Cubosomal Drug Delivery System: Promising Approach for Ocular Drug Delivery of Corticosteroid

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Abstract:

Background: Over a century ago, uveitis, sclerokeratitis, pemphigus, and keratoconjunctivitis were treated with systemic administration of hypophyseal-derived adrenocorticotropic hormone. Although expanding research has effectively improved the use of corticosteroids locally, various side effects prevent patient compliance. Scientists are continuously developing different strategies and logical and dynamic approaches to enhance the acceptance of corticosteroid drug delivery.

Objective: Over recent decades, the number of problems associated with using corticosteroids was overcome by changing the route of systemic drug delivery to ocular drug delivery. The recent development of lipid-based drug delivery has made it possible to enhance the bioavailability of corticosteroid ocular drug delivery. These novel methods may overcome the ocular barriers and side effects of conventional eye drops. Many of these formulations are presently undergoing clinical trials, and few are clinically effective.

Result: This review presents an overview of corticosteroid-loaded cubosomal drug delivery as a non-invasive, nanoparticulate topical ocular delivery system.

Conclusion: This novel formulation is easy to formulate, has a high residence time, sustains drug release, and enhances the bioavailability of corticosteroids. On this basis sheds light on the anatomy of the eye, various barriers to ocular drug delivery, various approaches for ocular drug delivery, mechanism of cubosomal absorption, polymers used,

methods, and evaluation parameters for the development of cubosome.

Key Words: Cubosomes, Ocular drug delivery, Corticosteroid, Novel drug delivery.

1. Introduction

The human visual system is a sensory organ facilitating vision by reflecting light. There are two categories of cells, i. e rod, and cone cells, which are involved in reflexive visual acuity and vision, including color distinction and light sensitivity. The mammalian iris can approximately differentiate between 10 million colors (1) and may be sensitive enough to detect a single photon (2). Eyes consist of dynamic watery fluid, which is called aqueous humor. This aqueous humor is secreted by the ciliary body and drained out through Schlemm's canal by tubercular meshwork (3). This aqueous humor maintains an ocular pressure, resulting in a convex shape from the foreground to the globe (4) (5).

In the overall population, approximately 80 million people are visually impaired or blind(6), which is growing at a rate of about 7 million people every year. "India is home to the world's largest number of blind people," this sentence says by - The Times of India- Oct 11, 2007. In India only, about 70 percent of people when crosses the age of 40 get affected by cataract (7), trachoma, and glaucoma, which progresses to visual defect or blindness (8) (9). Yearly the cases of blindness are rising by 2 million, which is anticipated to double in the year 2020 (10). According to the World Health Organization, Age-related Macular degeneration is in third position after cataracts and glaucoma, which progress further to blindness (11). The risk factors associated with AMD are dry eyes, genetic tendencies, arterial hypertension, ultraviolet rays, and consumption of a non-balanced diet (WHO 2018) (12). Hence, the WHO, with an international expert panel, is developing a suitable approach for treating AMD, and there is an increasing demand for cost-effective therapeutics (13); proper treatment and various novel drug delivery systems improve patient compliance. Topical drug delivery is the most effective method for delivering drugs to both anterior and posterior regions. However, intravitreal and periocular pathways are also recommended for drug delivery in some critical conditions. Traditional approaches do not effectively target the pharmacological site of action of the eye, which necessitates the development of novel delivery strategies for eyes.

The novel drug delivery system should be (i) Less invasive so that it will minimize ocular tissue injury, infection, and pain associated with drug delivery, (ii) patient-safe, and (iii) selectively tailored to prevent unwanted side effects.

This is achieved by developing novel drug delivery methods, such as drug-loaded cubosomal vesicles that concentrate the drug at an ocular site. Cubosomal encapsulation is a relatively new technique of drug encapsulation. Cubosomes are nano-structured (particle size 10-500nm), Biocontinuous cubic liquid crystalline phase (14). Cubosome systems encapsulate hydrophilic and lipophilic drugs, hydrophilic drugs are encapsulated inside vesicles, and lipophilic drugs are partitioned between hydrophilic domains.

1.1 Anatomy and physiology of the eye:

The human eye structure is spherical but somewhat flattened in the anteroposterior direction with a 23-24 mm diameter. Between the lens and retina, the inner portion of the eye is filled with gelatinous fluid called vitreous humor.

The ocular globe is divided into; the anterior chamber and the posterior chamber. The anterior chamber consists of the external cornea, conjunctiva, iris-ciliary body, aqueous humor, and lens, and the posterior chamber consists of the outer sclera, choroid, retina, and vitreous humor (15).

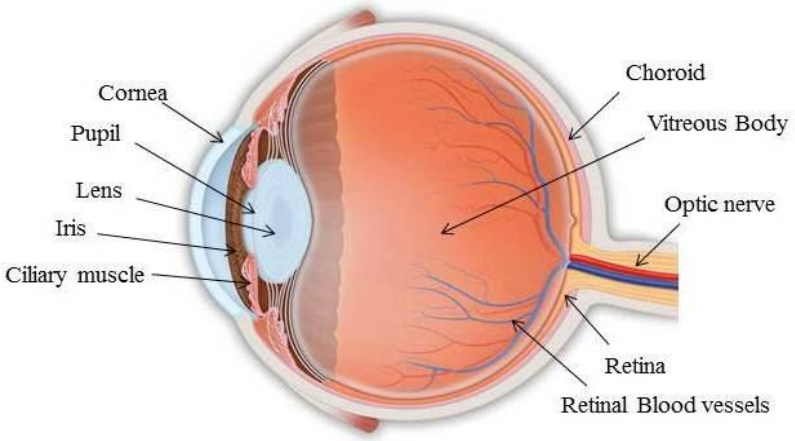

Figure 1: Anatomy of the human eye.

The eye's outer layer is called the cornea, which is clear and transparent but contains no vasculature. The cornea has three layers: epithelium, stroma, and endothelium. These membranes are divided by Bowman's layer and Descemet's membrane. The corneal endothelium consists of regularly arranged polygonal cells that do not regenerate themselves. Thus, the mammalian corneal endothelial cell density decreases with age, and the cells stretch to compensate for dead cells. The joining region of the cornea and the sclera is covered by a thin vascularized mucus membrane with a clear transparent surface called the conjunctiva. The clear transparent surface of the conjunctiva contains the goblet cells. These goblet cells are responsible for mucus secretion. The white portion of the eye

is called the sclera. The sclera constitutes 70 % of water along with collagen fibers which provide structural integrity and shape to the sclera and eye (16).

The space between the sclera and choroid is called suprachoroidal space. Suprachoroidal provides a path for aqueous humor drainage. When the drug is injected into the suprachoroidal space, the area expands, allowing the incorporation of large amounts of fluid. But this route is associated with disadvantages like fluid injected in this area is drained from the eye rapidly.

Mucus: The mucus covers the upper eyelid. The mucus acts as the primary coating of the precorneal tear film. The outer layer of the tear film contains a mixture of lipids. The Meibomian gland in the eyelids secretes this mixture. The aqueous middle layer consists of many proteins (secreted mainly by the lachrymal gland) dispersed in a salt solution.

The aqueous humor: The chambers of an anterior segment are filled with aqueous fluid, which creates intraocular pressure (IOP). The cornea binds the anterior chamber front and back, some portion of the sclera and iris, the lens, and part of the ciliary body. The iris and the lens limit the posterior chamber.

Vitreous humor: It is a transparent gelatinous liquid that transmits light to the retina while maintaining the eyeball's structure and size.

1.2 Barriers to ocular absorption

Drug administration through the anterior portion is the most appropriate non-invasive route. The total area of the conjunctival sac pulled away is about 2.5ml, but when the eyelid returns to its normal position, it decreases to approximately 1ml. But drugs administered through the ocular route are absorbed less than 5% because of various obstacles. Some of the barriers are below (17);

1.2.1 Tear

The tear is considered a precorneal barrier because tears form the outermost precorneal film of 3 μm thickness (Due to the three-layer lipid, aqueous, and mucin layer; from exterior to interior) and have turnover (approximately one μL/min). Dilution, rapid clearance, and drug molecule binding to tear proteins all served as significant barriers to reducing the effects of the medications administered.

1.2.3 Cornea

It is transparent, a well-defined vascular part consisting of five layers with different polarity, i.e., epithelium, stroma, endothelium, Bowman membrane, and Descemet's layer.

The cornea acts as a mechanical barrier. This prevents the transport of exogenous substances in the eye (Figure 3). Also, the cornea acts as a rate-limiting layer for drug permeation. The corneal epithelium has lipophilic nature. The tight junction among all cornea cells limits the entry of drugs from the tear film. Molecular sizes do not pose many barriers, but larger than 10 Å molecules cannot cross the cornea at a measurable rate. The stroma comprises 90% of the total cornea, consisting extracellular matrix. The collagen fibrils' lamellar arrangement and highly hydrated structure are barriers to lipophilic drugs. The separating layer between the stroma and aqueous humor is the Corneal endothelium (innermost monolayer of hexagonal-shaped cells). But from a leaky endothelial junction, permeation of macromolecules between the aqueous humor and stroma is observed. Bowman membrane and Descemet's layer have less resistance for permeation of drug.

