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# DEVELOPMENT AND ASSESSMENT OF A GEL CONTAINING MOXIFLOXACIN HYDROCHLORIDE ENCAPSULATED IN CUBOSOMES FOR TARGETED DELIVERY TO THE EYE

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**Abstract**

The objective of this study was to create and assess a sustained-release cubogel formulation of moxifloxacin hydrochloride (MX) for the treatment of conjunctivitis. A cubosome carrying the antibiotic MX was created utilising a top-down method by utilising different quantities of glycerol monooleate and poloxamer 407. In addition, MX cubogels were created by scattering optimised cubosomes in a cold in-situ gelling method. According to Higuchi's release kinetic model, formulations MA4, MA8, MA11, and MA14 released 95.84%, 95.77%, 97.45%, and 97.68% of MX after a duration of 12 hours. The in-vitro corneal permeation investigation shown that the goat cornea absorbed 81% more MX cubogel compared to traditional formulations. Based on the findings of antibacterial and histological tests, it can be concluded that the chosen formulations are suitable for safe administration in the eyes. The study determined that MX cubogel exhibits enhanced permeability and prolonged release properties, making it a potential substitute for traditional eye drops.

**Keywords*:*** Conjunctivitis**,** Cubosome, Glycerol monooleate, Poloxamer 40, Moxifloxacin HCL

## Introduction:

The eye serves as a portal to the essence of the human spirit due to its intricate and unique nature within the human anatomy. The division primarily consists of the anterior and posterior segments of the human eye. Each of these primary constituents is linked to a certain ocular condition. Conjunctivitis, glaucoma, blepharitis, and cataracts are some examples of disorders that can impact the front part of the eye, known as the anterior segment. Conjunctivitis is the inflammation of the conjunctiva caused by a bacterial or viral infection. Ophthalmic drug delivery systems can be categorised into traditional and advanced systems (1,2).

Moxifloxacin hydrochloride, a fourth-generation broad-spectrum fluoroquinolone antibiotic, is mainly prescribed for the treatment of bacterial keratitis and conjunctivitis. Various ophthalmic formulations of moxifloxacin, including ointments, eye drop solutions, gels, and ocular inserts,


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have been studied to extend the duration of drug retention in the eye following topical application. These compositions have slightly extended the duration of corneal contact, but they have not been well accepted due to patients not following the prescribed treatment and experiencing visual problems caused by ointments and inserts. Just 5% of the administered dose of moxifloxacin (MX) eye drops actually reaches the ocular tissue, while the remaining amount is expelled through a protective barrier in the eye.

The outcome of tear formation, nasolacrimal drainage, protein binding, systemic absorption, enzymatic breakdown, and Blood Retinal Barrier (BRB) is a diminished bioavailability. (3).

In recent times, the advancement of different delivery systems has led to an increase in the duration that drugs stay in the eye, their capacity to penetrate ocular barriers, and their availability for ophthalmic use. Several examples of these systems include prodrugs, stimuli-responsive in-situ gel, and various drug delivery vehicles such as liposomes, nano or microparticles, Niosomes, dendrimers, microneedles, and cubosomes (2). Cubosomes are a unique mechanism for trapping pharmaceuticals in ocular dosage forms because they have a large interior surface area, high thermal stability, high thermodynamic stability, and the ability to encapsulate hydrophobic, hydrophilic, and amphiphilic compounds.

Cubosomes have been more popular as ocular nanocarriers in recent years because of their biocompatibility and bio adhesive properties. Cubosomes are a unique sort of crystalline liquid phase that exhibit cubic crystallographic symmetry. They are created by dispersing self-assembled amphiphilic lipid molecules in water-based solutions (4,5). Hence, considering its physicochemical properties, cubosomes exhibit great potential as a suitable option for delivering drugs to the eyes. A commonly used method to prolong the amount of time a drug stays in the front part of the eye is to distribute the drug-containing vesicular system into the gel that forms in the eye.

The system. Multiple studies have shown that the use of cubosomal in-situ gel has resulted in enhanced patient compliance and absorption rates (4,6).

The term "cubogel" refers to a Cubosome that is spread throughout an in-situ gel. The cubogel formulations have a dual action. The in-situ gel helps to prolong the contact duration of the formulations with the cul-de-sac region, while the cubosomes enhance the permeability of drugs to the cornea (7).

Moxifloxacin administration by cubosomal ocular in-situ gel has various advantages over traditional drug delivery methods. These include protection against gastrointestinal degradation, the first-pass effect, changes in blood levels, and loss of the medication through lachrymal drainage. Multiple studies have shown that cubosomes offer superior benefits compared to chemical permeability enhancers, prodrugs stimuli-responsive in-situ gel, and other drug delivery carriers such as liposomes, nano- or microparticles, Niosomes, dendrimers, and microneedles. (8,9).The aim of this study was to create and analyse a moxifloxacin cubosomal ocular in-situ gel (MX-Cub) using different polymer compositions. The goal was to enhance the bioavailability of the drug and improve patient compliance by preventing hepatic first-pass metabolism, lachrymal drainage, and gastric degradation(10).



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**Material and Method:**

**Materials:**

Glyceryl Mono Oleate (GMO) and Moxifloxacin Hydrochloride were purchased from Yarrow Chem Products, located in Mumbai, India. SD Fine Chemicals, located in Bangalore, India, generously donated Poloxamer 407, Chitosan, and Carbopol 940. All other reagents utilised were of analytical grade.

**Methods:**

**Production of Moxifloxacin Cubosomes using the Top-Down Technique**

Different quantities of genetically modified organisms (GMOs) were measured and subjected to heating at a temperature of 50°C until they became fluid and could flow easily. The substances were administered sequentially into preheated poloxamer 407 solutions, which were kept at a constant temperature of 50°C using a water bath. Afterwards, a carefully measured quantity of the MX medication was added to this mixture and properly mixed. The transparent lipid solution was added slowly and incrementally to preheated distilled water (50°C) while constantly swirling on a magnetic stirrer (EIE Instruments Pvt Ltd, EIE-223ML). The combination underwent mechanical agitation at a speed of 1500 revolutions per minute for a duration of 2 hours. After the lipid phase was fully added, the solution was left undisturbed for a day to reach equilibrium. A biphasic system was generated, which was agitated by stirring. After undergoing a 24-hour equilibration period, milky white Cubosome dispersions were generated. The formulation of these prepared cubosomal dispersions is outlined 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 |
| Distilledwater(mL) | 92.5 | 92.5 | 92.5 | 92.5 | 90 | 90 | 90 | 90 | 87.5 | 87.5 | 87.5 | 87.5 |

**Optimization of Cubosomes**

A total of 12 distinct polymeric formulations of MX cubosomes, comprising GMO and poloxamer, were synthesised. Formulation F7 was selected as the optimal formulation based on the particle size, zeta potential, and entrapment efficiency of the developed formulations. In order to facilitate further investigation, the F7 was employed to prepare MX cubosomes.



