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Research Article

Formulation and Evaluation of Moxifloxacin Hydrochloride Loaded Cubosomal Gel for Ocular Delivery

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Abstract

This study aimed to develop and evaluate an ocular, prolonged-release cubogel formulation of moxifloxacin hydrochloride (MX) to treat conjunctivitis. Using glycerol monooleate and poloxamer 407 in varying concentrations, a cubosome containing the antibiotic MX was developed using a top-down approach. Further, by dispersing optimized cubosomes in a cold in-situ gelling system, MX cubogels were prepared. Following Higuchi's release kinetic model, formulations MA4, MA8, MA11, and MA14 released 95.84, 95.77, 97.45, and 97.68% of MX after 12 hours. The in-vitro corneal permeation study showed that the goat cornea absorbed 81% of MX cubogel than conventional formulations. From the results of antibacterial and histopathological studies, the selected formulations were safe for ocular administration. This study concluded that MX cubogel may be a suitable alternative to conventional eye drops due to its increased permeability and sustained release characteristics.

Keywords: Conjunctivitis, Cubosome, Glycerol monooleate, Moxifloxacin hydrochloride Poloxamer 407

INTRODUCTION

The eye is the window to the human soul because it is a complex and distinctive component of the human body. The majority of the division is made up of the anterior and posterior segments of the human eye. Each of these major components is connected to a particular eye disorder. Conjunctivitis, glaucoma, blepharitis, and cataracts are a few conditions that can affect the anterior segment of the eye. Conjunctivitis occurs when a bacterial or viral attack on the conjunctiva results in eye inflammation. Numerous ophthalmic drug delivery systems can be divided into conventional and cutting-edge drug delivery systems [1,2].

Broad-spectrum fluoroquinolone antibiotic of the fourth generation, moxifloxacin hydrochloride, is primarily used to treat bacterial keratitis and conjunctivitis. Different ophthalmic dosage forms of moxifloxacin, such as ointments, eye drop solutions, gels, and ocular inserts, have been investigated to prolong the time medications remain in the eye after being applied topically. These compositions have somewhat prolonged the corneal contact period but have not been well received due to patient noncompliance and vision impairment brought on by ointments and inserts. Only 5% of the instilled dose of moxifloxacin (MX) eye drops reaches the ocular tissue, and the remainder is eliminated through an ocular barrier as a result of tear production, nasolacrimal drainage, protein binding, systemic absorption, enzymatic breakdown, and Blood Retinal Barrier (BRB), which results in poor bioavailability [3].

The development of various delivery systems has increased ocular residence time, drug penetration through ocular barriers, and ophthalmic bioavailability recently. Some of these systems are prodrugs, stimuli-responsive in-situ gel, and drug delivery vehicles like liposomes, nano or microparticles, niosomes, dendrimers, microneedles, and cubosomes.[2] Due to their high internal surface area, high heat stability, high thermodynamic stability, and capacity to encapsulate hydrophobic, hydrophilic, and amphiphilic substances, cubosomes are one of the unique systems for entrapment drugs for ocular dosage forms.

In recent years, cubosomes have drawn increasing interest as ophthalmic nanocarriers due to their biocompatibility and bioadhesive qualities. Cubosomes are a special type of crystalline liquid phase with cubic crystallographic symmetry formed by the dispersion of self-assembled amphiphilic lipid molecules in aqueous media [4,5]. Therefore, based on its physicochemical characteristics, cubosomes are excellent candidates for the ocular drug delivery system. One common technique to extend the precorneal residence time is to disperse the drug-loaded vesicular system into the in-situ gel

system. Several literatures revealed that cubosomal in-situ gel improved patient compliance and absorption [4,6].

A cubosome dispersed in the in-situ gel is known as cubogel. These cubogel formulations exhibit a dual mechanism, wherein in-situ gel aids in extending the time that formulations are in contact with the cul-de-sac region, and cubosomes boost drug permeability to the cornea [7].

Compared to conventional drug delivery methods, moxifloxacin administration through cubosomal ocular in-situ gel has several benefits, including preventing gastric degradation, the first-pass effect, blood level fluctuations, and lachrymal drainage drug loss. Several literatures revealed that these cubosomes have advantages over chemical permeability enhancers, prodrugs stimuli-responsive in-situ gel, and drug delivery carriers such as liposomes and nano- or microparticles, niosomes, dendrimers and microneedles. [8,9]

Therefore, the objective of this study was to develop and characterize the moxifloxacin cubosomal ocular in-situ gel (MX-Cub) using various polymer compositions in order to increase the bioavailability and patient compliance by avoiding hepatic first-pass metabolism, lachrymal drainage, and gastric degradation.

MATERIALS AND METHODS

Materials

Glyceryl Mono Oleate (GMO) and Moxifloxacin Hydrochloride were bought from Yarrow Chem Products, Mumbai, India. SD Fine Chemicals Bangalore, India gifted Poloxamer 407, Chitosan, and Carbopol 940. All other reagents used were of analytical grade.

Methods

Preparation of Moxifloxacin Cubosomes by Top-Down Technique

Variable amounts of GMO were weighed and heated to 50°C until they flowed freely. These were injected drop by drop into preheated poloxamer 407 solutions maintained at 50°C using a water bath. Subsequently, a precisely weighed amount of the MX drug was introduced to this mixture and thoroughly combined. The resulting clear lipid solution was then added gradually, drop by drop, into preheated distilled water (50°C) while stirring continuously on a magnetic stirrer (EIE Instruments Pvt Ltd, EIE-223ML). This mixture was subjected to mechanical stirring at 1500 rpm for 2 hours. Once the lipid phase was completely added, the solution was set aside for a day to achieve equilibration. A two-phase system formed, which was disrupted by stirring. After equilibrating for 24 hours, milky white cubosome dispersions were produced [10]. The composition of these prepared cubosomal dispersions is presented in **Table 1.**

Table 1: Formulation of Moxifloxacin Loaded Cubosomes

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
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	1000	250	500	750	1000	250	500	750	1000
Distilled water (mL)	92.5	92.5	92.5	92.5	90	90	90	90	87.5	87.5	87.5	87.5

Optimization of Cubosomes

12 different polymeric formulations of MX cubosomes containing GMO and poloxamer were produced. The formulation F7 was chosen as the best formulation based on the prepared formulation's particle size, zeta potential, and entrapment effectiveness. To prepare MX cubosomes for additional research, this F7 was used.

