RESEARCH PAPER

Formulation and Evaluation of Carteolol Hydrochloride-Loaded Cubosomal pH-Sensitive In-Situ Gel for Glaucoma Therapy

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ABSTRACT

Background: Glaucoma is a chronic optic neuropathy that requires effective intraocular pressure control. However, the hydrophilic nature and rapid nasolacrimal drainage of conventional Carteolol HCl eye drops limit ocular bioavailability to less than 5%. This study developed a novel pH-sensitive in-situ gel loaded with Carteolol HCl cubosomes to enhance precorneal residence and sustain drug delivery. **Method:** Nine cubosomal formulations (F1–F9) were synthesized via melt dispersion-emulsification using glyceryl monooleate and Poloxamer 407. The optimized dispersion was incorporated into a pH-sensitive matrix composed of carbopol 934 and HPMC. The formulations were characterized by particle size, zeta potential, entrapment efficiency, and TEM. The evaluation included gelling capacity, in vitro release kinetics, and three-month stability studies. **Results:** The optimized formulation (F5) exhibited a particle size of 157.9 nm, high entrapment efficiency (85.73 ± 1.08%), and drug content of 91.46 ± 1.14%. TEM confirmed a spherical, bicontinuous cubic architecture. F5 was incorporated into Carbopol 934 and HPMC-based gels, in which the optimized IG2 formulation exhibited a rapid sol-to-gel transition (18 s) at physiological pH (7.4). IG2 provided controlled drug release (61.46 ± 2.^{12}% over 12 h) via anomalous non-Fickian transport. Sterility and three-month stability studies confirmed safety and physical integrity of the formulation. **Conclusion**: The results suggest that the developed Carteolol HCl-loaded cubosomal in-situ gel formulation offers promising potential for improving ocular bioavailability and patient compliance in the long-term management of glaucoma.

Keywords

Cubosomes, Carteolol Hydrochloride, Glaucoma, In-situ Gel, Carbopol 934, Glyceryl Monooleate, Sustained Release, Ophthalmic Drug Delivery

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INTRODUCTION

Glaucoma is a chronic neurodegenerative optic neuropathy characterized by progressive retinal ganglion cell (RGC) loss and irreversible vision impairment, primarily driven by elevated intraocular pressure (IOP). It currently affects approximately 80 million people worldwide and is the second leading cause of permanent blindness globally, with an estimated 111.8 million cases by 2040 [(1)]. In India alone, nearly 12 million individuals are affected, accounting for 12.8% of the global burden of blindness. Pharmacological IOP reduction remains the cornerstone of glaucoma management, with beta-blockers being the most widely prescribed first-line agents.

Carteolol hydrochloride (HCl), a non-selective beta-adrenergic antagonist with intrinsic sympathomimetic activity (ISA), effectively reduces IOP by 20–30% through suppression of aqueous humor secretion [(2)]. Its partial agonist activity confers a favorable cardiovascular profile with a lower risk of bradycardia and bronchospasm than timolol, making it preferable in patients with mild cardiorespiratory comorbidities. Despite these advantages, Carteolol HCl is only available as a conventional aqueous ophthalmic solution (1% and 2%), which is severely limited by ocular barriers.

Topical ophthalmic bioavailability is typically less than 5% [(3)] because of precorneal constraints, including the

small conjunctival sac volume (~7–10 µl), nasolacrimal drainage, reflex lacrimation, and tear film dilution [4]. The hydrophilicity of Carteolol HCl (log P = 0.24) further restricts transcorneal permeation across the lipophilic corneal epithelium. Collectively, these barriers necessitate twice-daily dosing and risk systemic cardiopulmonary side effects via nasolacrimal drainage, compromising long-term patient compliance[5,6].

To address these challenges, cubosomes bicontinuous cubic liquid crystalline nanoparticles (100–300 nm) formed by the self-assembly of glyceryl monooleate (GMO) with Poloxamer 407 as a stabilizer, offer a promising platform [7]. Their unique honeycomb-like internal architecture provides an exceptionally high internal surface area (200–400 m^2/g), enabling the encapsulation of both hydrophilic and lipophilic drugs with sustained release over 6–24 h and enhanced corneal permeation via lipid bilayer fusion [8,9]. Integration of cubosomes into a pH-sensitive in-situ gel system using Carbopol 934 further prolongs precorneal residence: the formulation remains a low-viscosity liquid at pH 4.0–4.5 for easy instillation and undergoes sol-to-gel transition upon contact with tear fluid (pH ~7.4), anchoring drug-loaded cubosomes to the corneal surface and minimizing drainage-related losses [10].

Therefore, this study aimed to develop, optimize, and evaluate Carteolol HCl-loaded cubosomes dispersed in a pH-sensitive in-situ gel as a novel ophthalmic drug delivery system for glaucoma. This dual-action strategy synergistically combines the sustained-release and permeation-enhancing properties of cubosomes with the mucoadhesive retention of in-situ gelling, offering improved IOP control, reduced systemic exposure, and enhanced patient compliance compared to conventional Carteolol HCl formulations.

MATERIALS AND METHODS

Materials

Carteolol hydrochloride was received as a gift sample from B.L. Chemical Products (Mumbai, India). Glyceryl monooleate (GMO) and Poloxamer 407 (Pluronic F-127) were purchased from Vishal Chemicals, Mumbai, India. Tween 80, carbopol 934, hydroxypropyl methylcellulose (HPMC), sodium chloride, benzalkonium chloride, sodium bicarbonate, and calcium chloride dihydrate were purchased from Research Lab Fine Chem Industries (Mumbai, India). All chemicals and reagents were of pharmaceutical or analytical grade.