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

MX cubogel was created by carefully choosing from a set of cubosomes that had been fine-tuned. Subsequently, the cubosome was diluted in the pre-existing in-situ gel. Chitosan was employed as a polymer, while carbopol P934 served as a co-polymer, to produce an in-situ gel. The specified amount of chitosan was dissolved.

The mixture was immersed in distilled water and agitated continuously until it achieved complete dissolution, resulting in the formation of solutions with concentrations ranging from 0.25 to 1 percent weight/volume (w/v). The next day, Carbopol P 934 was added to the top of this mixture to provide hydration. A centrifugal mixer was employed to agitate the liquid. The specified amount of carbopol was evenly distributed in the intended concentration following one hour of uninterrupted agitation to produce the chitosan/carbopol solutions. The solution was gradually introduced into the beaker holding 40ml of distilled water while stirring constantly at a speed of 400-600 rpm. After two hours of uninterrupted agitation, a transparent gel was formed. The in-situ gel that was previously prepared was mixed with a cubosome dispersion using a magnetic stirrer for a duration of 30 minutes. Consequently, the final product is referred to as cubogel. The process included the utilisation of a solution containing 0.47 percent weight/volume of sodium chloride for adjusting the tonicity, and a solution containing 0.1 percent weight/volume of benzalkonium chloride as a preservative (11). Table 2 presents the composition of the MX cubogel.

**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** | **MA 10** | **MA 11** | **MA 12** | **MA 13** | **MA 14** | **MA 15** | **MA 16** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MX Cubosoms(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 |
| *Benzakonim 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 |
| SodiumChloride(%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 to100ml | | | | | | | | | | | | | | | |



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**Evaluations:**

**Characterization of Moxifloxacin Loaded Cubosomes**

**Visual Examination:**

After approximately one week of preparation, the dispersions were visually inspected to assess their optical characteristics, including colour, turbidity, homogeneity, and the presence of macroscopic particles.

**Particle Size:**

The Zeta Seizer, a device manufactured by Malvern Instrument Ltd in Malvern, UK, was employed to measure the average particle size (in nanometers) of different cubosomal dispersions loaded with MX, using a dynamic light scattering technique. The samples were diluted in particle-free filtered water (12).

**Poly Dispersity Index (PDI):**

PDI 12,13 can be used to determine the uniformity and efficacy of particle surface changes across the particle sample as well as the aggregation of nanoparticles. The UK-based company, Malvern software, was utilised to analyse the combined outcomes from a particle size analyser in order to ascertain the PDI (Polydispersity Index) (12,13).

**Zeta Potential:**

The zeta potential values of the several MX-loaded cubosomal dispersions were determined using a zeta sizer equipment manufactured by Malvern Instruments Ltd, located in Malvern, UK. The zeta potential was employed to analyse the surface charge of the nanoparticles in the produced cubosomal dispersion. This is essential for assessing the colloidal dispersion's long-term stability (10,12). The elevated zeta potential levels generate sufficient electrical repulsion to inhibit particle aggregation.

**Particle Morphology:**

The shape of cubosomal dispersion was analysed using a Transmission Electron Microscope (TEM, Jeol/JEM 2100, Tokyo, Japan).

**Entrapment Efficiency:**

The drug entrapment efficiency (EE) of each of the 12 cubosomal dispersions was determined by centrifuging measured quantities at 5,000 rpm for 20–30 min. The filtrate was analysed using a UV-visible spectrophotometer (Shimadzu UV1800, Japan) to measure its absorption at the maximum wavelength of 287 nm.

**Characterization Studies of Cubogel**

**Visual Clarity and Appearance:**

Clarity is an essential characteristic of ophthalmic medicines. The clarity of all created formulations was assessed through visual observation on a black-and-white backdrop (15,16).



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**Determination of pH:**

The pH of an ophthalmic formulation should be carefully selected to provide both formulation stability and patient comfort during administration [8,15]. The optimal pH range for ophthalmic formulations should be maintained within the range of 5 to 7. The pH of the produced formulations was evaluated using a digital pH metre.

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

To evaluate the gelation capability of the system, a small amount of formulation was added to a vial containing 2 mL of simulated tear fluid that had been recently prepared and equilibrated at a temperature of 37 °C. The purpose of the experiment was to visually evaluate the production of gel. The duration of gelation was observed. Grades were allocated based on the viscosity of the gel and its rate of formation over a period of time. Grades were determined based on the rate of gel dissolution within a few minutes (+), the duration of gelation after a few hours (++), the duration of gelation over an extended length of time (+++), and the stiffness of the gel (8,16).

**Gelation Temperature:**

The gelation temperature was determined by immersing a test tube containing a 2 ml cold sample solution in a water bath maintained at a temperature of 37.5 °C for a duration of two minutes. By inserting the thermometer into the test tube, we were able to measure the precise temperature at which the solution underwent gelation. The gelation limit was investigated up to a temperature of 50°C. The gel was said to have developed when the formulation exhibited non-flowing behaviour. The experiment was conducted three times, and the outcomes were recorded.

**Rheological Studies:**

At the standard body temperature, the solutions were allowed to solidify before their thickness was measured at 37 °C using a Brookfield viscometer (LMDV 100) model with spindle number 62 rotating at 100 revolutions per minute. A mean of three findings was calculated (11).

**Drug Content:**

The drug concentration of MX hydrochloride was determined by diluting 1 mL of the formulation with 50 mL of newly prepared simulated tear fluid with a pH of 7.4. A 5 mL sample was extracted, and then diluted with artificial tear fluid to reach a final amount of 50 mL. The diluted sample was subsequently analysed using a UV spectrophotometer at a wavelength of 287 nm (17).

**In-vitro Drug Release Study:**

The liberation of MX from the in-situ gel was assessed by observing the movement of the drug through a cellophane membrane using a Franz diffusion cell. A volume of one millilitre of the formulation was momentarily introduced into the donor compartment located above the membrane. The receptor compartment (STF) contained 25 ml of stimulated tear fluid as the receptor media. The temperature was consistently maintained at 37±0.5 degrees Celsius, while the magnetic stirrer was adjusted at a speed of 50 revolutions per minute. At specified time intervals, 5 ml of the release medium was extracted and mixed with the receptor medium. The



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receptor compartment was then replenished with an equivalent volume of new receptor medium (19). The drug concentrations in the release media were measured using spectrophotometry (Shimadzu UV1800, Japan) at a wavelength of 287 nm at different time intervals.

