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DEVELOPMENT AND CHARACTERIZATION OF EYE DROP CONTAINING MOXIFLOXACIN HYDROCHLORIDE FOR BETTER THERAPEUTIC EFFECT

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ABSTRACT

This study aimed to develop and assess eye drops containing 4th generation fluoroquinolone antibiotic as viable therapy for ocular infection. A wide range of bacterial eye infections can be treated with fluoroquinolones, a class of broad-spectrum antibiotics effective against both gram-positive and gram-negative bacteria. To improve medication stability and ocular tolerability, the formulation procedure involved choosing suitable excipients, regulating the pH, and guaranteeing isotonicity. Comprehensive physicochemical assessments, including viscosity, pH, and sterility tests, were performed on the created eye drops. Ex-vivo cornea permeation studies were used to identify the pharmaceutical release profile using simulated tear fluid, and common eye pathogens were used to assess the antibacterial activity. The results indicate that because the produced fluoroquinolone eye drops have strong antibacterial action, steady drug release, and stability, they may be used clinically to treat ocular infections. To assess the safety and therapeutic efficacy in vivo, more research is advised.

KEYWORDS: Fluoroquinolones, Eye drops, Ocular infections, Antibacterial activity, Controlled drug release.

INTRODUCTION

Easily accessible location for topical medication is the eye. Medications are frequently injected into the eye system to provide a focused effect inside or on the surface of the eye.[1] Ocular medication delivery has proven to be a substantial difficulty for researchers because of the unique structure and physiology of the eye, which involves several barriers such as blood retinal barriers, lachrymal fluid-eye barriers, and drug loss from the ocular surface.

The formulator's main task is to overcome these obstacles without endangering any tissue.[2] The cornea, which is made up of endothelium, stroma, and epithelium, is the eye's anterior layer. Nonetheless, this layer functions as a mechanical barrier that prevents medication molecules from being delivered. The epithelium and endothelium are thought to hinder the flow of hydrophilic molecules because of their high fat content. Because of its high-water content, the stroma is impenetrable to molecules that are lipophilic.[3] The corneal barrier also contributes significantly to limited ocular bioavailability, it indicates that fewer than 5% of drugs taken by mouth can penetrate the cornea and get into the tissues inside the eye.

The following are some drawbacks to utilizing traditional dose forms:

- Reduced absorption due to limited permeability.
- Quick eradication.
- Regular instillation.
- Low precorneal residence time results in decreased bioavailability.
- Drug drainage from the nasolacrimal duct.[4]

Traditional ocular dose forms have numerous drawbacks, including inefficient absorption, insufficient duration of stay within the conjunctival sac, and precorneal loss due to tear dynamics. More efforts are currently being directed toward creating innovative drug delivery systems for ocular dosage forms that can ensure a localized effect for a longer duration and increase the permeability of medications that are weakly permeable to the cornea. To achieve these objectives, which include extending the period of corneal residence, optimizing corneal permeability, and extending drug release, a variety of vesicular systems have been investigated.[5] Some limitations of these systems include their control the amount of medication that enters the cornea, which could lead to insufficient drug concentration at the site of action.

The two main strategies to maximize the systemic bioavailability of medications given ocularly are as follows in order to get over these issues as follows:

- Extend the duration of ocular residence.
- A greater absorption by the eye.[6]

Increasing ocular residence time: The drainage systems and lacrimal secretions work to swiftly eliminate materials and foreign objects from the corneal epithelium. The anterior segment of the optical chamber should be filled with drops to lengthen the residence period or postpone clearing. Additionally, products can be made with polymers like polyacrylic acid (Carbopol), hydroxypropyl methylcellulose, or methylcellulose, which raise the viscosity of the solution and serve as bio adhesives in the tissue of the eyes.[7]

Increase ocular absorption: Enhancers work by speeding up the pace at which medications pass through the epithelium. To improve absorption, they modify the epithelial cells structural makeup without causing the cells to die. Dimethyl sulfoxide, decamethonium, EDTA, and glycocholate are a few examples. These characteristics are ideal for absorption enhancements.

- They ought to effectively boost the medication's absorption.
- Must not result in permanent damage.
- It must not irritate.
- Should function well in small amounts.
- The effect should to be transient and completely reversible.
- The absence of a lag effect is necessary when absorption is needed.
- Needs to be steady.[8]

With a methoxy group at position C-8 and a significant C-7 side chain, doxifloxacin is a 4th generation fluoroquinolone. Moxifloxacin (0.5%w/v) eye drops need to be administered six times a day, or more frequently in cases of severe disease same like other fluoroquinolones. The dosage is 1-2 drops. Moxifloxacin has bactericidal properties and inhibits DNA gyrase (topoisomerase II) and topoisomerase IV, two essential enzymes involved in bacterial DNA replication, transcription, repair, and recombination. When treating experimental keratitis in rabbits, levofloxacin or ciprofloxacin are less effective than moxifloxacin. It has been discovered that moxifloxacin penetrates rabbits inflamed ocular tissue more effectively than ciprofloxacin, lomefloxacin, ofloxacin, or levofloxacin.[9]

The advent of new generations of fluoroquinolones, such Doxifloxacin HCl, has made the treatment of gram-positive bacterial infections conceivable. This medication has greater power than anybody else topical antibiotic, enabling it to completely destroy Staphylococcus species that are resistant to methicillin. Moxifloxacin HCl reaches the ocular tissues, including the tear film, cornea, anterior chamber, and ciliary body, at a very high level because it is biphasic, meaning it is soluble in both lipid and aqueous solutions. Therefore, it was considered combining the drugs advantages with mucoadhesive/pH-sensitive polymers, like Carbopol and hydroxypropyl methylcellulose (a substance that increases viscosity), to create a formulation that might work better than the drug's traditional eye drops. The mixture may be used to treat bacterial keratitis, bacterial endophthalmitis, keratoconjunctivitis, and acute and subacute conjunctivitis, among other external diseases of the eye.

