International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 6 Nov-Dec 2022, pp: 1851-1865 www.ijprajournal.com ISSN: 2456-4494

A Review of Liposomal Ocular Drug Delivery System

[1]chandra, [2]poovarasu, [3]jaya Prakash, [4]thilakar, [5]sathish, [6]govindharaj
[(1)]Assistant Professor, Psv College Of Pharmaceutical Science And Research Krishnagiri , india
[(2,3,4,5,6)] UG Students, Psv College Of Pharmaceutical Science And Research Krishnagiri , india
correspondingauthour:chandra

Submitted: 17-12-2022 Accepted: 28-12-2022

ABSTRACT
Delivery of drugs to eye is a great challenge for pharmaceutical researchers because a number of barriers in the eye prevent the actual dose from reaching the site and also from being maintained in its therapeutic concentration at the location. A number of delivery systems for the eye have been developed in the past couple of years that are not only new but also safe and reliable and help to overcome all those barriers in the eye which are responsible for the very less bioavailability of drugs. The novel drug delivery systems are non-irritant to the eye and produce an enhanced retention time in the eye, thus providing greater efficacy and bioavailability. The liposomal systems for delivery of drugs to eye are advantageous as they have the ability to entrap both hydrophobic and hydrophilic drugs and are suitable for delivery to both the anterior and posterior segment of the eye.

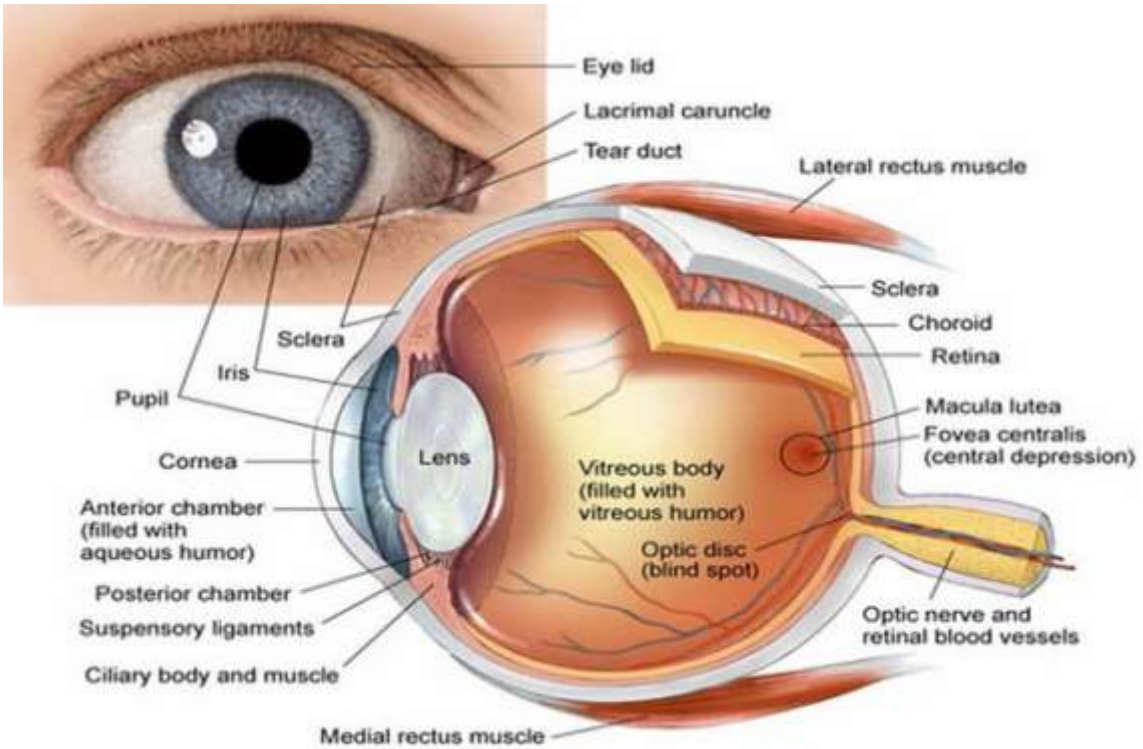
Key word: liposomes, classification, preparation, formulation, evaluation, application.

IMPORTANCE OF NOVEL DRUG DELIVERY IN OCULAR DRUG DELIVERY SYSTEM

- The effective delivery of drugs to the eye is still a major challenge to the researchers for the chief reason that the eye offers several physiological barriers, which act as a hindrance, and restrict the ocular delivery of drugs at the desired site.
- Scientists are focusing upon newer areas of research, in order to further improve and enhance the various drug delivery systems so that the drug may be specifically and suitably delivered at the target site.
- Various novel drug delivery systems for the eye are currently under the process of clinical trials. They have been made in such a manner that the duration of action is enhanced and a sustained release of the drug is thereby obtained.
- Considering the fact that there exists a room for prospective progresses in the areas of effective delivery of drugs and diagnostics, numerous benefits are still provided in the field of nanotechnology, as it allows not only an enhanced site-specific delivery of drugs but also a release of the drug in a controlled manner.
- the sole purpose for this site-specific delivery of drugs, as well as its release in a controlled manner, is mainly to achieve an improved pharmacokinetics and pharmacodynamics profile of the drug,
- To reduce the associated toxicity at the site, and for a better immunogenicity and recognition of the system within the body, for an enhanced therapeutic efficacy.

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 6 Nov-Dec 2022, pp: 1851-1865 www.ijprajournal.com ISSN: 2456-4494

OCULAR ANATOMY AND PHYSIOLOGY



Right Eye (viewed from above)

The understanding of ocular anatomy and physiology is very essential before designing a drug delivery system for the eye. Human eye is a very small, yet, sensitive organ, which poses numerous challenges and barriers, for the effective delivery of drugs. Basically, the human eye can be roughly divided into two major segments:

(a)Anterior segment and
(b)Posterior segment.

