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LIPOSOMES: A NOVEL DRUG DELIVERY SYSTEM TO TREAT OCULAR DISEASES

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ABSTRACT

Liposomal drug carriers now provide a wide range of options for ocular delivery. The delivery of medications to ocular tissues is a tough job for formulation experts. Liposomes have several benefits, allowing them to be employed in a wide range of applications. Among other things, they can serve as useful drug carriers in preclinical and clinical trials. Physiological obstacles (nasolacrimal discharge, blink), anatomic obstacles (static and dynamic), enzymatic activity, and metabolism obstacles all obstruct drug transport towards anterior and posterior regions. Liposomes are categorized according to their structure, inventions, Intravitreal Implants, classification, preparation methods, advantages, disadvantages, applications, and formulations. Furthermore, the recent inventions those are related with ocular drug delivery technologies also been elaborated. Manuscript also includes the application of lipid nanoparticles those are used in the treatment of ocular disorders.

KEYWORDS: Liposomes, Ocular drug delivery, lipid nanoparticles, posterior segment, anterior segment, applications.

INTRODUCTION

Pharmaceutical researchers encounter a huge challenge when it comes to delivering pharmaceuticals to the eye since a number of impediments in the eye prevent the genuine dose from reaching the site and maintaining in its therapeutic concentration.[1] The eye is distinguished by its intricate structure and strong resistance to medicines and other external

agents.[2] The medicine's ocular administration is primarily used to treat ophthalmic illnesses and is not considered a method of achieving systemic pharmacological activity.[3] In recent years, several novel ocular delivery techniques have been devised, as well as safe and trustworthy ones, to assist overcome all of the obstacles in the eye that contribute to limited drug bioavailability. The new drug delivery systems are less irritating towards the eyes and also have a long contact duration inside the eye, leading to increased efficacy and absorption.[4] Delivering medication through the ocular route is difficult due to underlying characteristics including rapid evacuation of eye-drops from the corneal surface, which leads to rapid nasolacrimal drainage, and transiting pharmaceuticals over the blood ocular wall and the cornea.[5] For the anterior eye segment, eye drop options are given to cure bacterial or fungal disorders, conjunctiva, tumours, dry eye condition, and glaucoma.[6]

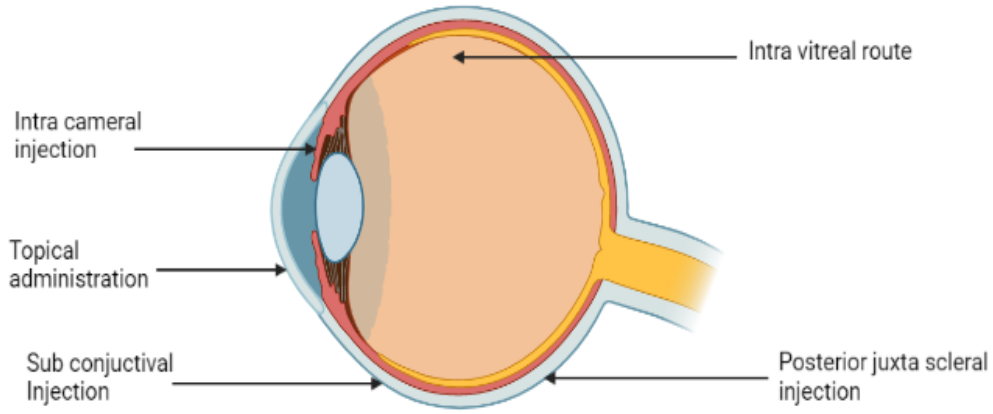

Figure 1: Routes of entry for ocular medication delivery.

With ocular drug delivery, obtaining optimal medicine concentration is difficult due to the eye's particular protective factors. A detailed understanding of eye's stationary and non - stationary obstacles is required for the creation of a drug carrier that achieves dose in the desired spot. An anterior segment of the eye, which is placed next to the vitreous humour, is made up of the cornea, pupil, watery liquid, iris, lens, and coloured part of the eye. The sclera, choroid, retina, vitreous humour, macula, and optic nerve make up the posterior segment, which covers the back two-thirds of the eye. Local, systemic, mode of occurrence, and intravitreal medication administration are the most prevalent methods for treating eye problems. The two primary obstacles for anterior and posterior sector ocular drug dispersion following systemic injection are the plasma and blood-retinal obstacles, respectively. The non-coloured ciliary epithelium and the iris blood vessel endothelium form a blood-aqueous

barrier that prevents dissolved substances from reaching the aqueous fluid. The blood retinal wall, which limits the entrance of solute into retina, is divided into two layers of cells: retina capillaries cells and retinal coloured epithelium cells. Liposomal delivery technologies for medication to the eye is favourable because they can collect either hydrophobic or hydrophilic medicines and can be delivered to both the anterior and posterior portions of the eye.[7,8] The retinal is the sensitive inner coating of the eye's posterior region, which would be divided from choroid by Bruch's membrane.[6] Fluid evacuation and diluting the eye drops, limited formulation residence time, inadequate corneal and conjunctival uptake of the medication, and drug losses in systemic circulation are the key drawbacks for topical ocular delivery. To optimise eye drops intake by improving penetration and reducing pharmaceutical losses, many resources are used. Another option is to utilise drug delivery systems to improve ocular absorption, such as nano or micro-systems capable of increasing the active substance's absorption and delivering a regulated and sustained drug release.[8] Non-invasive drug delivery, such as oral drugs, eye ointments, and external eye drops, has traditionally been treated a variety of eye disorders, but the majority of them are unsuccessful and only apply in initial minor symptoms.[9] Drug delivery systems try for the highest spatiotemporal efficiency while delivering therapeutic amounts of an active drug.[36] The convenience and stability of using external and non-invasive procedures for drug administration to posterior part of an eye over injections are advantages of using external and non-invasive approaches of drug carrier to posterior part of an eye. In order to promote the product, the delivery system must be cost-effective, dependable, and adaptable to pharmaceuticals with a wide range of Physico-chemical features.[37] In ocular drug delivery, the pro-drug idea is used to increase overall absorption of topically injected drugs, which is a key challenge in the area.[13,38] The use of nanoparticles in medication formulations can improve drug absorption in the ocular tissues.[14] Colloidal drug delivery systems are more effective in increasing ocular medication absorption while reducing systemic absorption and negative effects.[15] Drug delivery techniques based on nanotechnology, such as micro suspensions, lipid nanoparticles, and polymeric micro systems[16] and liposomes have solved many solubility issues with poorly soluble medications such as dexamethasone, budesonide, ganciclovir, and others.[17]

The majority of ophthalmic medications are administered topically as eye drops. In the absence of any attempts to change this, the timing of delivery of drugs out of an eye drop fits the trend, with kinetics that resemble first order, this translates toward "pulse-entry" of the drug leads to a rapid decline inside the amount in the tears. Efficient eye drop therapy can be

obtained by providing a suitably big pulse with a long-lasting effect, or by delivering a less concentrated pulse more often.[18]

Some of the innovative ocular medication delivery technologies have improved pulse entrance and increased drug absorption in the cornea.