Preparation of Moxifloxacin Loaded Cubosomal Ocular *in-situ* Gel

MX cubogel was made by selecting from a group of cubosomes that had been optimized. Then, the cubosome was diluted in the *in-situ* gel that had already been made. In order to create an *in-situ* gel, chitosan was used as a polymer, and carbopol P934 as a co-polymer. The required quantity of chitosan was dissolved in distilled water and stirred continuously until it was completely dissolved to create the solutions (0.25-1 percent w/v). Carbopol P 934 was applied to the top of this mixture the following day to hydrate it. An overhead stirrer was used to stir the liquid. The required quantity of carbopol was dispersed in the desired concentration after an hour of continuous stirring to create the chitosan/carbopol solutions. The solution was slowly added to the beaker containing 40ml of distilled water while continuously stirring at 400-600 rpm. Two hours of continuous swirling resulted in the formation of a clear gel. Using a magnetic stirrer, the previously created in-situ gel was briefly mixed with cubosome dispersion for 30 minutes. As a result, the finished good is known as cubogel. This involved using 0.47 percent w/v NaCl for tonicity adjustment and 0.1 percent w/v benzalkonium chloride as a preservative [11]. **Table 2** displays the MX cubogel's composition.

Table 2: Formulation of Moxifloxacin loaded cubosomal in-situ gel

Ingredients	MA 1	MA 2	MA 3	MA 4	MA 5	MA 6	MA 7	MA 8	MA 9	MA1 0	MA1 1	MA1 2	MA1 3	MA1 4	MA1 5	MA1 6
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	1.0	1.0	1.0	1.0
Carbopol (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
Benzakonium 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	Up to 100 ml															

Evaluations

Characterization of Moxifloxacin Loaded Cubosomes

Visual Examination

About a week after preparation, the dispersions were visually examined for optical appearance (color, turbidity, homogeneity, and macroscopic particles) [10].

Particle Size

Using a dynamic light scattering technique, the Zeta Seizer (Malvern Instrument Ltd, Malvern, UK) was used to detect the mean particle size (nm) of the various MX-loaded prepared cubosomal dispersions. Samples were diluted in purified water that was free of particles [12].

Poly Dispersity Index (PDI)

The consistency and effectiveness of particle surface modifications throughout the particle sample and nanoparticle aggregation can all be detected using PDI [12,13]. Malvern software, based in the UK, was used to analyze the cumulative results from a particle size analyzer to determine the PDI.

Zeta Potential

The zeta potential values of the various produced MX-loaded cubosomal dispersions were measured using a zeta sizer device (Malvern Instruments Ltd, Malvern, UK). Zeta potential was used to examine the surface charge of the nanoparticles in the created cubosomal dispersion, which is crucial for determining the long-term stability of the colloidal dispersion [10,12]. The high zeta potential values provide enough electric repulsion to prevent the aggregation of the particles.

Particle Morphology

Using a Transmission Electron Microscope (TEM, Jeol/JEM 2100, Tokyo, Japan), the morphology of cubosomal dispersion was examined [10].

Entrapment Efficiency

Measured amounts of each of the 12 cubosomal dispersions were centrifuged at 5,000 rpm for 20–30 min to determine the drug entrapment efficiency (EE). Using a UV-visible spectrophotometer (Shimadzu UV1800, Japan), the filtrate was determined at the absorption maximum (max) of 287 nm.

Characterization Studies of Cubogel

Visual Clarity and Appearance

One of the most crucial qualities of ophthalmic preparations is clarity. Visual observation of all developed formulations against a black-and-white background was used to assess their clarity [15,16].

Determination of *pH*

An ophthalmic formulation's pH should be such that it guarantees formulation stability while also causing no irritation in the patient when administered [8,15]. The pH range for ophthalmic formulations should be between 5 and 7. Using a digital pH meter, the developed formulations were assessed for pH.

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

A drop of formulation was put into a vial with 2 mL of freshly made simulated tear fluid equilibrated at 37 °C to test the system's ability to gel. It was carried out to assess gel formation visually. It was noted how much time gelation required. Different grades were assigned depending on the gel's consistency and how quickly it formed over time. Grades were assigned according to how quickly the gel dissolves after a few minutes (+), how long the gelation lasts after a few hours (++), how long it lasts for an extended period (+++), and how stiff the gel is [8, 16].

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 two minutes. 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. 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

At physiological temperature, the prepared solutions were allowed to gel before having their viscosity measured at 37 °C with a Brookfield viscometer (LMDV 100) model in spindle number 62 at 100 rpm. An average of three results were calculated.[11]

Drug Content

The drug content of MX hydrochloride was ascertained by diluting 1 mL of the formulation to 50 mL with freshly made simulated tear fluid 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 at a wavelength of 287 nm [17].

***In-vitro* Drug Release Study**

The release of MX from the in-situ gel was measured by the diffusion of the drug across a cellophane membrane using a Franz diffusion cell. One milliliter of the formulation was briefly placed in the donor compartment above the membrane. 25 ml of stimulated tear fluid served as receptor media in the receptor compartment (STF). The temperature was maintained at 37±0.5degrees Celsius, and the magnetic stirrer was set to 50 revolutions per minute. At predetermined intervals, 5 ml of the release medium was withdrawn and diluted with the receptor medium, and the receptor compartment was refilled with an equal volume of fresh receptor medium [19]. The drug concentrations in the release medium were analyzed spectrophotometrically (Shimadzu UV1800, Japan) at 287 nm at various time intervals.

Release Kinetics and Mechanisms

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). Comparing the obtained r^2 values led to selecting the model that best fit the data.[13]

***In-vitro* Corneal Permeation Study**

These studies were conducted on optimized formulations. The primary distinction was the substitution of the cellophane membrane, utilized in the in-vitro release study, with a goat cornea for the in-vitro corneal permeation studies. The in-vitro corneal permeability study data provided the basis for calculating the cumulative percentage of drug permeated, flux (J), and the apparent permeability coefficient [18] (Papp).