Preparation of Carteolol HCl-Loaded Cubosomal Dispersion

The cubosomal dispersions loaded with carteolol were prepared using the "melt dispersion emulsification method." The cubic phase-forming lipid (glyceryl monooleate,GMO) and the colloidal stabilizer (Poloxamer 407) were then accurately weighed, mixed together in a glass vial, and melted in a thermostatic water bath at 60 ± 2 °C to a uniform lipid blend[3,11,13]. Then, the amount of Carteolol HCl required was dissolved in the molten lipid phase with constant magnetic stirring to ensure a uniform distribution of the drug. The aqueous phase was separately prepared with simulated tear fluid (STF with pH of 7.4) having Tween 80 as emulsifier and was heated at 60 ± 2 °C, which was near the melting point of the lipid phase.[14] The aqueous phase was then heated and added dropwise to the drug-loaded lipid melt with continuous magnetic stirring (1000-1800 RPM) for approximately one hour to obtain a coarse cubosomal suspension. The dispersion was then cooled slowly to room temperature under stirring to ensure that stable nanoparticles were formed, transferred into sealed glass vials, and stored at room temperature until further use. A central composite design of experiments was utilized to optimize the formulation and processing parameters for the Carteolol HCl cubosomes. In this statistical model, the concentrations of glyceryl monooleate and Poloxamer 407 were defined as independent variables, whereas the vesicle size and percentage entrapment efficiency (% EE) were evaluated as critical dependent responses. The ranges established for these variables during the optimization process are listed in Table 1.

Table 1. Composition of Optimized Formulation for Carteolol HCL Cubosomes (20ml)

Optimization Formulation Paramerters	
GMO	**1500 mg**
Poloxamer 407	**150 mg**
Tween 80	**0.2 ml**
Temperature	**60 ± 2 °C**
Stirring Speed	**1400 RPM**
Stirring Time	**1 hr**
Drug (Carteolol Hcl)	**200 mg**

Preparation of pH-Sensitive In-situ Ophthalmic Gel

An in-situ gel was prepared using a stepwise cold method [16], which is a pH-sensitive process. Carbopol 934 was the main active ingredient that was pH-dependent, whereas HPMC was the viscosity-enhancing co-polymer. Two formulations (IG1 and IG2) were prepared at different concentrations of both polymers as described in Table 2. HPMC was dispersed in hot distilled water at 80 °C, under stirring at 500 rpm, then cooled and stored overnight at 4 °C for complete hydration. Carbopol 934 was slowly added to cold distilled water, stirred slightly to hydrate, and kept at room temperature for 2 h to ensure a uniform translucent dispersion. The HPMC solution was then slowly added to the Carbopol dispersion [17] with gentle stirring for 30 minutes at 200-300 rpm, and sodium chloride and benzalkonium chloride (0.01% w/v) were added as tonicity adjusters and preservatives, respectively. The pH was adjusted to 5.0–6.0 with 0.1 N sodium hydroxide, and the pH was continuously monitored using a calibrated pH meter. The formulation was a free-flowing sol that could be instilled into the eye. The optimized dispersion of cubosomes (1:1) was then added slowly to the gel base under mild stirring at 4 °C to avoid early gelation and low-speed stirring was performed for 20 min to obtain a homogeneous dispersion. Mild sonication was applied to minimize aggregation. The volume was adjusted to 20 mL with distilled water, and the in-situ gel was collected, placed in sterile glass containers, and kept at room temperature for evaluation. [19]

Formulation Code	Carbopol 934 (% w/v)	HPMC (% w/v)	Optimized Cubosome Dispersion	Benzalkonium Chloride (% w/v)
IG1	0.50	0.50	1%	0.01
IG2	0.75	0.375	1%	0.01

Table 2. Composition of pH-sensitive in-situ gel formulations (IG1 and IG2) containing optimized Carteolol HCl cubosomal dispersion.

CHARACTERIZATION OF CUBOSOMAL DISPERSION

Determination of particle size, PDI & zeta potential

The particle size, polydispersity index (PDI) and zeta potential of Carteolol HCl-loaded cubosomal dispersions were measured by dynamic light scattering (DLS) at 25 °C using a Malvern Zetasizer (ZS XPLORER, Malvern Panalytical, UK) and diluted suitably with distilled water. Measurements were performed in a disposable cuvette (PCS1115) at a 90° detector angle and a zeta cell (ZEN1002) for the zeta potential.

Morphology of optimized cubosomal dispersion

The morphology of the cubosomal formulations was examined using TEM. The sample was removed and applied to a carbon-coated copper grid, left to adsorb for 30 s, blotted, and negatively stained with 2% w/v phosphotungstic acid for 30 s. The grid was then air dried at room temperature and scanned to ensure the characteristic cubic morphology and homogeneity of the nanoparticles.

Determination of Entrapment Efficiency

An indirect ultracentrifugation method was used to determine the entrapment efficiency (%EE) [20]. Each cubosomal dispersion was centrifuged at 4500 rpm for 20 min to sediment the drug-loaded nanoparticles and the unentrapped drug in the supernatant. [15] The clear supernatant was carefully collected and the drug-free solution was used as a blank to quantify the concentration of the free Carteolol HCl spectrophotometrically at λmax = 228 nm. The following equation was used to determine the entrapment efficiency:

$$\%EE = [(\text{Total drug} - \text{Free drug}) / \text{Total drug}] \times 100.$$

Determination of Drug Content

The drug content of the prepared Carteolol HCL-loaded cubosomal dispersions was determined using UV spectrophotometry. An appropriate amount for each

formulation was taken and subjected to a Simulated Tear Fluid (STF, pH 7.4) which was used to disrupt and release the entrapped drug completely and efficiently.

The mixture was then sonicated to aid full lysis of the cubosomal vesicles and uniform dissolution of the drug. The resulting solution was filtered through a 0.45 µm membrane filter to eliminate the residues of undissolved excipients. The clear filtrate was diluted as needed using the same solvent system. [21]

A blank was prepared and the absorbance of the prepared sample was measured at 228 nm. The drug content was determined by constructing a calibration curve for Carteolol HCL and calculating the drug content as a percentage of the theoretical drug content using the following formula:

Drug Content (%) = (Actual drug content / Theoretical drug content) × 100

Characterization of Cubosomal pH-Sensitive In-situ Gel

Appearance and Clarity

All cubogel formulations were visually inspected against white and black backgrounds under proper lighting for physical appearance, clarity and homogeneity [22]. The formulations were checked for the presence of particulate matter, turbidity or phase separation. A satisfactory formulation would be a uniform dispersion containing no visible particles and of an acceptable clarity or opalescence.