2. MATERIAL AND METHODS

Materials

Drug: Moxifloxacin HCL (gift by senses pharmaceutical private limited), cyclodextrin ((gift by senses pharmaceutical private limited), hydroxy propyl methyl cellulose (gift by senses pharmaceutical private limited), sodium citrate dihydrate (gift by senses pharmaceutical private limited) sodium chloride (gift by senses pharmaceutical private limited) benzalkonium chloride (gift by senses pharmaceutical private limited) and water for injection (gift by senses pharmaceutical private limited).

Selection of vehicle

Moxifloxacin HCl solubility was tested in a different solvents, including ethanol, acetone, distilled water, phosphate buffer 7.4 pH and 2-propanol. Research showed that moxifloxacin HCl dissolved more readily in water than in any other solvent. The sample was analysed using UV spectroscopy to determine its solubility quantitatively. After scanning the wavelength between 400 and 200 nm, 288 nm was discovered to have the greatest absorption.[11]

FTIR studies

Using KBR pellets measuring 0.1 mm, Fourier Transformer Infrared Spectroscopy (FTIR) was used to determine the drug excipient compatibility analysis. To verify the interaction, the infrared spectra of the medicine in its pure form, Moxifloxacin HCl, and the combination of moxifloxacin HCl and all the excipients are compared.[12]

Differential scanning calorimetry (DSC) characterization

Using a calorimeter, the pure drug and physical combination were thermally characterized. The samples were put in aluminium pans that were sealed. From 200^0 C to 3000^0 C, the samples were scanned at 200^0 C/min.

Preparation

Moxifloxacin HCl eye drop is prepared by conventional method. Dissolve required quantity of Cyclodextrin in approximately 500 mL of WFI (Water for Injection) with continuous stirring until fully dissolved. Add required quantity of Moxifloxacin to the Cyclodextrin solution and stir until completely dissolved, forming an inclusion complex. Dissolve required quantity of Sodium Citrate in the solution. This acts as a buffering agent. Slowly add required quantity of Hydroxypropyl Methylcellulose (HPMC) to the solution while stirring to avoid clumping. Continue stirring until fully dissolved. Add 0.1ml of Benzalkonium Chloride to the solution, ensuring it is fully dissolved. This serves as a preservative. Add 9 g of Sodium Chloride to the solution to adjust the tonicity, making the formulation isotonic with tears. Add additional Water for Injection to bring the total volume to 1-liter (1000 mL). Ensure thorough mixing to achieve a homogenous solution. Dispense the solution into suitable glass containers or bottles that can withstand autoclaving. Autoclave the solution at 121°C (250°F) for 15-20 minutes. Ensure that the autoclave cycle is appropriate for the volume and type of containers used. Allow the solution to cool to room temperature.[13] The composition of these prepared eye drops is shown in Table 1.

The composition of these prepared eye drops is shown in Table 1(quantity prepared to 100ml).[14]

Ingredients	Moxifloxacin HCl	β Cyclo- dextrin	HPMC	Sodium citrate	Sodium chloride	Benzalkonium chloride	WFI
F1	0.5% w/v	0.1g	0.65g	0.1g	0.9% w/v	0.001% v/v	QF
F2	0.5% w/v	0.3g	0.1g	0.3g	0.9% w/v	0.001% v/v	QF
F3	0.5% w/v	1.05g	0.3g	0.1g	0.9% w/v	0.001% v/v	QF
F4	0.5% w/v	0.3	1.05g	0.5g	0.9% w/v	0.001% v/v	QF
F5	0.5% w/v	2g	1g	0.3g	0.9% w/v	0.001% v/v	QF
F6	0.5% w/v	0.1g	0.65g	0.5g	0.9% w/v	0.001% v/v	QF
F7	0.5% w/v	1.05g	1g	0.1g	0.9% w/v	0.001% v/v	QF
F8	0.5% w/v	2g	0.65g	0.1g	0.9% w/v	0.001% v/v	QF
F9	0.5% w/v	0.1g	1g	0.3g	0.9% w/v	0.001% v/v	QF
F10	0.5% w/v	0.3g	2g	0.3g	0.9% w/v	0.001% v/v	QF
F11	0.5% w/v	2g	0.65g	0.5g	0.9% w/v	0.001% v/v	QF
F12	0.5% w/v	1.05g	1g	0.5g	0.9% w/v	0.001% v/v	QF
F13	0.5% w/v	0.1g	0.3g	0.3g	0.9% w/v	0.001% v/v	QF

* HPMC: Hydroxy propyl methyl cellulose, WFI: Water for injection, QF quantity sufficient

Optimization of moxifloxacin HCl eye drops

Thirteen distinct compositions of polymers Eye drops including cyclodextrin, sodium citrate, and HPMC were made with moxifloxacin HCl. Based on the formulation's pH, viscosity, and particle size, formulation F2 was selected as the optimal formulation.