**Release Kinetics and Mechanisms:**

The data obtained from in-vitro drug release studies of the optimised formulations were analysed using various mathematical models to determine the kinetics of drug release from the in-situ gel formulation. These models included the zero-order model (which plots the cumulative percentage of drug released against time), the first-order model (which plots the logarithm of the cumulative percentage of drug remaining against time), Higuchi's model (which plots the cumulative percentage of drug released against the square root of time), and the Korsmeyer-Peppas model (which plots the logarithm of the percentage cumulative drug release against the logarithm of time). The process of comparing the acquired r2 values allowed for the selection of the model that most accurately matched the data (13).

**In-vitro Corneal Permeation Study:**

The research was carried out on formulations that had been optimised. The main difference was the replacement of the cellophane membrane, which was used in the in-vitro release study, with a goat cornea for the in-vitro corneal permeation studies. The findings from the in-vitro corneal permeability investigation served as the foundation for determining the cumulative percentage of medication penetrated, the flux (J), and the apparent permeability coefficient (Papp) (18).

**Sterility Testing:**

Sterility is a crucial need for any ophthalmic preparation. The objective of sterility testing is to determine the presence of viable microorganisms in the manufactured ophthalmic drugs. Sterility tests were conducted for the Fluid ThioGlycollate Medium (FTGM) to cultivate aerobic organisms.

The bacteria Staphylococcus aureus and Bacteroidesvulgatus, as well as the fungus Candida albicans, were grown using a medium called Soybean casein digest media (SCDM). The entire study was conducted within a laminar air flow hood, ensuring aseptic conditions. Prior to utilisation, glassware underwent autoclaving. Isopropyl alcohol was used to sterilise any components that could not be autoclaved, ensuring the removal of microorganisms.

**Antibacterial Study:**

The antibacterial efficacy of the MX cubosomal in-situ gel was evaluated using the agar cup plate method. The bacterium chosen for this test was Staphylococcus aureus. Four samples were assessed to ascertain the minimum inhibitory concentration. The test samples were meticulously inserted into these cups and then marked. The samples underwent a diffusion process for a duration of 2 hours, followed by incubation of the petri dish at a temperature of 37°C for a period of 24 hours. After the period of incubation, the areas of inhibition surrounding each cup were measured and then compared to a control sample labelled as (19).



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**Ocular Tolerance Studies**

**Histopathology Study:**

The tolerability of the goat cornea was assessed ex-vivo by histologically evaluating cross-sections of removed corneas using light microscopy. The potential corneal irritation produced by the new composition was carefully monitored. The corneas were subjected to the formulation and a positive control (1% w/v; SLS) for a duration of one hour. Following the incubation period, the samples were washed with STF and immediately immersed in a 10% (v/v) formalin solution for preservation. The corneal tissue was subjected to alcohol treatment, subsequently immersed in molten paraffin, and finally formed into a block. Sections were obtained by cutting perpendicular to the long axis of the tissue, stained with haematoxylin and eosin, and examined under a microscope for any changes in the structure of the tissue (20,21).

**Results:**

A total of twelve unique MX-Cub formulations were created utilising a top-down approach. The selection of this methodology was based on its superiority over the bottom-up method in terms of time and energy efficiency, as well as its ability to provide a higher yield at a lower cost.

The visual assessment of the dispersion's visual characteristics, including colour, turbidity, and presence of aggregates, was conducted. The samples had a characteristic emulsion-like appearance, appearing milky and lacking any noticeable particles.

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

Table 3 displays the findings for particle size, polydispersity index, zeta potential, and EE.

**Table 3: Characterization of MX-Loaded Cubosomes**

| Formulations | Particle Size(nm) | Zeta Potential(-mv) | PDI | EntrapmentEfficiency |
|---|---|---|---|---|
| **F1** | 306.5±1.41 | 37.1±0.11 | 0.680±0.02 | 75.32±0.12 |
| **F2** | 384.8±3.60 | 39.2±0.29 | 0.612±0.05 | 71.60±0.23 |
| **F3** | 355.9±3.12 | 34.0±0.18 | 0.583±0.03 | 69.86±0.16 |
| **F4** | 410.8±4.53 | 33.4±0.19 | 0.513±0.02 | 68.83±0.21 |
| **F5** | 398.7±2.46 | 38.5±0.39 | 0.712±0.03 | 84.78±0.69 |
| **F6** | 402.5±5.32 | 39.8±0.71 | 0.673±0.02 | 84.01±8.82 |
| **F7** | 431.8±3.61 | 35.9±0.03 | 0.609±0.09 | 85.03±0.76 |
| **F8** | 484.5±3.28 | 31.5±0. | 0.594±0. | 81.09±0.92 |



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|---|---|---|---|---|
| **F9** | 541.7±4.31 | 29.7±0.65 | 0.753±0.03 | 79.87±0.81 |
| **F10** | 559.7±1.41 | 30.1±0.68 | 0.712±004 | 77.12±0.82 |
| **F11** | 643.8±2.82 | 28.7±0.04 | 0.694±0.03 | 75.24±0.54 |
| **F12** | 714.2±3.72 | 27.9±0.09 | 0.648±0.02 | 72.12±0.32 |

The particle size studies confirmed that the dispersed particles fall within the cubosomal range of 10-500nm.The optimal formulation exhibited a low Polydispersity Index (PDI) of 0.609, indicating a uniform particle size distribution. Figure 1 displays a graphical representation of the PDI for the best formulation F7.
The zeta potential values for the formulations (F1-F12) varied between -27.9±0.09 mV and -39.8±0.71 mV. Increased magnitude of the zeta potential indicates improved stability. According to the information presented in Figure 2,

The zeta potential value for F7 is -35.9, which suggests that the cubosomes are stable. Figure 2 displays a graphical representation of the zeta potential for the most effective formulation, F7(9).
The encapsulation efficiency of the several MX-loaded cubosomal dispersions exhibited a drug content range of 68.83±0.21% to 84.96±0.69%. The findings demonstrated a positive correlation between the increase in lipid and surfactant content and the corresponding rise in entrapment efficiency.

