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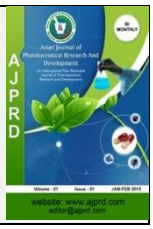

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Review Article

Liposomes –A Overview

Akshay Kumar Patel*, Shivanand K Mutta, Rajkumar Prasad Yadav

Department of Pharmaceutics, Mallige College of Pharmacy, Bangalore-560090, Karnataka, India

A B S T R A C T

Liposomes are sphere-shaped vesicles made up of one or more bilayers of phospholipids. The ability of delayed vesicles to transport medications, vaccines, diagnostic specialists, and other bioactive operators has accelerated development in the liposomal drug delivery system. The liposomal delivery system's pharmacyelements and pharmacokinetics properties have been altered, resulting in a higher therapeutic index and lower overall toxicity. There are many factors to consider, including size, size distribution, surface electrical potential, lamella count, and encapsulation efficacy. The use of surface modification in the development of liposomes with various mechanisms, kinetic properties, and biodistribution was discovered to be beneficial. Drug delivery, drug targeting, controlled release, and improved solubility have all been studied extensively with liposomes.

Keywords: Types of Liposomes, Mechanism of Liposomes, Drug Release, Evaluation of Liposomes, Application of liposomes

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***Address for Correspondence:**

Akshay Kumar Patel, Department of Pharmaceutics, Mallige College of Pharmacy, Bangalore-560090, Karnataka, India

INTRODUCTION

The aim of a novel drug delivery system is to have some control over drug release in the body, whether that control is temporal, spatial, or both. Novel drug delivery seeks to either sustain drug activity at a predetermined pace or reduce adverse side effects by maintaining a relatively steady, efficient drug level throughout the body. Liposomes are colloidal, vesicular structures composed of one or more lipid bilayers surrounding an equal numbers of aqueous compartments.[1]Liposomes range in size from the tiniest vesicle (diameter 20nm) to liposomes that can be seen under a light microscope and have a diameter of 1m or greater, roughly equivalent to the dimensions of living cells. A liposome can transport drugs in one or three compartments (water soluble agents in the central aqueous core, lipid soluble agents in the membrane, peptide and small proteins at the lipid aqueous interface).[2]Membranes are usually made of phospholipids, which are molecules that have a head group and a tail group. Water attracts the head, while water repels the tail, which is made up of a long hydrocarbon chain. A liposome is a self-forming structure that consists of one or more concentric lipid bilayers separated by aqueous buffer compartments and is an artificially prepared spherical vesicle composed of a lipid bilayer.[4]

Composition of liposome:[4]

Phospholipids:

These structures are mainly made up of phospholipids. PC also known as lecithin, is a biocompatible phospholipid present in plants and animals that is commonly used in liposomal preparation. Furthermore, other compounds, such as cholesterol, are also used in conjunction with phospholipids.

Cholesterol:

Cholesterol molecules in the membrane increases separation between choline head groups which reduces the normal hydrogen bonding and electrostatic interaction.

Structure:

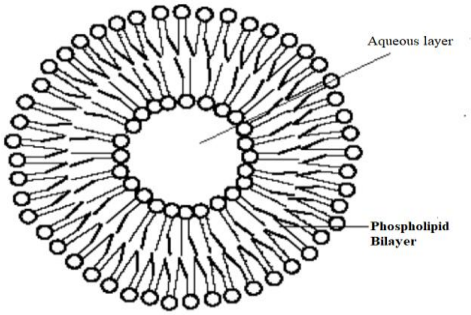


Figure 1: Structure of liposomes [4]

Liposomes are spherical structures with a diameter ranging from 15nm to 1000nm. Membrane proteins, for example, may have targeting ligands attached to their surfaces to guide them to the appropriate sites within the cell. The liposome can be used to deliver nutrients and prescription drugs to the body. The sphere-like shell encased a liquid interior that could contain peptides and proteins, hormones, enzymes, antibiotics, antifungal, and anticancer agents, among other things. Liposome properties and behaviour vary significantly depending on lipid composition, surface charge, scale, and preparation process. Since they are made up of naturally occurring substances, liposomes have the distinct benefit of being both nontoxic and biodegradable.