Sterility Testing

One of the most important prerequisites for an ophthalmic preparation is sterility. The goal of the sterility testing is to find out whether the manufactured ophthalmic medications include live microorganisms. In this sterility tests were carried out for Fluid Thio Glycollate Medium (FTGM) is used to culture aerobic bacteria (*Staphylococcus aureus*) and anaerobic bacteria (*Bacteroides vulgatus*), as well as fungi (*Candida albicans*) Soybean casein digest medium (SCDM) was utilized. The entire study was carried out in a laminar air flow hood under aseptic conditions. Before being used, glassware was autoclaved. All non-autoclavable materials were thoroughly wiped with isopropyl alcohol to make them free from microorganisms

Antibacterial Study

The antibacterial properties of the MX cubosomal in-situ gel 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 labelled. The samples were allowed to diffuse for 2 hours before incubating the petri dish at 37°C for 24 hours. Following incubation, the inhibition zones around each cup were measured and compared to a control sample [19.]

Ocular Tolerance Studies

Histopathology Study

Ex-vivo ocular tolerability was evaluated by histologically examining excised goat cornea cross-sections using light microscopy. Potential irritation caused by the new formulation on the cornea was closely observed. Corneas were exposed to the formulation and a positive control (1% w/v; SLS) for an hour. After the incubation, they were rinsed with STF and promptly preserved in a 10% (v/v) formalin solution. The corneal tissue underwent alcohol treatment, was embedded in melted paraffin, and then solidified into a block. Cross-sectional slices were prepared, dyed with haematoxylin and eosin, and microscopically inspected for any alterations in tissue structure [20,21].

RESULTS

Twelve distinct MX-Cub formulations were prepared using a top-down method. This technique was chosen due to its advantages over the bottom-up method, such as reduced time and energy consumption and a more cost-effective yield.

The visualappearance of the dispersion, such as color, turbidit y, and aggregate presence, was evaluated visually. Typically, the samples appeared emulsion-like and milky without any discernible aggregates.

Particle Size, Poly Dispersity Index, Zeta Potential, and Entrapment Efficiency

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

Table 3: Characterization of MX-Loaded 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

From the particle size measurements verified that the dispersion particles fit within the cubosomal range of 10-500nm.The ideal formulation demonstrated a low PDI of 0.609, indicating a consistent particle size distribution. A graphical representation of the PDI for the optimal formulation F7 can be seen in **Figure 1.**

The zeta potential for the formulations (F1-F12) ranged from -27.9± 0.09 mv to -39.8± 0.71 mv. A greater absolute value of the zeta potential suggests enhanced stability. As seen in Figure 2, the zeta potential value for F7 is -35.9, indicating stable cubosomes [9]. A graphical depiction of the zeta potential for the optimal formulation F7 is presented in **Figure 2.**

The encapsulation efficiency of the various MX-loaded cubosomal dispersions showed a drug content range between 68.83±0.21% and 84.96±0.69%. The results indicated that as the lipid and surfactant content increased, so did the entrapment efficiency.

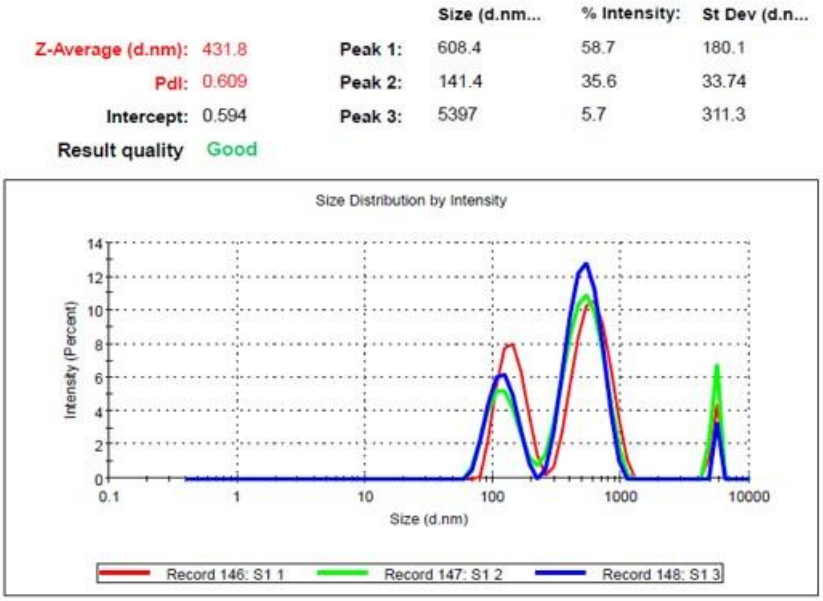


Figure 1: PDI of optimized cubosome dispersion (F_7)

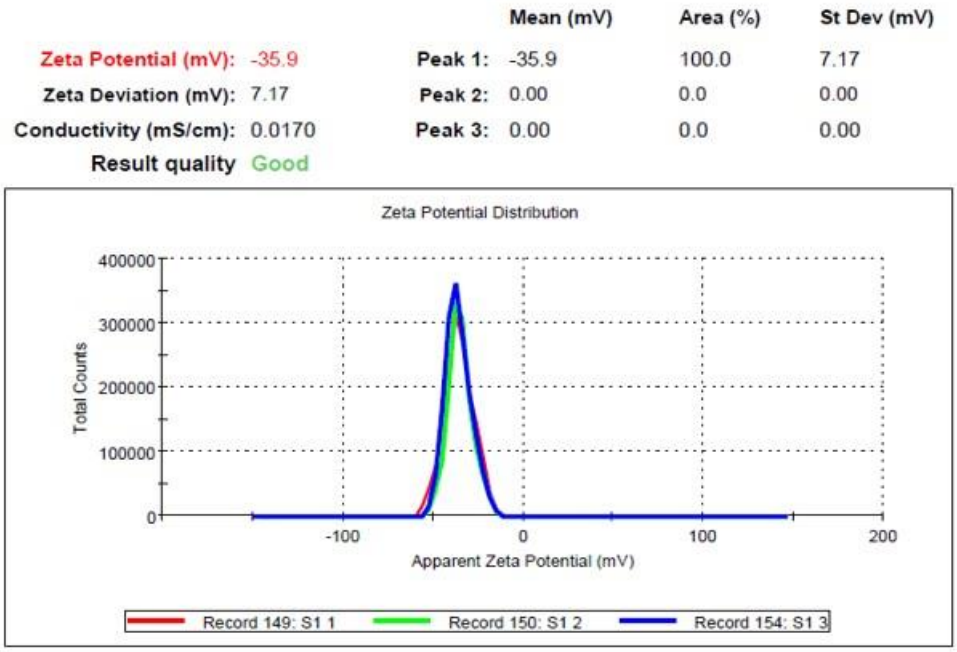


Figure 2: Zeta Potential of optimized cubosome dispersion (F_7)

Particle Morphology

The prepared dispersion's morphology was examined using TEM to confirm the formation of cubic structures and the resulting photomicrographs are shown in **Figure 3(a, b, c).** The prepared cubosomes are nano-sized, as shown by transmission electron micrographs.