Determination of pH

The pH of each Cubogel formulation was determined at room temperature using a calibrated digital pH meter that was standardized with pH 4.0 and 7.0 buffer solutions before use. The electrode was dipped into the formulation and the reading was taken after equilibration.

Rheological Study

The viscosity of the cubogel formulations was determined using a Brookfield viscometer at room temperature with spindle No. 62 [23] at different spindle rotational speeds of 10, 20, 30, 50, and 100 rpm. The volume of the sample was inserted into a sample chamber and the readings were obtainedafter equilibration at each speed to describe the flow behavior.

Gelation Study

The gelling capacity and the gelling time were observed by the addition of 1 drop of each formulation to 2 mL of STF (pH 7.4) at 37 ± 2 °C. [24] The gel formation time and integrity were observed visually. The gelling capacity was ranked as: (−) no gelation; (+) gelation within s, dissolving rapidly; (++) immediate gelation, maintained for hours; (+++) immediate gelation, maintained for hours.

Gelation Strength

Gel strength was measured using a texture analyzer by recording the time (s), required for a probe to penetrate the gelled matrix to a depth of 5 cm. The longer the penetration time, the stronger the gel. [19] For cubosomal in situ gels, values between 20 and 50 s are considered optimal;[16] gels falling below this range erode too rapidly, while those exceeding it may cause ocular discomfort.

In-vitro drug release

The optimized cubogel (1 g) was used in a Franz diffusion cell with a pre-hydrated dialysis membrane (MWCO 12-14 kDa) for the drug release study. STF (pH 7.4, 20 mL) served as the receptor medium at 35 ± 0.5 °C with stirring at 100 rpm. Aliquots (1 mL) were withdrawn at 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 10, and 12 h, replaced with fresh STF, and analyzed spectrophotometrically at 228 nm. [16,25] The obtained drug release profiles were subsequently evaluated using kinetic models, such as the Higuchi and Korsmeyer-Peppas equations, to determine the diffusion rate constants and release mechanisms.

Ex Vivo Corneal Permeation Study

Fresh goat corneas obtained from a local abattoir (within 2 h of slaughter, stored in ice-cold PBS with gentamicin sulfate, 100 µg/mL) were used to evaluate corneal permeation of Carteolol HCl from F5 cubosomal dispersion, IG1, IG2, and a 1% w/v aqueous solution [23] (reference control). Corneas with 2–3 mm scleral rim were mounted in Franz diffusion cells (area: 0.785 cm²) with the epithelial side facing the donor compartment and the endothelial side bathed in simulated tear fluid (STF, pH 7.4, 35 ± 0.5°C, 100 rpm).[15] Following 30 min equilibration, each formulation was applied to the donor compartment, and 1 mL samples were withdrawn at predetermined time intervals (0.5–12 h), replaced with fresh STF, and analyzed spectrophotometrically at 228 nm. The cumulative drug permeation per unit area (µg/cm²) was plotted against time, and the steady-state flux (J), permeability coefficient (Kp = J/Cd), and enhancement ratio (ER) relative to the aqueous solution were derived.

Sterility Testing

Sterility testing was performed to detect viable microorganisms in the optimized cubosomal in situ gel formulations using membrane filtration. Fluid Thioglycolate Medium (FTM) was prepared by dissolving 29.75 g in 1000 ml distilled water, and Soybean Casein Digest Medium (SCDM) was prepared by dissolving 30 g in 1000 ml distilled water; both were sterilized by autoclaving at 121°C, 15 lbs pressure. The filtration assembly with a 0.45 µm membrane (47 mm diameter) and peptone solution (1 g/L) as diluting fluid was similarly sterilized. [26] A 1% gel solution in isopropyl myristate was filtered aseptically through the membrane, followed by three successive washings with 100 ml each. Each membrane was cut in half and immersed in SCDM (incubated at 25°C) and FTM (incubated at 35°C) for 14 days under strict aseptic conditions.[23]

Stability study

The stability of the optimized Cubogel was determined in sealed amber glass containers for three months at 25 ± 2 °C / 60 ± 5 % RH according to the guidelines of ICH Q1A(R2) [14]. Samples were taken at 0, 1, 2 and 3 months and tested for physical characteristics, pH, gelling, and viscosity.Storage stability was compared with the results of baseline samples.

RESULT & DISCUSSION

Nine batches (F1–F9) of Carteolol HCl-loaded cubosomal pH-sensitive gel were successfully prepared using the melt dispersion-emulsification method. The prepared Carteolol HCl loaded cubosomal in-situ gel was systematically evaluated for physicochemical characteristics, drug release behavior, and formulation stability to establish its suitability for ophthalmic delivery.

Particle Size, Poly Dispersity Index, Zeta Potential

Cubosomes in nine different formulations (F1 to F9) were obtained by examining the particle size, PDI and zeta potential through the studies on varying concentrations of GMO, Poloxamer 407 and Tween 80. The particle size ranges from 12.26 nm and 564.51 nm. (Table 3) Formulation F1 (12.26 nm) fell below the characteristic cubosome range, likely representing micellar rather than true cubic liquid crystalline structures due to insufficient GMO to sustain the bicontinuous phase. Formulations F8 and F9, with sizes of 328.64 nm and 564.51 nm respectively, exceeded the acceptable threshold for ocular nanoparticles, compromising corneal permeation efficiency and predisposing the dispersion to aggregation. The particle size of F3–F7 were in the range of 100–300 nm, which is consistent with the well-formed cubosomal nanoparticles reported in literature for ophthalmic applications. Among these F5 showed the best result with particle size 157.9 nm (PDI: 0.31) which has a narrow size distribution suitable for ocular drug delivery. The zeta potential of F5 was −32.5 mV, which was the highest magnitude among all batches indicating high interparticle electrostatic repulsion which shows that it is physically stable over extended periods of time. The absolute Zeta potential values of F1 (−12.4 mV), F3 (−9.3 mV) and F7 (−14.6 mV) were well below the commonly accepted |±30| mV threshold, indicating a higher likelihood to agglomerate and coalesce upon storage. The favorable combination of particle size, PDI, and zeta potential led to the selection of F5 as the optimized cubosomal formulation for incorporation into the in-situ gel matrix.