Recent inventions for ocular Drug-Delivery technologies

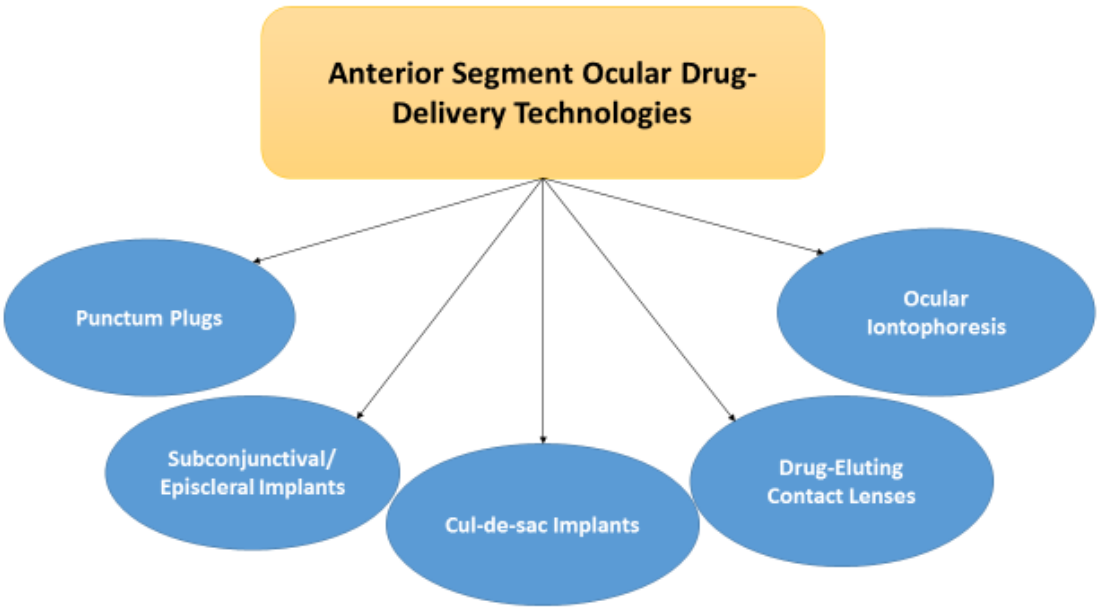

Figure 2: Inventions for anterior segment.

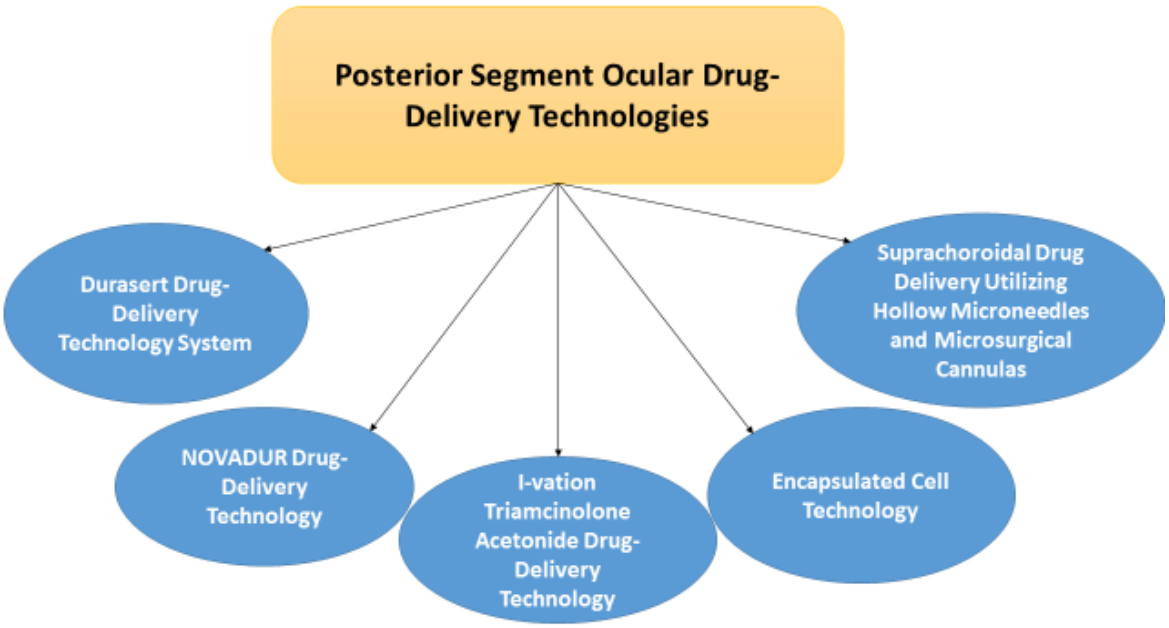

Figure 3: Inventions for posterior segment.