- Anterior segment is the exterior part of the eye, which forms the outer surface. It is positioned in front of the vitreous humor. It mainly consists of structures like cornea, pupil, conjunctiva, ciliary body, lens, aqueous humor, and iris.
- Posterior segment is the part of the eye, which lies behind and cannot be viewed directly. It forms the interior structure of the eye. The posterior segment consists mainly of the sclera, retinal pigment epithelium, choroid, neural retina, vitreous humor, macula, and optical nerve. For efficient ocular delivery, the drug must overcome the major barriers offered by the eye, in order to protect the eye from the toxicants. These barriers of the eye are specific to the site, depending upon the route of administration of drugs.

Barriers to ocular delivery of drugs may be broadly classified into three major types:

(a) Pre-corneal barrier,
(b) Static barriers and
(c) (c)Dynamic barriers.

Pre-corneal barriers

•The corneal layers particularly the (epithelium & stroma are considered as major barriers for ocular drug delivery. It is vital to understand that the permeant should have an amphipathic nature in order to permeate through these layers

Static barriers

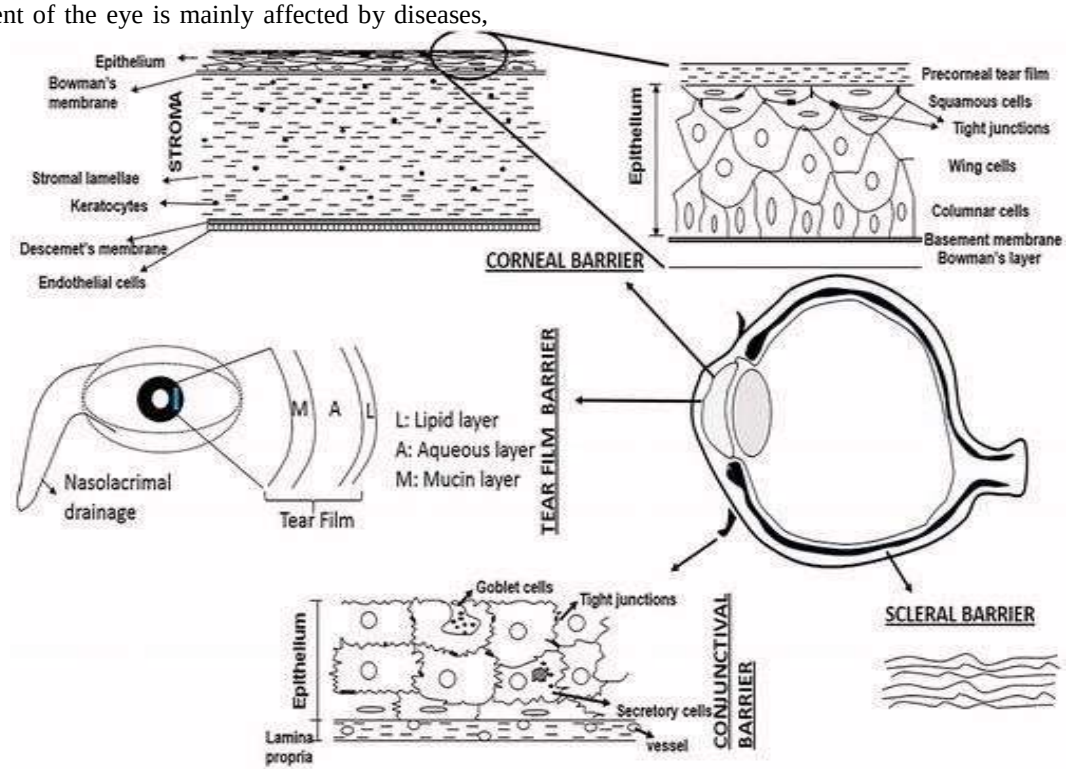
•Static barriers of the eye are mainly the various layers of the cornea, sclera, and the retina, inclusive of the blood aqueous barrier as well as the blood-retinal barrier.

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 6 Nov-Dec 2022, pp: 1851-1865 www.ijprajournal.com ISSN: 2456-4494

Dynamic barriers

•Dynamic barriers offered by the eye are mainly the choroidal and the conjunctival blood flow, dilution of tears, and the lymphatic clearance.

Anterior segment of the eye may be affected by certain diseases like glaucoma, allergic conjunctivitis, anterior uveitis, and cataract, in addition to numerous other diseases. Posterior segment of the eye is mainly affected by diseases, the main causative factor of which is limited to age. The diseases of the posterior segment of the eye are a predominant reason for causing visual impairment in the industrialized nations of the world. Examples of diseases affecting the posterior segment of the eye are age related macular degradation (AMD), diabetic retinopathy, and so on.



NOVEL DRUG DELIVERY SYSTEM FOR OCULAR THERAPY

- Novel drug delivery system is required for ocular delivery of drugs because the conventional ocular dosage forms suffer from numerous disadvantages.
- The major problem that is suffered by such conventional therapies is least ocular bioavailability, due to which majority of the drug gets lost, and a minor percentage of it actually reaches the desired site.

REVIEW OF LITERATURE

Dina Fathalla et al., formulated and evaluated liposomal gels for sustained ocular delivery of latanoprost using two different methods, namely thin film hydration and reverse phase evaporation techniques. The objective of their work was to develop a liposome-based delivery system for the sustained ocular delivery of latanoprost, a prostaglandin analog commonly used in the management of glaucoma. Latanoprost was incorporated into different liposomes that were evaluated using variety of techniques Fourier transform infrared and differential scanning calorimetry studies confirmed the interaction between the drug and different excipients in the vesicles, which resulted in drug encapsulation efficiency ≥ 90%. Drug encapsulation efficiency increased with the drug/lipid ratio and encapsulation efficiency ~98% was obtained at drug/lipid ratio of 50%. Vesicles incorporated into Pluronic® F127 gel had sustained drug release where ~45% of the encapsulated drug was released in 2 days.

Latanoprost liposomal gels had neither irritation nor toxic effects on the rabbits' eyes. Further, they had a sustained reduction in the rabbit's intraocular pressure over a period of 3

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 6 Nov-Dec 2022, pp: 1851-1865 www.ijprajournal.com ISSN: 2456-4494

days, which was significantly longer than that achieved by the commercial latanoprost eye drops.

