have been found to possess beneficial effects. The drug verteprofin that is found to be effective against eye disorders has been recently approved as liposomal formulations. The liposomes increase both cell mediated and humoral immunity which is evidenced by the fact that intramuscular injection of liposomal immunoadjuvant released encapsulated antigen which get passively accumulated in the regional lymph node. They are used for drug delivery to sites of action. They are used as models for artificial cells [31]. The use of liposomes for transformation or transfection of DNA into a host cell is known as lipofection [32]. 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 [33].

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.

CONCLUSION

Liposome vesicles seem to be potential carriers of different drugs that could be used for therapeutic applications. So many factors contribute to their success as drug delivery vehicles. The drugs which are difficult to administer intravenously are solubilized as liposomes. The structures of liposomes have major characteristics including low toxicity, biocompatibility, lower clearance rates, the ability to target cancer tissues and controlled release of drugs. 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. Based on the size, lamellar number and form and formulation of constitutes, there are several types of liposomes. Though many commercial liposomes have already been discovered, registered and introduced in pharmaceutical and cosmetic 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 tumor and various disorders. More investigations are necessary to expand the desirable aspects of drug delivery systems in clinical trials.

REFERENCES

[1] Kumar, K.P.S., Bhowmik, D., Deb, L. 2012: Recent Trends in Liposomes Used As Novel Drug Delivery System. The Pharma Innovation, 1, pp.29-38.

[2] Gregoriadis, G., 1976: The carrier potential of liposomes in biology and medicine. Part 2, The New England Journal of Medicine, 295, pp.765–770.

[3] Lian, T, Ho, R.J., 2001: Trends and developments in liposome drug delivery systems. Journal of Pharmaceutical Sciences, 90, pp.667-680.

[4] Subash Chandran, M.P., Pandey,V.P., 2014: Liposomes as drug delivery – An overview, International journal of research in pharmaceutical and nano sciences, 2014;3(2), pp.88-100

[5] Jan, V., Emilio, B., Robert, A., Anthony, H., 1994: Tissue distribution and therapeutic effect of intravenous free or encapsulated liposomal doxorubicin on human prostate carcinoma

xenografts. Journal of Cancer, 73(5), pp.1478–1484.

[6] Gregoriadis, G., Florence, A.T., 1993: Liposomes in drug delivery. Clinical, diagnostic and ophthalmic potential, Drugs, 45, pp.15-28.

[7] Maluta, S. Mufamadi, Viness, P., 2011: A review on composite liposomal technologies for specialized drug delivery, Journal of Drug Delivery, 2011, pp.51-70.

[8] Knepp, V.M., Szoka, F.C., Guy, R., 1990: Controlled drug release from a novel liposomal delivery system. II. Transdermal delivery Characteristics. Journal of Controlled Release, 12, pp.25-30.

[9] Marushchak, D., Gretskaya, N., Mikhalyov, I., Johansson, L.B., 2007: Self-aggregation--an intrinsic property of G(M1) in lipid bilayers. Molecular Membrane Biology. 24(2), pp.102-12.

[10] Pandey. V.P., Subash Chandran, M.P., 2014: Liposome drug delivery, World journal of pharmacy and pharmaceutical sciences, 4(1), pp.282-286.

[11] Gajanan, R., Sarje, Parvin, P., 2012: Recent advances on liposomal drug delivery system: a review. International Journal of Universal Pharmacy and Bio Sciences. 1(1), pp.14-25.

[12] Bo, Y., Robert, J.L., James, L., 2009: Microfluidic methods for production of liposomes. Methods in Enzymology, 465, pp.129–141.

[13] Abolfazl, A., Rogaie, R.S., Soodabeh, D., 2013: Liposome: classification, preparation, and applications. Nanoscale Research Letters, 8, pp.102.

[14] Szoka, F., Papahadjopoulos, D., 1980: Comparative Properties and Methods of Preparation of Lipid Vesicles (Liposomes). Annual Review of Biophysics and Bioengineering, 9, pp.467-508.

[15] Liu, L., Yonetaini, T., 1994: Preparation and characterization of liposome-encapsulated haemoglobin by a freeze-thaw method. Journal of Microencapsulation, 11(4), pp.409-421.