The formulation F7 was chosen as the best formulation based on the prepared formulations' particle size, zeta potential and EE. This formulation was used to prepare MX in-situ gel.

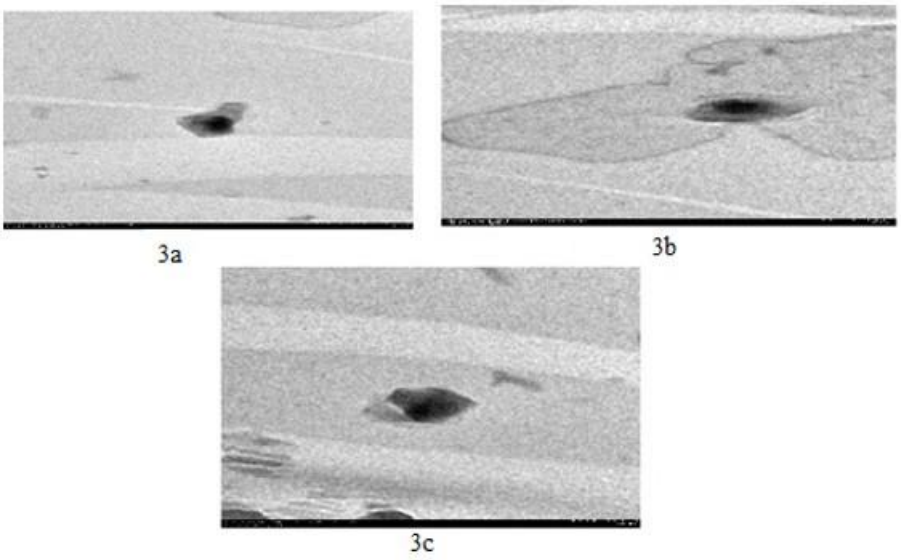


Figure 3 (a, b, c): The TEM image of optimized cubosome dispersion (F_7)

Evaluation of Moxifloxacin Loaded Cubosomal Ocular *in-situ* Gel

Chitosan was used as a polymer, and Carbopol 940 as a co-polymer to develop an in-situ gelling system. While the concentration of the co-polymer was changed, the polymer concentration remained constant at 0.25, 0.5, 0.75, and 1 percent w/v (0.1 percent - 0.4 percent). Clarity, pH, in-vitro gelation study, viscosity, rheological studies, antimicrobial studies, in-vitro release study, and in-vitro permeation study of the prepared in-situ gels were assessed [18].

Clarity test, pH, Gelling Capacity Test, 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.**

Table 4: Clarity Test, pH, Gelling Capacity Test, Gelation Temperature, Rheological Studies, and Drug Content of Moxifloxacin Loaded Cubosomal *in-situ* Gel

Formulations	Clarity	Gelation Temperature (°c)	pH	Gelation Time (Sec)	Drug Content (%)	Gelling Capacity	Viscosity (cps)	*In-vitro* Drug Release (%)
MA1	Clear	No gelation Up to 45°c	6.8	_	92.6±0.87	-	264.3±1.87	82.43±1.82
MA2	Clear	40°C	6.1	198.67	94.2±1.76	+	288.6±0.97	85.16±0.67
MA3	Clear	40°C	7.0	164.56	94.8±0.97	+	312.4±0.89	89.76±0.76
MA4	Clear	39°C	7.2	101.67	96.2±0.87	++	336.8±1.23	95.84±0.12
MA5	Clear	No gelation Up to 45°c	6.9	_	94.8±0.72	-	358.3±0.76	88.23±1.76
MA6	Clear	37°C	7.1	128.98	93.7±0.69	++	389.2±0.83	91.12±0.98
MA7	Clear	35°C	7.3	109.65	95.1±0.98	++	412.9±0.98	93.67±0.76
MA8	Clear	34°C	7.4	102.45	97.2±0.87	+++	453.7±1.24	95.77±0.43
MA9	Clear	36°C	6.9	108.65	95.3±0.76	++	528.7±0.87	92.76±0.85
MA10	Clear	34°C	6.7	103.54	94.7±0.94	+++	573.2±0.56	93.43±0.96
MA11	Clear	32°C	7.3	98.70	96.1±0.65	+++	664.9±0.76	97.45±0.32
MA12	Stiff	29°C	7.2	45.67	94.5±0.87	++++	709.7±0.54	92.45±1.23
MA13	Clear	32°C	6.8	100.87	95.8±0.56	++	776.8±0.98	94.28±0.98
MA14	Clear	31°C	7.2	86.79	96.2±0.87	+++	804.3±0.89	97.68±0.06
MA15	Stiff	28°C	7.1	45.35	94.5±1.23	+++	889.3±0.95	93.14±0.94
MA16	Stiff	28°C	6.9	43.54	93.6±1.87	++++	917.8±0.85	88.65±1.23

Following the in-situ gel formulation guidelines, a clarity test was conducted against both white and black backgrounds. [8] Various ratios of chitosan were mixed with the co-polymer carbopol 940p to produce in-situ gel compositions. The pH plays a crucial role in ophthalmic formulations. The recorded pH levels ranged between 6.1 and 7.4. Gelation temperature, ranging from 28° to 40°C for most formulations, dictates the gel's stability. Formulations MA1 and MA5, which contained lower polymer concentrations, showed no gelation up to 45°C. The drug content of moxifloxacin in-situ gel ophthalmic formulations was assessed using the UV method. The drug content values were between 92.6-96.2%.