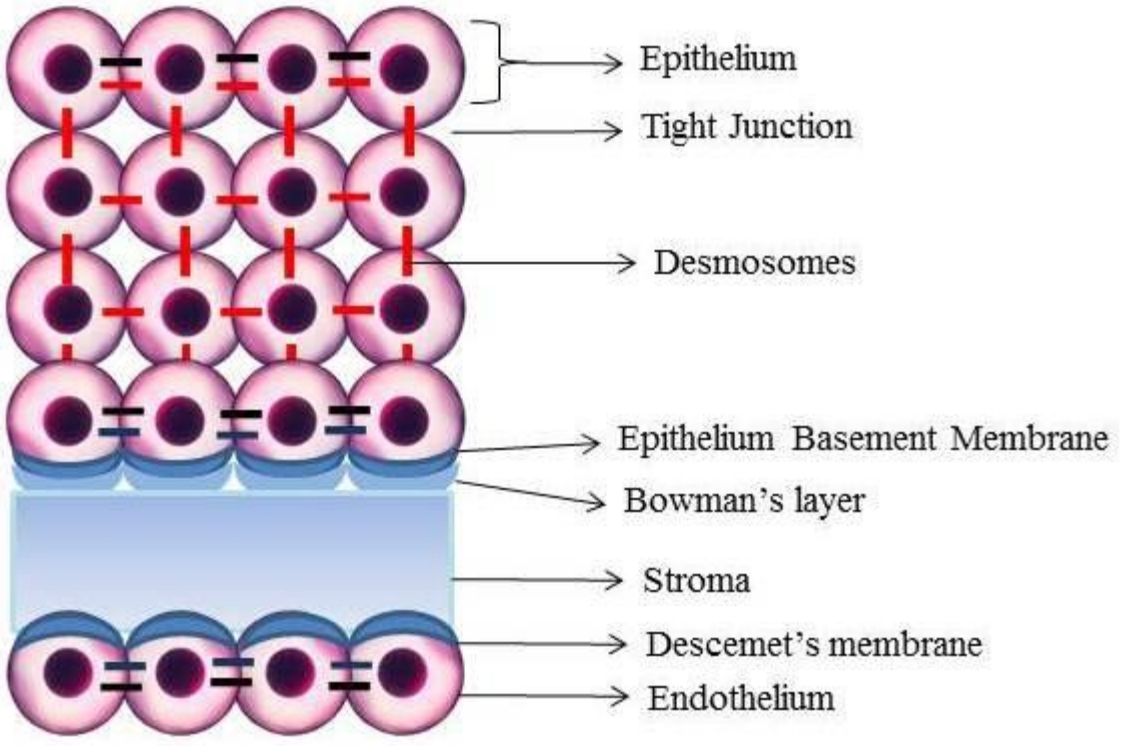

Figure 2 : The corneal structure with its cellular organization of various transport-limiting barriers.

1.2.4 **Conjunctiva**

Conjunctiva is a thin and transparent membrane that covers one-third of the eyeball and acts to form and maintain the tear film. Conjunctiva/ episclera has a dense network of capillary and lymphatics. When blood and lymph administer at the conjunctiva/ episclera, it get cleared by pinocytosis or paracellular transport due to a dense network of veins and lymphatics. In rat eyes, the lymphatics remove ten percent of the low-molecular-weight

hydrophilic molecule (sodium fluorescein) injected in the sub-conjunctiva within the first hour, causing systemic exposure. This effect is observed because interstitial fluid is returned to the systemic circulation after filtration via lymph nodes.

1.2.5 **Sclera**

Collagen fibers and proteoglycans are also discovered in the sclera's extracellular matrix. Scleral permeability is proportional to molecular radius and reduced approximately exponentially with increasing molecular radius. Due to the variable thickness of the sclera, the region near the equator at 12–17 mm behind the corneoscleral limbus is the best location for transscleral drug delivery. An increase in hydrophilicity increases drug diffusion because drugs may diffuse through the aqueous medium in the pores of the fiber matrix pores.

1.2.6 **Choroid/Bruch's Membrane**

Choroid is a highly vascularised body tissue that supplies blood to the retina. It has a ten-fold higher blood flow-per-unit-tissue weight compared to the brain. The retina's and choroid's thickness can be measured noninvasively using Optical Coherence Tomography (OCT), which indicates that choroidal thickness decreases with age. Choroid thickness can vary due to a variety of ocular diseases.

1.2.7 **Retina**

The drugs will pass through the vitreous to the posterior chamber and then be metabolized through aqueous turnover and uveal blood flow. The retina and the vitreous are separated by internal-limiting-membrane (ILM), which acts as a barrier for drug diffusion from the vitreous to the retina. Drug molecules larger than 100 kDa cannot cross the retinal layers into the subretinal space.

1.2.8 **Blood-Retinal Barrier**

Blood-retinal barrier (BRB) is made up of close junctions of retinal-capillary-endothelial-cells (vesicles function through endocytosis or transcytosis), which prevent drugs from being transported from the blood into the retina (18).

The function of BRB is the uptake and disposal of nutrients and metabolites, which are maintained by Müller cells and retinal capillary vessels. The role of Müller cells is to support neuronal activity, control and homeostasis of K+ and other ions signaling

molecules, and control extracellular pH.

The choroid is a perfused tissue relative to the retina, which resulting drugs can quickly reach the choroid by systemic drug administration. Since the choriocapillaris is smaller, drug molecules in the bloodstream soon equilibrate with the choroid's extravascular space. As a result, BRB prevents drugs from entering the retina from the choroid.

Some of the dynamic barriers include;

- Reflex blinking (5–7 blinks/min).
- The tear turnover-The basal tear flow is 1.2 ml/min (0.5– 2.2 ml/min). Reflex stimulation can raise lachrymation by 100 times, up to 300 ml/min, allowing the drug to drain from the eye after delivery quickly.
- Nasolacrimal drainage.
- Pathological conditions affecting the conjunctiva may limit the holding capacity of the conjunctival sac (19).
- 2-3 ml mucus is secreted daily, which covers the ocular surface and becomes a barrier for drug absorption (20).

All the above conditions significantly decrease the bioavailability of the drug. Furthermore, the drug's molecular size, morphology, solubility, and lipophilicity; function as a barrier to ocular administration (21); some investigations have also revealed the importance of precorneal tear film as a significant barrier to drug absorption (22).

1.3 Drug delivery methods for the eyes

Various methods are employed to deliver a drug into the ocular cavity.

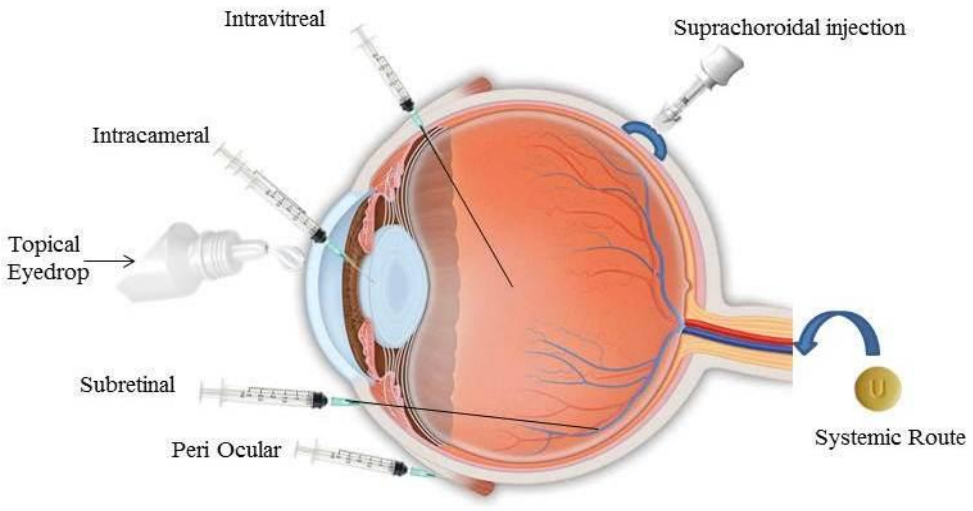

Figure 3 : Image of an eye showing Various drug delivery routes

1.3.1 In Anterior Segment:

Eye-Drops: The drug is directly instilled in the eye cavity. However, because of drawbacks such as shorter retention times, new technologies are now being used to extend the retention time of topically applied drugs.

Contact Lens: A contact lens was previously pre-soaked in an antihistamine drug, ketotifen. Pre-soaking protects contact lens wearers from allergic conjunctivitis. But now a day's, several approaches have been investigated to increase patient compliance.

Cul-de-sac Inserts: Ocusert® consists of two layers of ethylene-vinyl acetate copolymer (EVA, outer layer) and pilocarpine in alginate gel (Inner layer) within di-(ethylhexyl) phthalate (release enhancer) sandwiched between EVA layers (Control release). But it has several drawbacks, including poor IOP control due to the challenge of inserting and ejection the implant, and irritation during insertion, resulting in poor patient compliance.

Lacrisert® is a rod-shaped, water-soluble cul-de-sac insert made of hydroxypropyl cellulose (Without preservatives and other ingredients). It is having 1.27 mm diameter and a 3.5 mm long shape specially prescribed for moderate to severe dry eye syndrome.