This F2 was used to make Moxifloxacin HCl eye drops for further study.

3. EVALUATION

Visual appearance and clarity

A visual inspection was conducted of the look. Visual inspection of the formulations in different lighting conditions and against white and black backgrounds was used to assess the clarity of the previous formulations.[14]

pH

With a resolution of 0.01 pH and a measurement range of 0 to 14 (0.1 mV in 5 s; thermal deviation: 0.002 pH/ºC), the micro-pH 2000 pH meter was used to measure the pH. Following the manufacturer's instructions, the pH meter was calibrated using the 4.00 and 7.50 pH buffer solutions prior to the readings.[15]

Viscosity

The viscosity was measured with a Brookfield viscometer at 25 ± 0.1 ˚C. The tested recipe was added to the plate in an amount of around 0.5ml. There was a 0.5 to 100 rpm range for the rotating speed. Only when the torque was within the permissible range of 10% to 100% were the results considered legitimate. Plotting viscosity and shear stress against shear rate was done. The power law model was used to study the rheological behavior.[16]

Drug content

A 100ml volumetric flask holding 1 ml of an eye drop sample was diluted with 10ml of simulated tear fluid in order to determine the drug concentration. To find the proportion of drug content, the absorbance at 288 nm was measured using a UV spectrophotometer.[17]

Particle Size

The Zeta Seizer (Malvern Instrument Ltd, Malvern, UK) was used to measure the mean particle size (nm) of the different Moxifloxacin HCL eye drops using a dynamic light scattering approach. Particle-free filtered water was used to dilute the samples.[18]

Isotonicity

To test the eye drops' tonicity, citrated blood was added, and the results were examined under a 45x microscope to determine whether the drops caused RBCs to expand or burst.[19]

Antibacterial activity

The antibacterial activity was assessed using an agar diffusion test and the cup plate technique. Through a solid agar media, the medication was permitted to diffuse. The produced formulations containing moxifloxacin and the control were created to have a standard minimum inhibitory concentration (MIC) of 2 µg/ml. An autoclave was used to create and sterilize 60 ml of nutritional agar medium at a pressure of 15lb/SQ-inch for eighteen minutes. After that, 0.5 ml of the microbe suspension was added to the medium, which was then maintained at a temperature of 52°C to 58°C. We'll carry out this in an aseptic manner. Each petri plate was filled with 20 cc of the microbial agar suspension right away. Following the media's solidification, sterile dilution solutions of distilled water and the generated formulations, appropriately diluted with sterile moxifloxacin hydrochloride (standard solutions) were added to the sterile nutritional agar petri plate cup. Test organisms for Escherichia coli and Staphylococcus aureus were previously planted here. The solutions were allowed to diffuse for two hours before the agar plates were incubated for twenty-four hours at 37°C. Every cup's zone of inhibition (ZOI) was measured and contrasted with the controls. A laminar airflow unit was used during the entire procedure. Every formulation solution underwent three tests. Throughout the investigation, both positive and negative controls were kept in place.[20]

In vitro diffusion study

In vitro diffusion studies of the formulations were carried out using a modified diffusion cell apparatus. A cellulose membrane (Mwt cut off: 12-14 kilodalton) was soaked overnight in SLF before placing it in between the receptor and donor compartment. 1 ml of the formulations (equivalent to 10 mg of the drug) was placed in a donor compartment. The receptor compartment was filled with 50 ml of SLF and stirred at 50 rpm using a magnetic stirrer bar. The temperature was maintained at 35-37±0.5 °C. 1 ml of the samples was collected from the receptor compartment at 0.25, 0.5, 1, 2, 3, 4, 5, 6, 7, and 8hr and replaced with an equivalent amount of the SLF. The analysis of drug release from the samples was conducted at a wavelength of 288 nm using a UV-Vis spectrophotometer.[21]

Ex-vivo cornea permeation

Ex-vivo release studies were conducted using a Franz diffusion cell, a model of a donor-receiver compartment with two chambers. The temperature was set at 37 ± 0.50 C on a magnetic stirrer. A goat cornea in contact with the receptor media received an even distribution of 1ml of the formulation, which was carefully quantified. The receptor medium was constantly shaken at a rate of 20 revolutions per minute in order to simulate eyelid blinking. Samples were removed on a regular basis and diluted with 10ml of STF. A UV Spectrophotometer set to 288 nm was used to quantify the drug content against a reference standard using artificial tear fluid as a blank.[22]

Sterility testing

One of the most important prerequisites for an ophthalmic preparation is sterile conditions. Determining if the manufactured ophthalmic drugs include living microorganisms is the aim of the sterility testing. Soybean casein digest medium (SCDM) was used in the sterility tests for Fluid Thio Glycollate Medium (FTGM) it is employed to grow fungi (Candida albicans), aerobic bacteria (Staphylococcus aureus), and anaerobic bacteria (Bacteroides vulgates). The entire investigation was conducted in an aseptic laminar air flow hood. Glassware was autoclaved before use. To ensure that no microbes remained on them, isopropyl alcohol was used to thoroughly wipe any non-autoclavable materials.[23]

Optimization of Moxifloxacin hydrochloride eye drop

The Moxifloxacin hydrochloride was optimized using Design Expert® (DOE) software by factorial design. The experiment included three independent factors, HPMC K4M, sodium citrate, and cyclodextrin concentrations, with three levels each. As presented in table 2, the responses considered were viscosity, gelling time, and in vitro drug release. 13 experiments were conducted using the Box-Behnken design outlined in table 3. The responses were then recorded and applied to the design to optimize the formulation.[24]

Table 2: Independent and dependent factors.