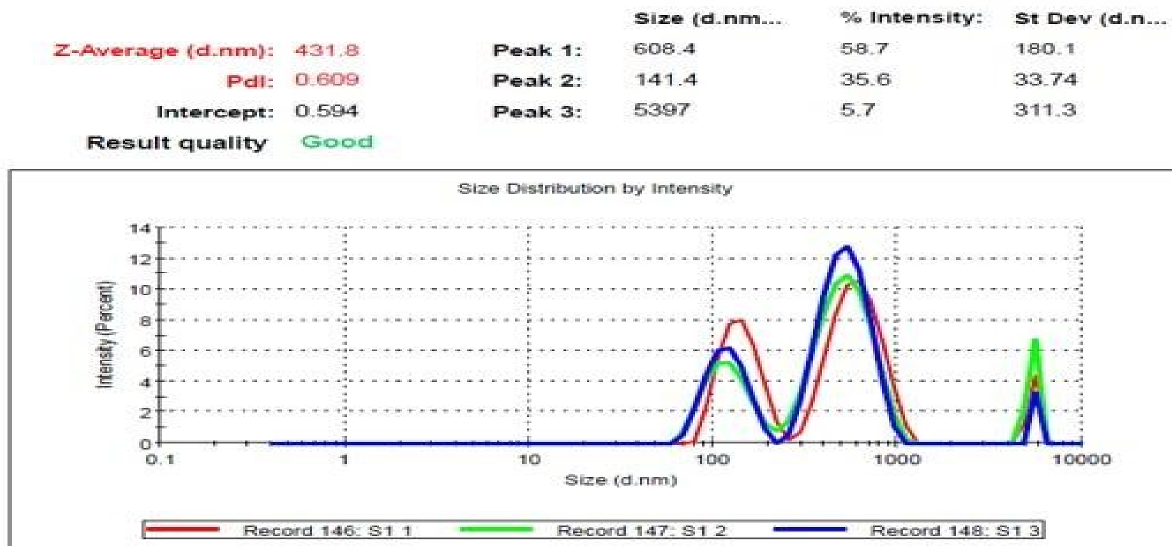


**Figure 1: PDI of optimized cubosome dispersion ($F_7$)**



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**Particle Morphology:**

The morphology of the generated dispersion was analysed using TEM to verify the presence of cubic structures. The obtained photomicrographs may be observed in Figure 3(a, b, c). The text The cubosomes that were created had a nano-sized structure, as demonstrated by the transmission electron micrographs.

Formulation F7 was selected as the optimal formulation based on the particle size, zeta potential, and encapsulation efficiency (EE) of the developed formulations. The aforementioned formulation was employed to synthesise MX in-situ gel.

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

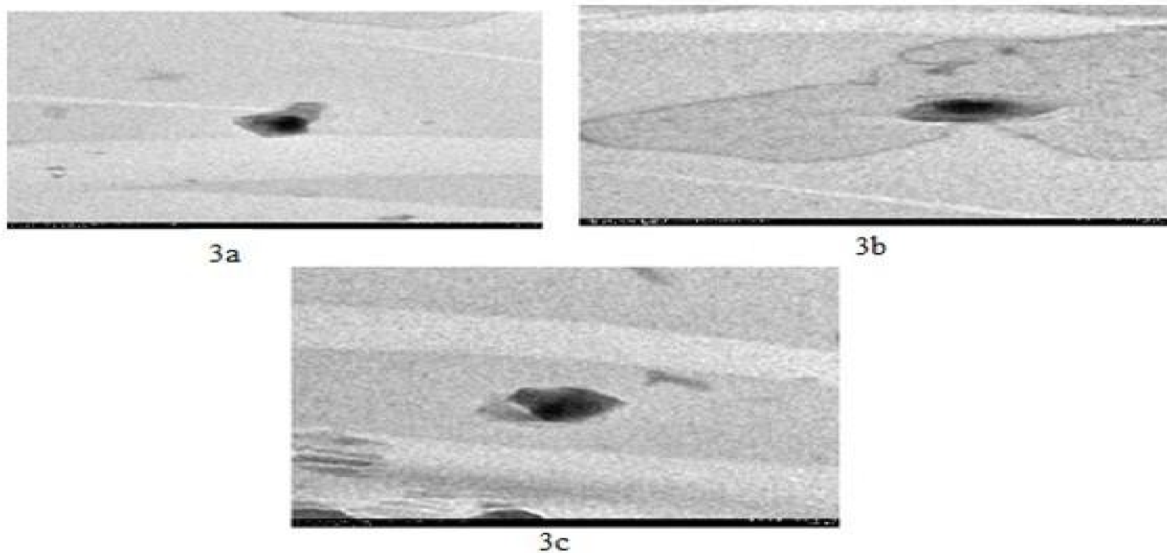


**Figure 3 (a, b, c): The TEM image of optimized cubosome dispersion ($F_7$)**

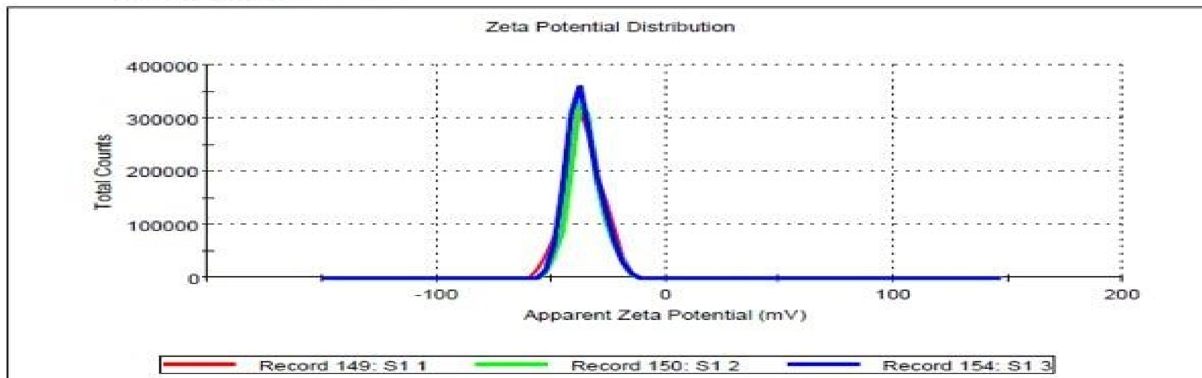


**Figure 2: Zeta Potential of optimized cubosome dispersion ($F_7$)**



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The in-situ gelling system was developed using chitosan as the polymer and Carbopol 940 as the co-polymer. The co-polymer concentration was varied, while the polymer concentration remained constant at 0.25%, 0.5%, 0.75%, and 1% w/v (0.1% - 0.4%). Clarity, pH, in-vitro gelation research, viscosity, rheological studies, antibacterial

An assessment was conducted on the studies, in-vitro release research, and in-vitro permeation study of the generated in-situ gels, which were evaluated at (18).