Punctual Plugs: A punctual plug is placed in the puncta to block medication drainage of the drug through the nasolacrimal duct system, improve the retention time, and increase absorption and efficacy after installing eye drops. Since it is filled with drugs, it is now called a drug delivery device.

Subconjunctival/Episcleral Implants: A subconjunctival insert comprises a PLGA tube with a drug core. One end of the tube is closed with a permeable polymer, while the other is covered with an impenetrable polymer, silicone (PVA). The release of the drug through the PVA end and the release rate are controlled by the internal diameter of the PLGA tube, which can last anywhere from 3 months to a year (23).

1.3.2 Drug Delivery Systems to Posterior Segment of the Eye:

Intravitreal Implants: These implants have a drug with polymer/s layers surrounding them. The rate of drug delivery might range from days to several years. The polymer permeability regulates drug release. Examples of intravitreal implants are Durasert™, Retisert®, and I-vation™ TA, etc.

Injectable Particulate Systems: When liposomes or any complex-formed drug is mixed with saline solution, it becomes a milky, slightly opaque color and forms gel. It also

contains biodegradable benzyl benzoate. They will last up to a year after a single intravitreal injection. Some examples of injectables are RETAAC, Cortiject®, and Visudyne®.

Eye-Drops: Eye drops are solution suspension or emulsion instilled into the eye cavity. Caking and poor dispersibility are drawbacks associated with poorly water-soluble drugs, which can impair bioavailability due to dosing errors. Filtration and sterilization can be difficult due to the broad range of particle sizes, so emulsion-based eye drops are favored over suspensions.

1.3.3 Physical Devices

Iontophoresis: Iontophoresis is a non-invasive technique for delivering drugs at an ocular site that prevents the complications of surgical implantation of regular, high-dose intravitreal injections. A weak direct current (DC) is used to move charged molecules through the sclera and through the choroid, retina, and vitreous, with the oppositely charged electrode inserted in every part of the body to complete circulation.

Micro- Electromechanical (MEMS) Intra-ocular Drug Delivery Device: A MEMS studied in managing chronic and refractory ocular diseases. MEMS can re-fill with the drug solution to avoid repeated surgeries for long-term therapy. The MEMS are made of biocompatible and versatile perylene. The Replenish system will last up to more than five years before needing replacement, much longer than current treatments.

1.4 Nano formulations and ocular drug delivery

Cubosomes are discrete, sub-micron, nano-structured, self-assembled particles of bicontinuous cubic liquid crystalline phase, which physically looks transparent (24); they are stable in excess water, so their dispersions are less viscous than the parent cubic phase (drug and polymer mixture) (12). The Father of the word "Cubosome" was Larsson since the structure resembles cubic molecular crystals (24), and its internal structure was confirmed in 1967 (25). Cubosomes have particle sizes ranging from 10-500 nm in diameter and square-shaped structures (26). Cubosomes refer to as "bicontinuous" because it has two distinct (continuous but non-intersecting) hydrophilic regions separated by the bilayer, which has a large interfacial area. Cubosomal systems are a unique concept for developing ocular dosage forms because they have lots of potential in the development of drug nano-formulation, including high drug load. After all, it has a high internal surface area, high heat Stability (27), high thermodynamic stability (24), and

can encapsulate hydrophobic, hydrophilic, and amphiphilic substances (28).

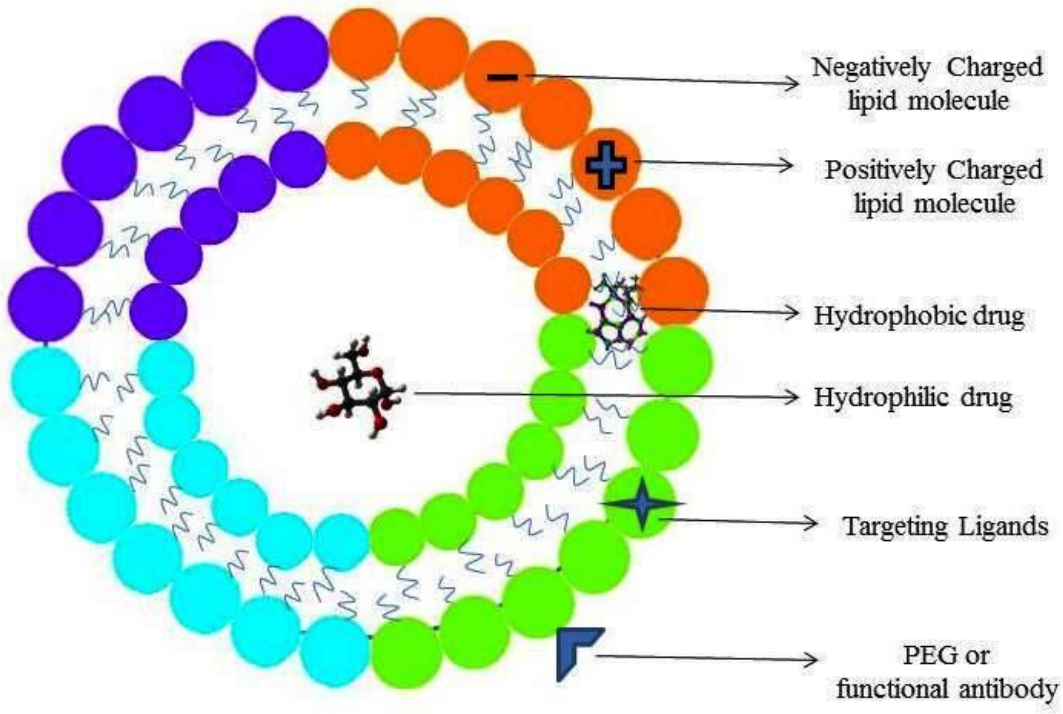

Figure 4: Two internal water channels are separated by honeycomb construction with various drug entrapment and target sites

Generally, ribosomes are composed of simple dispersion of lipids and surfactants (polymers) with drugs, which may be polar or non-polar; hence cubosomes are said to be amphiphilic structures. The class of amphiphilic materials like mono-glycerides, glycerate surfactants, phosphatidylethanolamines, and urea derivatives has a natural tendency to form non-lamellar nano-structures (29). The amphiphilic molecules act by hydrophobic effect in a polar solvent to spontaneously recognize and combine to create a liquid crystal of the nanometre scale. Then formed cubosomes are incorporated into a product and applied as ocular drops, creams, or gels, which are absorbed/released via diffusion. Because of the much smaller length scale of cubosomes, which makes them difficult to directly use for controlled release, the diffusivity from the eye of solubilized molecules (drug) is reduced by about 33% in bulk cubic phases. However, changing cubosome charge, viscosity, and structure can improve release kinetics (30). Cubosomes can be modified with protein to increase stability for a longer duration.

The universal application for delivering drug molecules associated with various

limitations is drug delivery vehicles hence bulk cubic phases designed to achieve controlled release frequently; the first patent on cubosomes was to specify its medical and controlled release applications (31). Corticosteroids are often used to treat ocular inflammatory disease for more than 40 years, first used intraocularly in 1974 by Peyman et al. (12). Corticosteroids interact with the steroid receptors in ocular cells to show angiostatic and anti-permeable properties. Then they cause either induction or repression of the specific genes and inhibit inflammatory symptoms like edema and vascular permeability (32). So they prescribed for posterior segment diseases, AMD, diabetic retinopathy, and macular edema (33). The most typical approach for the administration of corticosteroids is the topical instillation of the formulation into a cul-de-sac of an eye to treating ocular diseases like conjunctivitis, uveitis, endophthalmitis, glaucomatous conditions, and post-operative pain.

To achieve maximum bioavailability of steroids, several formulations have been developed like gels or hydrogel, in situ gelling systems, ocuserts, and polymeric vehicles, but each system carries its side effects (34) (35) :

Table 1: Dosage form along with its limitation

Dosage form	Limitations
Hydrogel	Skin infections, drug oozing (36)(37)
Gel dosage form	Blur vision, Ocular irritation (38)
Ocusert	Minor surgery procedure (39)
Conventional eye drop suspension	Ocular discomfort, less bioavailability (40)

To overcome problems, cubosomal drug delivery of corticosteroids is considered one of the challenging approaches to overcome this problem.

1.5 Mechanisms of permeation of cubosomes through ocular surface

The drug transport mechanism across the biological membrane depends on the condition and composition of the carrier and the anatomy and physiology of the ocular system. Trans and para-membrane transport are two mechanisms involved in ocular membrane transport. This mechanism is manipulated by using a drug carriers system, in which drugs can be incorporated either in the core or as an integral part of the vesicles.

The migration of drug molecules across a membrane by passing between or through two cells is known as paracellular diffusion (Passive Process). The drug's size and shape, as well as the pore size of the cell, are all factors in the passive diffusion process (drug

molecule). The passage of a drug across a cell is known as transcellular diffusion. In intestinal absorption, the drug is attached to the cellular enzymes or/or apical regional efflux pumps, resulting in transcellular diffusion. Transcellular diffusion is either passive or facilitated, or active. Transcellular movement is a common route in which the drug passes through the passage of the drug. Paracellular pathways are followed by highly polar drugs, which are transported through the cells. When we topically apply the cubosomal eye drops, the encapsulated corticosteroid can be effectively delivered to the cornea and anterior sclera. Interactions of vesicles with the corneal epithelium may have increased the probability of transcorneal drug penetration and enhanced intraocular drug supply via the non-corneal route. Conjunctival and scleral penetration is important in delivering poorly absorbed drugs.