Factors	Levels	
	Low	High
Independent variables		
HMC K4M (g)	0.3	1
Beta-Cyclodextrin (g)	0.1	2
Sodium citrate (g)	0.1	0.5
Dependent variables	Desirability Goal	
Viscosity	Minimize	

Particle Size	In range
In-vitro drug release	Maximize
pH	Maximize

Table 3: Experimental runs of 32 factorial design of Moxifloxacin hydrochloride eye drop formulation.

Formulation code	Cyclodextrin	Hpmc	Sodium citrate
MOX 1	0.1g	0.65g	0.1g
MOX 2	2g	0.3g	0.3g
MOX 3	1.05g	0.3g	0.1g
MOX 4	1.05g	0.65g	0.3g
MOX 5	2g	1g	0.3g
MOX 6	0.1g	0.65g	0.5g
MOX 7	1.05g	1g	0.1g
MOX 8	2g	0.65g	0.1g
MOX 9	0.1g	1g	0.3g
MOX 10	1.05g	0.3g	0.5g
MOX 11	2g	0.65g	0.5g
MOX 12	1.05g	1g	0.5g
MOX 13	0.1g	0.3g	0.3g

4. RESULTS AND DISCUSSIONS

Estimation of Moxifloxacin HCl by UV Spectrophotometer

The medication (µg/ml) solution was examined for UV absorption at wavelengths of 200–400 nm. After recording the spectrum, the absorbance maxima (max) at 288 nm were visible.

Construction of Calibration Curve of Moxifloxacin HCl

Plotting the drug's calibration curve in simulated tear fluid (pH 7.4) involved measuring the absorbance of solutions with varying doses (1–10 µg/ml). The coefficient of correlation was 0.999, the slope was 0.0636, and the Beers and Lamberts were determined to be between 1 and 10 µg/ml as shown in fig.1.

Intercept slope

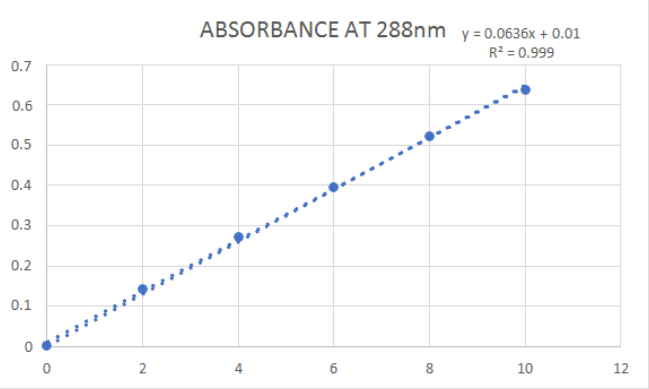


Figure 1: Calibration Curve.

FTIR Studies

Figures 2, 3, 4, and 5 display the FTIR spectra of pure drugs and drug-polymer mixtures.

The peaks of the pure drug and drug polymer mixture did not significantly change, according to the spectral analysis. Therefore, no particular interaction between the medication and the polymers in the formulations was seen.

