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Original Article

Development and Characterization of Moxifloxacin Hydrochloride Cubogel for Enhanced Ocular Drug Delivery

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ABSTRACT

Purpose: The study aimed to formulate and evaluate an ocular effective prolonged release cubogel formulation of Moxifloxacin Hydrochloride (MX-Cubogel) to treat conjunctivitis. **Materials and Methods:** Initially, a Moxifloxacin-loaded cubosome (MX-Cubosome) was prepared using a top-down technique using glyceryl monooleate and poloxamer 407 in different concentrations. The optimized MX-Cubosome was selected based on the physicochemical characteristics and was used to prepare MX-Cubogel by dispersing the cubosome into an *in situ* gelling system using the cold method. Physicochemical characterization, drug-polymer interaction studies, and histopathological studies were performed. **Results:** Twelve MX-Cubosomal dispersions were prepared. The cubosomes were consistent, aggregate-free, and milky white. Particle sizes ranged from 306.5 to 714.2 nm, with a uniform size distribution. Zeta potentials were between -27.9 and -39.8 mv and encapsulation efficiencies ranged from 69% to 85%. Electron microscopy confirmed the nano-sized structure of the cubosomes. The MX-Cubogels were clear, with no visible impurities. Four formulations (MB11, MB12, MB15 and MB16) gelled immediately and remained stable over time. The gelation temperatures of the majority of formulations were between 28ºC and 43ºC. MX-Cubogels had a drug content of 92.5% to 97.7%. Four formulations (MB_4, MB_7, MB_{10} and MB_{13}) released more than 95% of the drug over 12 hr. The antibacterial activity of the MX-Cubogels was demonstrated by an inhibition zone of 32 mm, which was greater than the standard (27 mm). Histological examination of goat corneas treated with selected formulations (MB_4, MB_7, MB_{10} and MB_{13}) showed normal structures, with no changes in the epidermal layer after cubosomal formulation penetration. **Conclusion:** The study demonstrates that MX-Cubosome dispersions can be successfully formulated as *in situ* gels (MX-Cubogel) with sustained release and antibacterial activity. The gels are also non-irritating to the cornea. MX-Cubogel might be a good alternative to conventional eye drops.

Keywords: Moxifloxacin Hydrochloride, Cubosomes, *in situ* Occular Gel, Histopathological Studies, Conjunctivitis.

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INTRODUCTION

The human visual system is a sensory organ that improves vision by reflecting light. The human eye's anterior and posterior portions comprise most of the division. Each of these major components is linked to a variety of eye diseases. Conjunctivitis, glaucoma, blepharitis, and cataracts are just a few conditions that can affect the anterior portion of the eye. Topical drug delivery is the most effective method for delivering drugs to both the anterior and posterior regions. The drops provide therapeutically effective concentrations in topical application, which has been the preferred route of administration for bacterial conjunctivitis and keratitis because they wash away bacteria and bacterial antigens; adverse systemic effects of the drugs are reduced or eliminated. Because conventional methods do not effectively target the pharmacological site of action of the eye, new delivery methods for the eyes must be developed.[1]

The novel medication delivery method should be (i) minimally invasive to reduce the risk of ocular tissue damage, infection, and pain, (ii) patient-safe, and (iii) specifically tailored to avoid unintended side effects. New dosage forms for medications have always been developed in order to make them more patient-friendly and effective.[2] New pharmaceutical formulations may also improve bioavailability and reduce side effects. This is accomplished by developing novel drug delivery methods, such as drug-loaded cubosomal vesicles that concentrate the drug at the ocular site.

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Cubosomal encapsulation is a relatively new drug encapsulation technique. Cubosomes are biocontinuous cubic liquid crystalline phase nanostructured (particle size 10-500 nm).[3] Cubosome systems encapsulate hydrophilic and lipophilic drugs, with hydrophilic drugs contained within vesicles and lipophilic drugs partitioned between hydrophilic domains. The drug transport mechanism of the cubosome across the biological membrane is determined by the carrier's condition and composition, as well as the anatomy and physiology of the ocular system. Ocular membrane transport involves mainly two mechanisms: trans and para-membrane transport. This mechanism is manipulated through the use of a drug carriers system, in which drugs can be incorporated either in the core or as an integral part of the vesicles.[4]

In situ forming gels are formulations that undergo a transition into a gel and can be dropped into the eye as a solution or suspension. *In situ* gelling systems are being developed to extend a drug's precorneal residence time, thereby increasing ocular bioavailability and patient compliance. Because of the precorneal residence time of some *in situ* gelling for several hr, *in situ*, gel is more acceptable for patients and has been the most appealing system.[5] When the cubosomal eye drops are applied topically, the encapsulated drug is effectively delivered to the cornea and anterior sclera. Vesicle interactions with the corneal epithelium could have increased the likelihood of transcorneal drug penetration and increased intraocular drug supply via the non-corneal route. These cubogel formulations have a dual mechanism in which the *in situ* gel helps to extend the contact time of the formulations on the cul-de-sac region, and the cubosome increases the drugs' corneal permeability.[6] MX delivery via cubosomal ocular *in situ* gel has several advantages over conventional drug delivery systems, including avoiding gastric degradation, the first pass effect, blood level fluctuations, and drug loss via lachrymal drainage.