1.6 Advantages of cubosomes:

Various common advantages of Cubosomes are;

1. Cubosomal drug delivery has a larger interior surface area and cubic crystal structures so, which significantly high drug payloads (24) **.**
2. Promising vehicles for different routes of administration.
3. They can be prepared by a simple method (41).
4. Having lipid biodegradability. **(**12)**.**
5. They can encapsulate all 3 types of hydrophilic, hydrophobic, and amphiphilic substances (42).
6. Bioactive compounds are released in a regulated and targeted manner (43)
7. Even after dilution, Cubosomes retain their stability, which is impossible with other liquid crystalline systems because they transform into micelles. Thus, being incorporated into formulations easily (41).
8. Protecting drugs from degradation and delivering them directly to the tumor site, i.e., site targeting action (44).
9. The cubic phases of cubosomes are colloidal dispersion which is thermodynamically stable for a longer time (43)
10. Improves the solubility of poorly water-soluble drugs.

1.7 The disadvantages of the cubosomes

1. They do not offer controlled drug delivery on their own compared with polymer-based drug delivery (24).

2. It's quite challenging to load hydrophilic drugs during the development of ribosomes because we use a large amount of water for the dispersion of GMO and Poloxamer already present in it.
3. Because of the high viscosity and lack of advanced types of machinery, large-scale production can be challenging (45) (43)

1.8 Polymers used to design cubosomes

In an aqueous solution, ribosomes are not significantly more colloidally stable than ordinary emulsions. A steric stabilizer was added to maintain colloidal stability. The van-der-Waals forces that cause flocculation and, as a result, coalescence and creaming in normal O/W emulsion systems are reasons for the destabilization of cubosome dispersions (46). Without disrupting the inner cubic structure, the stabilizer must prevent unfavorable interactions between hydrophobic domains between particles. Some stabilizers create a steric-repulsive barrier and an electrostatic-repulsive barrier between particles. The charged stabilizer is employed to provide an electrostatic barrier for flocculating cubosome (47). Charged surfactant molecules have a greater proclivity for disruption of the cubosomal internal phase. Charged nanoparticles, especially negatively charged, possess a short half-life compared to neutral nanoparticles.

In contrast, positively charged nanoparticles have been discovered to be poisonous and quickly eliminated from systemic circulation. Some polymers show repellency to surfaces, providing stealth and steric hindrance. Polyethylene glycol (PEG)/ polyethylene oxide (PEO): It is an H+ acceptor but not an H+ donor. It is an uncharged, hydrophilic polymer having low toxicity and low immunogenicity. The use of PEG in nano-formulation helps to improve the pharmacokinetics and pharmacodynamics properties of nano-formulation. The concentration of the stabilizer significantly impacts the steric stabilization property of nanoparticles. A "mushroom" surface conformation of the stabilizer on the particle surface is often the result of low stabilizer surface coverage. Increased density of PEG chains on the surface of the particles shows "brush" conformation, which is effective in stabilization and protein repellence action. Recently known that 10% w/w is the standard concentration of stabilizers used to formulate aggregate-free dispersion of cubosome dispersions. This, in 1976, says that To achieve optimal stabilizer stability, there must be equilibrium between the anchoring unit (i.e., hydrophobic head) and the extending unit (i.e., hydrophilic tail). Heller and Pugh discovered in 1954 that increasing the length and concentration of PEG improved their

stability. Lee et al. later verified this in 1989, using ™ (Pluronic®L63, P65, P105, F68, F88, F108) and Poloxamine™(Tetronic®908) (increasing hydrophilic PEG chain lengths on polystyrene beads). PEG is considered an ideal hydrophilic domain for steric stabilizers in lyotropic liquid-crystalline nanoparticles; little is known about the optimal PEG chain length for achieving optimum steric stabilization effectiveness in ribosomes (25).

1.9 Methods of preparation of cubosomes

Cubosomes have been prepared using many different approaches;

1.9.1 Top-down Approach :

In this method, the emulsification of a mixture of glyceryl-monooleate and the surfactant in water was used to make the ribosomes. To summarize, the dispersing step, which consisted of glyceryl monooleate (GMO) with Poloxamer 407 (P407), was melted at 70°C in a thermostatically operated water bath, then drug solubilized for 5 seconds using a vortex mixer.

After that, insulin syringes were used to inject the mixture into preheated aqueous phase at 70°C. The rotor homogenizer was used for emulsification for 5 minutes using at 10,000 rpm. For further investigation, the formulated dispersion was cooled and held at room temperature (48) (49).

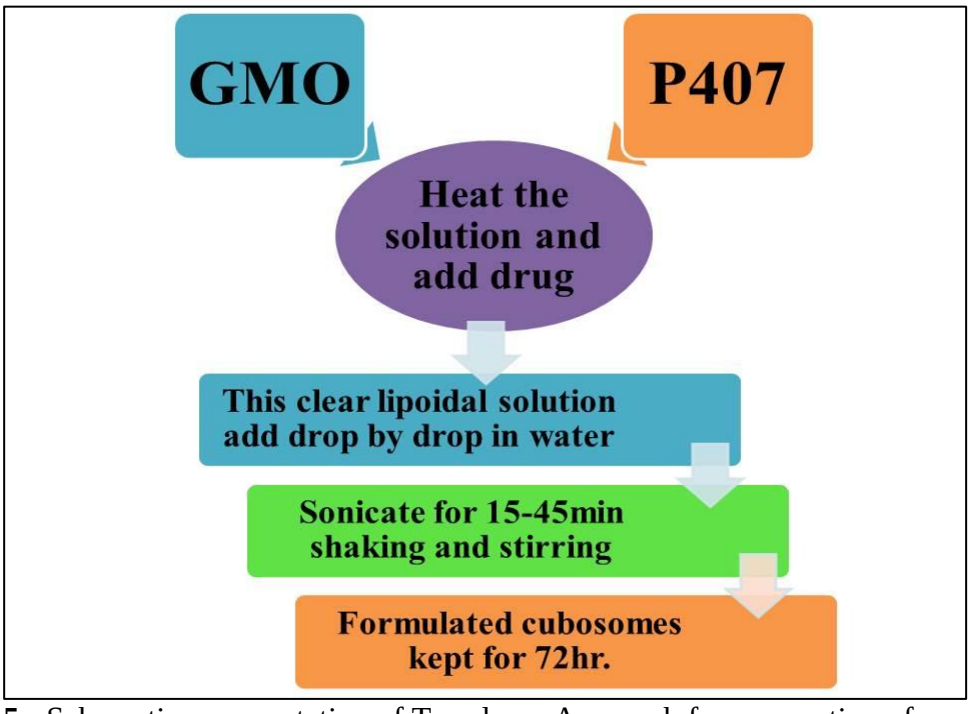

Figure 5 : Schematic representation of Top-down Approach for preparation of

cubosomes

1.9.2 Fragmentation method (50) (51):

Drug-loaded cubosomes were formulated by fragmenting bulk gelly cubic consistency phase containing GMO and Poloxamer 407. GMO with Poloxamer 407 was fully melted in a water bath at 60°C, and then the drug was applied to dissolve/blend while stirring/ vortex-mixing continuously to get a homogeneous solution. After 48 hours gel like cubic phase formulation was formed at room temperature. Add approximately 20 mL of water in cubic gel and stir with the help of a mechanical stirrer. After that, dispersion is fragmented by using an intermittent probe sonicator with a 400W energy input with 9S-18S pulse mode under cooling in a 20°C water bath for 10 minutes. The formulated milky coarse dispersion was homogenized in a high-pressure homogenizer to produce opalescent cubic nanoparticle dispersion. The final liquid crystal nanoparticle dispersion was kept at room temperature for further research.