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

**Table 4 displays the outcomes of the clarity test, pH measurement, gelling capacity assessment, gelation temperature analysis, rheological tests, and drug content evaluation.**

| Formulations | Clarity | Gelation Temperature(°c) | pH | Gelation Time(Sec) | DrugContent (%) | Gelling Capacity | Viscosity (cps) | *In-vitro*Drug Release(%) |
|---|---|---|---|---|---|---|---|---|
| **MA1** | Clear | No gelation Up to45°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 | Nogelation Upto45°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. | 98.70 | 96.1±0.65 | +++ | 664.9±0. | 97.45±0.32 |


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|---|---|---|---|---|---|---|---|---|
| | | | 3 | | | | 76 | |
| **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 |

A clarity test was conducted according to the in-situ gel formulation criteria, using both white and black backgrounds. The user's text is "[8]". Different proportions of chitosan were combined with the co-polymer carbopol 940p to create in-situ gel compositions. The pH is essential in ophthalmic formulations. The measured pH values varied from 6.1 to 7.4. The gel's stability is determined by its gelation temperature, which typically falls between 28°C and 40°C for most formulations. Formulations MA1 and MA5, characterised by lower polymer concentrations, did not undergo gelation even when exposed to temperatures up to 45°C. The concentration of moxifloxacin in in-situ gel ophthalmic formulations was determined using the UV technique. The drug content readings ranged from 92.6% to 96.2%.

**In-vitro Drug Release:**

Table 4 contains information on the in-vitro release percentages of MX from different cubosomal gel dispersions that were generated. The values vary from 82±1.82% to 97±0.06%. The formulations MA4, MA8, MA11, and MA14 exhibited drug diffusion rates that surpassed 95%. Figure 4 presents a graphical depiction of the release of substances from cubosomal formulations in a laboratory setting.

The drug release in a controlled laboratory setting was thoroughly explained using the Higuchi and Korsmeyer-Peppas equation kinetics. Using Microsoft Excel 2003, a linear regression analysis was employed to calculate the release rates k and n for each model. Table 5 presents a concise overview of the results obtained from the linear regression analysis, which includes the regression coefficients.



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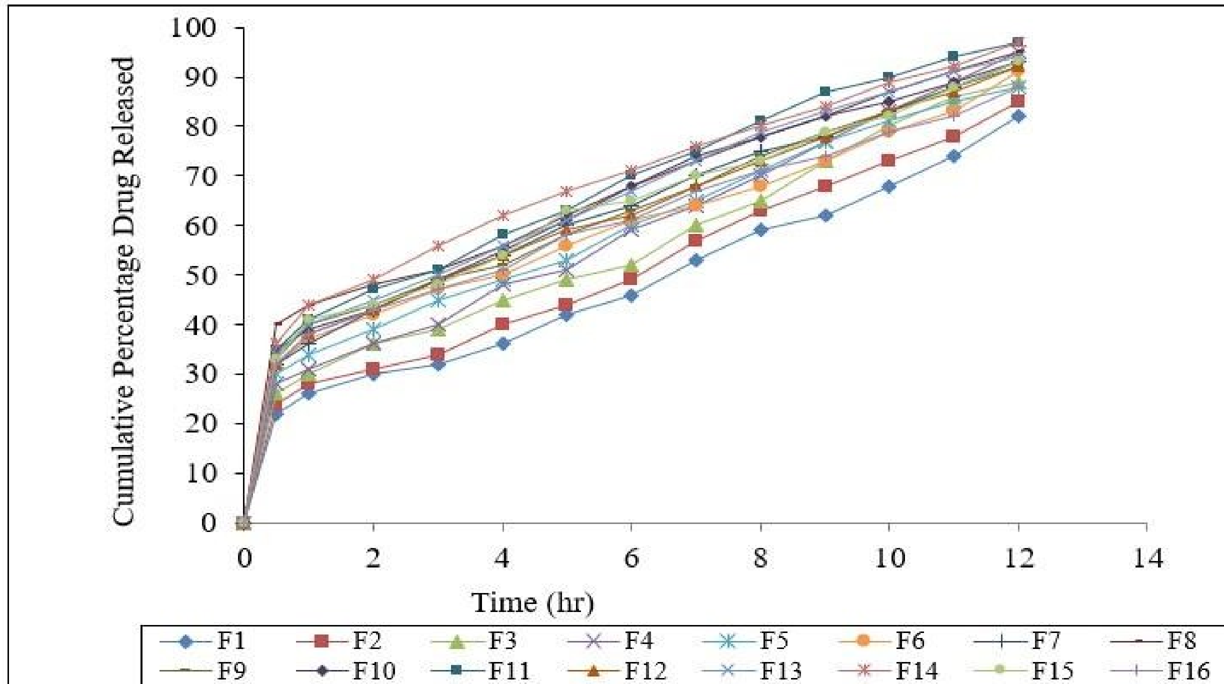


**Figure 4: In-vitro release of cubosomal in-situ gel formulations**

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

| **Formulations** | **Higuchi** | | **Korsmeyer-Peppas** | | **Mechanismofdrugrelease** |
|---|---|---|---|---|---|
| | $R^2$ | **k(min-1/2)** | $R^2$ | **n** | |
| **MA4** | 0.968 | 6.301 | 0.505 | 0.099 | Higuchi |
| **MA8** | 0.896 | 5.742 | 0.422 | 0.011 | Higuchi |
| **MA11** | 0.922 | 6.239 | 0.453 | 0.010 | Higuchi |
| **MA14** | 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.