Advantages of liposomes:

- Have individualised drug delivery
- Non-toxic, versatile, biocompatible, and fully biodegradable
- Increases medication effectiveness and therapeutic index
- Aid in reducing harmful substance exposure to sensitive tissues
- The size of the container may be adjusted to accommodate smaller or larger drug molecules.
- Medications that are both water and lipid soluble can be used.
- It's possible to use it in a variety of ways.

Disadvantages of liposomes:

- Low solubility
- Short half-life
- Degradation, hydrolysis, leakage, and fusion of phospholipids
- High production cost

MECHANISM OF TRANSPORTATION THROUGH LIPOSOMES

Endocytosis by phagocytic cells of the reticuloendothelial system, such as macrophages and neutrophils, is one of four pathways by which liposomes communicate with cells. Adsorption to the cell surface can occur through nonspecific weak hydrophobic or electrostatic forces or through unique interactions with cell-surface components. Fusion with the plasma cell membrane occurs when the liposome's lipid bilayer is inserted into the plasma membrane and the liposomal material is released into the cytoplasm at the same time. Liposomal lipids are passed to cellular or subcellular membranes, or vice versa, without the liposome contents being associated. It's always difficult to tell which mechanism is in operation, and several mechanisms can be active at the same time.

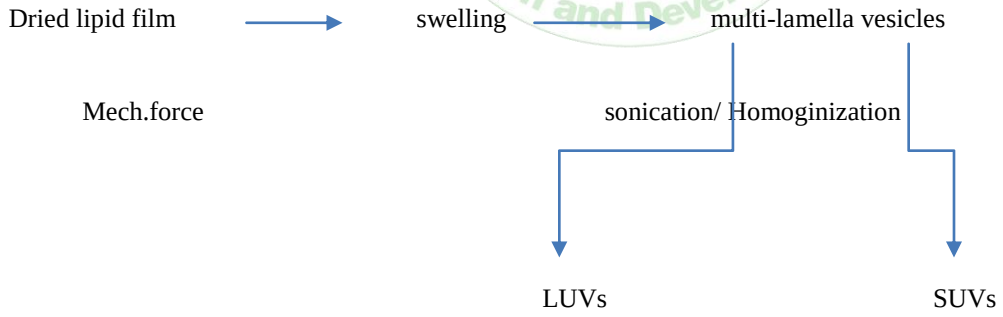


Figure 2: Mechanism of liposomes Formation

Classification of liposome:

Liposomes are classified below as

1. Structure based
2. Method of preparation
3. Composition and application

1. Structure based Liposomes:

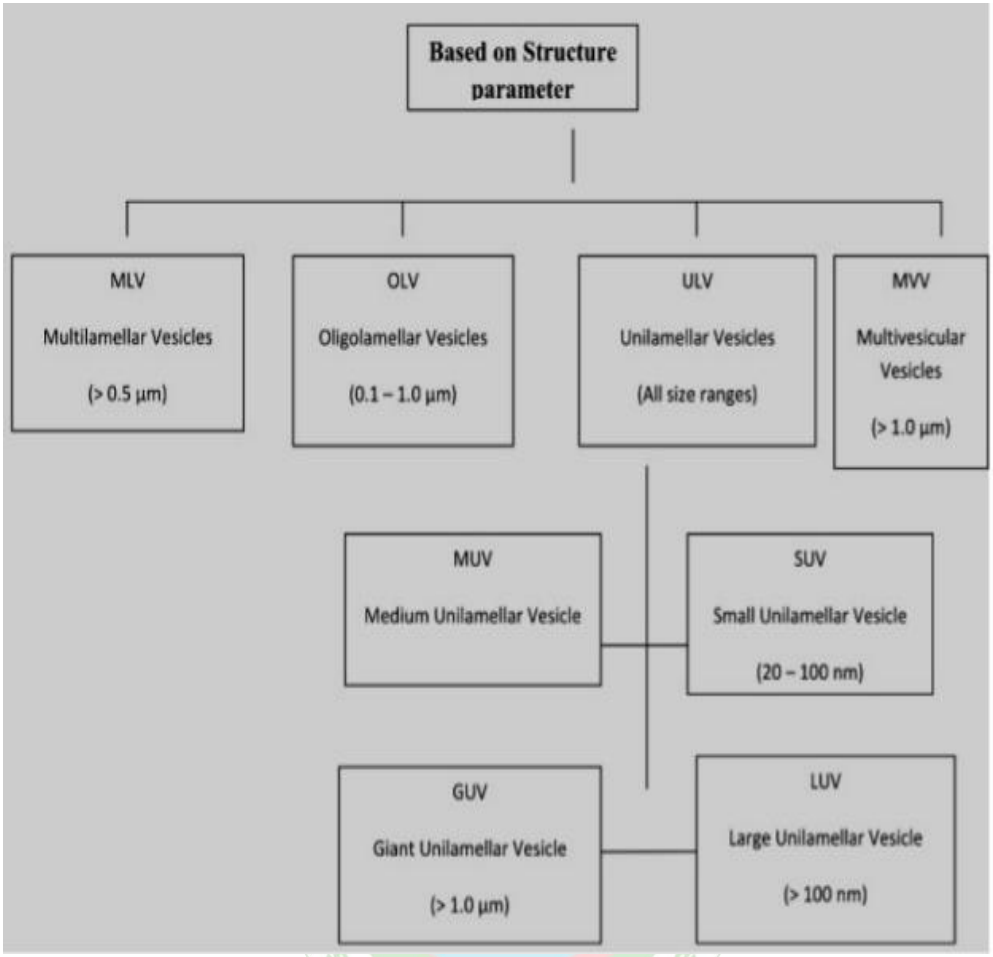


Figure 3: Structure based Liposomes

Method of preparation:

1. Passive loading techniques
2. Active loading technique

Passive loading techniques

1. Mechanical dispersion method.
2. Solvent dispersion method.
3. Detergent removal method (removal of non-encapsulated material

1. Mechanical dispersion method

- Sonication
- French pressure cell: extrusion
- Freeze-thawed liposomes
- Lipid film hydration by hand shaking, non-hand. shaking or freeze drying
- Micro-emulsification.
- Membrane extrusion
- Dried reconstituted vesicles
- Freeze-thawed liposomes

2. Solvent dispersion method:

- Ethanol injection
- Ether injection
- Double emulsion
- Reverse phase evaporation vesicles
- Stable pluri lamella vesicle

3. Detergent removal method (removal of non-encapsulated material

- Detergent (cholate, alkylglycoside, triton x-100)removal from mixed micelles by
- Dialysis
- Column chromatography
- Dilution
- Reconstituted sendai virus enveloped

Passive loading techanique:

Mechanical dispersion method [7,12,13]

1. Lipid film hydration by hand shaking, non-hand shaking or freeze drying:

To ensure a homogeneous mixture of lipids, the lipids must be dissolved and blended in an organic solvent when producing liposomes of mixed lipid composition. Typically, chloroform or a chloroform:methanol mixture is used in this method. The aim is to create a simple lipid solution that allows for full lipid mixing. Typically, 10–20 mg lipid/ml organic solvent is used to make lipid solutions, but higher concentrations can be used if lipid solubility and mixing are appropriate. The

organic solvent is extracted after the lipids have been thoroughly mixed in it, resulting in a lipid film. For use of minimal amounts of organic solvent. In a fume hood, the solvent is evaporated using a dry nitrogen source. The organic solvent can be extracted by rotary evaporation for larger amounts, leaving a thin lipid layer on the sides of the round bottom flask. By putting the vial or flask on a vacuum pump overnight, the lipid film is thoroughly dried to eliminate any remaining organic solvent. If the use of chloroform is objectionable, an alternative is to dissolve the lipids in tertiary butanol or cyclohexane. The lipid solution is transferred to containers and frozen by swirling them in a dry ice-acetone or alcohol (ethanol or methanol) bath. When using the bath technique, make sure that the container can withstand rapid temperature changes without cracking. After complete freezing, the frozen lipid cake is placed on a vacuum pump and lyophilized until dry (1–3 days depending on volume). The thickness of the lipid cake should not be greater than the diameter of the lyophilization pot. The vacuum pump may be used to extract dry lipid films or cake; the jar should be tightly closed, tapped, and frozen until ready to hydrate.