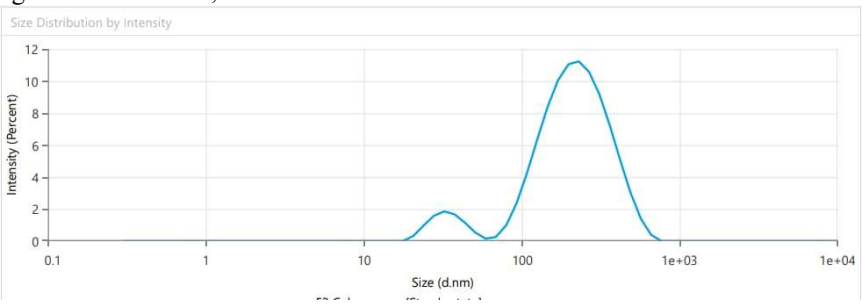


Fig. 1. Particle Size & PDI of Optimized Batch(F5)

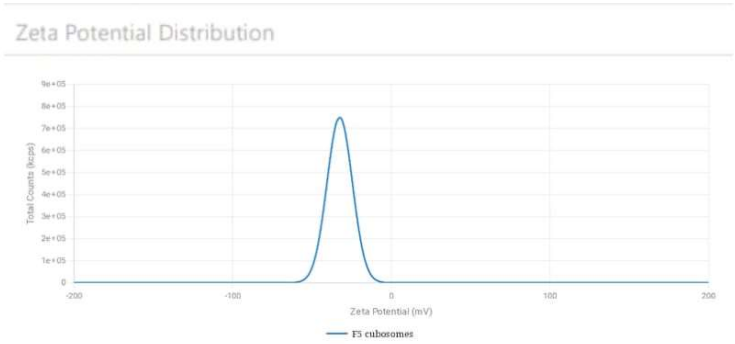


Fig. 2. Zeta Potential of Optimized Batch(F5)

Entrapment Efficiency (%EE) and Drug Content (%DC)

Entrapment efficiency of all the nine formulations ranged from 58.42 ± 1.24% (F1) to 85.73 ± 1.08% (F5). Drug content ranged from 72.34 ± 1.45% (F1) to 91.46 ± 1.14% (F5) (Table 3). The large increase in %EE from early to mid-series formulations indicates that there was more GMO available to form a structured lipid bilayer that could take up the drug. GMO can form a bicontinuous cubic phase and thus provide a large internal surface area with connected aqueous channels. [27] Thus, it is possible that both hydrophilic and partially lipophilic drug molecules can be incorporated simultaneously. This structural feature is crucial for the observed high entrapment in F5.

The relatively lower %EE of F8 and F9 with respect to their higher concentration of GMO can be explained by the possibility that an excessive presence of lipid might distort the cubic phase, which in turn would result in a relatively low %EE. Most of the formulations exhibited a significant positive relationship between %EE and %DC. This implies that as %EE increased, the %DC was also relatively higher, meaning the drug molecules had been more homogeneously dispersed within the cubosomal matrix. However, F4 exhibited a relatively high %DC of 83.27 ± 1.28% but a lower %EE of 62.26 ± 1.52%, probably suggesting a relatively low proportion of the drug was physically entrapped, whereas a higher quantity might have been present on the surface of the cubosomal vesicles. F5, on the other hand, showed the optimal cubosomal characteristics (%EE of 85.73 ± 1.08%) and (%DC of 91.46 ± 1.14%) for ocular cubosomal formulations (%EE > 70% and %DC > 85%), suggesting a higher quantity of drug entrapped within the matrix and a lower quantity of lost drug on the surface of the cubosomal vesicles.

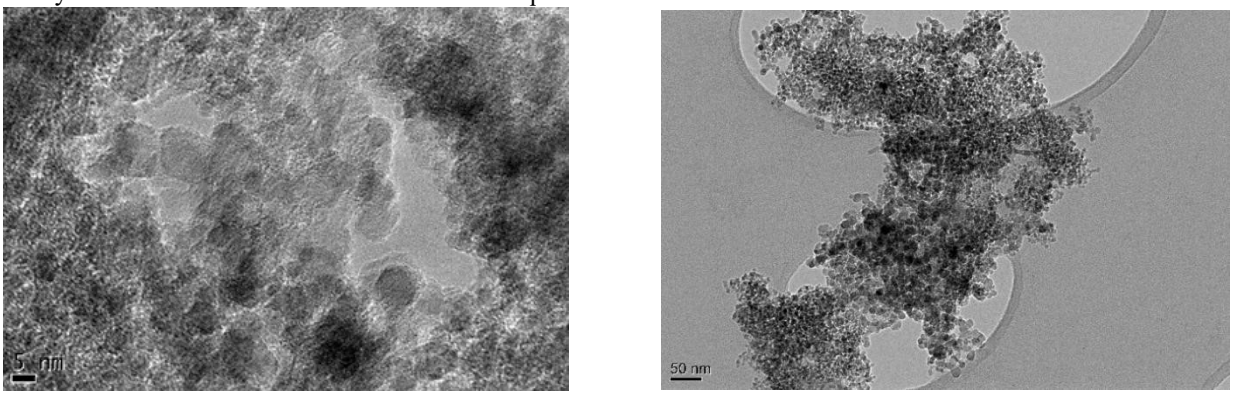
Table 3. Particle size, PDI & zeta potential, EE%, Drug content of Carteolol HCl cubosomal dispersions (F1–F9). *Optimized formulation.