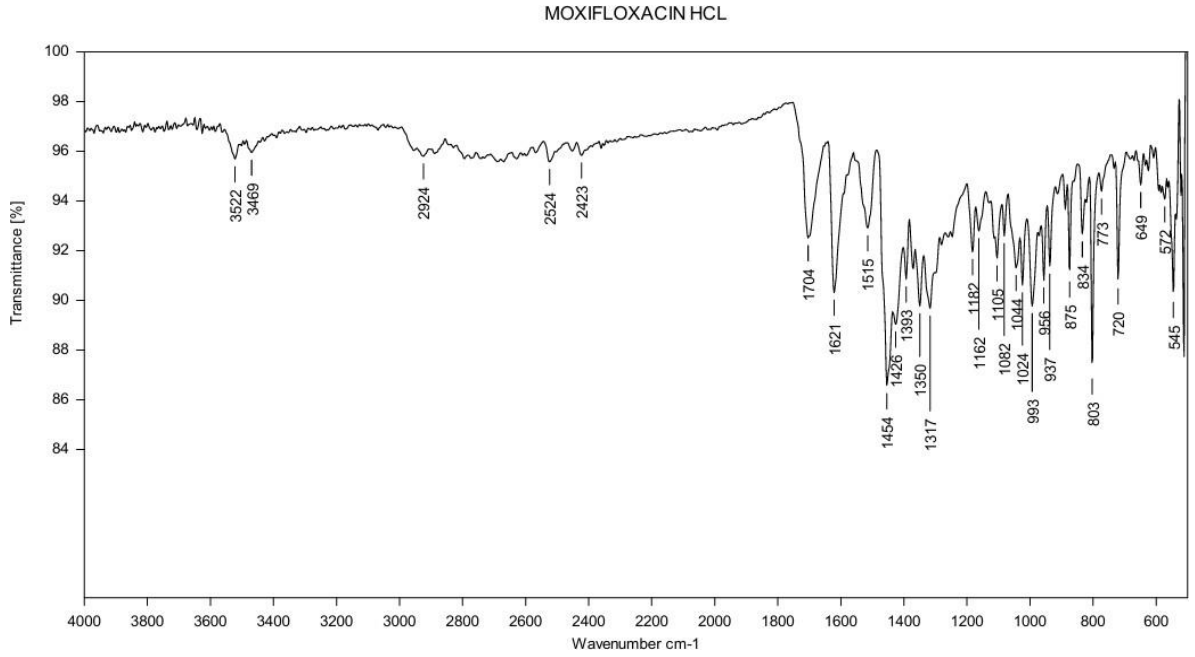


Figure 2: FTIR of Moxifloxacin HCl.

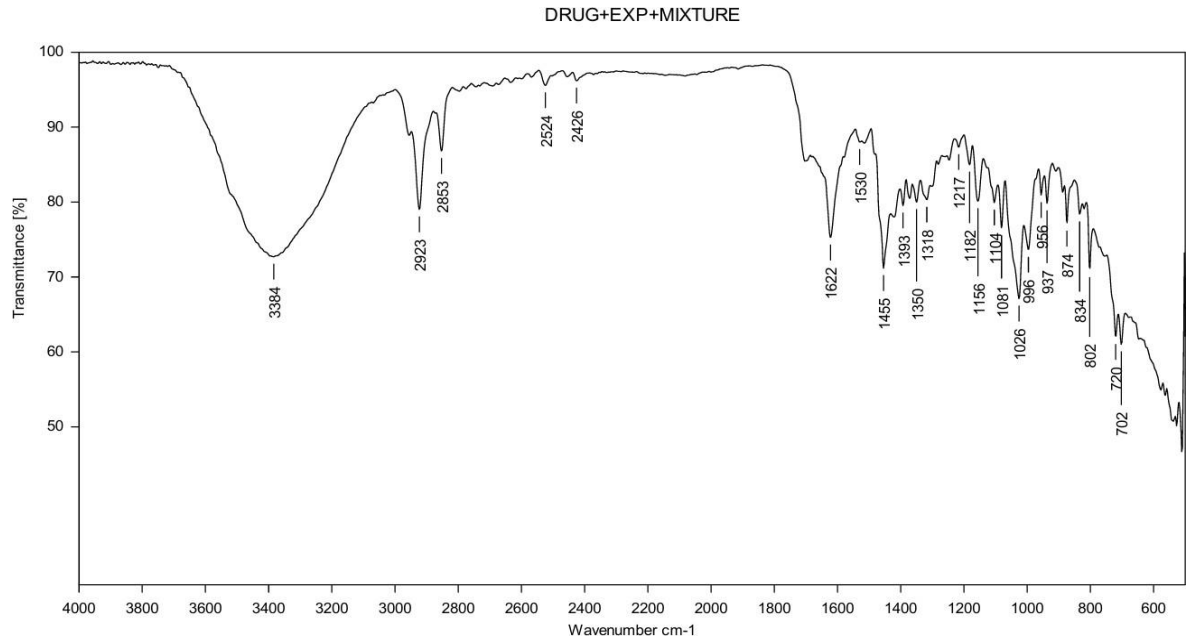


Figure 3: FTIR Spectra of Moxifloxacin HCL, beta -cyclodextrin, HPMC, sodium citrate, sodium chloride, benzalkonium chloride.

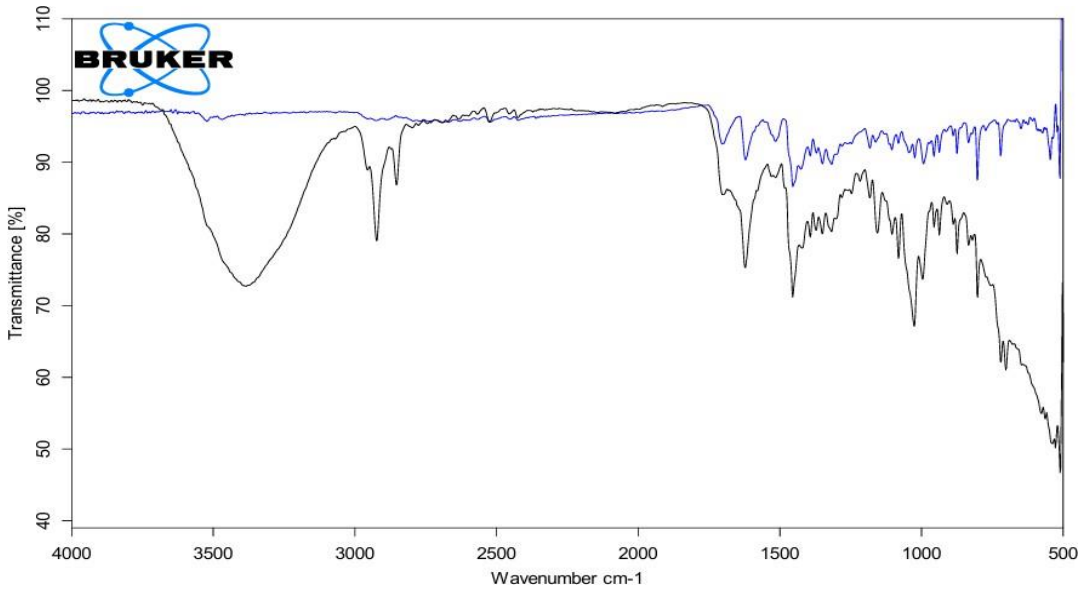


Figure 4: FT-IR mixture comparison of drug and excipients.