Micro emulsification:[6]

SLV is prepared using this process. Microemulsifying lipid compositions with high shearing stress produced by a high pressure homogenizer is possible.

Sonication:

It is the most commonly used tool for SUV preparation. MLV are sonicated in a passive environment using a bath sonicator or a probe sonicator. The key drawbacks of this approach are the poor encapsulation effectiveness and phospholipid and compound degradation.

A.Probe sonication:

It is directly engrossed in the dispersion of liposomes. The energy input into lipid dispersion during probe sonication is extremely high. Local heat is generated by the coupling of energy at the tip. As a result, the vessels must be immersed in ice or water. More than 5% of the lipid can be de-esterified by sonication for up to 1 hour. Titanium can slough off and pollute the solution using this process.

I.Bath sonication:

The probe sonicators have been largely replaced. Controlling the temperature of the liquid dispersion is easier with this process. It can be kept healthy in a sterile container or in an inert environment.

II. French pressure cell:

It requires the gentle handling of unstable materials and the extrusion of MLV through a narrow orifice. The benefit of this approach is that the liposomes formed are larger than sonicated SUVs. However, maintaining a high temperature is difficult, and working volumes are limited.

A.Freeze-thawed liposomes:

The product of the SUV's fusion through the freezing and thawing processes. By increasing the phospholipid concentration and the medium's ionic pressure, certain forms of synthesis are effectively inhibited.

B.Membrane extrusion:

In the high-pressure extrusion process, MLVs prepared by thin-film hydration are repeatedly passed through filters made of polycarbonate membranes, reducing the liposome size. The liposomes are rendered using a thin-film hydration process and then extruded for ten cycles to obtain uniformly formed extruded liposomes.

C. Dried reconstituted vesicles:

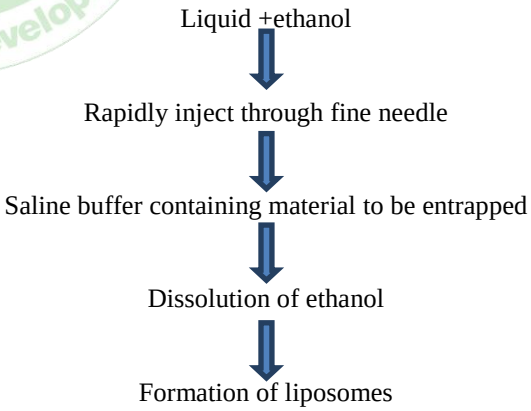
The preformed liposomes are rehydrated in an aqueous fluid containing an active ingredient, and then the mixture is dehydrated.

Solvent dispersion method

Ether Injection Method: [13,14]

At 55–65°, an aqueous solution of the substance to be encapsulated is steadily injected with lipids dissolved in diethyl ether or an ether/methanol mixture. The formation of liposomes is caused by the subsequent removal of ether under vacuum. The method's main downside is that the liposomes formed are heterogeneous in nature (70–190 nm) and that the substance to be encapsulated will be exposed to a higher temperature.

Ethanol injection:[13,14]



In the ethanol injection, take liquid and ethanol in beaker. It will inject rapidly with fine needle and the after saline buffer solution containing material to be entrapped. Then dissolve in ethanol and formed liposomes.

Double emulsion vesicles:[7]

The external portion of the liposome layer is formed in this strategy by emulsifying a natural arrangement in water at second stretches between two points. Multicompartment vesicles are obtained when the

natural arrangement, which currently contains water beads, is placed in an overabundance watery medium and mechanical scattering is performed. The resulting arranged scattering is depicted as a W/O/W structure. Multi-compartment vesicles are obtained when a natural arrangement, which currently contains water beads, is placed in an abundant fluid medium and mechanical scattering is performed. The resulting arranged scattering is depicted as a W/O/W structure. Monolayers of phospholipids encircling each water compartment are intently limited by one another at this point in the progression. The next move is to use a mechanical vortex blender to overpower the water beads and allow them to break down to a certain degree. The lipid monolayer that encased the fallen vesicle is then transferred to neighbouring unblemished vesicles, forming the external flyer of massive unilamellar Liposomes players. The framed vesicles are unilamellar and have a diameter of 0.5 micrometers. The percentage of embodiment is discovered to be 50%.