***In-vitro* Drug Release**

Data concerning *in-vitro* release percentages of MX from various prepared cubosomal gel dispersions can be found in **Table 4.** These values range from 82±1.82% to 97±0.06%. Notably, the formulations MA4, MA8, MA11, and MA14 demonstrated drug diffusion rates exceeding 95%. A visual representation of the in-vitro release from cubosomal formulations is provided in **Figure 4.**

The *in-vitro* drug release was described in detail based on Higuchi and Korsmeyer-Peppas equation kinetics. With the aid of Microsoft Excel 2003, a linear regression analysis was used to determine each model's release rates k and n. **Table 5** summarizes the outcomes of the linear regression analysis, including the regression coefficients.

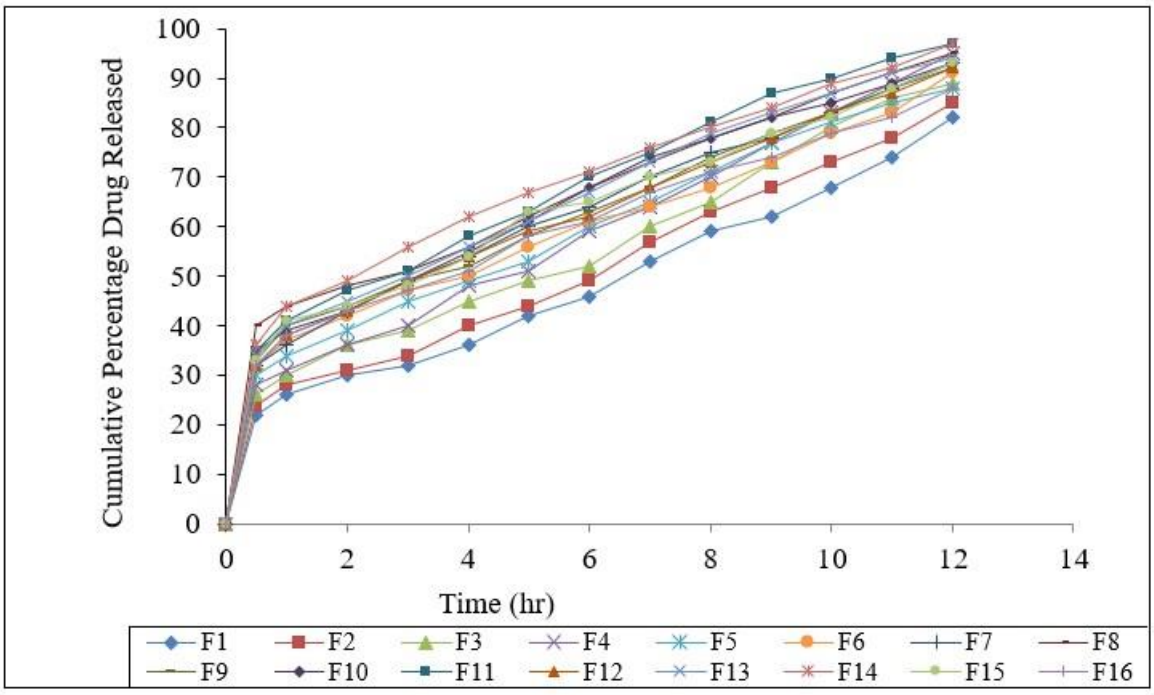


Figure 4: In-vitro release of cubosomal in-situ gel formulations

Table 5: Release Kinetics of Moxifloxacin Loaded Cubosomal *in-situ* Gel

Formulations	Higuchi		Korsmeyer-Peppas		Mechanism of drug release
	R^2	k ($min^{-1/2}$)	R^2	n	
MA_4	0.968	6.301	0.505	0.099	Higuchi
MA_8	0.896	5.742	0.422	0.011	Higuchi
MA_{11}	0.922	6.239	0.453	0.010	Higuchi
MA_{14}	0.892	5.918	0.424	0.011	Higuchi

***In-vitro* Corneal Permeation Study**

The data for the *in-vitro* drug permeation study is given in **Figure 5.** The permeation of moxifloxacin hydrochloride depicts a similar pattern to the *in-vitro* drug release of the same.

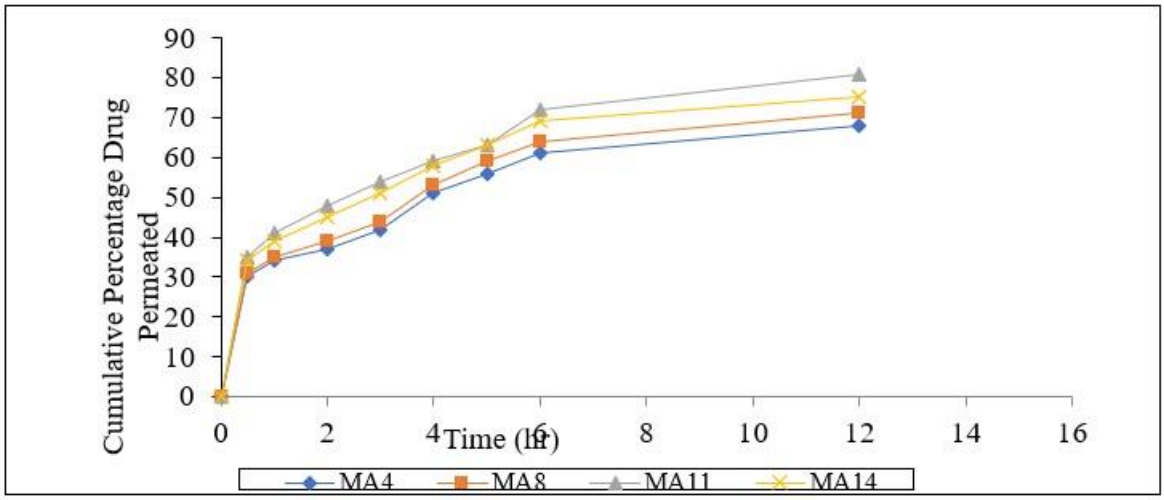


Figure 5. *In vitro* Permeation of Optimised Cubosomal Gel Formulations

Sterility Testing

One of the requirements for ophthalmic preparations is sterility. Microbes in the preparation may irritate, inflame, or infect the eye. After 14 days, there was no growth (no turbidity) in the formulations that had been incubated in media that was appropriate for the growth and proliferation of both aerobic and anaerobic bacteria and fungus. When compared to the positive control sample (which displayed turbidity), the negative control and test samples remained transparent. The "positive control" tube had macroscopic evidence of microbial growth (turbidity), while the "test" and "negative control" tubes exhibited no signs of microbial growth, according to the results of sterility testing in the optimized formulations. The results demonstrated the cubogel's efficacy by autoclaving and suggested that it passed the sterility test for both aerobic and anaerobic bacteria and fungi.