Clinically used intravitreal implants

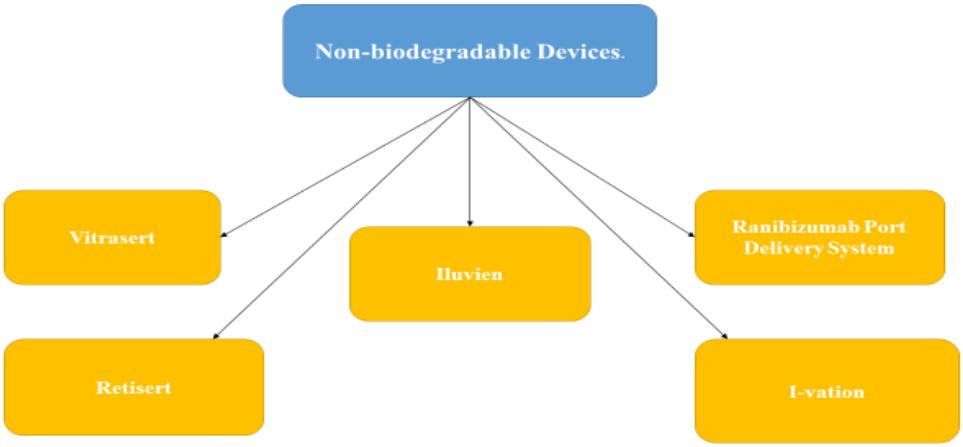

Figure 4: Non- biodegradable implants.

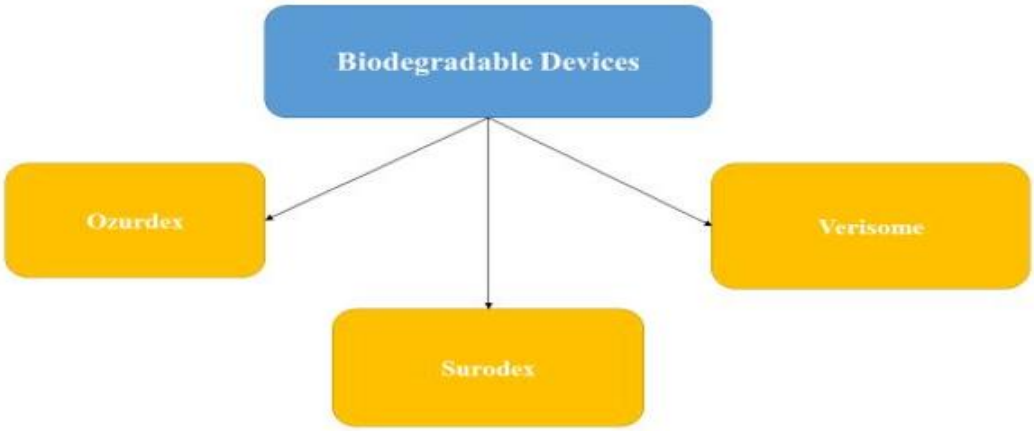

Figure 5: Biodegradable implants.

Factors limiting ocular bioavailability of drugs

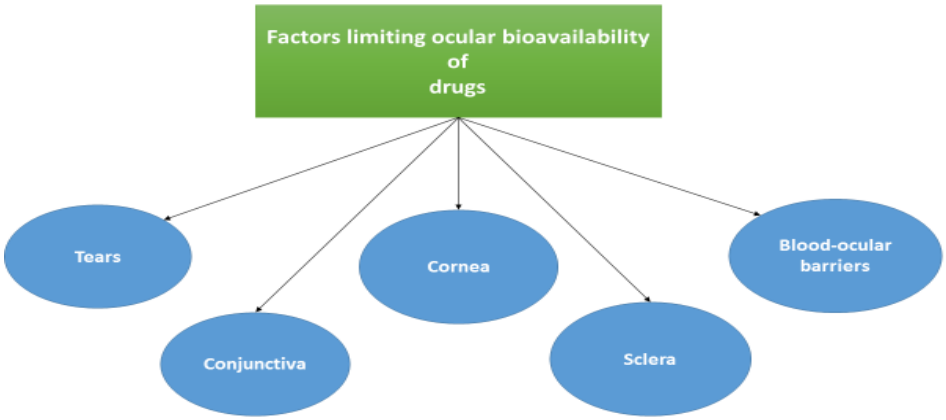

Figure 6: Factors limiting ocular bioavailability of drug.

Liposomes

In 1965, liposomes were initially employed as medication delivery vehicles.[22] (Fre'zard 1999) Liposomes were lipid bilayers isolated by water sections in artificial vesicles.[23] Liposomes have historically been utilised as drug delivery vehicles, with various formulations having received FDA approval and being tested in clinical studies.[24] Liposomes are lipid multilayer spherical vesicles with aqueous core and repulsive ring.[25] In the pharmaceutical industry, liposomal formulations are being studied intensively as secure and efficient carrier system.[26] Liposomes are colloidal vesicles composed with one or many lipid bilayers enclosed by an equivalent amount of aqueous cores.[27] When phospholipids are dispersed in water, liposomes develop spontaneously, forming a sealed construct with an internal aquatic environment enclosed by phospholipid bilayer borders.[28] Liposomes, which seem to be micro composed of cell membrane lipid bilayer enclosing the aqueous layer, for a multitude of purposes, are a viable approach for ocular medication orders, including increased drug uptake residence time.[29] Liposomes are tiny vesicles made up of concentric lipid bilayers separated by aqueous buffer compartments. The existence of several bilayers distinguishes multilamellar vesicles (MLV).[30] Positive charge liposomes have a greater propensity for attaching the corneal surface in neutral or negative charge lipid nanoparticles.[31] In retinal bodies, their half-lives can also be prolonged without generating toxicity. However, due to their weak bioadhesion, their ability to infiltrate underlying tissue is restricted. A variety containing biological macromolecules, like polymers and specific ligands, have been used in combination with liposomes to improve trans-shipment efficiency.[34]

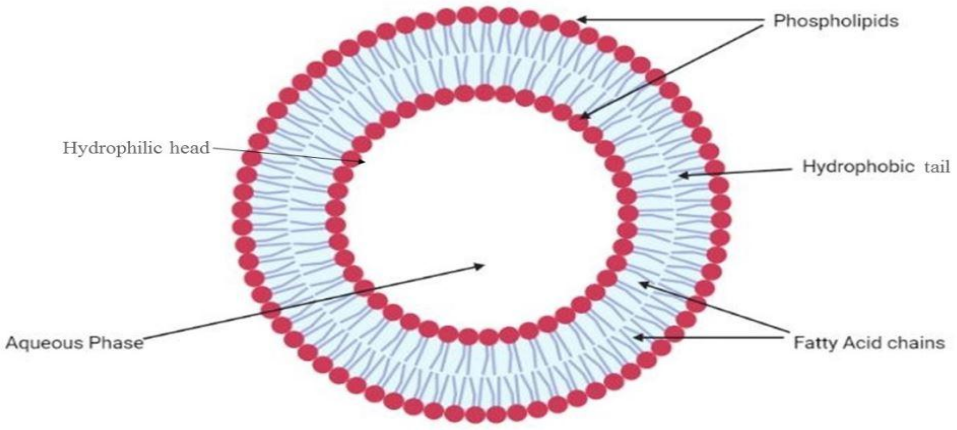

Figure 7: Diagrammatical representation for hydrophilic (polar) Head and Hydrophobic (non- polar) tail.