Reverse phase evaporation vesicles:[7,13,14]

Natural solvents containing phospholipids, such as diethyl ether/isopropyl ether or a combination of diethyl ether and chloroform (1:1 v/v) and a blend of chloroform-methanol (2:1 v/v), use the reverse stage disappearing technique. The natural and fluid stages should be indistinguishable, resulting in an oil/water emulsion. To increase the efficacy of liposome formulations, phosphate cushion saline or citrus Na2HPO4 cushion is applied to the fluid level. The formation of liposomes is encouraged by the rotational vanishing of natural solvents under vacuum. The strategy's primary advantage is its extremely high exemplification pace. The technique's key drawback is the likelihood of residual dissolvable in the description, as well as scaling issues.

Detergent Removal Method:[1,13]

The detergents were used to solubilize lipids at their essential micelle concentrations. When the detergent is extracted, the micelles become more phospholipid-rich, eventually combining to form LUVs. The benefits of the detergent dialysis process include high reproducibility and the development of uniformly sized liposomes. The main drawback of the method is the retention of traces of detergent within the liposomes.

I.Detergent Dialysis:[1]

Diachema, AG, and Switzerland sell a pilot plant called LIPOPREPR II-CIS. At higher lipid concentrations (80 mg/ml), the output potential is 30 ml liposomes per minute. When the lipid concentration is 10-20 mg/ml and the lipid concentration is 100 mg/ml, up to a liter of liposomes can be made.

II.Gel-permeation chromatography:[1]

The detergent is depleted using size special chromatography in this process. Liposomes are unable to reach the pores of the beads in a column. They seep into the gaps between the beads. Liposomes and detergent monomers can be isolated very well at low flow rates. Since the swollen polysaccharide beads adsorb large amounts of amphiphilic lipids, pretreatment is needed. Pretreatment is accomplished by using empty liposome suspensions to pre-saturate the gel filtration column with lipids.

III. Dilution:

Dilution of aqueous detergent and phospholipid micellar solution with buffers. Micellar size and poly dispersion are fundamentally increased. Poly dispersed micelles to vesicles occurs when the device is diluted beyond the mixed micellar phase. Gel filtration can be performed with Sephadex G-50, Sephadex G-I00 (Sigma-Aldrich, MO, USA), and Sepharose 2B-6B.

IV. Dailysis:[7]

Diachema, AG, and Switzerland have access to a pilot plant under the trademark LIPOPREPR II-CIS. At higher lipid fixation (80 mg/ml), the maximum production rate is 30 ml liposomes per minute. When lipid fixation is 10-20 mg/ml, however, multiple litres of liposomes can be produced. Dianorm-Generate supports LIPOPREPR in the United States.

Liposome-based products on the market:[12]

Table no 1: Liposome-based products on the market

Molecule	Treated disease	Product
Doxorubicin	Kaposi's sarcoma and AIDS-related cancers. Ovarian cancer and multiple myeloma.	DOXIL
Amphotericin B	Systemic fungal infections	AMBISOME
Inactivated hepatitis A virus	Hepatitis A	EPAXAL
Lidocaine	Anaesthesia for skin itching, burning or pain.	LMX4 LMX5

Evaluations of Liposomes [7,10,11]

Physical characteristics

Vesicular size & size Distribution:

Various techniques for ensuring scale and size conveyance are portrayed in the prose. Light microscopy and fluorescent microscopy are used in these techniques. Electron microscopy, laser light scattering, photon correlation spectroscopy, gel

permeation and exclusion, and the Zetasizer are some of the techniques used. Electron microscopy is the most precise method, but it is also the most time-consuming.

Microscopic Techniques:

Optical microscopy:

Bright field, gap, and fluorescent magnifying lenses can be used to overcome the scale of enormous vesicles (>1m).