Antibacterial Study

The diagrammatic representation of the antibacterial study is shown in **Figure 6.** The antibacterial efficiency of the selected sustained release moxifloxacin HCl formulations was calculated against staphylococcus aureus. Standard and ophthalmic formulation inhibition zones were 28 mm and 31mm. The zone of inhibition of standard and ophthalmic formulations was almost similar. The inhibition zones were evaluated after 24 h, and a reduction in the growth of microorganisms was observed.

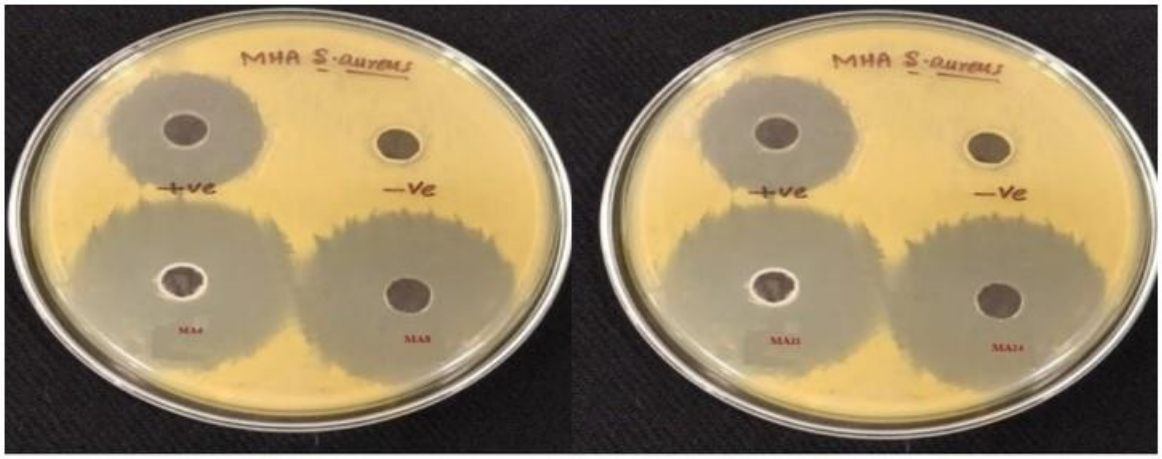


Figure 6. Antibacterial Study of Formulations MA4, MA8, MA11 and MA 14

Ocular Tolerance Studies

Histopathology Study

The histopathology results are revealed in **Figure 7.** The performance of histological examination of goat cornea with the selected formulations (MA4, MA8, MA11, and MA14) revealed the existence of normal ocular structures observed within the cubosomal formulations. No changes were observed in the epidermal layer after the internalization of cubosomal formulation inside the cornea.

FTIR spectra of cubosomes and cubosomal gel are shown in **Figures 8 and 9.**

It can be deduced from the Figures above that no distinctive peaks appeared or vanished.

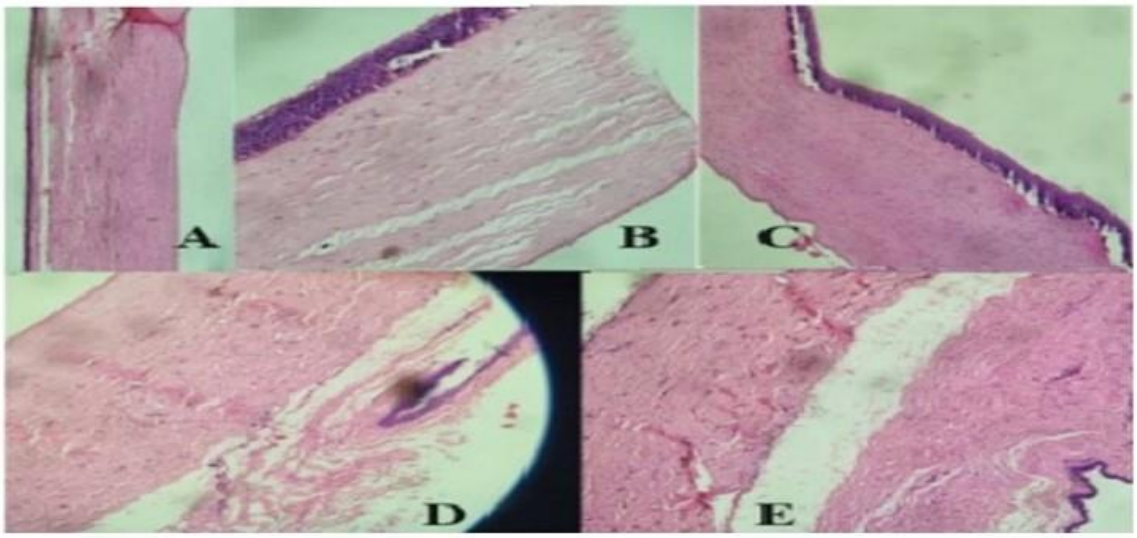


Figure 7. Histopathological Study of Formulations Positive Control, MA4, MA8, MA11 and MA13