The primary goal of this study was to develop and characterize a cubosomal ocular *in situ* gel of MX using various polymer compositions to improve bioavailability by avoiding hepatic first pass metabolism, lachrymal drainage, and gastric degradation and thus improve patient compliance.

MATERIALS AND METHODS

Materials

MX Hydrochloride and Glyceryl Mono Oleate (GMO) were purchased from Yarrow Chem Products, Mumbai, India. SD Fine Chemicals, Bangalore, India, gifted poloxamer 407. Chitosan and Sodium Alginate were gift samples from SD Fine Chemicals, Bangalore, India. All other reagents used were of analytical grade.

Methods

Preparation of MX- Hydrochloride Cubosome by Top-down Technique

To prepare MX-Cubosome, different amounts of GMO were weighed first and then heated to 50°C until liquefied. This liquid GMO was then gradually added, dropwise, into a solution of poloxamer 407 preheated to the same temperature of 50°C. After combining the 2, an exact amount of the MX drug was weighed, added to the mix, and stirred until fully integrated. This resulted in a clear lipid solution, which was then slowly incorporated, drop by drop, into preheated distilled water at 50°C while continuously stirring. This mixture was then stirred mechanically at 1500 rpm for 2 hr. Following the incorporation of the lipid phase, the solution was left undisturbed for a day to allow for equilibration, during which a two-phase system formed. After 24 hr of equilibration, the process yielded a milky white cubosome dispersion.[7,8] The specific composition of the resulting cubosomal dispersions can be found in Table 1.

Optimization of Cubosomes

Different polymeric combinations of GMO and poloxamer were used to prepare 12 formulations of MX-Cubosomes. F7 was chosen as the best formulation based on the prepared formulations' particle size, zeta potential, and entrapment effectiveness. To prepare MX-Cubosomes for additional research, this F7 was utilized.

Preparation of MX-Hydrochloride Loaded Cubosomal Ocular *in situ* gel (MX-Cubogel)

MX-cubogel was developed by selecting from an optimized batch of cubosomes. Different *in situ* gel base solutions were prepared by dissolving the polymeric material, specifically sodium alginate, and chitosan, in deionized water and continuously mixing it on a magnetic stirrer until the polymer was completely dissolved. In

Table 1: Formulation of MX- Hydrochloride Cubosomes.

Ingredients	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9	F_{10}	F_{11}	F_{12}
Moxifloxacin Hydrochloride (mg)	5	5	5	5	5	5	5	5	5	5	5	5
GMO (%w/v)	2.5	2.5	2.5	2.5	5	5	5	5	7.5	7.5	7.5	7.5
Poloxamer (mg)	250	500	750	1	250	500	750	1	250	500	750	1
Distilled water (mL)	92.5	92.5	92.5	92.5	90	90	90	90	87.5	87.5	87.5	87.5

the cold mechanical process, chitosan was used as a polymer to create an *in situ* gel, and sodium alginate was used as a co-polymer. The solutions (0.25-1% w/v) were created by dissolving the required chitosan in distilled water and continuously stirring until completely dissolved. A suitable amount of sodium alginate was dissolved in a suitable amount of water. An overhead stirrer was used to stir the fluid. The chitosan/sodium alginate solutions were created by continuously stirring for one hour until the desired concentration of sodium alginate was dispersed. A quantity equivalent of cubosomes containing MX was added to the prepared homogeneous polymeric dispersion solutions. The solution was gradually added to a beaker containing 30 mL of distilled water while continuously stirring at 400-600 rpm. The liquid was constantly swirled for 2 hr until a clear gel formed. As a result, the finished product is known as cubogel. As a preservative, 0.1% w/v benzalkonium chloride was used, along with 0.47% w/v NaCl for tonicity adjustment.[9,10] Table 2 shows the MX-Cubogel composition.

Evaluations

Characterization of MX Cubosomes

Visual Examination

About one week after preparation, the dispersions were visually inspected for optical appearance (color, turbidity, homogeneity, and macroscopic particles).[8]

Particle Size and Zeta Potential

The mean particle size (nm) of the various MX-loaded prepared cubosomal dispersions was determined using the dynamic light scattering technique on a Zeta Seizer (Malvern Instrument Ltd., Malvern, UK). Particle-free purified water was used to dilute the samples.[11] All measurements were made in triplicate at a scattering angle of 90º and a temperature of 25ºC. The zeta potential values of the various MX-Cubosome dispersions were determined using a zeta sizer (Malvern Instruments Ltd., Malvern, UK).

Polydispersity Index

The Polydispersity Index (PDI) calculates the average homogeneity of a particle solution, with higher PDI values indicating a more uniform size distribution in the particle sample.[12,13] PDI was calculated by performing a cumulative analysis of particle size analyzer results with Malvern software in the United Kingdom.

Particle Morphology

A Transmission Electron Microscopy (TEM, Jeol/JEM 2100, Tokyo, Japan) was used to examine the morphology of cubosomal dispersion. A droplet of MX-Cubosome dispersion was placed on a carbon-coated copper grid and stained with a 0.2% sodium phosphotungstate solution; the excess fluid was then removed using absorbent filter paper, and the sample was allowed to dry for 15 min at room temperature to study cubosome morphology.[14]

Entrapment Efficiency

The drug Entrapment Efficiency (EE) was determined by centrifuging (Rotex Centrifuge MR/66) measurable amounts of each of the 12 cubosomal dispersions at 5,000 rpm for 20-30 min. Filtrate was measured using a UV-visible spectrophotometer[12,14] (Shimadzu UV1800, Japan) at absorption Maxima (max) 287 nm, and entrapment efficiency was calculated using the following equation:

$$EE\ (\%) = Ct - Cf/Ct \times 100$$

Characterizations of MX-Cubogel

Visual Clarity and Appearance

Visual observation against a black and white background[15] was used to assess the clarity of all developed formulations.