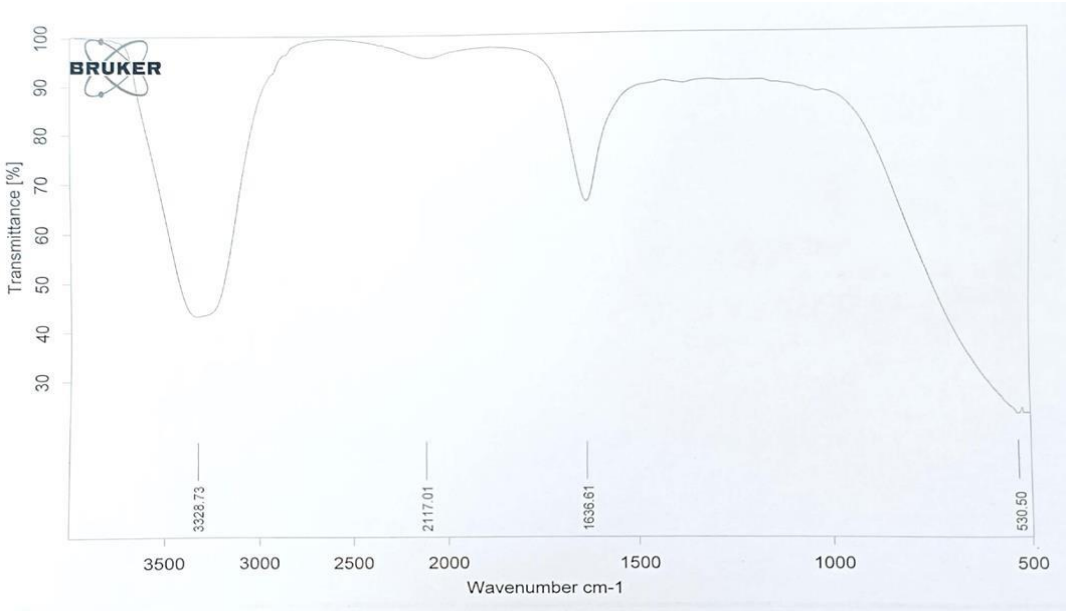


Figure 5: FTIR of excipients (cyclodextrin, hydroxy propyl methyl cellulose, sodium citrate, sodium chloride, benzalkonium chloride).

Table 4: Reported and observed IR frequency of Moxifloxacin HCl and its physical mixture.

FUNCTIONAL GROUP	STANDARD PEAKS	OBSERVED PEAKS (cm^{-1})	
		MOXIFLOXACIN HCL API	API+ EXCIPIENTS
O-H Stretching	3403.99	3400.33	3997.98
C-H stretching(alkanes)	2921.94	2923.43	2914.17
N-H stretching(amines)		2524.31	2516.67
C=O stretching (carbonyl group)	1710.43	1704.65	1711.32
N-H bending (primary amines)	1628.81	1621.05	1616.71

C-H bending (alkanes)	1450.53	1455.42	1454.78
O-H bending(phenols)/NO2 groups	1423.82	1393.40	1350.65
C-O stretching (alcohols/esters)	1167.89	1182.37	1156.08
C-N stretching (amines)	1150.35	1105.06	1091.72
C-F stretching (fluorinated compounds)	1010.78	1026.37	997.53
Aromatic C-H out-of-plane bending	850.67	874.47	834.91
C-Cl stretching (alkyl halides)	734.98	720.31	702.64