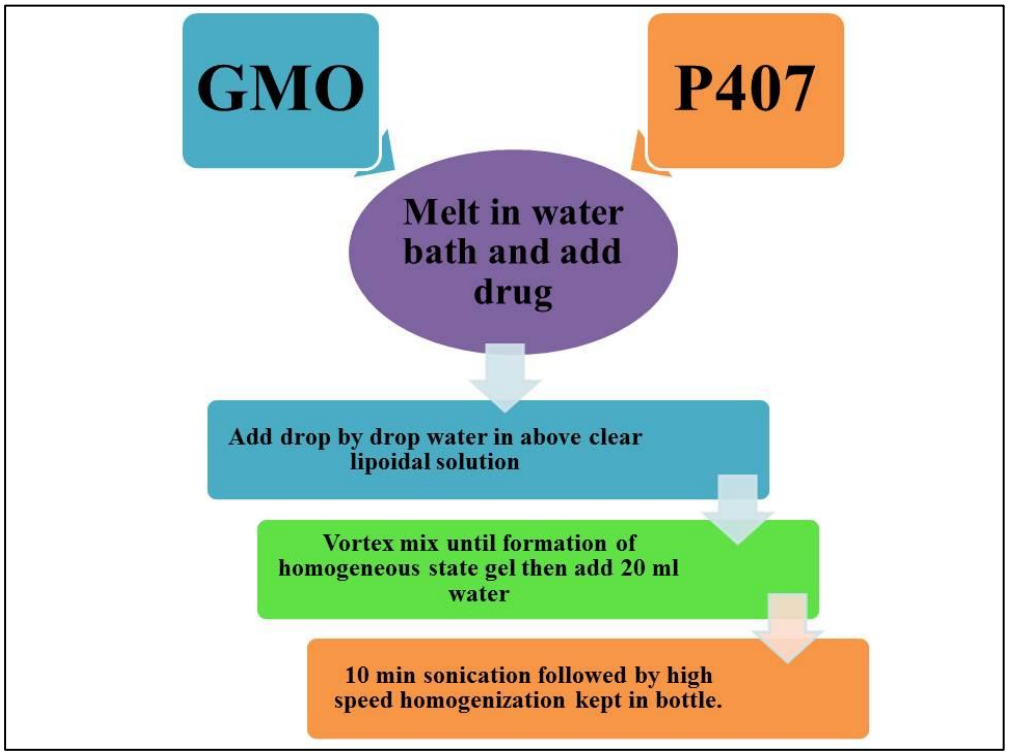

Figure 6 : Schematic representation of fragmentation method of preparation for cubosome

1.9.3 Adapted coarse method (52):

Formulation of drug-loaded cubosomes, an adapted coarse method, was used. GMO and P407 are dissolved entirely in 15 mL of chloroform. After the evaporation of chloroform (60 rpm and 602°C under pressure), a thin film is formed at the bottom of the flask. A 50

mL buffer (to dissolve the drug) was added to the dry lipid film to make coarse dispersions. The lipid film was mixed with the water phase with a sonicator for 15 min in a water bath under hot water (80±2°C). To prepare uniform dispersions, the heated mixture was quickly transferred to a beaker and homogenized for 1 minute at a speed of 13 500 rpm. The mixture was allowed to cool to ambient temperature before being stored in a container.

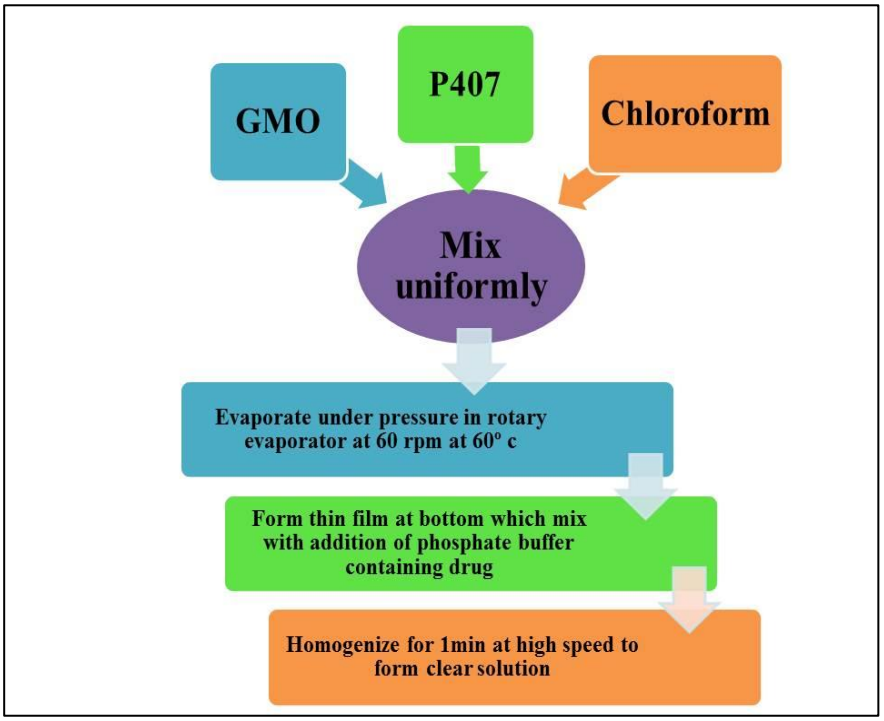

Figure 7 : Schematic representation of the Adapted coarse method of preparation for cubosome

1.9.4 Method D(53) (42)

In this method, Glyceryl monooleate/ poloxamer 407 (GMO/P407) bulk cubic gel was prepared by fragmentation method. The homogeneous solution of GMO and P407 (ratio of 18:1 (w/w)) was formed by melting a mixture in a hot water bath at 60°C. 2ml deionized water was used to dissolve the drug. Subsequently, the drug solution was slowly added into the GMO/P407 melt, and 1 min of vortex mixing was carried out to make a homogeneous mixture. After 48 hours, intermittent probe sonication in 20mL water was used for 10 minutes in a pulse mode with a 400 energy input. A high-pressure homogenizer at specific high pressures and cycles is used to generate opalescent cubosome dispersion. The formed cubic-phase gel was stored at room temperature for future research.

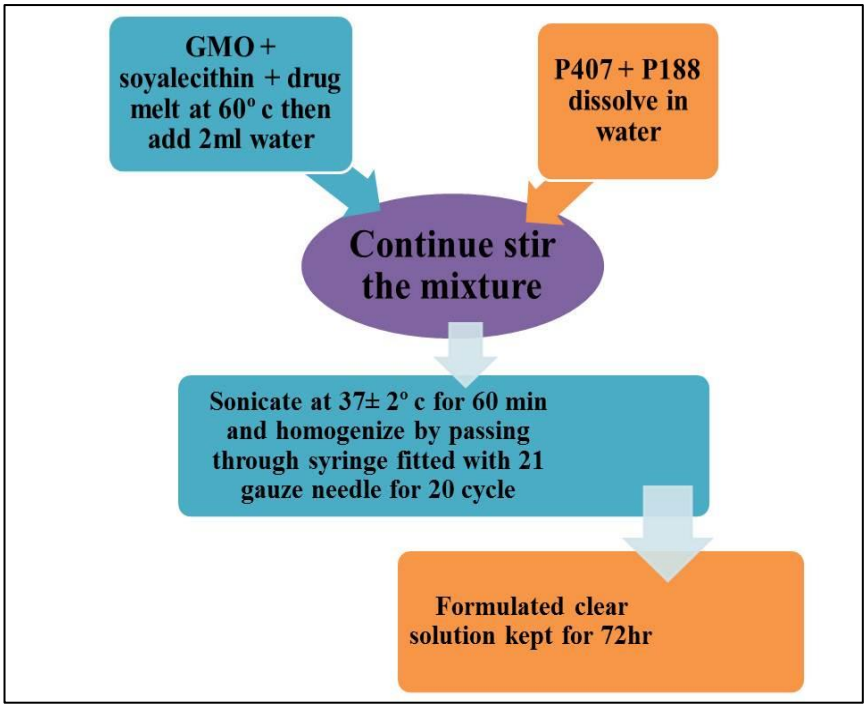

Figure 8 : Schematic representation of method D of preparation for cubosome

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1.10 Characterizations of ocular cubosomes

a variety of ways for the evaluation of cubosomes;

1. Transmission electron microscopy.
2. X-ray Diffraction Measurements/ Small-angle X-ray scattering.
3. Particle size - size distribution.
4. Encapsulation efficiency (EE)
5. *In vitro* trans-corneal permeation study/ *In vitro* release analysis.
6. Stability study.
7. Selection of animal model for the ocular study
8. Histological examination.
9. *In vivo* pharmacokinetics:

1.10.1 Transmission electron microscopy-(TEM)

The morphological composition of ribosomes was studied using TEM due to TEM's high magnification power. The following is the procedure for TEM analysis. The prepared formulation was loaded onto a membrane-coated mesh and then negatively stained for 2 minutes with a 2 % (w/v) phosphotungstic acid solution before drying at room temperature.

1.10.2 Measurements/ Small-angle X-ray scattering (SAXS)

SAXS analysis was performed by using the small-angle instrument SAXSess mc2; Anton Paar GmbH, which used a 2D CCD detector featuring a 2,084 × 2,084 array with 24 × 24 μm2 pixel size and a sample-detector distance of 311 mm to detect signals. The sample was placed in a capillary tube holder and equilibrated at 25ºC for 45 min before being exposed to X-Ray beams under a vacuum for 90 minutes. The two-dimensional scattering files from the CCD detector were converted to a 1-dimensional scattering function (q), explained by the equation $q= (4\pi/\lambda)$ (sin $\theta/2$), where q is the length of the scattering vector, λ being the wavelength and θ, the scattering angle. The relative locations of the Bragg peaks in the scattering curves20 are used to evaluate cubic space groups of ribosomes. Bragg peak reflections were detected after the scattering patterns were transformed into plots of intensity Vs. S-value. Using the characteristic spacing ratios, these concepts were applied to the various cubosomal phases:

Cubic P-type (Im3m- Primitive): √2: √4: √6 ...; cubic D-type (Pn3m- Diamond): √2: √3: √4...; and cubic G-type (Ia3a- Gyroid): √6: √8: √14...21

1.10.3 Particle size polydispersity index and zeta potential

Photon correlation spectroscopy is used to evaluate the Ps, PDI, and Zp of cubosomes. The particle size of ophthalmic formulations affects their dispersion significantly. In general, Cubosome particle sizes that penetrate the cornea will be less than 200 nm. An approximate stability threshold is a Zp value of >20 or 20 mV. The negative zeta potential of P407 adsorbed on the cubosome surface, acting as a coating sheet, stabilizes the cubosome surface and prevents aggregation. (54).