Ehab I. Taha et al., designed and evaluated liposomal colloidal systems for ocular delivery of ciprofloxacin. The aim of their study was enhancing ocular drug delivery for protective mechanism of eye is limited the bioavailability of drug. In this study several liposomal formulations containing ciprofloxacin have been formulated using reverse phase evaporation technique with final dispersion of pH 7.4. Different types of phospholipids including Phosphatidylcholine, Dipalmitoyl phosphatidylcholine and Dimyristoyl-sn- glycero-3-phosphocholine were utilized. The effect of formulation factors such as type of phospholipid, cholesterol content, incorporation of positively charging inducing agents and ultra-sonication on the properties of the liposomal vesicles was studied. Bioavailability of selected liposomal formulations in rabbit eye aqueous humor has been investigated and compared with that of commercially available ciprofloxacin eye drops (Ciprocin). Pharmacokinetic parameters including Cmax, Tmax, elimination rate constant, t1/2, MRT and AUC0–1, were determined. The investigated formulations showed more than three folds of improvement in ciprofloxacin ocular bioavailability compared with the commercial product [21].

P.Divakar et al., formulated and in vitro evaluated liposomes containing metformin hydrochloride. Liposomal suspensions were prepared using film hydration technique using varying concentrations of phosphatidylcholine and cholesterol and optimize the ideal combination for required drug release. The percentage drug release at the end was found to be 64.0- 83.0 % at 4 hours. Drug excipient compatibility was determined by using U.V spectroscopy, FT-IR spectral studies. The results of in vitro drug release studies showed that release from liposomal formulation was slow and sustained for >12 hours period. The formulations followed first order kinetics and release mechanism was non-fickian diffusion from all the formulations [22].

Ravindrakamble et al., developed and characterized liposomal drug delivery system for Nimesulide by various techniques such as ethanol evaporation and rotary evaporator method. The encapsulation of Nimesulide into liposomes significantly improves their properties. The average particle size, percent drug entrapment, drug release at the end was found to be 270-703µm, 49-58 %, and 65.71 % at 9 hours in case of ethanol injection method while in case of rotary evaporator it was found to be 1-12µm, 69-86% and 76.97% at 9 hours respectively. The Zeta potential for Nimesulide loaded liposomes of ethanol injection method (batch- 1) and rotary evaporator method (batch – 3) were - 21.23 and -26.78 mV respectively. The result obtained in this study rotary evaporator technique was better for Nimesulide liposomes preparation on the basis of stability,

Devi R et al., prepared and evaluated the topical liposomes of Fluconazole by thin film hydration technique using different ratios of soya phosphatidylcholine and cholesterol. The in-vitro diffusion study was carried out by dialysis membrane using both open ended tubes. The study was carried out in 40 ml of phosphate buffer solution pH 7.4. The percentage cumulative release from the optimized batch i.e. F7 with drug: lecithin: cholesterol ratio 1: 10: 5, found to be 75.02% release in 8 hours. The magnitude of drug retention within the vesicles on storage under defined conditions ultimately governs shelf life of the developed formulations

Fluconazole is a bis-triazole compound that exhibits a broad-spectrum antifungal activity belonging to the group of triazoles. It is effective against many fungal species including Candida **Price et al**. 1994. It acts as a fungistatic agent. The high penetration into the aqueous humour and low toxicity of fluconazole, make it a good candidate for consideration as a topical ocular antifungal agent [23].

Abbasoglu et al. 2001, The azole antifungal drugs inhibit biosynthesis of ergosterol, the major sterol found in the fungal cell membrane, which is essential to the regulation of membrane fluidity and integrity and to fungal growth and proliferation **Ambrosini et al**. 1998. Although topical fluconazole solutions in experimental Candida keratitis have proved to be effective, with good penetration into the cornea and aqueous humour, liposomal preparations were still thought to provide a potentially longer contact time, allowing greater penetration of the fluconazole **Behrens- Baumann et al**. 1990; Yee et al. 1997; **Yilmaz &Maden**2005).

The objective of this study was to prepare a liposomal fluconazole corneal drug delivery system for the purpose of increasing contact time and prolong the antifungal action of the drug in comparison with fluconazole solutionA reproducible model of Candida keratitis in rabbits was performed to study the antifungal activity of

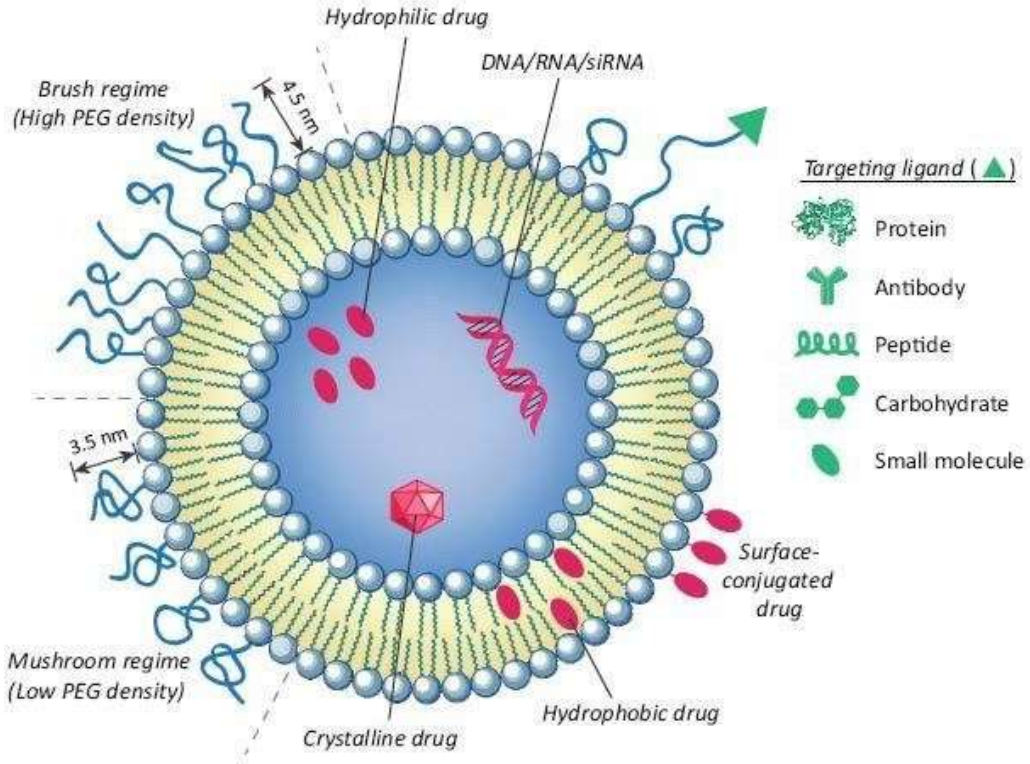
International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 6 Nov-Dec 2022, pp: 1851-1865 www.ijprajournal.com ISSN: 2456-4494

selected fluconazole liposomal formulations compared with that of fluconazole solution[24].