Table 2: Formulation of MX-Cubogel.

Ingredients	Formulations															
	MB_1	MB_2	MB_3	MB_4	MB_5	MB_6	MB_7	MB_8	MB_9	MB_{10}	MB_{11}	MB_{12}	MB_{13}	MB_{14}	MB_{15}	MB_{16}
MX-Cubosomes (mL).	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	10
Chitosan (gm).	0.25.	0.25	0.25	0.25	0.5	0.5	0.5	0.5	0.75	0.75	0.75	0.75	1	1	1	1
Sodium Alginate (gm).	0.1	0.2	0.3	0.4	0.1	0.2	0.3	0.4	0.1	0.2	0.3	0.4	0.1	0.2	0.3	0.4
Benzalkonium Chloride (%w/v).	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Sodium Chloride (%w/v).	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
Distilled water (mL).	Up to 100 mL															

Determination of pH

A digital pH meter (pH 100 B Ecobasic) was used to test the pH of the developed formulations.[16]

Gelling Capacity Test (Sol-to-gel Transition/*in vitro* Gelation Study)

In order to assess the gelling time of the prepared formulation, its gelling capacity was determined by placing a drop of the formulation into a beaker containing 5 mL of freshly prepared simulated tear fluid that had been equilibrated at 37°C. The gelling time of the formulation was visually monitored. The time required for the process of gelation was recorded. Grades were allocated in accordance with the gel's consistency and the rate at which it solidified over time.The grades were given as follows: gel after a few minutes and dissolves rapidly (+), immediate gelation and remains for a few hours (++), immediate gelation remains for an extended time (+++), and very stiff gel (++++).[17]

Gelation Temperature

The gelation temperature was ascertained by dipping the test tube containing the 2 mL cold sample solution in a water bath that was kept at a temperature of 37.5°C for 2 min. By putting the thermometer inside the test tube, we could record the temperature at which the solution turned into gel. Up to 50°C, the gelation limit was examined.[16] The gel was said to have formed when the formulation did not flow. The experiment was carried out thrice, and the results were noted.

Rheological Studies

After allowing the prepared solutions to gel at physiological temperature, viscosity was measured at 37°C using a Brookfield viscometer (LMDV 100) model in spindle number 62 at 100 rpm. The average of 3 determinations was taken.[17]

Drug Content

A glass rod was used to crush the formed gel completely, which was then vigorously shaken until it was completely dispersed and a clear solution was produced. The drug content of MX hydrochloride was ascertained by diluting 1 mL of the formulation to 50 mL with freshly made Simulated Tear Fluid (STF) having pH 7.4. A sample of 5 mL was taken out, diluted to a final volume of 50 mL with artificial tear fluid, and then subjected to UV spectrophotometer analysis (Shimadzu UV1800, Japan) at a wavelength of 287 nm (Sonjoy 2012).

In vitro Drug Release Study

Franz diffusion cell apparatus was utilized to assess the release mechanism of the drug from an *in situ* gel. 1 mL of the drug formulation was applied to the donor area, slightly above the membrane to begin the experiment. To replicate the drug's diffusion into the eye, 25 milliliters of artificial tear fluid were added to the receptor section. The temperature was kept constant at 37°C (plus or minus 0.5°C) for the experiment, and to ensure even mixing, a magnetic stirrer was used at 50 rpm. At specific intervals, 5 mL of fluid were removed from the receptor compartment to measure the drug concentration. This sample was then diluted with additional synthetic tear fluid to maintain the volume and concentration of the receptor compartment, an equal amount of fresh synthetic tear fluid was added after each sample was taken. The concentration of MX in these samples was determined using a spectrophotometer (Shimadzu UV1800, Japan), which measured the absorbance at a wavelength of 287 nm.[18]

Mechanism of Release Kinetics

Data from *in vitro* drug release studies of optimized formulations were fitted into a zero-order (cumulative percentage of drug released vs. time), first-order (log cumulative percentage of drug remaining vs. time), Higuchi's model (cumulative percentage of drug released vs. square root of time, SQRT), and Korsmeyer-Peppas model in order to understand the kinetics of drug release from *in situ* gel formulation (log percentage cumulative drug release vs. log time). By comparing the obtained r^2 values, the best-fit model was selected.[14]

In vitro Corneal Permeation Study

The same procedure used for *in vitro* drug release studies was applied to corneal permeation investigations. This investigation is done for formulations that are optimized. The sole difference is that *in vitro* corneal permeation studies,[6] goat cornea was used instead of the cellophane membrane used in *in vitro* release studies. The permeation across the corneal membrane was studied using corneas from goats. Franz diffusion cells were used in the investigation so that the corneal side would always be in contact with the formulation inside the donor compartment. The cumulative percentage of drug permeated, flux (J), and apparent permeability coefficient were computed using data from the *in vitro* corneal permeability study (Papp).