1.10.4 Encapsulation efficiency

The high EE suggests that most drugs are entrapped in cubosomes (54). The less encapsulation efficiency for hydrophilic drugs results in limited encapsulation capacity of the water channel in the cubosomes (54).

The following equations were used to measure the drug encapsulation efficiency:

Encapsulation efficiency (%) = (W initial drug−W free drug) / W initial drug X 100

Where,

W initial is the total amount of drug added into the formulation initially,

W free is the amount of drug in the filtrate. (14)

1.10.5 *In vitro/ Ex vivo* trans-corneal permeation/ release study (55)

Using a modified Franz diffusion cell apparatus, *Ex vivo* corneal penetration was performed.

Corneas (excised from eyeballs after rabbits' euthanasia following the guidelines or already slaughtered goat) were gently rinsed with saline. The evaluation was performed on a cornea to ensure it was free of wrinkles or pores. Place the cornea between the donor-receptor chambers. 10mL freshly prepared SOF (pH 7.4) containing 2% SLS (to maintain sink condition) was magnetically stirred at 100rpm and kept at a temperature of 35±2°C. After a 15-minute pre-incubation period, the formulation's precise volume (1mL) was placed in the cornea's donor chamber. At fixed time intervals (0.5, 1, 2, 3, 4, 6, 8, 10, 12, and 24 hours), samples (0.5 mL) were removed from the permeation medium and replaced with fresh medium to maintain a constant volume. HPLC or UV spectrometry is used to evaluate the drug concentration in a withdrawal sample.

1.10.6 Effect of terminal sterilization/ Stability Study

The optimized formulation is put in a type I glass bottle with a screw cap and sterilized according to the protocol. After the sterilization study, the physical appearance and values of EE%, PS, PDI, and ZP were compared with the unsterilized formulation as a reference. After sterilization, the sample was placed in a stability chamber for 1, 3, 5, 8, and 12 months to be studied for stability. Repeat the above procedure to evaluate the stability effect(14).

1.10.7 Selection of animal model for the ocular study

When a drug is administered through the ocular route, its pharmacokinetics (aqueous

humor) and precorneal retention time must be assessed. Surgery puncture (from the anterior chamber in sedated animals) or aqueous humor aspiration in sacrificed animals are used to assess drug pharmacokinetics. The precorneal retention period is investigated after removing lachrymal fluid from the conjunctival sac (capillary action) at certain time intervals. A less invasive method is used to measure pharmacological responses in vivo (miosis, mydriasis, IOP, aqueous humor flow measurement) to assess drug concentration levels in ocular tissues.

1.10.7.1 *Ex vivo/In vitro* models:

To put a limit on the use of animals, some alternative *Ex-vivo* models have been introduced:

Excised cornea tissue: As per the Ola Camber method, rabbit cornea is used for ocular study, considering that the cornea is a non-vascularized system, so excision is sometimes performed on dead animals, and corneas are preserved in sterile culture with antibiotics.

Reconstructed tissue culture: Human corneas constituting all three types of cells (epithelial, stromal, and endothelial) are reconstructed. Three-dimensional cornea constructs are obtained in two different ways; either by corneal cells from the newborn porcine or immortalized epithelial cells and native stromal fibroblasts grown in Transwell cell culture designed organotypic human cornea.

Immortalized cell lines: This method has advantages; immortalized cell lines may develop continuously and keep uniform growth characteristics.

Table 1: List of Cell lines with types of cells used.

Cell line	Used Cells
SIRC	an immortalized cell line from rabbit cornea.
RCE cell line	SV40 immortalized rabbit corneal epithelial cells.
HCE-T or HCE-2	SV40 immortalized human corneal epithelial
IOBANHC cell line	an immortalized epithelial cell line derived from human conjunctiva.
CJ4.1A and CJ4.3C	Immortalized conjunctiva epithelial cells from Fischer 344 rats.
HCDEC	Primary human conjunctiva-derived epithelial cell

Draize technique: It's one of the best-approved methods by the Food and Drug Administration to study ocular corneal toxicity dosage forms. Here rabbit is used as a test model**.** Some alternative tests for evaluation of toxicity studies are;

Chorioallantoic membrane (CAM) test: The vascularized pulmonary layer covering a chick growing within an egg is known as the chorioallantoic membrane (CAM). In this test, a section of CAM is isolated and incubated with the test formulation and then evaluated visually and scored during the incubation cycle.

Cornea opacity & permeability test (BCOP): The European Union Reference Lab has authorized this test as an alternative to animal testing (EURL-ECVAM). In this method, bovine corneas were isolated and mounted on holders containing two compartments containing culture medium. The anterior compartment is a compartment toward (the epithelium side) which receives the test formulation. After ten minutes of exposure, corneal opacity was calculated by measuring Na-fluorescein permeability.

Corneal hydration levels test (CHL): The CHL was evaluated after the *Ex vivo* penetration experiment. As Ex vivo, a corneal hydration level is a critical metric for assessing the influence of exogenous drugs on the cornea. The typical hydration range is 76 to 83 percent, while an HL of more than 83 percent suggests some degree of harm. The part exposed to each cornea's diffusion medium was weighed (Wa). Then, it was dried in an oven at 70°C for 12 h and reweighed (Wb). The CHL can be calculated using the following equation.

$HL\ (\%) = (1 - Wb/Wa) \times 100\%$.

Isolated eyes: The Isolate Chicken Eye (ICE) test has been approved as an alternative method to the Draize test by the EURLECVAM. In this method, isolated eyes of rabbits or chickens were used. The swelling of the cornea, the opacity of the cornea, and fluorescein retention were assessed after incubation of isolated eyes of rabbits or chicken eyes with the test samples.

Commercial kits (EYTEX, EpiOcular): In the area of retinal pharmacotherapy, commercial kits are used for ocular toxicity study

Table 2: Commercial kits for ocular toxicity study along with their characteristics

Cell	Characteristics
Immortalized human RPE (ARPE-19) cells	Investigation of morphology, the expression of retina-specific indicators, and their barriers.
Monolayers of ARPE-	Development of TDDS to the posterior segment of the eye.

19 cells

1.10.8 In vivo pharmacokinetics

Rabbits are often used as model animals in In Vivo pharmacokinetic studies. The common procedure employed to perform pharmacokinetic study is a comparative study of marketed formulation Vs. Optimized formulation. Two groups of rabbits were created randomly: one that received commercially available eye drops and another that received optimized cubosome eye drops. The rabbits were anesthetized with sodium pentobarbital (30 mg/kg) injected into the marginal ear vein and secured with a rabbit bag during the experiment. After administration of 50 µL formulation (both), 100 µL aqueous humor was extracted with 1 mL insulin needle after 15,30,60,120,240, and 360 min and placed in a centrifuge tube. With a 1 mL insulin needle, 50 mL formulation (both) was administered, and after 15,30,60,120,240, and 360 minutes 100 mL aqueous humor was extracted and put in a centrifuge tube. Centrifugation was carried out at 4000 rpm for 10 min, and the supernatant layer was evaluated using the HPLC method. The non-compartmental approach was used to calculate the pharmacokinetic parameters using the WinNonlin pharmacokinetic software(54).

1.10.9 Histological examination

After all animal studies were completed, the rabbit/mice eyeballs were removed and immersed in Davidson solution to see whether the corneal structure remained intact after administration. To isolate the tissue specimens, the rabbit eyeballs were immersed in a 4 percent paraformaldehyde (PFA) solution for 24 hours. The samples were then stained with hematoxylin-eosin and observed with a microscope.

1.11 Ocular drug delivery of corticosteroids

In age-related macular degeneration, proper treatment with corticosteroids is needed with minimum side effects. Hence, currently, encapsulation properties, high drug payloads with stabilization of peptides or proteins in cubic phase structures, and formation of self-assembled using amphiphilic materials when present in an aqueous environment become the critical parameter of drug delivery systems for corticosteroids.

As such, the first patent describing cubosome usage specifies numerous medical and controlled release applications (30), and hence it may be helpful for the treatment of Age-related macular degeneration for encapsulation of steroidal agents.

Cubosomes, dispersed colloidal particles of cubic phase liquid crystals, are commonly prepared from a glycerol monooleate (GMO)-water mixture through high-pressure emulsification, using poloxamer 407 as a stabilizer (56). Their ability to incorporate both types of drugs, i.e., hydrophilic and hydrophobic drugs, and their biocompatibility and bioadhesive properties stimulated interest in ocular delivery of corticosteroid delivery with minimum adverse effect. The side effect of corticosteroid agents is minimized because sustained action of drug delivery reduces the frequency of dosing, and as such, it is lipoidal drug delivery; it may cure the significant symptoms of ocular disease, i.e., dry eye.