Formulation	Particle Size (nm)	PDI	Zeta Potential (mV)	Entrapment Efficiency (%)	Drug Content (%)
F1	12.26	0.23	-12.4	58.42 ± 1.24	72.34 ± 1.45
F2	98.45	0.42	-18.7	64.85 ± 1.38	76.18 ± 1.32
F3	121.72	0.38	-9.3	78.94 ± 1.16	84.52 ± 1.61
F4	143.56	0.34	-26.8	62.26 ± 1.52	83.27 ± 1.28
F5*	157.9	0.31	-32.5	85.73 ± 1.08	91.46 ± 1.14
F6	176.84	0.42	-29.4	82.41 ± 1.34	88.63 ± 1.37
F7	201.37	0.46	-14.6	76.58 ± 1.47	81.74 ± 1.52
F8	328.64	0.48	-19.8	69.32 ± 1.63	78.39 ± 1.68
F9	564.51	0.53	-15.2	71.87 ± 1.58	79.85 ± 1.43

Morphology of optimized cubosomal dispersion

The optimized F5 cubosomal dispersion was directly visualized by TEM demonstrating the structure and internal architecture of the nanoparticles. The images

taken at the nanoscale (50 nm) showed mostly discrete spherical nanoparticles with a diameter of 100–200 nm, consistent with the DLS value of particle size of 157.9 nm. The TEM image dimensions were in good agreement with the DLS measured dimensions, leading to the conclusion that particle size data obtained from the dispersion was reliable and that the dispersion was composed of well-formed individual nanoparticles, not aggregates. The high-resolution imaging at 5 nm revealed a striking view of the biocontinuous cubic liquid crystalline phase, whose internal structure is a honeycomb network of interconnected aqueous channels separated by curved lipid bilayers of GMO. This ordered nanostructure has been a hallmark of true cubosome and is the distinguishing characteristic between simpler lipid vesicles or micellar systems. [28] A well-defined internal architecture is directly related to a controlled and sustained release of the drug in vitro, the biocontinuous phase showing a large interfacial area and a tortuous diffusion pathway. [12] These TEM morphologies combined showed the successful formation of cubosomes and cubic phase ordering without any significant aggregation of the particles.



(A) (B)

Fig 3. TEM image of Optimized Batch(F5) (A) spherical nanoparticle morphology at 50 nm scale, (B) internal bicontinuous cubic phase structure at 5 nm scale

Appearance and pH Measurement

The successful incorporation of F5 cubosomes in Carbopol – HPMC gel matrix is supported by the appearance of IG1 and IG2 as a homogeneous and milky white dispersion without particulate or phase separation. The opalescent property was due to the scattering of light from the suspended lipid nanoparticles. The result of higher concentration of Carbopol 934 is slightly higher viscosity in case of IG2. Both formulations had physiologically acceptable pH (~6.0) which is optimal for the eye, thus instilling without any excessive irritation. [29] The uniformity of the appearance and the pH value indicated formulation stability, polymer compatibility and was suggested as a good preparation method. (Table 4)

Gelation Study

Both IG1 and IG2 demonstrated the ability of the Carbopol 934 system to transform from the sol to gel state at a simulated tear fluid (pH 7.4). The gelation time of IG1 was 28 s and the gel strength was moderate, and IG2 had a short gelation time of 18 s and strong gelling ability and good gel retention. The enhanced performance of IG2 was correlated with the greater amount of Carbopol 934 which resulted in a higher level of polymer ionization and matrix expansion. The properties refer to enhanced Ocular Residence Time (ORT), reduced drainage and enhanced Ocular Bioavailability. [21] (Table 4)

The gel strength of both formulations fell within the acceptable range of 20 to 50 s. IG1 showed a gel strength of 22 s, while IG2 recorded a higher value of 45 s. The stronger gel network of IG2 is well supported by its higher gelling capacity (+++), elevated gelling pH of 7.3, and greater viscosity, all of which point to a more robust and cohesive matrix formation upon contact with tear fluid. In comparison, IG1, with a gel strength of 22 s, sits closer to the lower end of the acceptable range, suggesting a softer network that may be relatively more prone to mechanical erosion. Overall, IG2 demonstrated superior gel strength, making it the optimized formulation with better potential for prolonged precorneal retention and sustained drug release.

Table 4. Physicochemical properties & Gelation characteristics of Carteolol HCl cubosomal in-situ gel formulations (IG1 and IG2).

Parameter	IG1	IG2
Appearance	Milky white, viscous liquid	Milky white, slightly more viscous liquid
pH	6.0 ± 0.08	6.0 ± 0.06
Gelling pH	7.1 ± 0.09	7.3 ± 0.06
Gelling Time	28 ± 1.4 sec	18 ± 1.2 sec
Gel Strength	22 sec	45 sec
Gelling Capacity	++	+++

Rheological Study

The viscosity of both IG1 and IG2 decreased with increasing shear rate (Table 5), which is typical of non-Newtonian ophthalmic gels, as termed pseudoplastic shear-thinning. [30] A higher concentration of Carbopol 934 was used in the formulation of IG2 which has a denser polymeric network than IG1, and as a consequence IG2 has a viscosity that is consistently higher than IG1. This behavior is rheological which helps in easy spreading during blinking and helps precorneal retention at rest. These properties of higher viscosity and mucoadhesion of IG2 are thought to lead to greater ocular residence time, longer duration of release, decreased nasolacrimal drainage and better therapeutic effect in glaucoma treatment.

Table 5. Viscosity (cP) of Carteolol HCl cubosomal in-situ gel formulations at varying shear rates (Brookfield viscometer, spindle No. 62, room temperature).