LIPOSOMES

Liposome are vesicles of lipid that have one or more phospholipid bilayers and enclose an aqueous core. They usually have size-range of 0.08–10.00µm. Based upon its size and the number of bilayers of phospholipid, liposomes may be classified into small uni-lamellar vesicles of size range between 10 and 100nm, large uni-lamellar vesicles of the size range between 100 and 300nm, and multi-lamellar vesicles that contain more than a single bilayer of phospholipid. Liposomes have shown to be an ideal ophthalmic drug delivery system because it can encapsulate hydrophilic as well as hydrophobic drugs and also shows a very good compatibility with the ocular tissues. Several research studies have also demonstrated that liposomal ocular delivery is effective for both anterior and posterior segments. y6

STRUCTURE OF LIPOSOME



There are two types of liposomes:

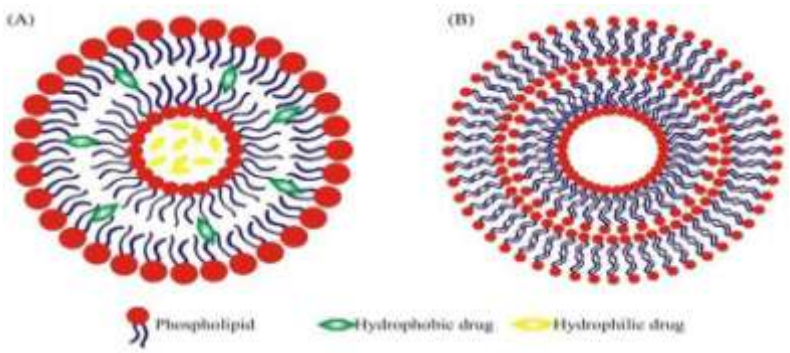
1. Based on structure
2. Based on composition and application.

Classification based on Structure

According to the size and number of bilayer membranes (lamellarity) forming vesicles, liposomes can be divided into the following categories:

- ❖ Small unilamellar vesicles (SUV): 20-100 nm.
- ❖ Large unilamellar vesicles (LUV): >100 nm.
- ❖ Giant unilamellar vesicles (GULV): >1000 nm.
- ❖ Oligo-lamellar vesicles (OLV): 100-1000 nm.
- ❖ Multi-lamellar large vesicles (MLV): >500 nm.
- ❖ Multi-vesicular vesicles: >1000 nm.

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 6 Nov-Dec 2022, pp: 1851-1865 www.ijprajournal.com ISSN: 2456-4494



STRUCTURE OF LIPOSOMES

Classification based on Composition and Application

Liposomes can be divided into several different types according to their composition and application, including:

oConventional liposomes
oImmuno-liposomes
oLong circulating liposomes
oCationic liposomes
oStimuli-responsive

Conventional liposomes

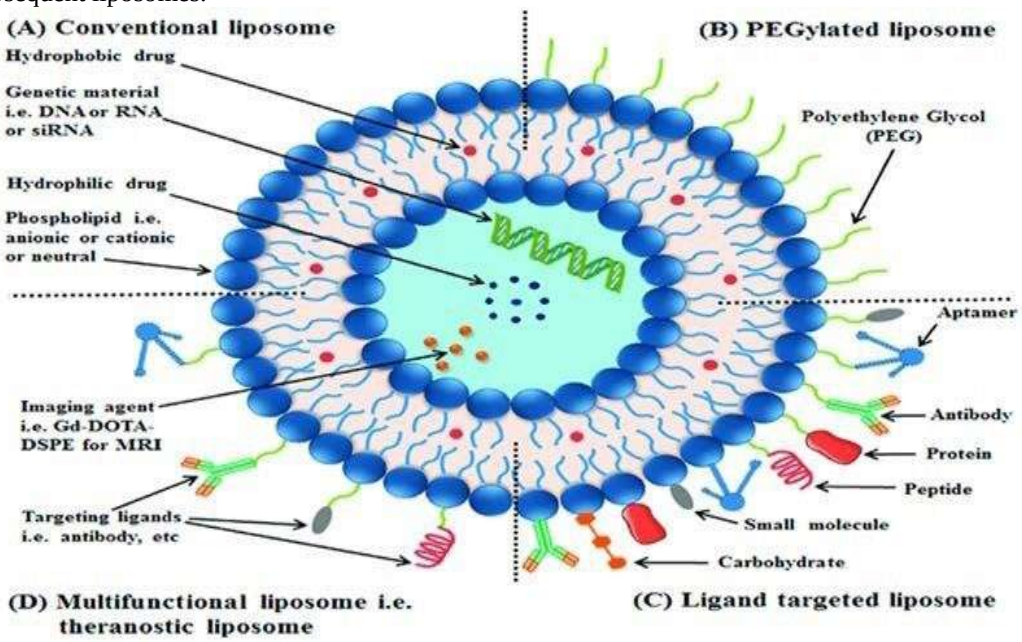
Conventional liposomes are the first generation of liposomes. They are lipid bilayer molecules surrounding the aqueous chamber and are the basis of all subsequent liposomes.

Immuno-liposomes

Immuno-liposomes are vesicles specially designed for active targeting of the drug substances inside the body.

Long circulating liposomes

The surface modification or PEG modification of liposomes is called PEGylation of liposomes, and the modified liposomes are called long circulating liposomes or stealth liposomes. Compared with conventional liposomes, PEG liposomes can avoid phagocytosis and circulate for a long time in systemic circulation.



Cationic liposomes

Cationic liposomes can be prepared by adding cationic phospholipid into bilayer membrane. This allows high rates of DNA incorporation, and for this reason, such liposomes may be more suitable for gene and antisense therapy.