Antibacterial Study

The antibacterial properties of the MX cubogel were tested using the agar cup plate method. *Staphylococcus aureus* was selected as the bacteria for this test. Four samples were evaluated to determine the minimal inhibitory concentration. Test samples were carefully introduced into these cups and subsequently labeled. The samples were allowed to diffuse for 2 hr before incubating the petri dish at 37°C for 24 hr. Following incubation, the inhibition zones around each cup were measured and compared to a control sample.[19]

Ocular Tolerance Studies

Histopathology Study

The *ex vivo* ocular tolerability was assessed through light microscopy to examine histological cross-sections of the excised cornea of the goat. The irritation potential of any corneal changes caused by the developed formulation was investigated further. After one hour of incubation in the formulations containing the positive control (0.9% w/v; NaCl), the corneas were rinsed with STF and promptly fixed with a 10% (v/v) formalin solution. Following alcohol treatment, the corneal tissue was immersed in molten paraffin and allowed to solidify into a block shape. Tissue structure modifications were detected through microscopy of prepared cross-sections and stained with hematoxylin and eosin.[20]

RESULTS

Twelve distinct formulations of MX-Cubosomes were prepared. This approach was chosen due to its numerous benefits compared to the bottom-up approach, including reduced time and energy requirements,[6] as well as a greater economic yield. Samples had a generally emulsion-like consistency and a milky appearance.

Evaluation of MX-Cubosomes

The dispersion was evaluated visually for its optical characteristics, including color, turbidity, and the presence of aggregates. A uniformly distributed sample, devoid of any discernible aggregates, with a milky white coloration, was obtained.

Particle Size, Polydispersity Index, Zeta Potential, and Entrapment Efficiency

The results of particle size, polydispersity index, zeta potential, and EE are shown in Table 3.

The prepared MX-Cubosome dispersions' particle size values ranged from 306.5±1.41 to 714.2±3.72 nm. Measurements of particle sizes were performed to verify that all of the dispersion's particles fall within the cubosomal range (10-500 nm). The results indicated that the PDI values ranged from 0.513±0.02 to 0.753±0.03 and were less than one, indicating appropriate uniformity and a high potential for corneal transportation.

The optimized formulation had a uniform particle size distribution and a low PDI of 0.609. The diagrammatic representation of PDI of the best formulation F_7 is shown in Figure 1.

Zeta potential is a marker for the stability of colloidal systems, reflecting the charge on the particles within these systems. Formulations F1 through F12 had zeta potentials that ranged from -27.9±0.09 to -39.8±0.71 mv. More stability is indicated by an increase in the zeta potential's absolute value. As seen in Figure 2, the zeta potential value for F7 is -35.9, indicating stable cubosomes. The diagrammatic representation of the zeta potential for the best formulation, $F_{7,}$ is shown in Figure 2.

The MX-Cubosome dispersions had varying EEs, ranging from 68.83±0.21% to 85.03±0.76%. The concentration of GMO and poloxamer 407 determined the drug content of MX cubic nanoparticles.

Particle Morphology

The morphology was investigated using TEM to verify the formation of cubic structures in the prepared dispersions, and the resulting photomicrographs are shown in Figure 3 (a, b, c). The transmission electron micrographs showed that the prepared cubosomes are nano-sized, confirming the particle size measurement results.

The optimal formulation, F7, was chosen based on the prepared formulations' particle size, zeta potential, and EE and was utilized to prepare MX cubosomal *in situ* gel.

Table 3: Characterization of MX-Cubosomes.

Formulations	Particle Size (nm)	Zeta Potential (-mv)	PDI	Entrapment Efficiency (%)
F_1	306.5±1.41	37.1±0.11	0.680±0.02	75.32±0.12
F_2	384.8±3.60	39.2±0.29	0.612±0.05	71.60±0.23
F_3	355.9±3.12	34.0±0.18	0.583±0.03	69.86±0.16
F_4	410.8±4.53	33.4±0.19	0.513±0.02	68.83±0.21
F_5	398.7±2.46	38.5±0.39	0.712±0.03	84.78±0.69
F_6	402.5±5.32	39.8±0.71	0.673±0.02	84.01±8.82
F_7	431.8±3.61	35.9±0.03	0.609±0.09	85.03±0.76
F_8	484.5±3.28	31.5±0.01	0.594±0.03	81.09±0.92
F_9	541.7±4.31	29.7±0.65	0.753±0.03	79.87±0.81
F_{10}	559.7±1.41	30.1±0.68	0.712±004	77.12±0.82
F_{11}	643.8±2.82	28.7±0.04	0.694±0.03	75.24±0.54
F_{12}	714.2±3.72	27.9±0.09	0.648±0.02	72.12±0.32

Evaluation of MX-Cubogel

Chitosan was utilized as the polymer, and sodium alginate as the co-polymer in the preparation of the *in situ* gelling system. While the co-polymer concentration was varied (0.1%-0.4%), the polymer concentration (0.25, 0.5, 0.75, and 1% w/v) was kept constant. Clarity, pH, *in vitro* gelation study, viscosity, rheological studies, antimicrobial studies, in vitro release studies, and *in vitro* permeation studies were performed.

Clarity Test, pH, Gelling Capacity, Gelation Temperature, Rheological Studies and Drug Content

The results of the clarity test, pH, gelling capacity, gelation temperature, rheological studies, and drug content are shown in Table 4.