Speed (rpm)	IG1 Viscosity (cP)	IG2 Viscosity (cP)
10	1124	1872
20	682	1035
50	516	774
60	318	474.6
100	172	256.2

In Vitro Drug Release Study

The drug release profile of F5 cubosomal dispersion is compared with those of IG1 and IG2 in vitro after 12 hours and the same is plotted. The in vitro drug release profile of F5 cubosomal dispersion and IG1 and IG2 are compared and plotted together after 12 hours. The Cubosomal architecture and the gel matrix were found to be crucial in controlling the release of Carteolol HCl in all three systems, as the release was sustained for a long period of time, while a conventional aqueous solution of Carteolol HCl would release immediately upon instillation. The F5 cubosomal dispersion released 76.82 ± 2.24% of the drug at 12 hours, and displayed a moderate initial release of 18.64 ± 0.92% at 0.5 hours, suggesting the rapid diffusion of surface-associated and/or the loosely entrapped drug, and then progressively slow diffusion of the drug molecules, passing through the tortuous internal channels of the bicontinuous cubic phase. The release kinetics were significantly influenced by the incorporation of F5 dispersion in the gel matrix. The result obtained for the release of IG1 revealed that 74.86 ± 2.38% release was achieved at 12 hours, which was significantly less than that obtained for cubosomal dispersion (85.85 ± 1.04%) and this could be attributed to the presence of an additional diffusional barrier due to the presence of a polymeric network of Carbopol–HPMC in IG1. IG2 showed the most controlled release with only 8.62 ± 0.72% released after 0.5 hours and 61.46 ± 2.12% after 12 hours. The initial burst and the more gradual release of IG2 are significantly lower, indicating a higher diffusional resistance due to the denser gel network created with the higher concentration of Carbopol 934. The release mechanism from IG2 resembles a two barrier system; the first barrier comprised of the structured lipid bilayer channels of cubosomes and the second barrier is represented by the entangled polymeric gel matrix releasing into the membrane resulting in an even and longer drug release profile. It may be a clinically relevant sustained release effect in glaucoma therapy as it enables twice-daily or once-daily dosing, which may be beneficial for long-term patient adherence, compared to the traditional multi-drop therapy.

Table 6. %CDR of Carteolol HCl from F5 cubosomal dispersion, IG1, and IG2 over 12 hours (Franz diffusion cell, STF pH 7.4, 35 ± 0.5 °C).

Time (h)	F5 Cubosome (%)	IG1 (%)	IG2 (%)
0.5	18.64 ± 0.92	12.34 ± 0.84	8.62 ± 0.72
1	26.48 ± 1.14	18.76 ± 1.12	13.48 ± 0.94
2	36.82 ± 1.38	26.43 ± 1.38	19.24 ± 1.16
4	48.64 ± 1.62	38.92 ± 1.64	28.76 ± 1.42
6	58.34 ± 1.84	49.18 ± 1.82	37.54 ± 1.68
8	67.48 ± 2.06	60.34 ± 2.14	46.82 ± 1.84
10	72.14 ± 2.18	68.42 ± 2.28	54.36 ± 1.96
12	76.82 ± 2.24	74.86 ± 2.38	61.46 ± 2.12

In Vitro Drug Release Kinetics

The cumulative release data of F5, IG1 and IG2 were fitted to zero-order, first-order, Higuchi and Korsmeyer-Peppas models to elucidate the mechanism of drug release and the highest R^2 value was considered as the best fit (Table 8). For all three formulations, the Korsmeyer-Peppas model was best fitted (F5: R^2 = 0.9980; IG1: R^2 = 0.9989; IG2: R^2 = 0.9976). The release exponent n for F5 was 0.4451, indicating a near-Fickian diffusion governed by concentration-gradient-driven transport through the tortuous aqueous channels of the cubic lipid phase. A gradual transition to anomalous non-Fickian transport was observed with gel incorporation, with n values of 0.5661 (IG1) and 0.6109 (IG2) suggesting the increasing contribution of polymer swelling and erosion along with cubosomal diffusion as the Carbopol 934 concentration increased. This was supported by a stepwise reduction in first order rate constants (F5: 0.1078 h^{-1}; IG1: 0.1067 h^{-1}; IG2: 0.0731 h^{-1}) and Korsmeyer-Peppas rate constants (F5: 26.18; IG1: 18.24; IG2: 13.00) indicating a progressive increase in diffusional resistance with increasing gel density. These findings support the dual-barrier mechanism of IG2 as the reason for its superior sustained release profile, which is consistent with the unusual drug release pattern of cubosomal ocular systems reported by Sayed et al. (2021) and Acharya et al. (2019).

Table 7. Drug release kinetic data of F5 cubosomal dispersion, IG1, IG2

Kinetic Model	F5 Cubosomal Dispersion	IG1	IG2
Zero Order(R^2)	0.9452	0.9828	0.9929
First Order	0.9934	0.9978	0.9978
Higuchi	0.9953	0.9969	0.9910
Korsmeyer-Peppas	**0.9980**	**0.9989**	**0.9976**
Best fit model	Korsmeyer-Peppas	Korsmeyer-Peppas	Korsmeyer-Peppas
Release mechanism	Fickian diffusion	Anomalous transport	Anomalous transport