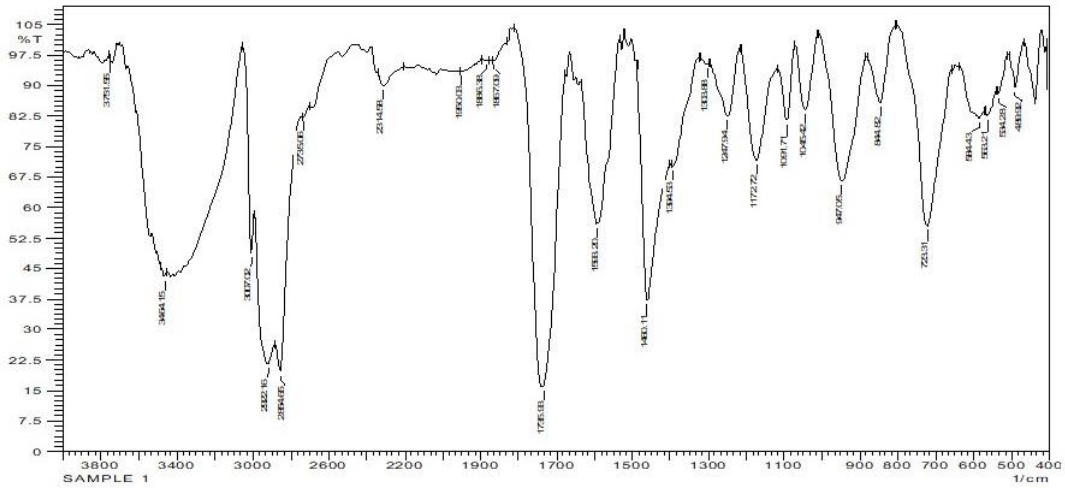


Figure 8. FTIR Spectra of Selected Cubosome Dispersion (F7)

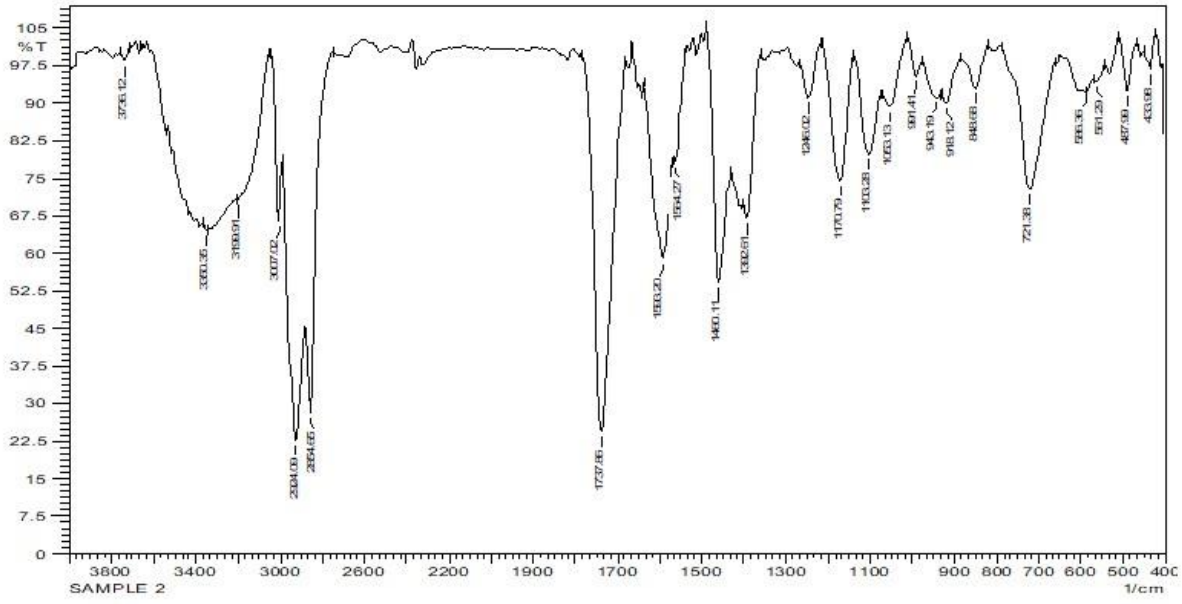


Figure 9. FTIR Spectra of Selected Cubosomal *in situ* Ocular Gel (MA11)

DSC of Cubosomes

Figures 10 and 11 depict the DSC thermogram of cubosomes and cubosomal gel. The melting point of cubosomes and cubosomal gel was indicated by a distinctive peak on the DSC thermogram at 50.35 °C and 49.46 °C, respectively.