Table 3 : Previously studied corticosteroid in ocular delivery

SN.	Researcher	Drug	Dosage form	Side effects
1	Lixia Luo et. al (57)	Dexamethasone	Encapsulates	Stability, homogeneity and mass production
2	Chee Wai Wong et al. (58)	Prednisolone phosphate and triamcinolone acetonide phosphate	Liposomes	
3	Darren J. Lee (59)	Dexamethasone	Drug delivery implants	Although small, the risk of endophthalmitis and blurred vision.
4	Xiaoyan Yang et al. (60)	Hydrocortisone Butyrate	Nanoparticles thermosensitive gel	Stability, initial burst phase
6	Yannis A. et al. (61)	Triamcinolone Acetonide	Intraocular injection	Endophthalmitis, Retinal and vitreous hemorrhage, Retinal detachment, Blurred vision
7	Corine R. Ghosn (61)	Dexamethasone	Intra-vitreal Implant	

1.12 Side effects of ocular drug delivery of corticosteroids which may be overcome by cubosomal drug delivery:

Corticosteroid drug delivery has various side effects, which cubosomal vesicular drug delivery systems will overcome. Natural blinking, induced lacrimation, regular tear turnover, quick precorneal clearance, and nasolacrimal drainage all result from washing the drug particles out of the eye. At the same time, cubosomes have bioadhesive properties that will overcome these side effects (62). Precorneal obstacles and corneal barrier together result in a significant loss for the drugs instilled cubosomes are nano in size as well as they can easily cross various barriers (57). Corticosteroid-containing gels, ocular implants, and in-situ gels are methods for improving precorneal residency, although they have drawbacks such as patient discomfort and obscured vision. Long-term use of steroids also causes cataracts and glaucoma, but corticosteroid-loaded cubosomes are transparent in appearance and have good bioavailability, so the small dose is effective (58). Corticosteroid eye drops are suspended in nature, so they irritate the eye due to their complex nature, but this is overcome by the incorporation of the drug into cubosomal vesicles.

Conclusion:

This report has reviewed the current understanding of the anatomy and physiology of the eye, along with various barriers to drug permeation in the ocular region. Due to this barrier, effective drug therapy, which targets desired ocular tissue, becomes a challenge for researchers, especially in the case of corticosteroids.

The corticosteroid has high demand due to its well-tolerated and effective vast application properties. It is prescribed for conditions like age-related diabetic retinopathy, uveitis, and other retinovascular diseases. But ocular administrations of corticosteroids are associated with multiple side effects like ocular irritation, drainage through ocular fluid, and less bioavailability. These side effects are overcome by implementing accurate drug delivery methods like cubosomes. Cubosome is bicontinuous cubic phase lyotropic liquid crystalline dispersion. Cubosomal ocular drug delivery has the property to overcome side effects associated with corticosteroidal visual therapy. The method of formulation of

cubosomes depends upon the property of drug moiety. Accordingly, evaluation parameters affect. Hence the research in the coming days will confirm that the development of corticosteroid-loaded cubosomes improves the bioavailability of the drug with minimum ocular side effects.

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References:

1. The M, Gomez D. Insect Colours and Visual Appearance in the Eyes of Their Predators. Vol. 38. 2010.
2. Badugu R, Szmacinski H, Reece EA, Jeng BH, Lakowicz R. Sensors and Actuators : B . Chemical Sodium-sensitive contact lens for diagnostics of ocular pathologies. Sensors Actuators B Chem [Internet]. 2021;331(July 2020):129434. Available from: https://doi.org/10.1016/j.snb.2021.129434
3. Berggren L. On the Appearance of Fluorescent Dyes in the Aqueous Humor After Intravenous Injection. Am J Ophthalmol [Internet]. 1929;42(4):595–602. Available from: http://dx.doi.org/10.1016/0002-9394(56)90786-3
4. Presland A. Ocular anatomy and physiology relevant to anesthesia. Anaesth Intensive Care Med [Internet]. 2016;1–6. Available from: http://dx.doi.org/10.1016/j.mpaic.2016.10.009
5. Montanino A, Angelillo M. Modeling with a meshfree approach the cornea-aqueous humor interaction during the air pu test. J Mech Behav Biomed Mater [Internet]. 2017; Available from: http://dx.doi.org/10.1016/j.jmbbm.2017.05.042
6. Guravaiah K, Sai Y. ScienceDirect Third Eye : Object Recognition and Speech Generation for Visually Third Eye : Object Recognition and Speech Generation for Visually Impaired Impaired. Procedia Comput Sci [Internet]. 2023;218:1144–55. Available from: https://doi.org/10.1016/j.procs.2023.01.093
7. Thomas R, Paul P, Rao GN, Jp M, Mathai A. PUBLIC HEALTH AND THE EYE Present Status of Eye Care in India. 2005;50(1).
8. Kumar S, Malik MA, Sooraj K, Sihota R, Kaur J. AC SC. Genomics

[Internet]. 2016; Available from: http://dx.doi.org/10.1016/j.ygeno.2016.11.003

9. Mwanza J, Tulenko SE, Barton K, Herndon LW, Mathenge E, Hall A, et al. Eight-Year Incidence of Open-Angle Glaucoma in the Tema Eye Survey. Ophthalmology [Internet]. 2018;1–9. Available from: https://doi.org/10.1016/j.ophtha.2018.10.016
10. Walker M, Turner HC, Coffeng LE, Bas MG. River Blindness : Mathematical Models for Control and Elimination. 2016;94.
11. Paez-escamilla M, Jhingan M, Gallagher DS, Singh SR, Fraser-bell S, Chhablani J. Jo ur of. Surv Ophthalmol [Internet]. 2020; Available from: https://doi.org/10.1016/j.survophthal.2020.08.005
12. Schwartz R, Warwick A, Olvera-barrios A, Pikoula M, Lee AY, Denaxas S, et al. Corresponding author : l P re of. Ophthalmol Retin [Internet]. 2021; Available from: https://doi.org/10.1016/j.oret.2021.04.001
13. Choradiya BR, Patil SB. A comprehensive review on nanoemulsion as an ophthalmic drug delivery system. J Mol Liq [Internet]. 2021;339:116751. Available from: https://doi.org/10.1016/j.molliq.2021.116751
14. Younes NF, Abdel-halim SA, Elassasy AI. Corneal targeted Sertaconazole nitrate loaded cubosomes : preparation, statistical optimization, in vitro characterization , ex vivo permeation and in vivo studies Nihal Farid Younes *, Sally Adel Abdel-Halim and Abdelhalim I . Elassasy. Int J Pharm [Internet]. 2018; Available from: https://doi.org/10.1016/j.ijpharm.2018.10.057
15. Mafee MF, Karimi A, Shah JD. Anatomy and Pathology of the Eye : Role of MR Imaging and CT. 2006;14:249–70.
16. Downie LE, Bandlitz S, Bergmanson JPG, Craig JP, Dutta D, Maldonado-codina C, et al. Contact Lens and Anterior Eye CLEAR - Anatomy and physiology of the anterior eye. Contact Lens Anterior Eye [Internet]. 2021;44(2):132–56. Available from: https://doi.org/10.1016/j.clae.2021.02.009
17. Cabrera FJ, Wang DC, Reddy K, Acharya G, Shin CS. Challenges and opportunities for drug delivery to the posterior of the eye. Drug Discov Today [Internet]. 2019;24(8):1679–84. Available from:

https://doi.org/10.1016/j.drudis.2019.05.035

18. Kang M, Kim D, Kang Y. Protective Effect of Nobiletin on High Glucose-Induced Blood-Retinal Barrier (BRB) Breakdown. Curr Dev Nutr [Internet]. 5:5140025. Available from: https://doi.org/10.1093/cdn/nzab033_025
19. Gan L, Wang J, Jiang M, Bartlett H, Ouyang D, Eperjesi F, et al. Recent advances in topical ophthalmic drug delivery with lipid-based nanocarriers. Drug Discov Today [Internet]. 2013;18(5–6):290–7. Available from: http://dx.doi.org/10.1016/j.drudis.2012.10.005
20. Ruponen M, Urtti A. European Journal of Pharmaceutics and Biopharmaceutics Undefined role of mucus as a barrier in ocular drug delivery. Eur J Pharm Biopharm [Internet]. 2015;(March). Available from: http://dx.doi.org/10.1016/j.ejpb.2015.02.032
21. Suri R, Beg S, Kohli K. Target strategies for drug delivery bypassing ocular barriers [Internet]. Journal of Drug Delivery Science and Technology. Elsevier B.V.; 2019. 101389 p. Available from: https://doi.org/10.1016/j.jddst.2019.101389
22. Eorgiev GEASG. The Precorneal Tear Film as a Fluid Shell : The Effect of Blinking and Saccades on Tear Film Distribution and Dynamics. 2014;1–15.
23. Carter BT, Luke SG. Jo ur na l P of. Int J Psychophysiol [Internet]. 2020; Available from: https://doi.org/10.1016/j.ijpsycho.2020.05.010
24. Sultana F, Arafat M, Sharmin S. An Overview of Nanogel Drug Delivery System. 2013;3(1):95–105.
25. Chong JYT, Mulet X, Boyd BJ. Steric Stabilizers for Cubic Phase Lyotropic Liquid Crystal Nanodispersions (Cubosomes) [Internet]. 1st ed. Advances in Planar Lipid Bilayers and Liposomes. Elsevier Inc.; 2015. 1–57 p. Available from: http://dx.doi.org/10.1016/bs.adplan.2014.11.001
26. Nanjwade BK, Yallappamaharaj R. Development of Cuboidal Nanomedicine by Nanotechnology. Austin J Nanomedicine Nanotechnol. 2014;2(4):1–8.
27. Jabłonowska E, Matyszewska D, Nazaruk E, Godlewska M, Gaweł D, Bilewicz R. BBA - General Subjects Lipid membranes exposed to

dispersions of phytantriol and monoolein cubosomes : Langmuir monolayer and HeLa cell membrane studies. BBA - Gen Subj. 2021;1865(1):129738.