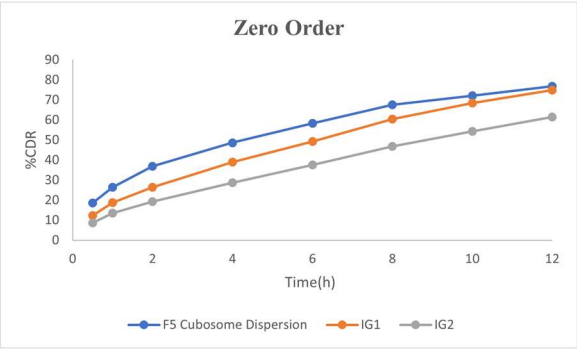


Fig. 4. Zero Order Plot

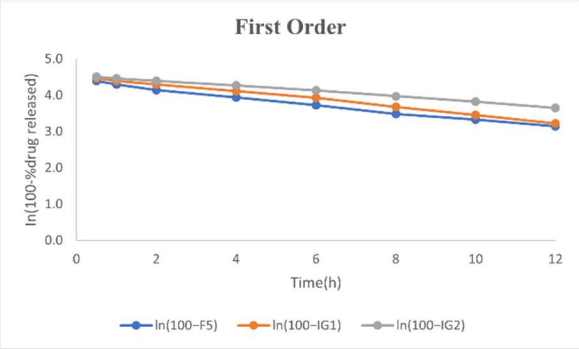


Fig. 5. First Order Plot

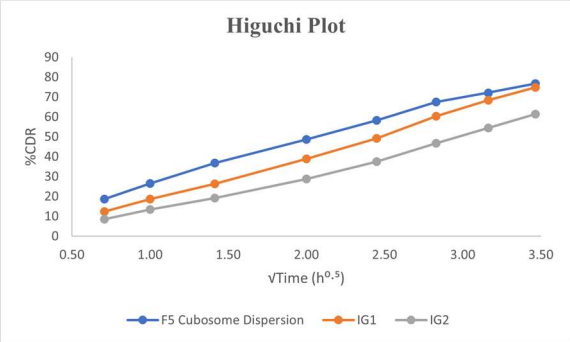


Fig. 6. Higuchi Plot

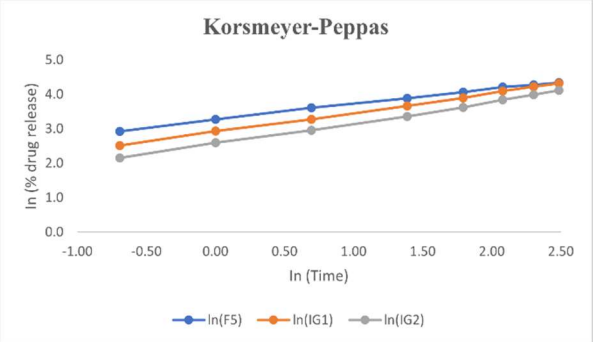


Fig. 7. Korsmeyer-Peppas Plot

Ex Vivo Corneal Permeation Study

Cumulative permeation across excised goat cornea over 12 hours followed a biphasic pattern with near-linear steady-state kinetics beyond 4 hours (Tables 9,10). Permeation ranked F5 (2859.87 µg/cm²) > IG1 (2484.08 µg/cm²) > CS (2253.50 µg/cm²) > IG2 (2099.36 µg/cm²), with corresponding flux of 141.46, 150.96, 115.10, and 139.81 µg/cm²/h and Kp of 14.15, 15.10, 11.51, and 13.98 × 10^{-3} cm/h.

F5 yielded the highest cumulative permeation (ER = 1.23) via lipid-fusion-driven transcorneal transport and mucoadhesive retention of negatively charged cubosomes (−32.5 mV). IG1 achieved the highest flux and Kp (ER = 1.31) through a sustained diffusion gradient within its moderately cross-linked matrix (0.50% w/v Carbopol 934). Although IG2 fell marginally below CS in total permeation, it retained an ER of 1.21 during the linear phase, indicating controlled, sustained delivery through the denser gel network (0.75% w/v Carbopol 934) clinically desirable for reducing systemic nasolacrimal absorption in glaucoma management.

Collectively, these findings confirm a dual-barrier mechanism of cubosomes as permeation modulators and the gel matrix as a tunable diffusion controller consistent with in vitro release data and supporting IG2 as a sustained-release ophthalmic platform for glaucoma therapy.[18]

Table 8. Cumulative corneal permeation (µg/cm²) of Carteolol HCl across excised goat cornea from conventional solution (CS), F5 cubosomal dispersion, IG1, and IG2 over 12 hours (Franz diffusion cell, STF pH 7.4, 35 ± 0.5°C).

Time (h)	CS (µg/cm²)	F5 (µg/cm²)	IG1 (µg/cm²)	IG2 (µg/cm²)
0.5	30.57	34.58	25.47	20.38
1	55.32	78.72	64.34	41.40
2	90.82	123.31	128.55	87.26
4	133.76	172.48	272.62	197.98
6	162.55	211.08	316.38	323.58
8	187.77	243.57	396.82	614.01
10	208.79	267.01	423.44	864.97

12	253.50	285.99	484.08	2099.36

Table 9. Ex vivo corneal permeation parameters of Carteolol HCl across excised goat cornea.

Formulation	Cumulative Permeation at 12 h (µg/cm²)	Steady-State Flux, J (µg/cm²/h)	Kp × 10^{-3} (cm/h)	Enhancement Ratio (ER)
Conventional Solution (CS)	2253.50	115.10	11.51	1.00 (reference)
F5 Cubosomal Dispersion	2859.87	141.46	14.15	1.23
IG1	2484.08	150.96	15.10	1.31
IG2	2099.36	139.81	13.98	1.21

Sterility Testing

Sterility is a critical requirement for ophthalmic preparations, as microbial contamination can cause ocular irritation, inflammation, or serious infection. The optimized cubosomal in situ gel formulations were tested for sterility as per guidelines, using Fluid Thioglycolate Medium (FTM) and Soybean Casein Digest Medium (SCDM) to detect aerobic bacteria, anaerobic bacteria, and fungi, respectively. All test samples and the negative control remained clear and transparent throughout the 14-day incubation period, while the positive control showed visible turbidity, confirming the validity of the test. The absence of any microbial growth in the test samples indicated that the cubosomal in situ gel formulations successfully passed the sterility test.