1. Negative stain Transmission Electron microscopy (TEM):

The use of negative stain TEM to determine the liposome size range at the lower end of the recurrence circulation is encouraged. Ammonium Molybdate, Uranyl Acetate, and Phosphotungstic corrosive are examples of negative stains used in TEM analysis.

I.Cryo- Transmission Electron microscopy Techniques (Cryo-TME):

It has been used to measure vesicle size and surface morphology, as well as to depict liposomal plans in which the drug is stacked by distant stacking to ensure their integrity. The process involves freezing the samples and then describing them using TEM.

II.Freeze fracture Electron microscopy:

Its primary objective is to assess the surface highlights and laterality. It can also be used to measure true vesicle breadth.

A.Diffraction Scattering Technique:

Laser light scattering:

For studying homogeneous colloidal particulate populations, laser-based, semi-portable light dispersing methods are useful. The time subordinate lucidity of light dissipated by a vesicle is used in this process. It may be used on architectures with a mean width of less than 1µm.

B. Hydrodynamic Techniques:

These methods incorporate Gel penetration, Field Flow Fractionation and Ultracentrifuge procedures.

2. Surface charge:

Charge on the vesicle surface is investigated using zeta potential and free stream electrophoresis. The versatility of liposomal scattering in a sufficient cushion determines the vesicle surface charge.

3. Lamellarity:

The shape of the vesicle was examined using electron microscopic techniques. Freeze Break Electron Microscopy and P31 Nuclear Magnetic Reverberation Analysis determine the lamellarity of vesicles.

4. Hydrodynamic Techniques:

Gel Permeation and Ultracentrifuge are two techniques used in this process. To distinguish SUVs from radial MLVs, exclusion chromatography on broad pure gels was used. Large vesicles with a diameter of 1 to 3 m normally do not penetrate the gel and end up on top of the column. A thin layer chromatography device based on agarose beads has been developed as a simple and fast method for estimating the size distribution of liposome preparations. However, it was not reported.

6. Zeta potential determination:

The electro mobility of the 90c angle was used to determine the zeta potential. The 3000 HS zeta-seizer equipment was used to perform the calculation in triplicate. For the possible determination, the sample was diluted with sufficient diluents.

Stability Studies:

At 4oC storage, all formulations of Metformin HCl liposomes were relatively stable. The original entrapped in liposomes drug leakage percent amounts were very small, and the amount retained in vesicle after one month had no noticeable difference from the amount immediately after preparation. However, all Metformin HCl liposome formulations were unstable at storage temperatures of 25°C. Furthermore, drug entrapment experiments revealed that at higher temperatures, there was more leakage.

APPLICATIONS OF LIPOSOMES[8,9,13,14]

Systemic Liposomal Drugs:

Liposomes may be used as effective drug delivery vehicles. Sterically stabilised liposomes are not readily absorbed by mononuclear phagocyte cells, have different biodistribution properties, and have been found to accumulate more readily in trauma, tumour, infection, and inflammation sites.

Topical Liposomal Drugs:

The ability of lipid vesicles with unique lipid composition to modify cell membrane fluidity is one of the skin treatment applications of liposomes, which is based on the similarity between the bilayer structure of lipid vesicles and natural membrane.

Cosmetic Applications:

Minoxidil, a vasodilator, is the active ingredient in products like "Regaine" that appear to prevent or delay hair loss. Liposomes have also been used in the treatment of hair loss. Skin care products containing empty or moisture-loaded liposomes minimise transdermal water loss and are beneficial in treating dry skin. They also boost lipid and water supply to the stratum corneum.

Food application:

Biopolymer matrices made of sugar, starch, gum, protein, synthetic, dextrin, and alginates are used in the majority of microencapsulation techniques currently used in the food industry. Liposomes, on the other hand, have only recently begun to gain prominence in food products.

Liposomes in anticancer therapy:

Different anticancer agents' liposome formulations were found to be less toxic than the free medication. Anthracyclines are drugs that avoid dividing cells from growing by intercalating DNA and thus destroy primarily rapidly dividing cells. These cells can be found in tumours, as well as gastrointestinal mucosa, hair, and blood cells, making this class of drugs extremely toxic.