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 6 Nov-Dec 2022, pp: 1851-1865 www.ijprajournal.com ISSN: 2456-4494

Stimuli-responsive

Liposomes can be easily functionalized through the introduction of functional materials, such as stimulus-response materials. Their structure, configuration, and other properties can be changed under certain in vivo or in vitro stimulation, such as the change of heat, light, magnetism, and pH value.

Type of liposome	Abbreviation	Composition
Conventional liposome	CL	Neutral or negatively charge phospholipids and cholesterol
Fuso-genic liposome	RSVE	Reconstituted sendai virus envelops
P^H sensitive liposomes	-	Phospholipids such as PER or DOPE with either CHEMS or OA
Cationic liposome	-	Cationic lipid with DOPE
Long circulatory liposome	LCL	Neutral high temp, cholesterol, and 5-10% PEG, DSP
Immuno-liposome	IL	CL or LCL with attached monoclonal antibody or recognition sequences

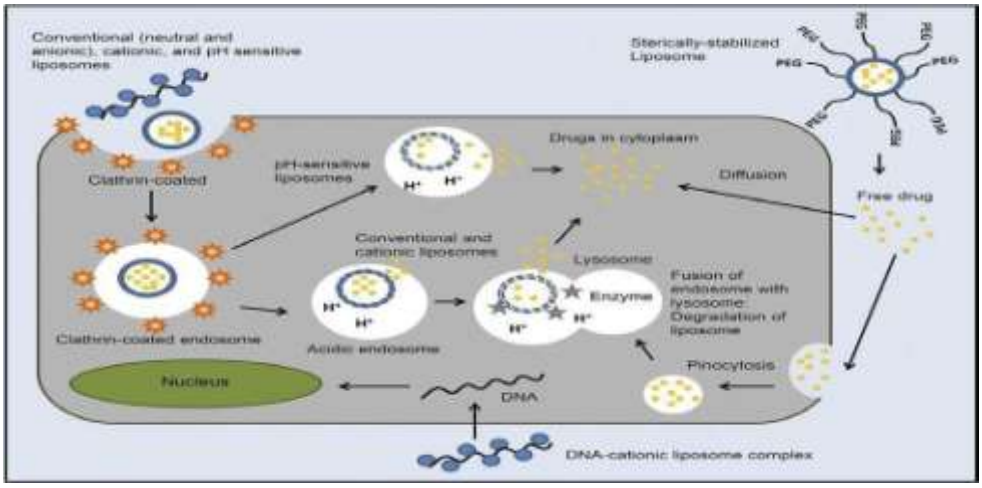
Mechanism Of Liposome Cell Interaction And Drug Release

Figure BELOW shows a pictorial representation of how the liposomes come into contact with the cell and the various mechanisms of release of the therapeutic agents from its phospholipid bilayer.

The liposomes that are loaded with drugs interact with the cell and release the therapeutic agents contained within it by the following mechanisms:

- Following administration, the liposomes on coming into contact with the cell may release its content onto the cell surface, which then enters the cytoplasm of the cell.
- Liposomes that are loaded with drug may also get adsorbed onto the surface of the cell either specifically or nonspecifically and release its content, after being destabilized by certain components of the cell membrane, and then enter the cell by the process of micropinocytosis.
- Liposomes may also fuse with the membrane of the cell and deliver the therapeutic agent into the cytoplasm.

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 6 Nov-Dec 2022, pp: 1851-1865 www.ijprajournal.com ISSN: 2456-4494



- The drug-loaded liposomes may also get endo-cytosed either directly or indirectly, thereby being delivered into the lysosome by the endosome, and releasing the drug into the cytoplasm, following provocation of destabilization of the endosome by the liposome.
- The liposomes also have the capability to undergo an exchange of its lipids with the lipids of the cellular membrane via the transfer-protein-mediated exchange.

METHODS OF LIPOSOMES PREPARATIONS

METHODS OF PREPARATION

PASSIVE LOADING TECHNIQUES

REMOTE LOADING TECHNIQUES

Mechanical dispersion Methods

Lipid film hydration by Hand shaking, non- Hand Shaking\freeze drying

Micro-emulsification

Sonication

French pressure cell

Membrane extrusion

Dried reconstituted vesicles

Solvent dispersion Methods

Ethanol injection

Ether injection

Double emulsion

Reverse phase evaporation vesicles

Stable pluri lamellar vesicles

Detergent Removal Methods

Detergent (cholate, alkyl-glycoside, triton x-100) removal from mixed micelles by;

Dialysis

Column chromatography

Dilution

Reconstituted Sendai virus enveloped

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 6 Nov-Dec 2022, pp: 1851-1865 www.ijprajournal.com ISSN: 2456-4494

METHODS OF LIPOSOMES

Generally, two methods are followed

A. Passive loading
B. Remote loading

Passive loading

This method involves the loading of the entrapped agents before (or) during the manufacture procedure.

Remote loading

Certain types of compounds with ionizable groups, and those, which display both lipid and water solubility.

Passive loading techniques

It includes three different groups of methods working on different principles.

1. Mechanical dispersion
2. Solvent dispersion
3. Detergent Solubilization

Mechanical/physical dispersion

Four basic methods of mechanical dispersion

- Hand-shaken multi-lamellar vesicles
- Non-shaken vesicles
- Pro-liposomes
- Freeze drying

Solvent dispersion methods

In this method, lipids are first dissolved in an organic solution, which then brought into contact with an aqueous phase containing material to be entrapped within the liposomes.

Detergent solubilizations

- In this method, the phospholipids are brought into contact with the aqueous phase via detergents, which associate with phospholipid molecule.
- The structures thus forms are known as micelles.
- As the detergent concentration increases further, the micelles ate reduced in size until they become saturated with detergent, where upon the concentration of free molecules equals the CMC and simple detergent micelles are formed.
- The basic feature is to remove the detergent form preformed mixed micelles containing phospholipid, where upon unilamellar vesicles form spontaneously.
- To remove the detergents, and all the mixed micelles to concentric bi-layered form, three methods can be employed:

i Dialysis
ii Column chromatography
iii Use of bio-beads.