Liposomes are generally 40 nm to 120 nm or more in size.[35] In these vesicular structures, a watery area was covered by natural or manufactured phospholipid bilayers.[38] Because of the large number of compounds accessible and the wide spectrum of therapeutic targets, lipid nanoparticles administration of small-molecule therapies is still a hot topic.[39] The molecules of medicine must reach certain areas inner the target tissue in order for the drug's effects to be maximised. Because drug molecules are often unable to effectively access their site of action, vehicles that can efficiently carry the needed amount of medication to the target site are required.[40] It's also worth looking into the drug delivery effectiveness of PEGylated liposomes containing cholesterol analogues for ocular medication treatment.[24] When compared to those given with uncoated C3G (Cyanidin-3-glycoside)-loaded liposomes, lipid peroxidation in the lens was significantly reduced.[41] With qualities such as enhanced permeation, bioactivity, and drug preservation, chitosan's smaller molecular mass might be a viable eye pharmaceutical carrier.[42] Muco-adhesive liposomes were created to prevent the massive loss of a topically administered medication solution.[43] Nanotechnology is critical to scientific and technical advancement in a variety of industries, particularly in the pharmaceutical industry. Commercially successful nanocarriers systems for superior properties such as targeting, extended / controllable release, high absorption, and so on are driving the broad applicability of nanotechnology in the pharmaceutical area.[44] Chemotherapy uses both pegylated liposome-encapsulated and non-pegylated liposomal forms.[45] Coating liposomes with SF (silk fibroin) assembled many single vesicular liposomes into a multi-vesicular liposome with a higher particle diameter in a highly structured and refined way, with no observable cytotoxicity.[46] It is vital to pay attention to several specific needs in order to maximise ophthalmic preparations. The most essential physical and chemical properties of the samples were tested, including pH, osmolarity, expansion, and viscosity.[47] The use of lipid nanoparticles as topical medication delivery methods with improved tissue penetration has attracted a lot of attention.[48] Liposomes might be a feasible option to eye drops, lowering the number of doses required and improving patient compliance.[49] Due to repulsive generated electrostatic forces, the negative charge liposome would be an ideal vehicle for medication delivery to the posterio portion of an eye.[50] Liposomes can lessen the toxicity of various active chemicals in the eye while also increasing their residence length.[51]

Classification of liposome

Liposomes are classified into three i.e. multilamellar vesicles (MLVs), large unilamellar vesicles (LUVs), and small unilamellar vesicles (SUVs).[52] In small unilamellar vesicles, a watery inner core is surrounded by a single lipid layer. A multilamellar vesicles are made up of many levels of lipid bilayers.[30,32,33] MLVs are metastable energy configurations with various features dependent on the liposomal formulation's polydispersity.[52] Figure 8 summarises the different types of liposomes and their sizes. The medicine actual size of lipid nanoparticles was determined by a number of factors, including the diameter of the nanoparticles, exactly the sort lipid used in their production, and the physicochemical characteristics of the medicinal agent. SUVs, for fact, are the smallest and so have a lower entrapment efficiency than MLVs. On the other side, LUVs offer a nice balance of size and medication loading capabilities.

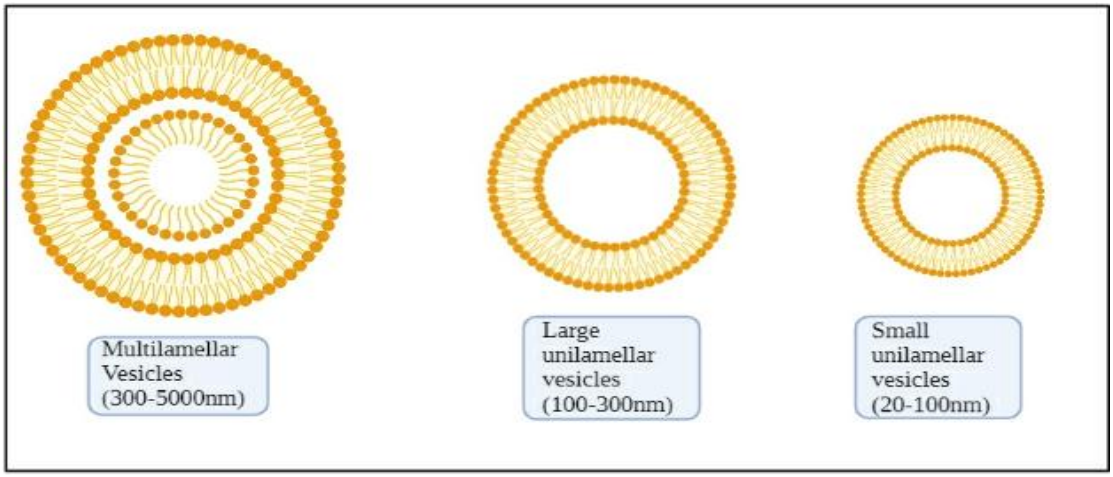

Figure 8: Classification of liposomes – MLV, LUV and SUV.