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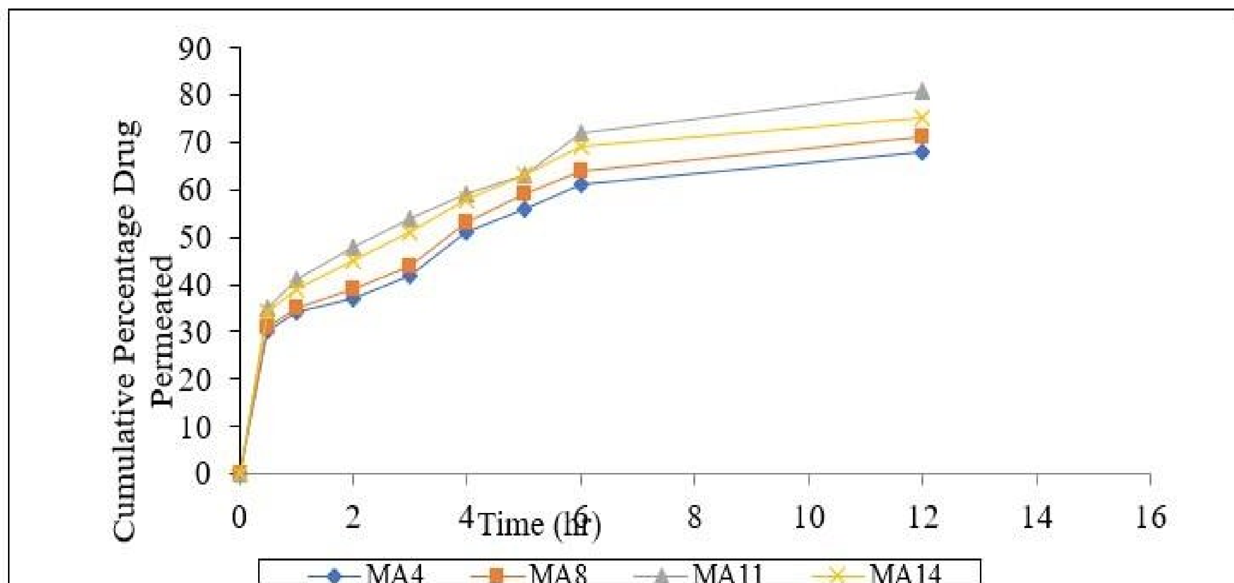


**Figure 5.** ***In vitro*** **Permeation of Optimised Cubosomal Gel Formulations**

**Sterility Testing:**

Sterility is a necessary condition for ophthalmic preparations. The presence of microorganisms in the preparation has the potential to cause irritation, inflammation, or infection in the eye. After a period of 14 days, there was no observable increase in the population (no turbidity) in the formulations that had been placed in a suitable growth medium for both aerobic and anaerobic bacteria and fungi. In comparison to the positive control sample, which exhibited turbidity, both the negative control and test samples-maintained transparency. Based on the findings of sterility testing in the optimised formulations, the "positive control" tube showed visible signs of microbial growth (turbidity), but the "test" and "negative control" tubes did not show any symptoms of microbial growth.

The results indicated that the cubogel was effective when subjected to autoclaving and showed that it passed the sterility test for both aerobic and anaerobic bacteria as well as fungi.

**Antibacterial Study:**

Figure 6 displays the diagram illustrating the antibacterial investigation. The antimicrobial efficacy of the chosen sustained release moxifloxacin HCl formulations was determined against staphylococcus aureus. The inhibition zones for the standard and ophthalmic formulations were 28 mm and 31 mm, respectively. The zone of inhibition seen in both the conventional and ophthalmic preparations was nearly identical. The inhibitory regions were assessed 24 hours later, and a decrease in the proliferation of bacteria was noted.



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

## Ocular Tolerance Studies

### Histopathology Study:

The histopathological results are displayed in Figure 7. The histological study of goat cornea using the selected formulations (MA4, MA8, MA11, and MA14) showed the presence of normal ocular structures within the cubosomal formulations. There were no observed alterations.Within the cornea, the cubosomal formulation is internalised into the epidermal layer.

Figures 8 and 9 display the FTIR spectra of cubosomes and cubosomal gel, respectively.The Figures above indicate that there were no discernible peaks that either appeared or disappeared.