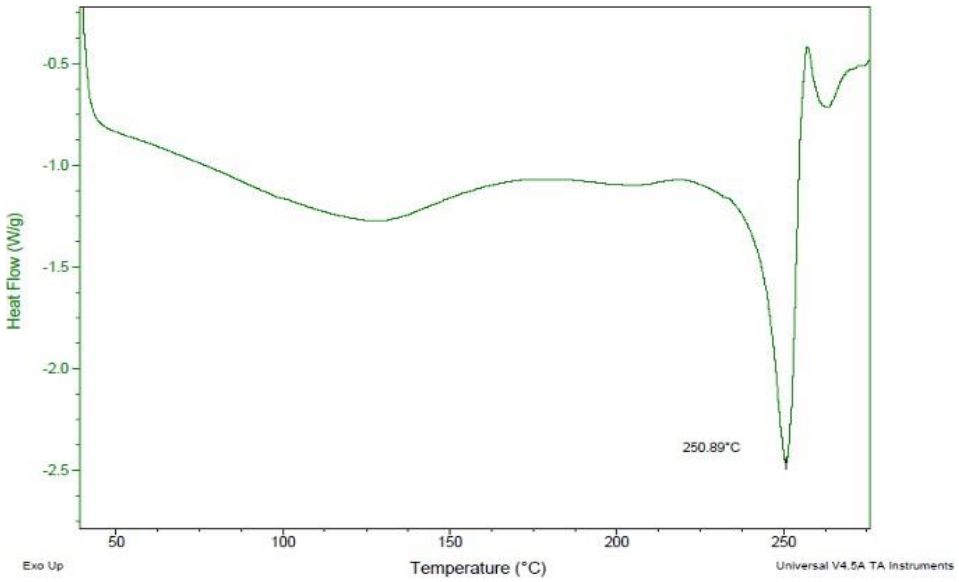


Figure No. 6: Thermal analysis of Moxifloxacin Hydrochloride.

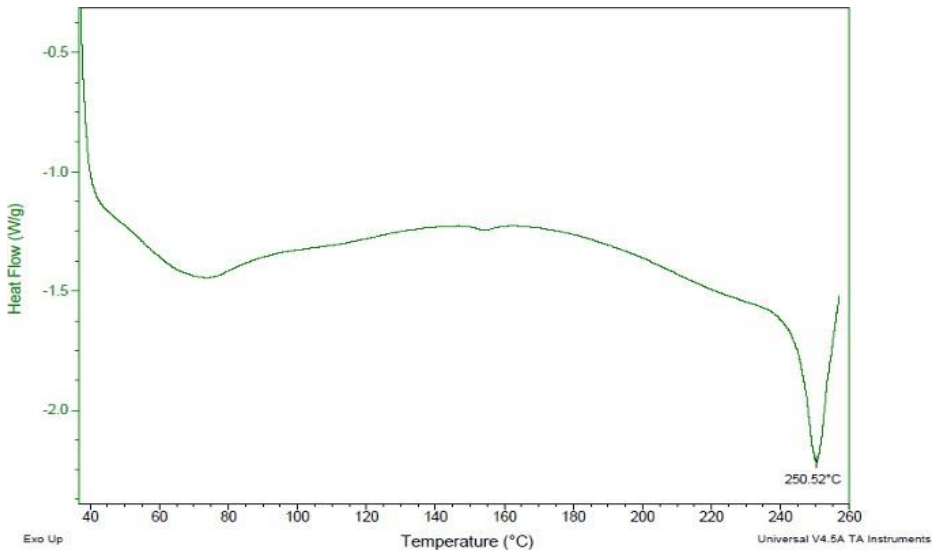


Figure 7: Thermal analysis of Moxifloxacin Hydrochloride+ Excipients.

Visual appearance, Drug content, clarity, pH

Each formulation had a pale-yellow colour and was determined to be transparent. The compositions pH values fell between 6.22 and 7.19. The percentage of drugs ranged between 84.9% and 105.37%. The results of prepared eye drops are shown table 3.

Viscosity

Viscosity of each formulation ranging from 44 to 248. The results of each formulation shown in table 3.

Particle size

In Table 3, the particle size results are displayed. The dispersion particles were confirmed to fit within the 10-500 nm range based on the particle size measurements. A constant particle size distribution was indicated by the optimal formulation's low PDI of 0.302. Figure 8 shows a graphical representation of the PDI for the best formulation, F2.