Liposomes can contain either lipophilic. Hydrophilic medicines become caught in the water phase, whereas hydrophobic medicines get trapped into lipid bilayers. Developing lipid nanoparticles using anionic or cationic lipids can increase the entrapment efficiency of ionic substances even further.[53] The most frequent constituents in liposomal formulations are phosphatidylcholine (PC) and additional elements like cholesterol or lipid-conjugated hydrophilic polymers. Cholesterol incorporation increases membrane stiffness, which enhances stability. Liposome stability is determined by the lipids bilayer's surface charge, volume, interface moisture, and mobility. Liposomes' interaction with the optical layer is determined by their surface charge. Nanocarriers with a positive charge penetrate the cornea quicker than those with a negative charge. When delivered systemically, neutral nanocarriers resist removal by the reticuloendothelial system (RES). On the other side, these vesicles have

a higher predisposition for self-aggregation. Negative and positive charge liposomes, from the other side, have a lower tendency to aggregate but are eliminated faster via RES cells because of greater contact of proteins in the blood. The diameter of lipid nanoparticles affect RES clearance as well. Due to a reduction in opsonization of lipid nanoparticles with serum protein, solid lipid nanoparticles with a diameter from less than 100nm have a substantially longer circulation period.[54] Phospholipids may create lipid bilayers due to their amphiphilic nature. This one-of-a-kind characteristic is used in the production of liposomes. In general, when phospholipids are hydrated, MLVs develop, which can then be converted into SUVs using sonication. Phospholipid micelles are generated when the concentration of surfactant in an aqueous solution exceeds the critical micelle concentration. Essential micelle numbers for amphiphiles are roughly four to five times larger than phospholipids, which produce liposomes after surfactant filtration and micelle aggregation, resulting in LUVs.[55] Liposomes have been created using a number of techniques. The most common processes are liquid evaporate, reversal stage volatilization, and soap filtration.[56] The encapsulated drug can be released from a liposome by passive transport, membrane breakdown, or membrane retaining. Drug molecules travel from the lipid membranes of the lipid nanoparticles to approach the additional vesicular layer of passive diffusion. The bulk, lipid content, and properties of the medicine determine the flow rate.[57,58,59] Because drug diffusion travels through a number of barriers in multi-layered liposomes, unilamellar liposomes have such a greater extraction efficiency than multilamellar liposomes. As a response, the drug's release has been postponed. Circulating blood phospholipase and increased lipoprotein may damage the phospholipid coats of liposomes, causing vesicle breakdown and the release of the encapsulated medication into the cells. The degree of breakdown of the lipid nanoparticles membrane determines the pace of drug release.[60] The dimensions, charge density, composition, particular ligand on the lipid nanoparticles exterior, and biological environment all impact liposome cell interactions. Adhesion, fusing, lipid transfer, and cell devouring are the four ways liposomes link with cells. Lipid membranes can be formed on the surface of the cell in a specific or non-specific manner, or they can be attached on living cells and release the encapsulated drug inside the cell. Liposomes can release encapsulated drugs in front of each cell membrane during adsorption, but these medications can however enter cells via micropinocytosis. Endocytosis, a process that can be selected or widespread, can also be used to absorb them inside the cell. Negative charge liposomes were shown to be more successful in absorbing into cells through the endocytosis process than neutral liposomes. Liposomes bind to receptors in lipid invaginations on membranes and pass through the membrane

through an endocytotic route. They can fuse with the endosomal membrane after endocytosis to generate an endosome that can be transported to lysosomes. Peptidase & hydrolase within lysosomes degrade lipid nanoparticles and their ingredients. Liposomes that are responsive to stimuli (in pH or temperature) were developed to avoid this breakdown and so increase cytoplasmic bioavailability. Solid lipid nanoparticles that are PH-sensitive can adhere to endosomal walls and discharge their contents further into cytoplasm. Liposomes can destabilise within the endosome and discharge their components, or they might disrupt the endosomal membrane and enable encapsulated material to leak into the cytoplasm.[61,62] We wanted to see how lipid membranes have been employed in the area of ocular medication delivery by various researchers over the last decade.

Techniques for making liposomes

Techniques for synthesizing in general shown in Figure 9.

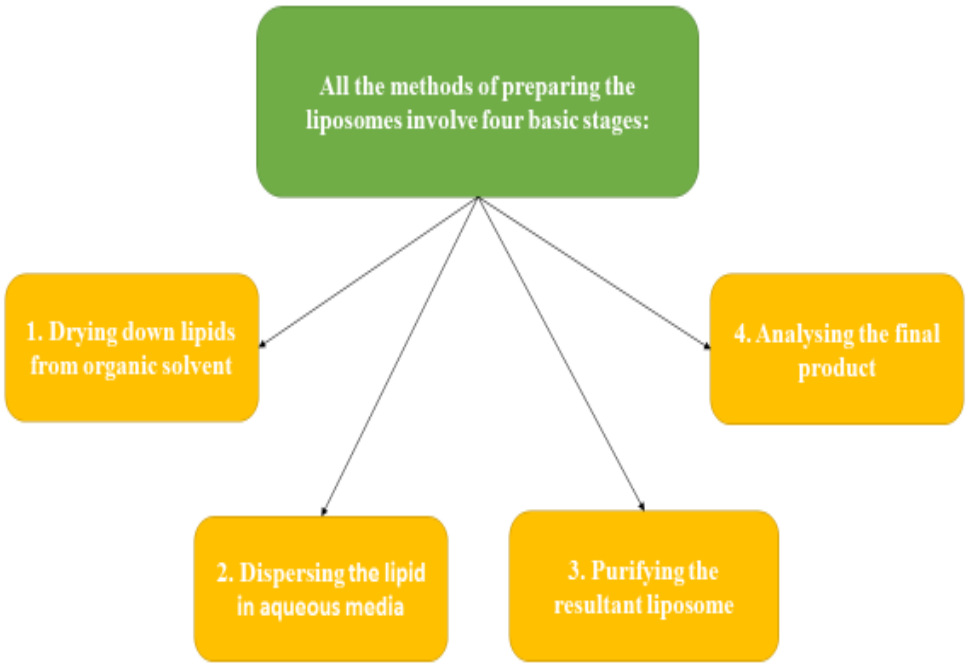

Figure 9: Different methods for preparation of liposomes.