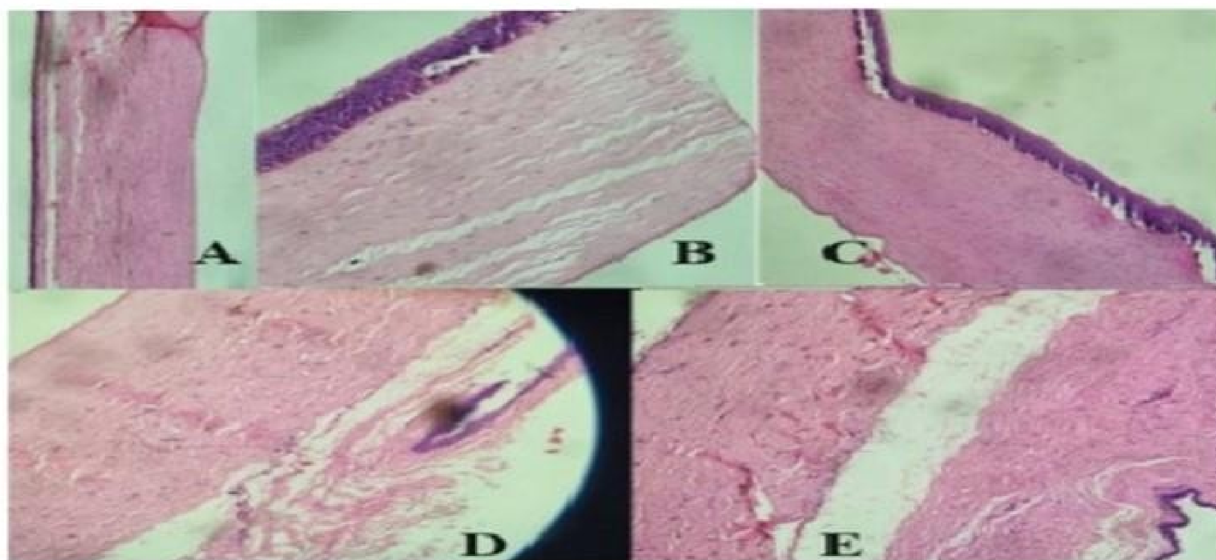


**Figure 7. Histopathological Study of Formulations Positive Control, MA4, MA8, MA11 and MA13**



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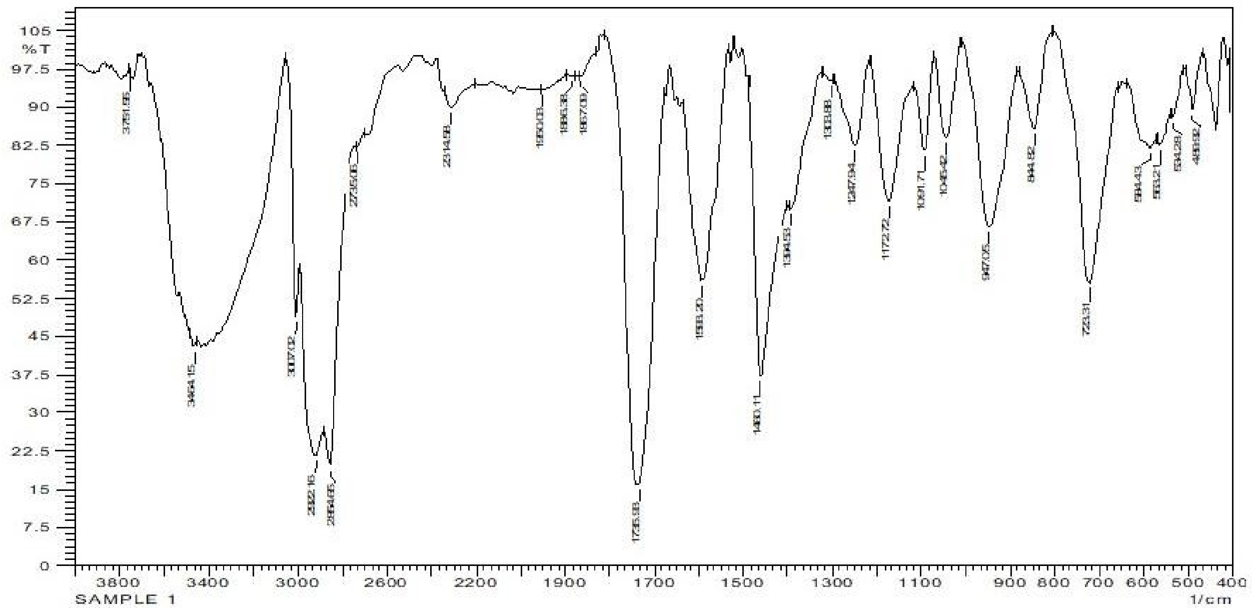


**Figure 8. FTIR Spectra of Selected Cubosome Dispersion (F7)**

## DSC of Cubosomes:

Figures 10 and 11 display the DSC thermogram of cubosomes and cubosomal gel. The melting point of cubosomes and cubosomal gel was identified by a distinct peak on the DSC thermogram at temperatures of 50.35 °C and 49.46 °C, respectively.

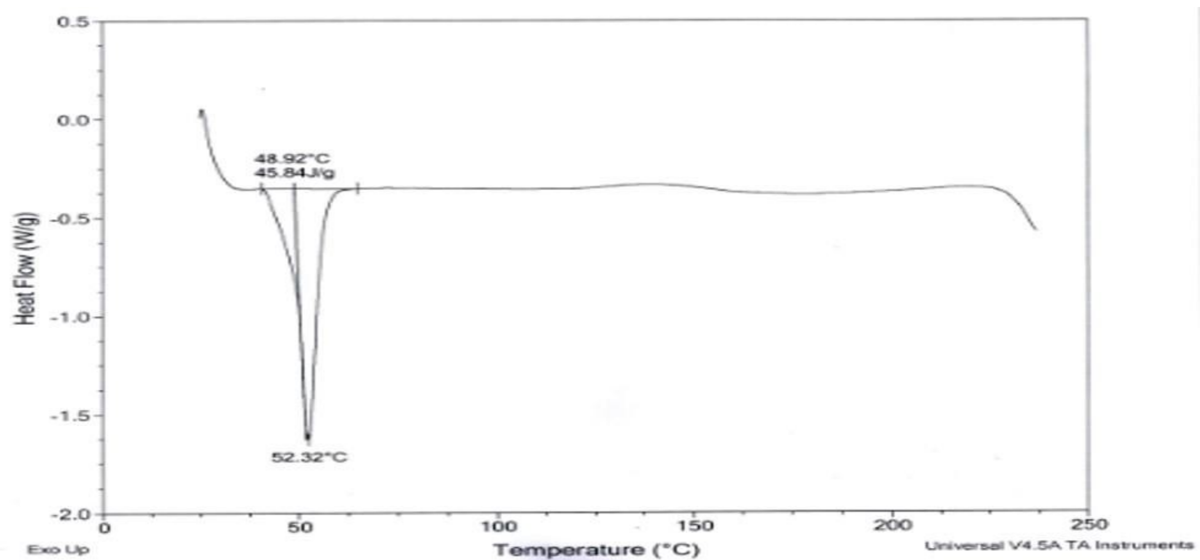


**Figure 10. DSC Thermogram of Optimized Cubosome Dispersion (F7)**



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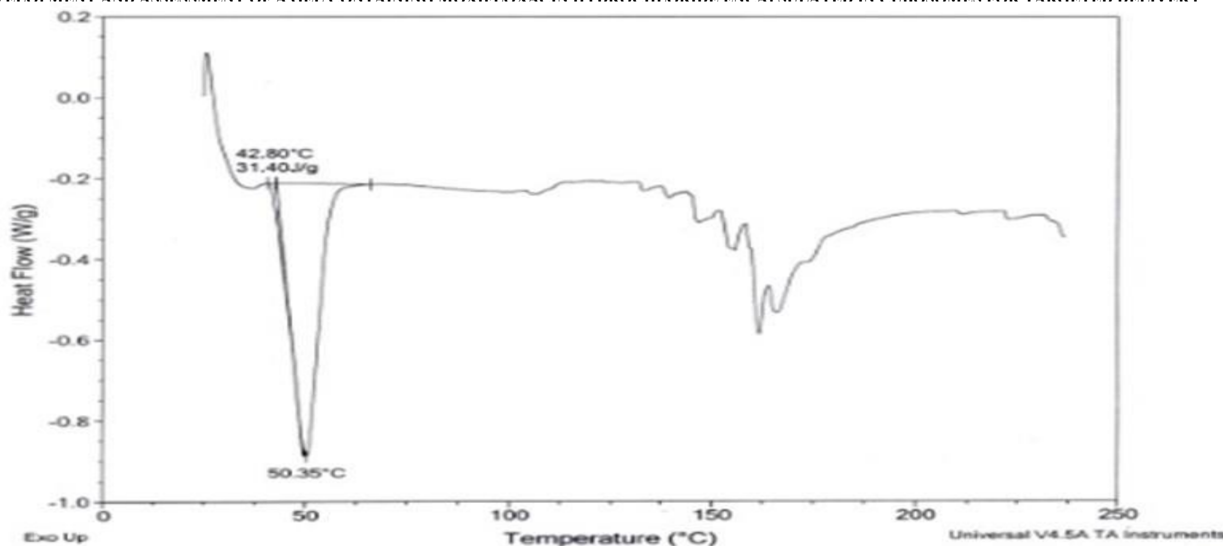


**Figures 11: DSC Thermogram of Selected Cubosomal *in situ* Ocular Gel (MA11)**

**Discussion:**

The present study involved the development of 12 formulations of Moxifloxacin Cubosomes utilising a top-down method, employing varying quantities of GMO and poloxamer. Among these 12 formulations, the F7 formulation was selected as the most optimal based on its particle size, zeta potential, and entrapment efficacy. The F7 was subsequently utilised in the production of MX cubogel.MX cubogel was chosen from a set of cubosomes (F7) that were enhanced utilising various polymeric combinations of chitosan and Carbopol.