Following the *in situ* gel formulation guidelines, a clarity test was conducted against both white and black backgrounds. Various ratios of chitosan were mixed with the co-polymer sodium alginate to produce *in situ* gel compositions. The pH plays a crucial role in ophthalmic formulations. The recorded pH levels ranged between 6.9 and 7.7. Gelation temperature, ranging from 28°C to 43°C for most formulations, dictates the gel's stability. The drug content of moxifloxacin *in situ* gel ophthalmic formulations was assessed using the UV method. The drug content values were between 92.5-97.7%. Formulations MB_1 and MB_5, which contained lower polymer concentrations, showed no gelation up to 50°C. Up to 50°C, the formulations MB_1 and MB_5 showed no gelation. After a few minutes, formulations MB_2, MB_3, MB_4, and MB_9 gel and dissolve quickly. After a few hours, the formulations MB_6, MB_7, MB_8, MB_{10}, MB_{13}, and MB_{14} had immediate gelation. The formulations MB_{11}, MB_{12}, MB_{15}, and MB_{16}, on the other hand, had immediate gelation but remained stable for an extended

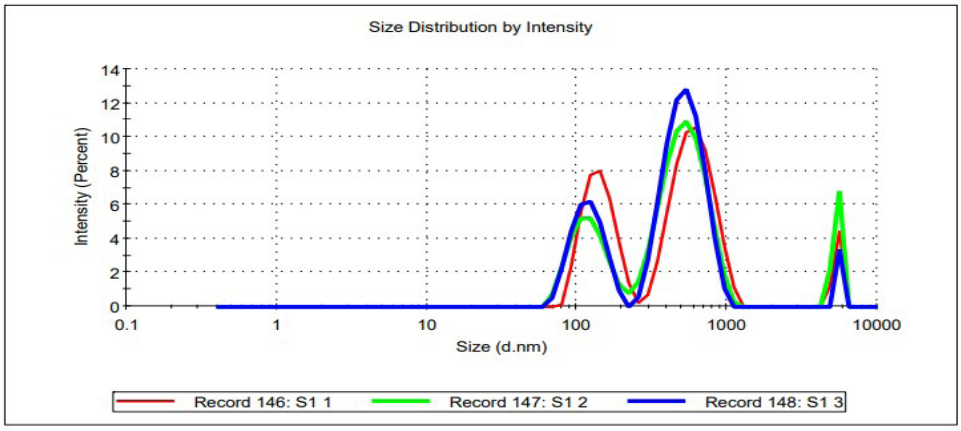

Figure 1: PDI of Optimized Cubosome Dispersion (F_7).

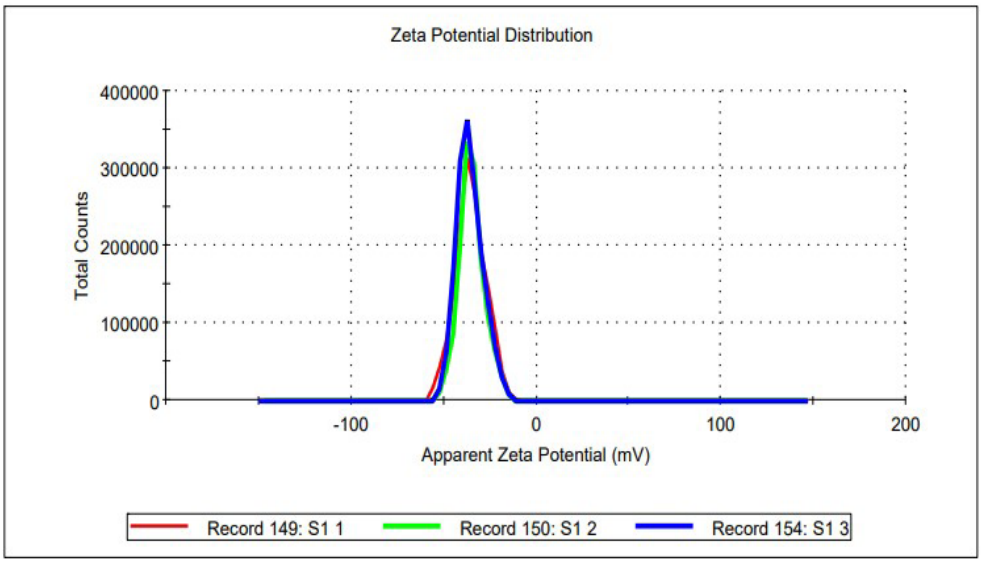

Figure 2: Zeta Potential of Optimized Cubosome Dispersion (F_7).

period. The viscosity of the formulations were ranged from 234.3±0.87 to 1317.8±0.75.

In vitro Release

At room temperature, the cubosomal *in situ* gel formulations of MX were solutions, but at body temperature and pH, they became stiff gels. *In vitro*, drug release studies were determined using the Franz diffusion cell. Four formulations (MB_4, MB_7, MB_{10} and MB_{13}) sustained drug release (>95%) beyond 12 hr, confirming the sustained release behaviour of *in situ* gel formulations.

Figure 4 displays the outcomes of *in vitro* drug release experiments on MX-Cubogel. The values range from 80+1.54% to 98+0.98%.