Stability Study

The physical stability of the optimized IG2 formulation was done by storing it at 25 ± 2 °C / 60 ± 5%RH for three months as per ICH Q1A(R2) guidelines and the evaluation was performed on 0, 1, 2 and 3 months (Table 10). The milky-white homogenous appearance of IG2 did not change throughout the study period, with no evidence of color variation, phase separation or particulate formation, suggesting the physicochemical compatibility of the cubosomal dispersion in this gel matrix under ambient storage conditions. There was no significant change in pH throughout the testing period, and the pH change was within the acceptable margin of error (≤ 0.1 pH units), indicating the formulation is adequately buffered and no degradation of the polymer or interaction between the polymer and the drug is expected to change the formulation's ability to release the drug at the appropriate pH. The pH responsive nature of Carbopol 934 network was retained with regards to its gelling capacity (++) after three months of storage. The viscosity measured at 10 rpm decreased by less than 1.1% (from 1872 cP to 1792 cP at 3 months) and this change is not considered to be functionally significant in terms of rheological performance or eye retention. Overall, these results suggest physical stability of the IG2 formulation for at least three months under the various storage conditions tested, and therefore warrant further preclinical and clinical development for its use as an in-situ glaucoma sustained-release ophthalmic formulation.

Table 10. Stability data for optimized Carteolol HCl cubosomal in-situ gel (IG2) stored at 25 ± 2 °C for three months

Parameter	0 Month	1 Month	2 Month	3 Month
Appearance	Milky white, clear	Milky white, clear	Milky white, clear	Milky white, clear
pH	6.0 ± 0.06	6.0 ± 0.08	5.5 ± 0.02	5.4 ± 0.06
Gelling Capacity	+++	+++	++	++
Viscosity (cP) at 10 rpm	1872	1865	1800	1792

DISCUSSION

Glaucoma is a major cause of irreversible blindness worldwide and is commonly treated using topical eye drops. Carteolol hydrochloride (HCl), a non-selective beta-blocker, is widely used to reduce intraocular pressure in glaucoma and ocular hypertension. However, conventional eye drops show poor ocular bioavailability (3–5%) due to low corneal permeability, rapid tear drainage, and short precorneal residence time, leading to frequent dosing and reduced patient compliance.

The present study aimed to develop a novel ophthalmic delivery system consisting of Carteolol HCl-loaded cubosomes incorporated into a pH-sensitive in-situ gel to achieve sustained ocular drug delivery.

Cubosome dispersions (F1–F9) were prepared using glyceryl monooleate (GMO) and Poloxamer 407 by the melt dispersion-emulsification method. All formulations were characterized for particle size, polydispersity index (PDI), zeta potential, entrapment efficiency, and drug content. The particle size of the prepared formulations ranged from 12.26 nm to 564.51 nm, while PDI values ranged from 0.23 to 0.53. Formulations F3 to F7 exhibited particle sizes within the ideal ocular cubosome range of 100–300 nm. Zeta potential values ranged from −9.3 mV to −32.5 mV, with F5 demonstrating the highest colloidal stability at −32.5 mV. Entrapment efficiency ranged from 58.42 ± 1.24% to 85.73 ± 1.08%, and drug content ranged from 72.34 ± 1.45% to 91.46 ± 1.14% across all batches. Among all batches, formulation F5 showed the most desirable characteristics with a particle size of 157.9 nm, PDI of 0.31, zeta potential of −32.5 mV, entrapment efficiency of 85.73 ± 1.08%, and drug content of 91.46 ± 1.14%, and was selected as the optimized formulation.

The optimized F5 cubosome dispersion was further incorporated into pH-sensitive in-situ gels using Carbopol 934 and HPMC in two different ratios (IG1 and IG2).

Both formulations were milky-white, homogeneous liquids at pH 6.0 and underwent rapid sol-to-gel transition at tear fluid pH (7.4). IG2 exhibited better gelling properties, higher viscosity, and stronger gel formation compared to IG1. Viscosity studies confirmed pseudoplastic (shear-thinning) behavior suitable for ocular application. Gelation resulted in an approximately 3.69-fold increase in viscosity at 10 rpm (pre-gel: 1872 cP; gelled state: 6913 cP), demonstrating effective pH-triggered sol-to-gel transition upon contact with simulated tear fluid.

In vitro drug release studies using a Franz diffusion cell demonstrated sustained drug release over 12 hours. F5 cubosome dispersion released 76.82 ± 2.24% drug, IG1 released 74.86 ± 2.38%, while IG2 showed the most controlled release with 61.46 ± 2.12% release and the lowest initial burst effect. These findings confirmed that incorporation of cubosomes into the in-situ gel further enhanced sustained drug delivery.

The kinetic modeling of drug release showed that all three formulations best fit the Korsmeyer-Peppas model and yielded the high R^2 values of 0.9980 (F5), 0.9989 (IG1), and 0.9976 (IG2). With increased concentration of Carbopol 934, the release exponent n values of 0.4451 (F5), 0.5661 (IG1) and 0.6109 (IG2) showed that the release process was near-Fickian for F5 and was anomalous, non-Fickian for IG1 and IG2, indicating increased role of polymer swelling and erosion and cubosomal diffusion with increasing Carbopol 934 concentration. Progressive decreases in Kkp (F5: 26.18 > IG1: 18.24 > IG2: 13.00) also confirmed that the diffusional resistance of the formulations increased gradually, which is consistent with the dual barrier mechanism for the better sustained release profile of IG2.

Sterility test carried out and all test samples and the negative control remained clear and transparent throughout the 14-day incubation period. The absence of any microbial growth in the test samples indicated that the cubosomal in situ gel formulations successfully passed the sterility test.

Stability studies of the optimized IG2 formulation conducted for three months under ICH conditions showed no significant changes in appearance, pH, gelling capacity, or viscosity, indicating good physical stability.

Overall, the prepared cubosome-loaded pH-sensitive in-situ gel demonstrated promising potential as an effective sustained ocular drug delivery system for glaucoma management.

CONCLUSION

The developed Carteolol HCl-loaded cubosomal in-situ gel offers a promising solution for the long-term management of glaucoma. By combining the nanostructured benefits of cubosomes with the triggered gelation of Carbopol 934, the system successfully achieves sustained drug release, enhanced stability, and improved potential for ocular bioavailability. These results suggest that the formulation could significantly reduce dosing frequency and improve patient compliance in glaucoma therapy.

DISCLOSURE STATEMENT

The authors declare no relevant affiliations or financial involvement with any organization or entity having a financial interest in or financial conflict with the subject

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