Liposomes in medicine and pharmacology:

The use of liposomes in medicine and pharmacology can be divided into diagnostic and therapeutic applications of liposomes containing different marker drugs, as well as their use as a tool, model, or reagent in basic studies of cell interaction, identification, and mode of action of certain substances. Changes in liposomal drug pharmacokinetics may result in increased drug bioavailability to specific target cells in the bloodstream, or, more specifically, to extravascular disease sites, such as tumours. The liposomal formulation of all-trans-retinoic acid is a recent advancement.

Liposomes in parasitic diseases and infections:

After being digested by phagocytic cells in the body after intravenous administration, traditional liposomes are ideal vehicles for delivering drug molecules to macrophages. Several parasitic diseases that usually occur in the cell of MPS are the best known examples of this ‘Trojan horse-like' process.

CONCLUSIONS

Hydrogels have long been known as extremely useful transporter systems and instruments for delivering targeted medications. Because of their increased drug delivery to unhealthy regions, liposomes are achieving clinical proposals. Liposomes are showing particular promise as intracellular delivery systems for anti-sense molecules, ribosomes, proteins/peptides, and DNA. Liposomes with enhanced drug delivery to disease locations, by ability of long circulation residence times, are now achieving clinical acceptance.

REFERENCE:

1. Bodke A.R, Smita S. Aher, Saudagar R.B. A Review on liposomes. Int J Pharm Chem Res. 2017; 3(2):120-27.
2. Uhumwangho M.U, Okor R.S. Current trends in the production and biomedical applications of liposomes: A review. J Med Bio Res. 2005; 4 (1): 9-21.
3. Gupta M, Vishwakarma A. An Updated Review On: Liposomes a drug Carrie. Asian J Pharm Edu Res.2018; 7(3):18-34.
4. Bunker A,Magarkar A, Viitala T. Rational design of liposomal drug delivery systems, a review: Combined experimental and computational studies of lipid membranes, liposomes and their PEGylation. Biochim Biophys Acta. 2016; 2334–52.
5. Popovska O, Simonovska J, Kavrakovski Z, Rafajlovska V. An overview: Methods for preparation and characterization of liposomes as drug delivery systems. Int J Pharm Phytopharm Res. 2013; 3 (3):182-89.
6. Mishra H, Chauhan V, Kumar K, Teotia D. A comprehensive review on Liposomes: a novel drug delivery system. J Drug Deliv Thera. 2018; 8(6):400-4.
7. Arpita1 S, Kumar S.V, Amresh G. Liposomes: A review. Int J Ind Herbs Drugs. 2020;6(6):1–6.
8. Verma A.K, Sachan A.K, Kumar S, Singh N, RaiA. K. Liposomes: An Advance tools for novel drug delivery system. Int Res J Pharm Appl Sci. 2014; 4(1):74-7.
9. Pradhan H, Kumar N, Saha S, Roy A. Liposome: Method of preparation advantages, evaluation and its application. J Appl Pharm Res. 2015; 3 (3):1- 8.
10. Satyavathi K, Bhojaraju P, Srikranthi M,Sudhakar P. Formulation and *in-vitro* evaluation of liposomal drug delivery system of cabazitaxel. J Pharm Drug Deliv Res. 2015; 4(2):1-5.
11. Subramanian L, Rani A.M, Rajasekaran T, Ramanathan M, Solairaj P. Formulation and *in-vitro* evaluation of liposomal drug delivery system of metformin HCl. J Drug Deliv Thera. 2019; 9(2):31-7.
12. Laouini A, Maalej J.C, Blouza L.I, Sfar S,Charcosset C, Fessi H. Preparation, characterization and applications of liposomes: State of the art. J Colloid Sci Bio.2012;1(1):147– 68
13. Akbarzadeh A, Sadabady R.R, Davaran S, Joo S.W, Zarghami N, Hanifehpour Y*et al.* Liposome: Classification, preparation, and applications. Nano Res Letters. 2013; 8(102):1-9.
14. Dwivedi C, Yadav R, Tiwari S.P, Satapathy T, Roy A. Role of liposome in novel drug delivery system. J Drug Deliv Thera.2014; 4(2):116-29.