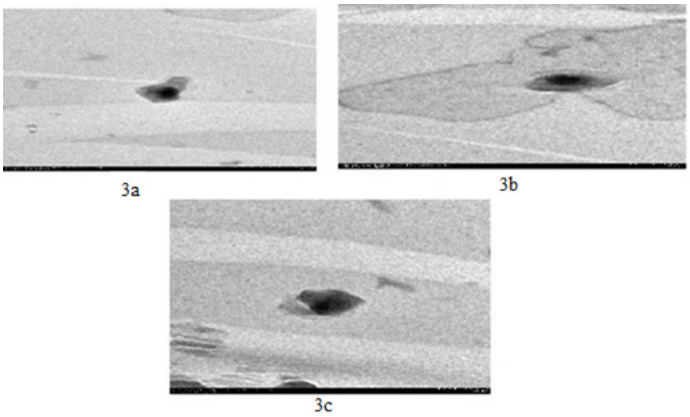

Figure 3: (a, b, c) TEM Image of Optimized Cubosome Dispersion (F_7).

The drug release of the formulations MB4, MB7, MB10, and MB13 is greater than 95%.

Based on the kinetics of the Higuchi and Korsmeyer-Peppas equations, the *in vitro* drug release was described in detail. Using Microsoft Excel 2003 software, a linear regression analysis was used to determine each model's release rates, 'k' and 'n'. Release kinetics was examined for the optimized cubogel formulations, as Table 5 illustrates.

To determine the drug release mechanism, the *in vitro* drug release data of MX-loaded cubogel formulations was subjected to a goodness of fit test using linear regression analysis in accordance with Higuchi's and Korsmeyer-Peppas models. All the formulations followed Higuchi kinetics and according to this, micropore diffusion may regulate the drug release from this formulation.

In vitro Corneal Permeation Study

The penetration ability of the *in situ* gel formulation was significantly higher than that of the other ophthalmic formulations. The enhanced corneal permeation of *in situ* gel could be attributed to the polymer's heightened mucoadhesive properties. Figure 5 provides the data for the *in vitro* drug permeation study. The pattern of MX permeation is comparable to the drug's *in vitro* release.

Table 4: Clarity Test, pH, Gelling Capacity, Gelation Temperature, Rheological Studies, and Drug Content of MX-Cubogel.

Formulations	Clarity	Gelation Temperature (°C)	pH	Gelation Time (Sec)	Drug Content (%)	Gelling Capacity	Viscosity (cps)
MB_1	Clear	No gelation Upto 50°C	6.9	_	93.8±0.87	-	234.3±0.87
MB_2	Clear	43°C	7.0	169.67	95.2±1.76	++	306.6±0.97
MB_3	Clear	40°C	7.1	123.56	96.8±0.97	++	412.4±0.89
MB_4	Clear	38°C	7.4	103.67	97.2±0.87	++	536.8±1.23
MB_5	Clear	No gelation Upto 50°C	7.2	_	95.8±0.72	-	258.3±0.76
MB_6	Clear	39°C	7.5	128.98	96.1±0.69	+++	389.2±0.83
MB_7	Clear	37°C	7.4	107.65	97.7±0.98	+++	472.9±0.98
MB_8	Clear	36°C	7.6	103.45	95.2±0.87	+++	553.7±1.24
MB_9	Clear	38°C	7.3	98.65	96.3±0.76	++	828.7±0.54
MB_{10}	Clear	37°C	7.5	99.54	97.7±0.94	+++	973.2±0.46
MB_{11}	Stiff	32°C	7.7	96.73	94.1±0.65	++++	1064.9±0.56
MB_{12}	Stiff	29°C	7.6	65.67	92.5±0.87	++++	1209.7±0.34
MB_{13}	Clear	36°C	7.4	96.87	97.1±0.56	+++	776.8±0.87
MB_{14}	Clear	30°C	7.2	86.79	93.2±0.87	+++	804.3±0.98
MB_{15}	Stiff	28°C	7.1	55.35	92.5±1.23	++++	1189.3±0.85
MB_{16}	Stiff	28°C	6.9	47.54	93.6±1.87	++++	1317.8±0.75

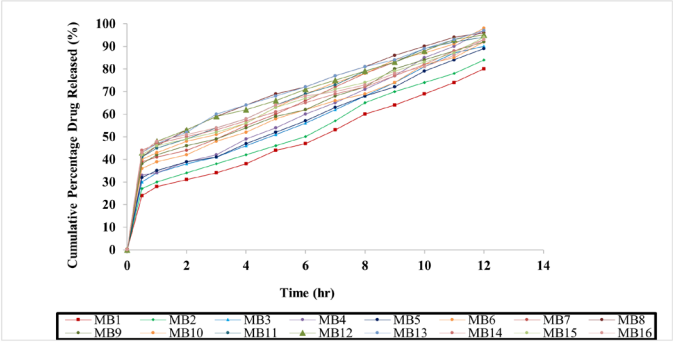

Figure 4: *In vitro* Drug Release of MX-Cubogel.