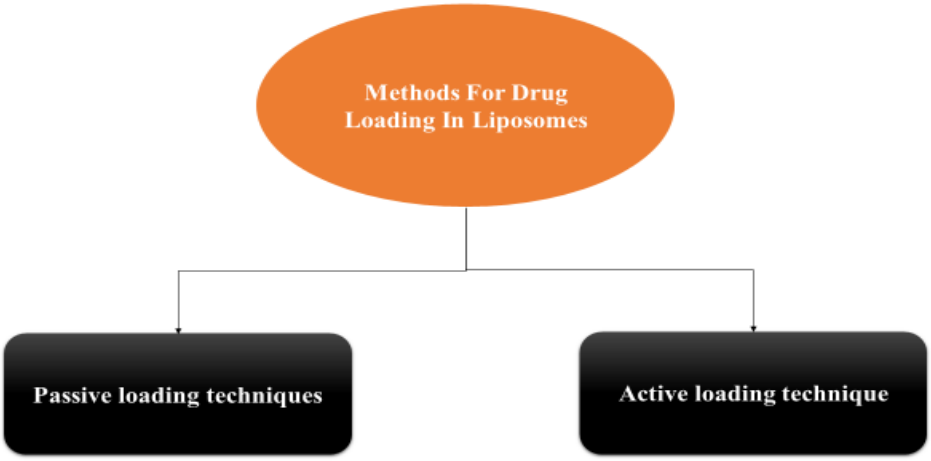

Figure 10: Drug loading method in liposomes.

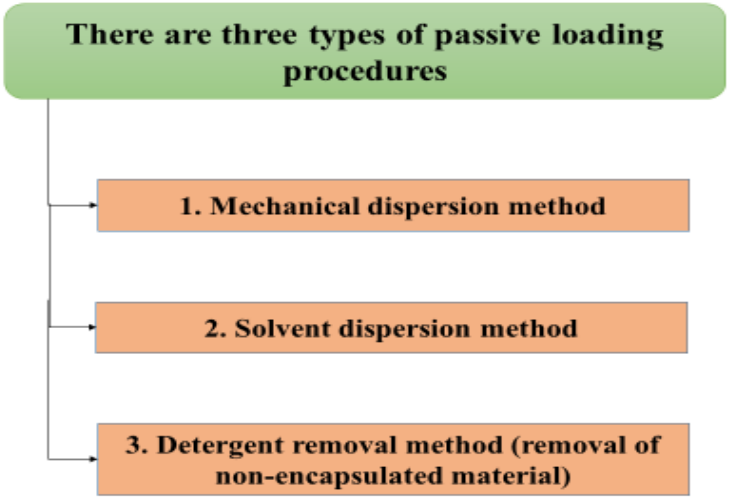

Figure 11: Different types of passive loading technique.

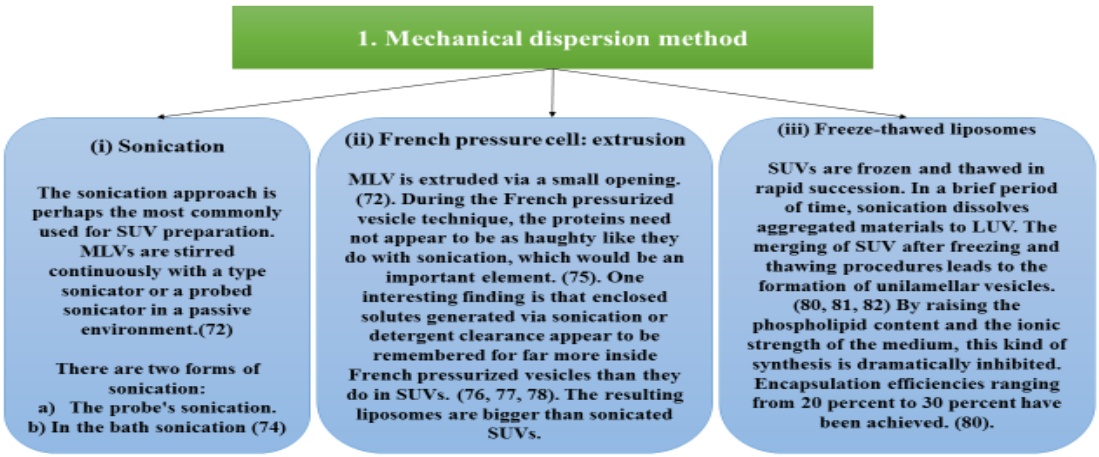

Figure 12: Mechanical dispersion method.

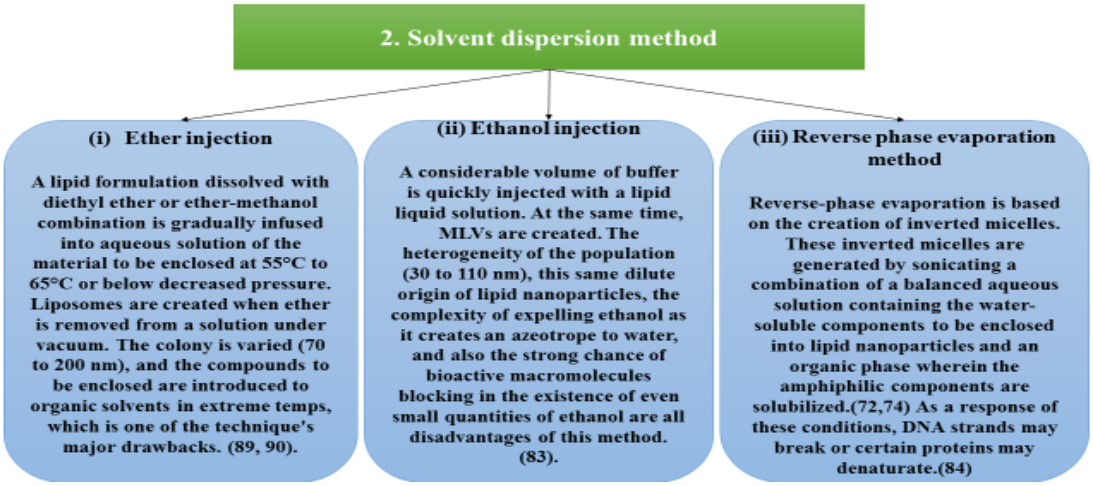

Figure 13: Solvent dispersion method.