[16] Subash Chandran, M.P., Pandey, V.P., 2015: Formulation and evaluation of glimepiride loaded liposomes, International journal of research in pharmaceutical sciences 6(4), pp.333-338

[17] Love, W.G., Amos, N., Williams, B.D., Kellaway, I.W., 1990: High performance liquid chromatographic analysis of liposome stability. Journal of Microencapsulation, 7(1), pp.105-12.

[18] Wim, J., Tom, T., Daan, J.A., 1986: Preparation of liposomes via detergent removal from mixed micelles by dilution. Pharmaceutisch Weekblad, 8(5), pp.259-265.

[19] Maria, L.I., Franco, D., Luigi, C., 2006: Stealth liposomes: review of the basic science, rationale, and clinical applications, existing and potential. International Journal of Nanomedicine, 1(3), pp.297–315.

[20] Paul, T.S., 1985: The partition of charged liposomes in aqueous two-phase systems, Journal of Molecular and Cellular Biochemistry, 68(2), pp.151-159.

[21] Ho, N.F.H., Ganesan, M.G., Weiner, N.D., Flynn, G.L., 1985: Mechanisms of topical delivery of liposomally entrapped drugs. Journal of Controlled Release, 2, pp.61-65.

[22] Moen, M.D., Lyseng-Williamson, K.A., Scott, L.J., 2009: Liposomal amphotericin B: a review of its use as empirical therapy in febrile neutropenia and in the treatment of invasive fungal infections. J.Drugs, 69(3), pp.361-92.

[23] Isacchi, B., Bergonzi, M.C., Grazioso, M., 2012: Artemisinin and artemisinin plus curcumin liposomal formulations: enhanced antimalarial efficacy against Plasmodium berghei-infected mice. European Journal of Pharmaceutics and Biopharmaceutics, 80(3), pp.528-34.

[24] Mehmet, D.B., Eser, A.E., Murat, Y.E., 2005: Topical use of liposomal copper palmitate formulation blocks porphyrin-induced photosensitivity in rats. Journal of Photochemistry and Photobiology B; Biology,80(2), pp.107-14.

[25] Monika, O., Pooja, C., Kuldeep, S.P., 2012: Liposome: A Novel Aerosol Carrier of Doxophylline in Treatment of Chronic Asthma & Chronic Obstructive Pulmonary Disease. International Journal of Biomedical Research, 3(7), pp.172.

[26] Subash Chandran, M.P., Pandey, V.P., 2016: Formulation and evaluation of gliclazide loaded liposomes, Der pharmacia letter. 8(11), pp.60-68.

[27] Subash Chandran, M.P., Pandey, V.P., 2016: In-vitro and in-vivo evaluation of glimepiride loaded liposomes, Der pharma chemical, 8(24), pp.22-26.

[28] Ho, R.J., Burke, R.L., Merigan, T.C., 1989: Antigen-presenting liposomes are effective in treatment of recurrent herpes simplex virus genitalis in guinea pigs. Journal of Virology, 63(7), pp.2951–2958.

[29] Laure, L., Serge, C,, Florence, A., 2009: New formulation of vasoactive intestinal peptide using liposomes in hyaluronic acid gel for uveitis, Journal of Controlled Release, 139(1), pp.22-30.

[30] Subash Chandran, M.P., Pandey, V.P., 2016: Formulation and evaluation of glimepiride loaded liposomes by ethanol injection method, Asian

journal of pharmaceutical and clinical research, 9(4), pp.192-195.

[31] Bangham, A.D., Hill, M.W., Miller, N.G.A., 1974: Preparation and Use of Liposomes as Models of Biological Membranes. Journal of Methods in Membrane Biology, 1, pp.1-68

[32] Chen, Y.W., Leaf, H., 1989: Highly efficient DNA delivery mediated by pH-sensitive immuneliposomes. Journal of Biochemistry, 28(24), pp.9508–9514.

[33] Biswajit, M., Balaram, P., Buddhadev, L., 2007: Sustained release of acyclovir from nano-liposomes and nano-niosomes: An invitro study. International Journal of Nanomedicine, 2(2), pp.213–225.