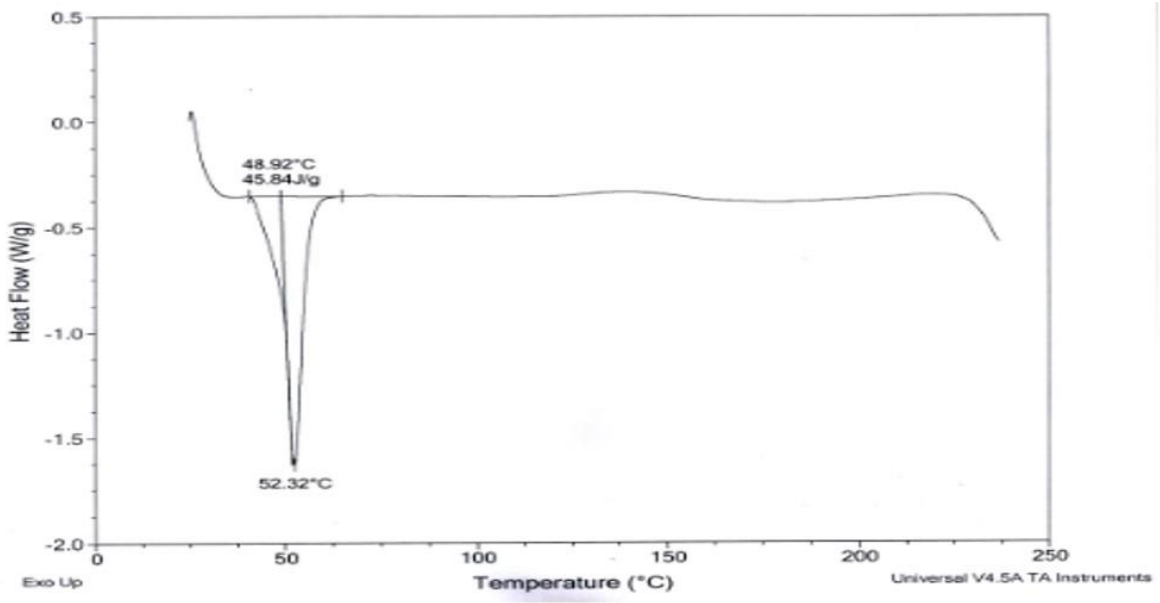
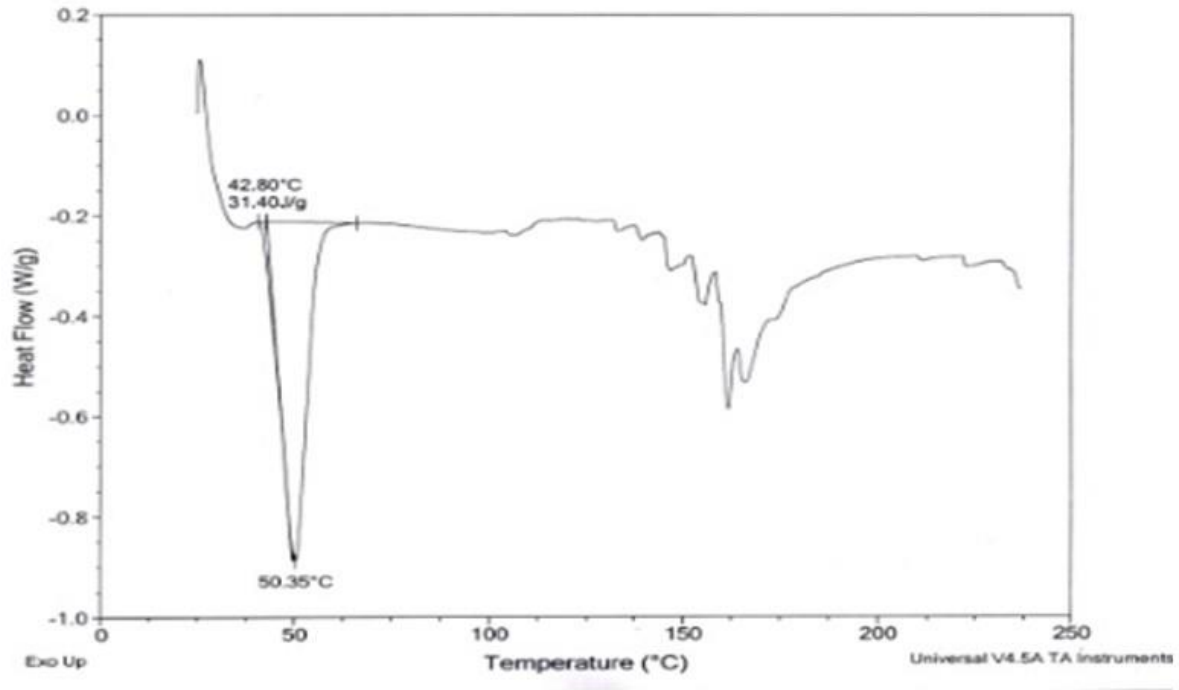


Figure 10. DSC Thermogram of Optimized Cubosome Dispersion (F7)



Figures 11. DSC Thermogram of Selected Cubosomal *in situ* Ocular Gel (MA11)

DISCUSSION

In the current study, 12 formulations of Moxifloxacin 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 carbopol.

From the particle size, polydispersity index, zeta potential, and EE of MX cubosomes, it was observed that as the GMO content increased, there was a corresponding decrease in particle size. This indicates good uniformity and a strong potential for corneal transportation. A rise in GMO content led to higher PS values for the MX-Cub. This phenomenon may arise from the minor shearing effect and a heightened propensity for cubosome aggregation at increased GMO levels. Furthermore, the particle size of cubosomes is inversely related to the concentration of Pluronic F 127 [15].

From the zeta potential results, the negative zeta potential values for cubosomes can be attributed to the adsorption of free fatty acid (specifically oleic 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.

It is essential to determine the drug entrapment efficiency to ensure the inclusion of the intended amount of Moxifloxacin in the cubosome dispersion. The amount of moxifloxacin in the cubic nanoparticles varied based on the concentrations of GMO and poloxamer 407. Notably, when the GMO content increased to 5%, the entrapment efficiency also increased. However, further increasing the GMO concentration led to a decrease in entrapment efficiency.

The particle morphology study supports the findings of particle size analysis.

All produced Moxifloxacin Loaded Cubosomal Ocular *in-situ* gel formulations were free of turbidity, suspended particles, and other contaminants, appearing clear or transparent. Consequently, all in-situ gel batches passed the clarity test.

Optimal gelling capacity ensures that after eye installation, the formulation swiftly transitions from sol-to-gel, maintaining its structure for an extended period. Visual observations revealed that gelling capacity depends on the concentration of the gelling and viscofying agents. Chitosan and Carbopol form complexes primarily through electrostatic interactions between their amino and carboxyl groups. Gelation time is influenced by co-polymer concentration. As the concentration of carbopol 940 rises, the sol-to-gel transition time shortens. Viscosity variations under different conditions are key in both application and in-vivo performance. The optimal formula should offer high viscosity in physiological conditions and low viscosity during storage. The in-situ gel's viscosity was studied using a Brookfield viscometer under varying shear stress conditions. The viscosity increased proportionally with modified chitosan and carbopol 940 concentrations. The drug content uniformity indicated that this indicated a uniform drug distribution.

The cubosomal in-situ gel formulations of MX transitioned from solutions at room temperature to stiff gels at body temperature and specific pH values. 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 carbopol in the gel may result in a gel network due to cationic influence from tears, further facilitating sustained drug release.

Chitosan utilized as a gelling agent in these formulations. It engages in electrostatic interactions with negatively charged mucus, granting the formulated gels improved adhesion to mucous membranes. Remarkably, over 12 hours, 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 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.

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 moxifloxacin HCl 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

In conclusion, this study successfully developed and evaluated a novel ocular, prolonged-release cubogel formulation of MX for treating conjunctivitis. The initially prepared and optimized cubosomes had favourable particle size, zeta potential, and EE. These cubosomes were then incorporated into a cold *in-situ* gelling system to prepare MX cubogels. The MX cubogels exhibited a sustained release of MX, up to and beyond 12 hours, according to Higuchi's release kinetic model. Additionally, in-vitro, corneal permeation, and antibacterial and ocular tolerance studies were favourable for an ophthalmic formulation. Taken together, these findings suggest that MX cubogel has the potential to be a promising alternative to conventional eye drops for the treatment of conjunctivitis. Its enhanced permeability and sustained release characteristics make it a viable option for improving efficacy and patient compliance in ocular drug delivery. Further research and clinical studies may validate its clinical utility in managing ocular infections.

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