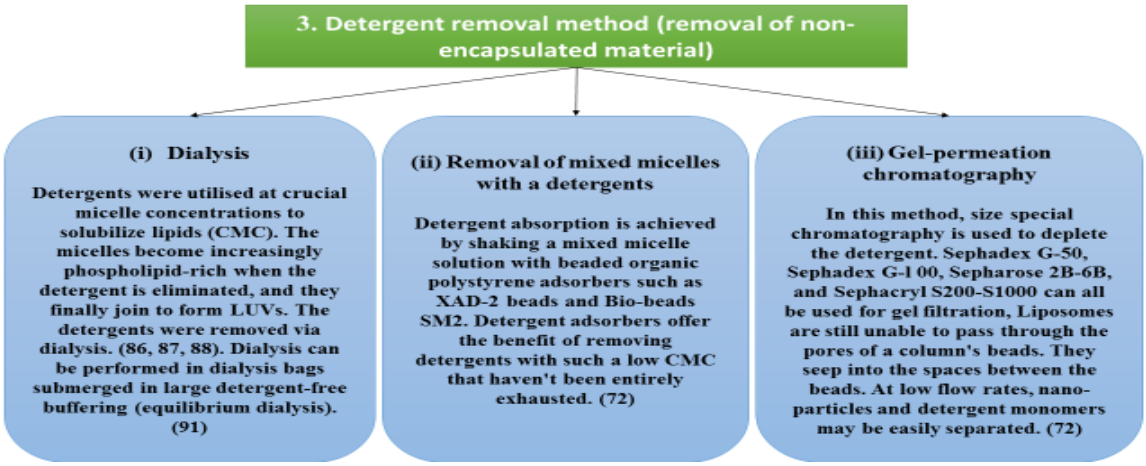

Figure 14: Detergent removal method.

Liposome characterization[63,64,65,67]

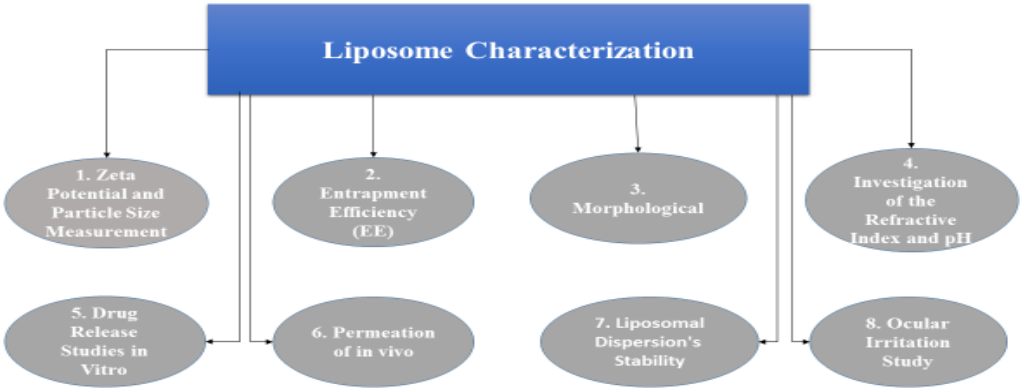

Figure 15: Characterization of liposomes.

Zeta Potential and Particle size measurement

The Malvern Zetasizer 2000 has been used to investigate the zeta potential as well as particle size distribution. Light scattering in a dynamic state was used to estimate particle sizes.[66] At room temperature, all measurements were repeated three times.

Entrapment Efficiency (EE)

The liposomal dispersion was decreased using buffer and spun at 15000 rpm for 30 minutes in an R-24 centrifuge. A UV spectrophotometer is used to determine the medication content in the supernatant. The EE is determined as a percentage of the total drug levels with in lipid nanoparticle dispersion. The effect of vibrating on EE is measured after 3 to 6 hours of shaking. The EE was calculated using the following equation:

$$EE = \text{total drug input (mg)} - \text{drug in supernatant (mg)} \times 100 / \text{Total drug input (mg)}$$

Morphological[63]

Liposome morphology is studied using a light microscopy. Each recipe included a drop of encapsulated suspension on a glass slide that was coated with a plastic cover for light microscope. On a stub coated with clean glass, a drop of distribution was inserted. The sample was sputter-coated with gold using an Apolaron E5100 sputter-coater. For the electron microscopy (TEM) inspection, a small sample of the liposomal distribution was negatively stained using uranyl acetate. 80 keV was used to analyse the samples.

Investigation of the refractive Index and pH

The index of refraction of selected formulations is investigated using an optical kind of refractometer. In order to determine the real pH of liposomal formulation at room temperature, digital pH metre is used.

Drug release studies in vitro

Float-A lyzer (1000 KD) Dialysis Gadgets facilitated in-vitro drug release by pouring PSA-loaded lipid nanoparticles in containers. A CA membrane is used to separate the dissolving media from the samples. Then vials are immersed in 300 ml of simulated tear fluid (STF) containing 1% SLS and a pH of 7.4. The heat and stirring rate is 37°C and 150 rpm, respectively. Then sample is suitably diluted, and HPLC is used to determine the amount of PA present.

Permeation of in vivo

To assess the in vivo penetration of the PSA packed liposome, migrating cells with a volume of 10 ml artificial tear fluid pH 7.4, 37°C, 200 rpm is utilised. The receptor chambers are covered by goat corneal membranes. The donor chambers are filled with 150 mg of PA-loaded lipid nanoparticles. To duplicate the lowest amount of tear thrown on the eye surface, the distribution media is poured towards the tip of the lipid nanoparticles. The Franz diffusing cells stirring speed is set to 600 revolutions per minute. A sample is obtained at regular intervals. After that, PSA penetration through the ocular membrane is examined.

Liposomal dispersion's stability

The PA-loaded lipid nanoparticles distribution and the blank lipid nanoparticles distribution is kept in 25°C. Every 15, 30, 60, and 90 days after preparation, they were visually viewed as well as microscopically examined.

Ocular irritation study[68,69]

To test chemicals, the HET-CAM method was used. The exposed CAM membrane was immediately treated with pure or diluted solutions, which were then examined under a microscope. The length of time it required for a reaction on the membrane to occur was recorded around 30, 120, and 300 sec. The compounds were sorted based on their irritation score (IS). The response effects were seen in the setting of bleeding, coagulation, and lysis. The research time was 300 seconds, and the test formulation was 0.3 ml. For comparison with the formulation, negative and positive controls were also examined.