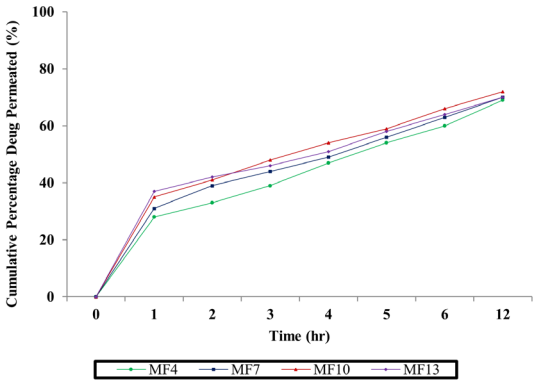

Figure 5: *In vitro* Permeation of MX-Cubogel.

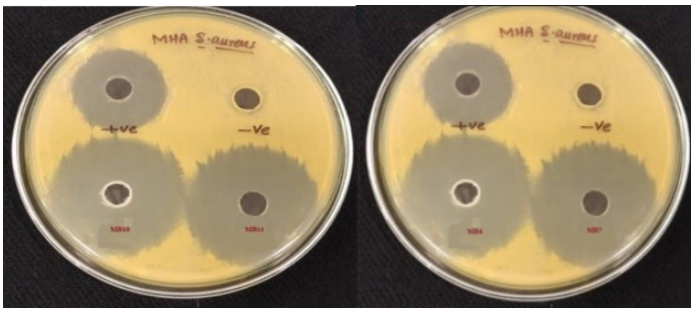

Figure 6: Antibacterial Study of MX-Cubogel (MB_4, MB_7, MB_{10} and MB_{13}).

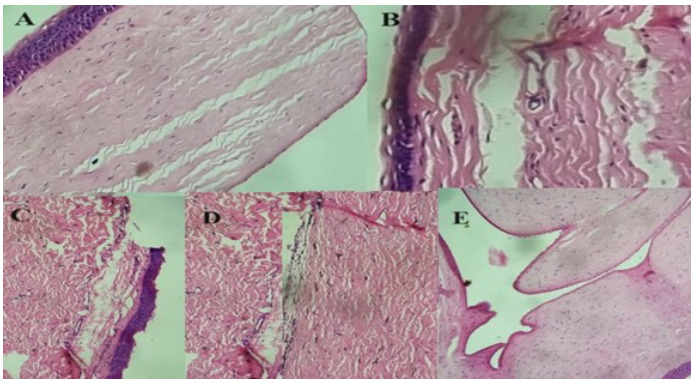

Figure 7: Light Microscopy of (A) Positive Control (1% w/v, SLS) and (B) MB_4, (C) MB_7, (D) MB_{10}, (E) MB_{13}.

Antibacterial Study

The diagrammatic representation of the antibacterial study is shown in Figure 6. The antibacterial efficiency of the selected sustained-release MX HCl formulations was calculated against *Staphylococcus aureus*. The zones of inhibition of standard and ophthalmic formulation were found to be 27 mm and 32 mm. The zone of inhibition of standard and ophthalmic formulations was almost similar. The inhibition zones were evaluated after 24 hr, and a reduction in the growth of microorganisms was observed.

Ocular Tolerance Studies

Histopathology Study

The histopathology results are revealed in Figure 7. The histological examination of goat cornea with the selected formulations (MB_4, MB_7, MB_{10} and MB_{13}) revealed the existence of normal ocular structures observed within the cubosomal formulations. No changes were observed in the epidermal layer after the internalization of the cubosomal formulation inside the cornea.

DISCUSSION

In the current study, 12 formulations of MX Cubosomes were developed by a top-down technique using different concentrations of GMO and poloxamer. Out of these 12 formulations, based on the particle size, zeta potential, and entrapment effectiveness, the F7 was chosen as the best formulation. This F7 was further used to prepare MX cubogel.

MX cubogel was selected from a group of cubosomes (F7) optimized using different polymeric combinations of chitosan and sodium alginate.

From the particle size, polydispersity index, zeta potential, and EE of MX cubosomes, it was observed that the particle size increases with increasing GMO content. The MX cubosome exhibited elevated particle size values after an escalation in the quantity of GMO. The increase in poloxamer 407 concentration is inversely correlated with cubosome particle size. This was because of condensed interfacial stability, which caused the nanoparticles to aggregate.

The zeta potential value indicates the electrical repulsion forces between the particles, strong repulsion forces prevent particles from aggregating and coalescing. From the zeta potential results, the negative zeta potential values for cubosomes can be attributed to the adsorption of free fatty acid found in trace amounts in GMOs. This negative charge arises from ionizing the carboxylic end group in the free fatty acid. Additionally, poloxamer 407 lends a negative charge to the cubic nano-crystals, stemming from interactions between the hydroxyl ions of poloxamer 407 and the aqueous environment.

Table 5: Release kinetics of MX-Loaded Cubosomal *in situ* Gel.

Formulations	Higuchi		Korsmeyer-Peppas		Mechanism of drug release
	R^2	k ($min^{-1/2}$)	R^2	n	
MB_4	0.953	6.180	0.439	0.027	Higuchi
MB_7	0.921	6.050	0.438	0.022	Higuchi
MB_{10}	0.903	5.885	0.433	0.021	Higuchi
MB_{13}	0.873	5.498	0.430	0.018	Higuchi

EE increases when GMO concentration increases to 5%, after which EE falls as GMO concentration rises. The particle morphology study supports the findings of particle size analysis.