Remote loading techniques

Load drug molecules into performed liposomes using pH gradients and potential difference across the liposomal membranes. A concentration difference in proton concentration across the membrane of liposomes can drive the loading of amphipathic molecules.

FORMULATION OF LIPOSOME

Preparation of liposomal ocular drug delivery system are used of drugs are Fluconazole.

Material

- Fluconazole
- Phosphatidylcholine from Soya-bean (PC), Cholesterol (Ch), Stearyl amine (SA), and DiacetylF. S. Habib, et al. 25 phosphate.
- Methyl alcohol
- Chloroform, diethyl ether, sodium hydrogen phosphate, disodium hydrogen phosphate and sodium chloride.
- All reagents were of analytical grade and 99% pure [7].
- Double distilled water, boiled and cooled was used throughout the experiments.
- Disposable syringe filter 0.22 & 0.45µm
- Thiopental® (0.5 gm/10 ml)
- **Inoculum**: Candida albicansNo. 4925 were used for animal inoculation
- Experimental animals: Forty male healthy, rabbits weighing approximately (1.5-2.5 Kg) each were used.

Formulation

- All the steps were performed under aseptic conditions. All glassware was sterilized by heating in hot air oven over 120°C for 2 hours. Boiled double distilled water was passed through a 0.22-µm disposable syringe filter (bacterial filter), and the entire procedures were performed under laminar air flow hood in presence of flame.

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 6 Nov-Dec 2022, pp: 1851-1865 www.ijprajournal.com ISSN: 2456-4494

- Fluconazole liposomes were prepared using the reverse-phase evaporation technique[8].
- The lipid components (phosphatidylcholine and cholesterol either alone or mixed with charge inducing agent such as Stearyl amine or diacetyl phosphate) expressed as weight ratios19&20 of the selected formulas.
- The different liposomal ingredients equivalent to 50 mg, were weighed into 250 ml long-necked quick fit round bottom flask and dissolved in 10 ml chloroform [9].
- The organic solvent was slowly evaporated under reduced pressure, using a rotary evaporator, at 40°C to reduce a thin lipid film.
- The lipid film was re-dissolved in 10 ml ether.
- Fluconazole solution in 10 ml acetone together with 5 ml distilled water was then added.
- The mixture was sonicated for one minute, swirled by hand, and re-sonicated for another minute.
- The organic solvents were evaporated on the rotary evaporator under reduced pressure.
- The liposomal suspension was kept overnight in the refrigerator at 5°C to mature [9].

Liposome formulae	Phosphatidylcholine (PC)	Cholesterol (Ch)	Stearulamine (SA)	Dicetlyphosphate (DP)
1	7 (31.82mg)	2(18.18 mg)	-	-
2	5 (25 mg)	5 (25 mg)	-	-
3	5 (22.72 mg)	5 (22.72 mg)	-	1 (4.54 mg)
4	5 (23.8 mg)	5 (23.8 mg)	0.50(2.38 mg)	-

Preparation of fluconazole loaded liposomes eye drops

Fluconazole eye drops were prepared under aseptic condition by diluting the optimized liposomes preparations with Sorensen`s modified phosphate buffer pH=7 containing 0.01% benzalkonium chloride as preservative so that, the eye drops contained the equivalent amount of 0.2 % of the drug [10].

Four liposome formulations were considered:

- Neutral liposomes PC:Ch weight ratio 7:4.
- Neutral liposomes PC:Ch weight ratio 5:5.
- Negatively charged liposomes PC:Ch:DP 5:5:1.
- Positively charged liposomes PC:Ch:S 5:5:0.5[11].

In-vivo antifungal evaluation (Experimental Candida keratitis in rabbits)

Animals: Forty adult rabbits were used in this study. Animals received standard dry food pellets and water. All eyes were initially examined by an ophthalmologist with hand-held torch. Only animals without any signs of ocular pathology were included.

Yeast: Candida albicansstrain No. 4925 was used for all experiments. This well- characterized strain has been used in a rabbit keratitis model, in which it proved (experimentally in Mycological center) to

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 6 Nov-Dec 2022, pp: 1851-1865 www.ijprajournal.com ISSN: 2456-4494

be highly invasive for the corneal stroma after surface inoculation[12].

Inoculation technique

The procedure is based on a model of experimental keratomycosis21. The rabbits were sedated by the intraperitoneal injection of 0.5 ml thiopental. Intra-stromal injection of 10µl of F. S. Habib, et al. inoculum (Candida albicans, containing 2.5 x 105 cell), was done in both eyes by inserting a sterile 27- gauge needle into the central corneal stroma tangential to the corneal surface to a depth of one half of the corneal thickness. The animals were excluded from the study if there was penetration of the inoculum into the anterior chamber or reflux of the inoculum was observed [13].

EVALUATION OF LIPOSOMES

1. Determination of percentage drug entrapment efficiency

Drug entrapment efficiency was calculated by using centrifugation method. 10ml of liposome suspension was taken and centrifuged at 15,000 rpm for 20 minutes. The supernatant liquid was collected and suitably diluted[1]. Then the absorbance was taken at 233 nm with the help of UV double beam spectrophotometer using pH 6.8 as a blank. The drug entrapment efficiency was calculated from the following formula[2].

$$\textbf{TotalEntrapementEfficiency} = \frac{\text{AMOUNT OF SUPERNANT LIQUID}}{\text{AMOUNT OF DRUG}} \times 100$$

2.Morphology analysis

The prepared Metformin HCL liposomes for all the formulations were viewed under for observing the vesicle formation and discreteness of dispersed vesicles. A slide was prepared by placing a drop of liposome dispersion on a glass slide and cover slip was placed over it and this slide was viewed under optical microscope at 40X magnification[3].

3.In vitro drug release study

Apparatus: USP TYPE II (paddle)
RPM : 50
Temperature: 37°C ± 0.5°C
Time : 30 min. interval up to 8 hours.

The in-vitro release for all the formulated Metformin HCL liposomes were carried out for 8 hours in phosphate buffer p H 6.8[4]. The studies were carried in USP dissolution apparatus II (Paddle) at 37ºC ± 0.5ºC and 50 rpm speed. 900 ml of phosphate buffer P^H 6.8 was used as a dissolution medium. Equivalent to 100 mg of Metformin HCL liposome was taken in a dissolution jar contains dissolution medium and the paddle was rotated at 50 rpm. 1 ml of samples were withdrawn at every 30 minutes. Up to 480 minutes and make up to 10 ml with pH 6.8 and analyzed for Metformin HCL content at 233 nm with P^H 6.8 as blank using double beam UV double beam spectrophotometer79[5].