Advantages

- Drug effectiveness and therapeutic index have improved.[71]
- The encapsulation improves drug stability.
- It is non-immunogenic, non-toxic, flexible, biocompatible, and biodegradable.
- The encapsulated agents' toxicity is reduced.
- Reduced exposure to hazardous drugs in sensitive tissues.
- The impact of avoiding a particular location.
- Pharmacokinetic effects have improved.[71]
- The structure's flexibility allows for the trapping of both water-soluble and insoluble medicines.[92]
- Controlling the effective release.

- Simple to put together.
- Provides both active and passive targeting.
- There is no cardiotoxicity because it does not collect in the heart.
- Prevent the medication from oxidising.[92]

Disadvantages

- Solubility is low.[71]
- It has a short half-life.
- Phospholipid oxidation as well as a hydrolysis.
- Encapsulated drug/molecule leakage and fusion
- Costs of manufacturing are high.
- Fewer stables. Liposomes are challenging to develop at an industrial scale due to their physiological and physicochemical instability.[51]

Application of lipid nanoparticles as ocular dosage[2]

Because of its advantages, the use of liposomes as a carrier system for eye medication has been studied.

a. It's a biocompatible, biodegradable nano-carrier.
b. By adhering to the ocular surface and increasing residence duration, it can help poorly absorbed medication molecules penetrate better.
c. It encloses both hydrophilic and hydrophobic pharmacological particles.
d. Lipid nanoparticles can help with pharmacokinetics, therapeutic impact, and toxicity associated with larger doses.
e. Because of their adaptability, lipid nanoparticle is widely explored in therapy of eye diseases.

Figure 16 summarises, liposomal compositions containing several therapeutic substances have recently been used.[2]

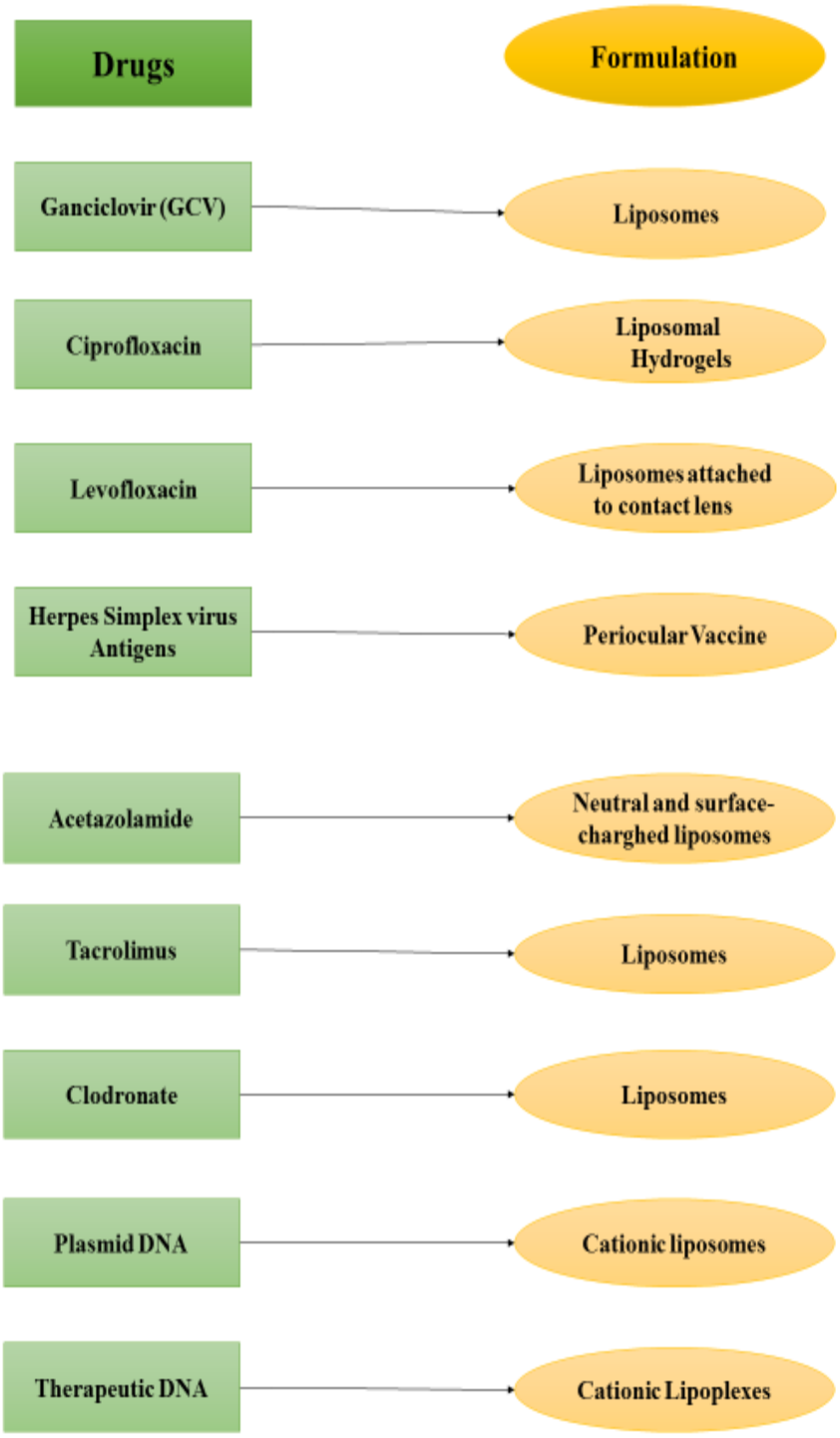

Figure 16: Summarization of liposomal compositions containing several therapeutic substances.

CONCLUSION

This study is done to improve the basic knowledge of liposomes to treat ocular disease. Both segment posterior as well as anterior were studied. Liposomes were studied according to its structure, inventions, Intravitreal Implants, classification, preparation methods, advantages,

disadvantages, applications, and formulations. Liposomes have been investigated extensively in the delivery of ophthalmic drugs. By delivering drugs in a regulated and targeted manner, these carriers have helped increase medication bioavailability.

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Conflict of interest

None.

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