The analysis of MX cubosomes revealed that a rise in GMO content resulted in a decrease in particle size, as demonstrated by the measurements of particle size, polydispersity index, zeta potential, and EE. This suggests a high level of consistency and a promising ability for corneal transplantation. An increase in genetically modified organism (GMO) content resulted in higher PS (performance score) values for the MX-Cub. This phenomenon may occur due to the little shearing impact and an enhanced tendency for Cubosome aggregation at higher levels of GMO. Moreover, the size of cubosomes is inversely proportional to the concentration of Pluronic F 127 (15).

The negative zeta potential values observed in cubosomes can be related to the adsorption of trace amounts of free fatty acid, notably oleic acid, prevalent in GMOs. The negative charge is generated by ionising the carboxylic end group in the free fatty acid. Moreover, poloxamer 407 imparts a negative charge to the cubic nano-crystals due to the interactions between the hydroxyl ions of poloxamer 407 and the surrounding aqueous environment.

It is crucial to assess the drug entrapment efficiency in order to confirm the presence of the desired quantity of Moxifloxacin in the cubosome dispersion. The quantity of moxifloxacin present in the cubic nanoparticles was dependent on the quantities of GMO and poloxamer 407. Significantly, as the GMO content reached 5%, there was a corresponding increase in the entrapment efficiency. Nevertheless, a higher concentration of GMOs resulted in a reduction in the trapping efficiency.



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The examination of particle morphology corroborates the results obtained from the analysis of particle size.

All the Moxifloxacin Loaded Cubosomal Ocular in-situ gel formulations that were developed did not have any turbidity, suspended particles, or other impurities. They appeared clear or transparent. Therefore, all in-situ gel batches successfully met the clarity test criteria.

The formulation's optimal gelling capacity allows it to quickly convert from a liquid to a gel state after being applied to the eye, and it maintains this gel structure for a long period of time. Visual observations indicate that the ability to form a gel depends on the concentration of the substances that cause gel formation and increase viscosity. The formation of complexes between Chitosan and Carbopol is predominantly driven by electrostatic interactions between their amino and carboxyl groups. The gelation time is affected by the concentration of the co-polymer. Increasing the concentration of carbopol 940 leads to a decrease in the time it takes for the sol-to-gel transition. The differences in viscosity under different settings are crucial for both application and in-vivo performance. An ideal formula should have high viscosity during physiological settings and low viscosity during storage. The viscosity of the in-situ gel was studied using a Brookfield viscometer while subjecting it to different levels of shear stress. The viscosity exhibited a direct correlation with the amounts of modified chitosan and carbopol 940. The drug content homogeneity demonstrated a consistent distribution of the medication.

The cubosomal in-situ gel formulations of MX undergo a shift from liquid solutions at room temperature to solid gels at body temperature and particular pH levels. Drug release experiments were performed utilising a Franz diffusion cell, which showed that the cubosomal in-situ gel had a much higher rate of drug release compared to conventional ocular formulations. The synergistic effect of poloxamer in the cubosome and carbopol in the gel can lead to the formation of a gel network, which is influenced by the cationic properties of tears. This gel network enhances the prolonged release of the drug.

Chitosan is used as a gelling agent in several formulations. The prepared gels exhibit enhanced adherence to mucous membranes due to their electrostatic interactions with negatively charged mucus. Surprisingly, after a span of 12 hours, more than 95% of MX was discharged from four different compositions. This indicates a consistent and prolonged release pattern for the in-situ gel compositions. The in-situ gel exhibited an initial rapid drug release, which can be beneficial for achieving therapeutic drug concentrations promptly. The rapid release of the medication can be attributed to its early migration towards the surface of the gel matrix. Follow-up observations revealed a reliable and continuous release of the medicine, leading to a potential decrease in the need for daily drug application. To ascertain the drug release mechanism, the in-vitro drug release data of MX-loaded cubogel formulations underwent a goodness of fit test using linear regression analysis. It was found that all the formulations adhered to Higuchi kinetics. According to this approach, the release of the drug from this formulation may be regulated via micropore diffusion.



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The in-situ gel formulation exhibited much greater penetration capacity compared to alternative ophthalmic formulations. The enhanced mucoadhesive properties of the polymer used in the composition of the in-situ gel may result in an increased ability to permeate the cornea.
The antibacterial analysis demonstrated a considerable rise in the zone of inhibition as the concentration of moxifloxacin HCl released from the in-situ gel rose. Furthermore, the study showed the absence of any microbial growth throughout the duration of the experiment.
The histopathology examination did not reveal any alterations in the epidermal layer following the internalisation of the cubosomal formulation within the cornea. These observations led to the conclusion that the created selected formulations were safe for administering to the eyes.

**Conclusion:**

Ultimately, this study effectively created and assessed a new ocular, extended-release cubogel formulation of MX for the treatment of conjunctivitis. The cubosomes that were first made and optimised exhibited desirable characteristics in terms of particle size, zeta potential, and EE (encapsulation efficiency). The cubosomes were subsequently integrated into a cold in-situ gelling apparatus in order to produce MX cubogels. The MX cubogels demonstrated a prolonged and continuous release of MX, lasting for at least 12 hours, as per Higuchi's release kinetic model. Furthermore, the results of in-vitro experiments, corneal permeability tests, and investigations on antibacterial properties and ocular tolerance all indicated positive outcomes for the ophthalmic formulation. Collectively, these results indicate that MX cubogel holds promise as a viable substitute for traditional eye drops in the management of conjunctivitis. Due to its improved capacity to pass through and its ability to release medication over a long period of time, it is a practical choice for enhancing the effectiveness of delivering drugs to the eyes and ensuring that patients follow their treatment. Additional research and clinical investigations could confirm its clinical usefulness in treating eye infections.

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