4.Particle size determination

The particle size determination is done by using Malven particle size analyzer. Groups of particles are dispersed in a liquid medium and measured as they are circulated between the flow cell, which is placed in the measurement unit, and a dispersion bath in the sampler. The dispersion bath incorporates a stirrer and an ultrasonic sonicator. A pump delivers the dispersed suspension to the flow cell. The pump is specially designed to ensure both liquid medium and the particles are circulated. It can be controlled from a PC. Organic solvents can be used as dispersion media80.

5.Stability studies

The behavior of the liposome to retain the drug was studied by storing the liposome at two different temperature conditions, i.e., 4ºC (refrigerator RF), 25°C±2ºC for a period of 1 month. The liposomal preparations were kept in sealed vials. At 30th day the samples were analyzed for the drug content following the same method described in % drug encapsulation efficiency and in vitro drug release. And also, the liposomes were studied for their morphology[6].

APPLICATION OF LIPOSOMES IN OPHTHALMIC DRUG DELIVERY

- Liposomes have been investigated for ophthalmic drug delivery since it offers advantages as a carrier system.
- It is a biodegradable and biocompatible Nano-carrier. It can enhance the permeation of poorly absorbed drug molecules by binding to the corneal surface and improving residence time.
- It can encapsulate both the hydrophilic and hydrophobic drug molecules. In addition, liposomes can improve pharmacokinetic profile, enhance therapeutic effect, and reduce toxicity associated with higher dose.

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 6 Nov-Dec 2022, pp: 1851-1865 www.ijprajournal.com ISSN: 2456-4494

- However, in the case of posterior segment disorders, improvement of intra-vitreal half-life and targeted drug delivery to the retina is necessary
- choroidal neovascularization (CNV), ocular histoplasmosis, or pathological myopia effectively [14].
- Intravitreal administration of liposomes has resulted in vitreal condensation, vitreal bodies in the lower part of eye, and retinal abnormalities.
- Rostaporfin requires less frequent administration compared to verteporfin. Liposome technology has been explored for ophthalmic drug delivery. However, there are some issues to be addressed such as formulation, and storage of liposomes is very difficult, and they are known to cause long-term side effects.
- Intra-vitreal administration of liposomes has resulted in vitreal condensation, vitreal bodies in the lower part of eye, and retinal abnormalities [15].

Topical Applications

In 1981, SAMOLIN et al. investigated the role of liposomes in ophthalmic drug delivery. Since then several investigators proposed strategies to enhance absorption of drugs having poor physicochemical properties. Studies performed by Schaeffer and KROHN suggested the role of charge and size in trans-corneal permeation. Investigators observed four-fold higher in vitro corneal flux from penicillin G-loaded SUVs. They reported corneal permeation in the order of SUV+ > MLV SUV− > SUV > MLV free drug. These studies explored the role of vesicle type on trans-corneal permeation across the excised rabbit cornea. On the contrary, liposomal formulation of hydrophilic drug, that is, dihydrostreptomycin sulfate, did not improve the corneal permeation. Considering these findings, it was evident that both vesicle type and physicochemical property of drug significantly affects the trans- corneal flux of the formulation [16].

Intra-vitreal Applications

Liposomes represent the first injectable systems for intra-vitreal administrations. Liposomes can provide sustained release for prolonged period. In addition, liposomal formulation can minimize the tissue toxicity and enhance the intra- vitreal half-life of drugs by decreasing rapid clearance from vitreous cavity.

Barza et al. delineated the effect of liposome size and pathological state of eye on the intra-vitreal elimination kinetics of carriers. Investigators observed that the clearance rate of SUVs was faster than LUVs. Moreover, intraocular inflammation also increases the intra-vitreal clearance rate. Recently ocular pharmacologists have utilized liposomal hydrogel and sterically (PEGylated) stabilized liposomes to address the drawbacks associated with intra-vitreal administrations of liposomes [17].

In an application, Rhodamine conjugated liposomes loaded with vasoactive intestinal peptide (VIP) were given intravenously to healthy rats to examine efficacy in the treatment of ocular inflammation. VIP is an immunomodulatory neuropeptide involved in the regulation of ocular immune response by modulating the activities of macrophages, T lymphocytes, and dendritic cells.

Intra-vitreal application of VIP loaded liposomes was proposed for the treatment of endotoxin-induced uveitis. Internalization of Rhodamine-conjugated liposomes (Rh-Lip) alone and loaded with VIP (VIP-Rh-Lip) was examined in male Lewis rats [18].

Intra-ocular and systemic bio-distributions of the liposomes were also determined. The authors reported that, after single intra-vitreal injection, liposomes were internalized by retinal Müller glial cells, resident time in the vitreous cavity due to rapid elimination through the lymphatic circulation. Investigators attempted to increase the half- life of VIP-loaded liposomes (VIP-LP) after intra-vitreal administration by suspending them in the hydrogel. HA which is the major component of vitreous was utilized for the studies.

The researchers incorporated liposomes in HA gel in order to attain sustained release of VIP from the liposomes. VIP-LP suspended in HA gel was retained in the vitreous cavity for 8 days after single intra-vitreal injection. Moreover, it was reported that formulation was effective in the treatment as evident by reduced clinical score and number of polymorphonuclearcells[19].

In a study tacrolimus loaded liposome were utilized for the treatment of uveoretinitis. The vesicles were prepared by reverse phase evaporation technique and subsequently evaluated for efficacy and safety following intra-Vitreal injection in rats. No change in the retinal function was observed in the liposome-treated rats.

Histopathological examination revealed reduced inflammatory response in comparison to

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 6 Nov-Dec 2022, pp: 1851-1865 www.ijprajournal.com ISSN: 2456-4494

free drug. Liposomes were able to maintain the vitreous concentration of more than 50 ng/mL for 2 weeks after single administration. Investigators concluded that tacrolimus-loaded liposomes were more effective than the drug alone. The formulation also reduced drug-related toxicity to inner retinal cells [20].