28. Yaghmur A, Mu H. Recent advances in drug delivery applications of cubosomes , exosomes, and solid lipid nanoparticles. Acta Pharm Sin B [Internet]. 2021;11(4):871–85. Available from: https://doi.org/10.1016/j.apsb.2021.02.013
29. Yaghmur A, Glatter O. Characterization and potential applications of nano-structured aqueous dispersions. Adv Colloid Interface Sci [Internet]. 2009;147–148(C):333–42. Available from: http://dx.doi.org/10.1016/j.cis.2008.07.007
30. Spicer PT, Small WB, Lynch ML, Burns JL. Dry powder precursors of cubic liquid crystalline nanoparticles (cubosomes ∗). 2002;297–311.
31. Damiani F, Salvati L, Biffi S, Bortot B, Harb H, Lutz-bueno V, et al. Journal of Colloid and Interface Science Potential of curcumin-loaded cubosomes for topical treatment of cervical cancer. J Colloid Interface Sci [Internet]. 2022;620:419–30. Available from: https://doi.org/10.1016/j.jcis.2022.04.031
32. Said M, Aboelwafa AA, Elshafeey AH, Elsayed I. Central composite optimization of ocular mucoadhesive cubosomes for enhanced bioavailability and controlled delivery of voriconazole. J Drug Deliv Sci Technol [Internet]. 2021;61(September):102075. Available from: https://doi.org/10.1016/j.jddst.2020.102075
33. Shibata T, Hashimoto N, Mase M. Brain Disorders Intracranial hemorrhage in posterior reversible encephalopathy syndrome due to corticosteroid pulse therapy. Brain Disord [Internet]. 2022;7(April):100040. Available from: https://doi.org/10.1016/j.dscb.2022.100040
34. Ali Z, Sharma PK, Warsi MH. Fabrication and Evaluation of Ketorolac Loaded Cubosome for Ocular Drug Delivery. 2016;6(09):204–8.
35. Eldeeb AE, Salah S, Ghorab M. AC SC. J Drug Deliv Sci Technol [Internet]. 2019; Available from: https://doi.org/10.1016/j.jddst.2019.04.036
36. Fang G, Yang X, Wang Q, Zhang A, Tang B. Materials Science & Engineering C Hydrogels-based ophthalmic drug delivery systems for

treatment of ocular diseases. Mater Sci Eng C [Internet]. 2021;127(April):112212. Available from: https://doi.org/10.1016/j.msec.2021.112212

37. Schilling AL, Kulahci Y, Moore J, Wang EW, Lee SE, Little SR. Jo ur l P re of. J Control Release [Internet]. 2020; Available from: https://doi.org/10.1016/j.jconrel.2020.10.062
38. Aboali FA, Habib DA, Elbedaiwy HM, Farid RM. Curcumin-loaded proteasomal gel as a bio-friendly alternative for the treatment of ocular inflammation : In-vitro and in-vivo assessment. Int J Pharm [Internet]. 2020;589(July):119835. Available from: https://doi.org/10.1016/j.ijpharm.2020.119835
39. Prima G Di, Licciardi M, Pavia FC, Ignazio A, Monte L, Cavallaro G, et al. Micro fi brillar polymeric ocular inserts for triamcinolone acetonide delivery. Int J Pharm [Internet]. 2019;567(April):118459. Available from: https://doi.org/10.1016/j.ijpharm.2019.118459
40. Huang WC, Cheng F, Wang YJ, Chen CC, Hu TL, Yin SC, et al. A corneal-penetrating eye drop formulation for enhanced therapeutic efficacy of soft corticosteroids against anterior uveitis. J Drug Deliv Sci Technol [Internet]. 2019;54(October):101341. Available from: https://doi.org/10.1016/j.jddst.2019.101341
41. Wang D, Liu H, Wang W. Chirality and chiral functional composites of bicontinuous cubic nano-structured cubosomes. Chinese Chem Lett [Internet]. 2021;(xxxx). Available from: https://doi.org/10.1016/j.cclet.2021.08.040
42. Yang Z, Tan Y, Chen M, Dian L, Shan Z, Peng X, et al. Development of Amphotericin B-Loaded Cubosomes Through the SolEmuls Technology for Enhancing the Oral Bioavailability. 2012;13(4).
43. Karami Z, Hamidi M. Cubosomes: Remarkable drug delivery potential. Drug Discov Today [Internet]. 2016;21(5):789–801. Available from: http://dx.doi.org/10.1016/j.drudis.2016.01.004
44. Varghese R, Salvi S, Sood P, Kulkarni B, Kumar D. Cubosomes in cancer drug delivery : A review. Colloid Interface Sci Commun [Internet]. 2022;46(September 2021):100561. Available from:

https://doi.org/10.1016/j.colcom.2021.100561

45. Article R. International Journal for Pharmaceutical Research Scholars (IJPRS). Int J Pharm Res Sch. 2014;3(2):540–50.
46. Wang H, Zetterlund PB, Boyer C, Spicer PT. Journal of Colloid and Interface Science Polymerization of cubosome and exosome templates to produce complex microparticle shapes. J Colloid Interface Sci [Internet]. 2019;546:240–50. Available from: https://doi.org/10.1016/j.jcis.2019.03.069
47. Lee E. Polymer Chemistry. 2019;3778–85.
48. Elgindy NA, Mehanna MM, Mohyeldin SM. Self-assembled nano-architecture liquid crystalline particles as a promising carrier for progesterone transdermal delivery. Int J Pharm [Internet]. 2016; Available from: http://dx.doi.org/10.1016/j.ijpharm.2016.01.049
49. Del C, Bessone V, Parinaz S, T LI, Quinteros DA, Loh W, et al. European Journal of Pharmaceutical Sciences Latanoprost-loaded phytantriol cubosomes for the treatment of glaucoma. 2021;160(January).
50. Yang Z, Peng X, Tan Y, Chen M, Zhu X, Feng M, et al. Optimization of the Preparation Process for an Oral Phytantriol-Based Amphotericin B Cubosomes. 2011;2011(February 2009).
51. Qin L. Characterization of cubosomes as a targeted and sustained transdermal delivery system for capsaicin. 2015;4209–18.
52. Bei D, Marszalek J, Youan BC. Formulation of Dacarbazine-Loaded Cubosomes — Part I : Influence of Formulation Variables. 2009;10(3):1032–9.
53. Sharma R, Kaur G, Kapoor DN. International Journal of Drug Development and Fluconazole Loaded Cubosomal Vesicles for Topical Delivery. 2015;7(3):32–41.
54. Huang J, Peng T, Li Y, Zhan Z, Zeng Y, Huang Y, et al. Ocular Cubosome Drug Delivery System for Timolol Maleate : Preparation , Characterization , Cytotoxicity , Ex Vivo , and In Vivo Evaluation. 2017;
55. Battaglia L, Gallarate M, Serpe L, Foglietta F, Muntoni E, Rodriguez AP, et al. Ocular delivery of solid lipid nanoparticles [Internet]. Lipid Nanocarriers for Drug Targeting. Elsevier Inc.; 2018. 269–312 p. Available

from: http://dx.doi.org/10.1016/B978-0-12-813687-4.00007-4

56. Sagalowicz L, Michel M, Adrian M, Frossard P, Rouvet M, Watzke HJ, et al. Crystallography of dispersed liquid crystalline phases studied by cryo-transmission electron microscopy. J Microsc. 2006;221(2):110–21.
57. Luo L, Yang J, Oh Y, Hartsock MJ, Xia S, Ding Z, et al. HHS Public Access. 2020;68–80.
58. Wong CW, Czarny B, Metselaar JM, Ho C, Ng SR, Barathi AV, et al. Evaluation of subconjunctival liposomal steroids for the treatment of experimental uveitis. Sci Rep. 2018;8(1):1–11.
59. Lee D. Intraocular Implants for the Treatment of Autoimmune Uveitis. J Funct Biomater. 2015;6(3):650–66.
60. Yang X, Trinh HM, Agrahari V, Sheng Y, Pal D, Mitra AK. Nanoparticle-Based Topical Ophthalmic Gel Formulation for Sustained Release of Hydrocortisone Butyrate. AAPS PharmSciTech. 2016;17(2):294–306.
61. Ghosn CR, Li Y, Orilla WC, Lin T, Wheeler L, Burke JA, et al. Treatment of experimental anterior and intermediate uveitis by a dexamethasone intravitreal implant. Investig Ophthalmol Vis Sci. 2011;52(6):2917–23.
62. Eldeeb AE, Salah S, Ghorab M. Formulation and evaluation of cubosomes drug delivery system for treatment of glaucoma: Ex-vivo permeation and in-vivo pharmacodynamic study. J Drug Deliv Sci Technol [Internet]. 2019;52(January):236–47. Available from: https://doi.org/10.1016/j.jddst.2019.04.036