One of the most important characteristics of ophthalmic preparations is clarity. A clarity test was performed immediately following the formation of the *in situ* gel formulation against a white and black background. No turbidity, suspended particles, or other contaminants were produced in any formulations, and they all appeared clear or transparent. As a result, all batches of *in situ* gel passed the clarity test.

One of the most important parameters in ophthalmic formulation is pH. The effects of pH on solubility and stability are 2 critical areas. The pH of an ophthalmic formulation should be such that it ensures formulation stability while also not irritating the patient upon administration.[9,16] The pH of ophthalmic formulations should be between 5 and 7. Chitosan undergoes a sol-gel transition at pH 6.5 when the medium changes from mildly acidic to neutral due to its cationic nature. When the pH of chitosan rises, it deionizes and forms a three-dimensional network. When the pH of the medium exceeds 5.5, sodium alginate undergoes a sol-gel transition in aqueous solution.[17] It is not irritating or toxic to people after topical application. The actual pH level measured was between 6.9 and 7.7.

The gelling capacity is the rate and amount of sol-to-gel conversion. The formulation should have a high gelling capacity so that after being injected into the eye as a liquid (drops), it will undergo a rapid sol-to-gel transition and retain its integrity for an extended period without dissolving or eroding. The primary mechanism underlying the formation of complexes between chitosan and sodium alginate is a novel ophthalmic gel-forming mucoadhesive polymer that gels in the presence of divalent-cations (calcium ion) in the lachrymal fluid (pH 7.4). Visual observations revealed that gelling capacity depends on the concentration of the gelling and viscofying agents. Chitosan and sodium alginate form complexes primarily through electrostatic interactions between their amino and carboxyl groups.

Except for formulations MB_1 and MB_5, the gelation temperature of the prepared *in situ* gelling systems was found to be in the range of 28°-43°C. MB_1 and MB_5 showed no sign of gelation up to 50°C, which could be attributed to the lower polymer concentration in those batches.

An ophthalmic formulation must have an optimal viscosity that allows for easy instillation as liquid drops into the eye and undergoes rapid gel transition upon installation in the eye. In the eye, rheological evaluation of all formulations revealed Newtonian flow before and pseudoplastic flow after gelling. Following gelling, the viscosity increased. The viscosity of the formulations was directly proportional to their polymeric content. It was observed that formulations containing a co-polymer and a polymer increased the viscosity of the formulation without impairing its clarity.

The UV method was used to determine the drug content. The drug content of the ophthalmic formulations of MX *in situ* gel was found to be satisfactory (ranging from 92.5 to 97.7%), indicating uniform drug distribution

In vitro drug release studies were conducted using a Franz diffusion cell, revealing that the cubosomal *in situ* gel exhibited significantly enhanced drug release compared to other ocular formulations. The combined action of poloxamer in the cubosome and sodium alginate in the gel may result in a gel network due to cationic influence from tears, further facilitating sustained drug release. Remarkably, over 12 hr, over 95% of MX was released from four distinct formulations. This suggests a sustained release profile for the *in situ* gel formulations. An initial rapid drug release, or "burst release", was noted for the *in situ* gel, which can be advantageous for quickly attaining therapeutic drug concentrations. This burst release is potentially attributed to the drug's initial movement toward the gel matrix's surface. Subsequent observations indicated a consistent, sustained drug release, which can reduce the frequency of drug application daily. In order to determine the mechanism of drug release, the *in vitro* drug release data of MX-loaded cubogel formulations was subjected to a goodness of fit test by linear regression analysis, and all the formulations followed Higuchi kinetics. According to this model, micropore diffusion may regulate the drug release from this formulation.

The aqueous stroma of the cornea serves as the key barrier for hydrophobic agents. In contrast, the epithelium of the cornea, which is lipidic, serves as the primary barrier for hydrophilic drugs. *In situ* gel formulation demonstrated much higher penetration ability than other ophthalmic formulations. The increased mucoadhesive nature of the polymer utilized in

the formulation of *in situ* gel may cause its improved corneal permeation capacity.

The antibacterial study confirmed that the zone of inhibition increased significantly as the amount of drug diffused from the *in situ* gel was increased, and it was confirmed that there was no microbial growth throughout the study.

No changes were observed in the epidermal layer after the internalization of cubosomal formulation inside the cornea after the histopathology study. Based on these observations, it was concluded that the prepared selected formulations were safe for ocular administration

CONCLUSION

The MX-Cubogel developed in this study shows promise as an ocular drug delivery system for treating conjunctivitis, potentially enhancing bioavailability by circumventing hepatic first pass effect, tear drainage, and gastric degradation. This improvement could lead to better patient compliance. The research demonstrated that MX-Cubosome dispersions can be effectively turned into *in situ* gels, delivering sustained medication release and strong antibacterial affects key factors in effective ocular treatments that provide lasting presence on the eye and combat pathogens. Importantly, histopathological studies indicate that the gels do not irritate the cornea, which is vital for patient comfort and adherence to the treatment plan, offering an advantage over traditional eye drops that may cause irritation or require more frequent application.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVATIONS

MX: Moxifloxacin Hydrochloride; GMO: Glyceryl Mono Oleate; Nacl: Sodium Chloride; PDI: Poly Dispersity Index; TEM: Transmission Electron Microscopy; EE: Entrapment Efficiency; STF: Simulated Tear Fluid; MIC: Minimal Inhibitory Concentration; ZOI: Zone of Inhibition.

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