COMMERICALLY AVAILABLE NOVEL FORMULATION (OR) MARKETED FORMULATION

Here, a few examples of liposomal formulations for the ocular space are enlisted, that are commercially available, and examples of ocular formulations that are already marketed, or under clinical trials.

DRUG	FORMULATION	RESULTS
GCV	Liposomes	In vitro trans-corneal permeation and in vivo ocular pharmacokinetics was improved
Ciprofloxacin	Liposomal hydrogel	Fivefold higher trans-corneal permeation than the liposomes alone
Levofloxacin	Liposomes attached to the contact lens	Drug was released following first- order kinetics for more than 6 days and formulation had showed activity against **S.aureus**
Herpes simplex virus antigens	Periocular vaccine	Treated rabbits showed anti-gB immune response and protected reactivation of HSV infection
Acetazolamide	Neutral and surface charged liposomes	Positively charged liposomes reduced IOP and exhibited prolonged effect than negatively charged liposomes
Tacrolimus	Liposomes	More than 50 ng/mL vitreous concentration was maintained for 2 weeks and reduced drug related toxicity.

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 6 Nov-Dec 2022, pp: 1851-1865 www.ijprajournal.com ISSN: 2456-4494

Vasoactive intestinal peptide	Rhodamine conjugated liposomes	Liposomes were internalized by retinal Muller glial cells, resident macrophages, majority of the liposomes reached the cervical lymph nodes and resulted in slower release and long-term expression inside the eye
Clodronate	Liposomes	Effectively inhibit infiltration of ED2-positive macrophages
Plasmid DNA	Cationic liposomes	Significantly increased transfection efficiency of DNA
Therapeutic DNA	Cationic lipoplexes	Achieved good vitreous mobility with moderately PEGylated cationic lipoplexes with size less than 500 nm

CONCLUSION

Liposomal carriers serve as a novel means for drug delivery to the eye. Based on the findings of the various researches that have been conducted, it can be concluded that, drugs, when delivered to the eye by novel methods, significant number of advantages are achieved. Unlike the major problem of least bioavailability that is associated with conventional dosage for ocular delivery, the newer versions significantly overcome it.

By these newer methods, an enhanced retention of the dose in the eye is obtained. Also, bio-adhesive substances used in the formulation prevent it from flowing away with the tears; hence, the drug is not lost. Maximum EE of the drug is obtained, with size range in Nano scale, which does not cause ocular irritation. These novel formulations usually offer an extended or a controlled release within the ocular tissues. Toxicity issues have not been reported so far in the formulations.

In-vivo bioavailability tests in various animals have also reported better activity compared to the conventional ones. Novel formulations offer a better patient compliance too, due to which they represent promising ocular drug delivery

REFERANCE

[1]. Tariq Jamshaid. Pharmaceutics & novel drug delivery systems. Pharmaceutical regulatory affairs, 2015; 4(3):74.
[2]. Naresh Kalra1 and G. Jeyabalan. Niosomes: a versatile drug delivery system.
[3]. Research journal of life sciences, bio-informatics, pharmaceutical and chemical science, 2016; 2(4): 44-54.
[4]. NagashreeKotturi. Novel drug delivery system. Research & Reviews: Journal of Pharmaceutics and Nanotechnology, 2015; 3(2): 32-36.
[5]. ShaktipalPatil, AmrapaliMhaiskar and DharmendraMundhada. A review on novel drug delivery system: a recent trend 2016; 6(2): 89-93.
[6]. R.R. Bhagwat and I.S. Vaidhya. Novel drug delivery systems: An overview. International journal of pharmaceutical sciences and research, 2013; 4(3): 970-982.
[7]. Kant shashi, Kumar satinder and Prashar bharath.2012; 3(7)
[8]. S. SuggyChrai, R. Murari and Imran Ahmad 14(11),2001

[9]. W Andreas, Karola-Uhl.1,9(2011)
[10]. M Mansoori, S Agrawal, S Jawade, M. I. Khan. Review on Liposome,(2012):453-464
[11]. AbdusSamad, Y. Sultana and M. Aqil.2007: 297-305
[12]. S.P. Vyas and R.K. Khar,(2011): 173-248
[13]. David B. Troy and Paul Beringer. Remington: 21st edition;(2006): 766-767
[14]. AbolfazlAkbarzadeh, SoodabehDavaran,Nosratollah,
[15]. Zarghami,YounesHanifehpour and KazemNejati-Koshki. Liposome: classification and applications. 2013; 8(102):1-9.
[16]. V.N.L Sirisha, I. BhavaniHarika, B. Sruthi, Namrata, P. Kirankumar, N. Vamsi Krishna, O. UmaMaheshwaraRao. Application of Liposomes in opthalmic drug delivery system2012; 2(5):
[17]. R. Lipowsky and E. Sackmann. Applications of Liposomes, Handbook of Biological Physics. 1995; 1: 491-519.
[18]. RutujaRavindraDeshmukh, Snehal Vijay Gawale, Arvind Ahire and Nikita DilipraoDerle. A review on: liposomes. 2016; 5(3):506-517.
[19]. Dina Fathalla, Ghareb M. Soliman and EhabA.Journal of Clinical & Experimental Ophthalmology. 2015; 6(1): 1-9.
[20]. S. Rathod and S. G. Deshpande. Design and evaluation of liposomal formulation of pilocarpine nitrate. Indian Journal of Pharmaceutical Science, 2009; 72(2):155-160.
[21]. Yilmaz S &Maden A (2005): Am J Ophthalmol 140: 454–458.
[22]. https://pubmed.ncbi.nlm.nih.gov/?term=Agarwal+R&cauthor_id=2511 6511
[23]. Thilannguyen, Thihiepnguyen and Dai hainguyen. Development and in vitro evaluation of liposomes using soy lecithin to encapsulated paclitaxel. International
[24]. Abbasoglu OE, Hosal BM, Sener B, Erdemoglu N &Gursel E (2001): Exp Eye Res 72: 147–151.
[25]. Behrens-Baumann W, Klinge B &Rűchel R (1990): Br J Ophthalmol 74: 40–42.