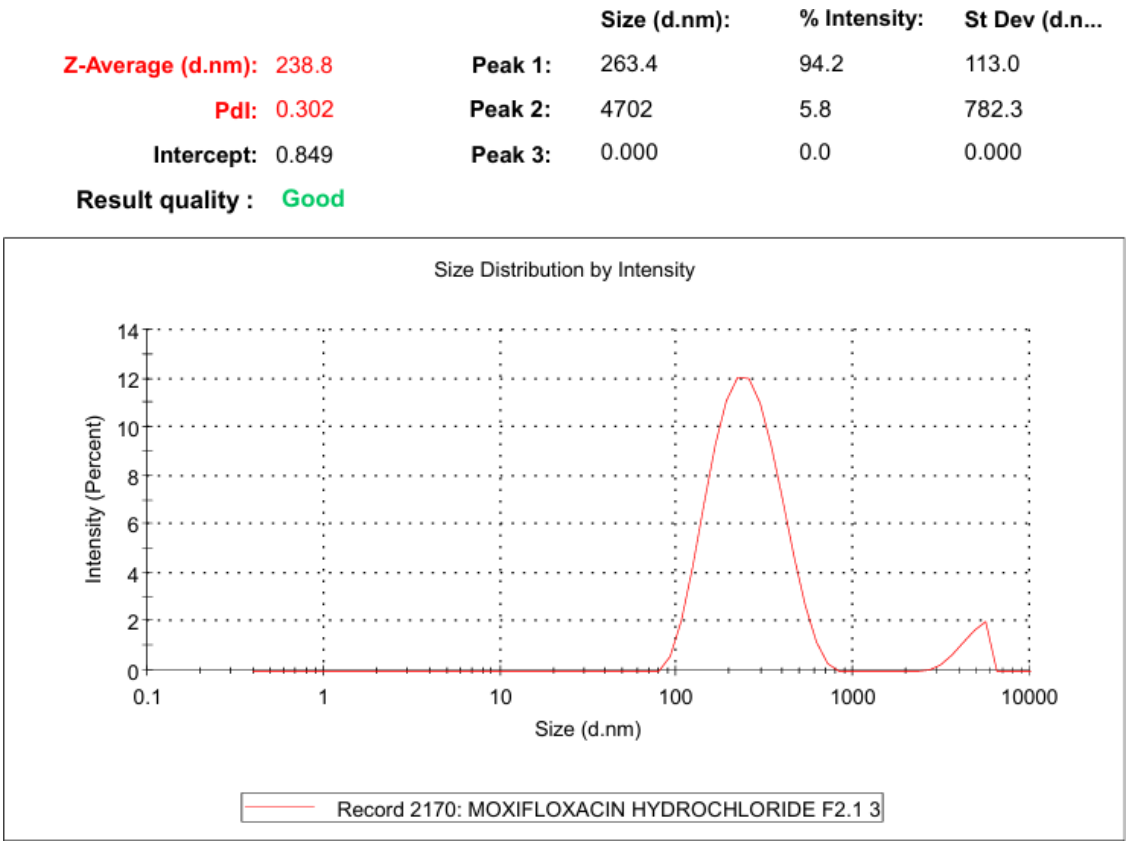


Figure 8: PDI of optimized eye drops.

Formulation Code	Visual Appearance	Clarity	pH	Viscosity (CPS)	Drug Content (%)	Particle Size (nm)
F1	Light yellow	Clear	7.19	165±0.34	85.39±0.55	296.1±1.57
F 2	Light yellow	Clear	7.14	57±0.45	100.01±0.43	270.8±0.98
F 3	Light yellow	Clear	6.58	154±0.98	95.49±0.62	270.1±0.39
F 4	Light yellow	Clear	7.14	236±0.37	91.45±0.43	270.8±0.98
F 5	Light	Clear	6.54	46±0.17	85.97±0.58	236.1±1.68

	yellow					
F 6	Light yellow	Clear	7.1	146±0.38	98.67±1.89	232.1±0.67
F 7	Light yellow	Clear	6.51	44±0.48	94.62±1.09	279.8±0.73
F 8	Light yellow	Clear	6.71	239±0.56	101.26±0.89	286.7±0.89
F 9	Light yellow	Clear	6.23	67±0.74	92.03±0.71	269.3±0.56
F 10	Light yellow	Clear	6.22	57±0.83	87.99±0.61	230.5±0.79
F 11	Light yellow	Clear	7.08	248±0.25	87.41±0.78	265.3±1.89
F 12	Light yellow	Clear	6.31	137±0.38	96.94±0.19	289±1.64
F 13	Light yellow	Clear	6.34	227±0.37	93.17±0.46	273.7±0.61

Table 5: Post formulation studies of eye drops[11] Ex-vivo cornea permeation

Figure 8 depicts the formulation F2's in vitro drug release. To determine the pattern of drug release table 4, the drug release data were submitted to a number of pharmacokinetic parameters, including zero order, first order, Higuchi square root, and krosmeyer Peppas model. For a duration of three hours, the formulation F2 exhibited good sustained release.

Table 6: Ex-vivo cornea permeation of F2.

Time	% drug release
0	0
30	6.06
60	14.86
90	26.42
120	41.97
150	60.6
180	83.39

Sterility studies

When the formulations were put through a sterility study by direct inoculation, all of them were found to be sterile and showed no signs of microbial development.

Isotonicity

Isotonicity testing of Moxifloxacin hydrochloride eye drops having BAK exhibited no change in the shape of blood cells (bulging or shrinkage), which reveals the isotonic nature of the formulation as showed below figure.

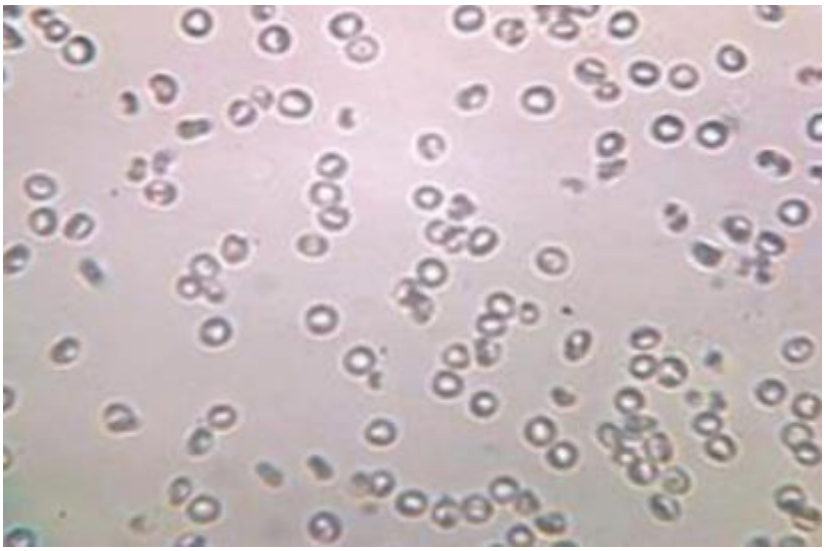


Figure No 9: Red blood cell with optimized formulation Moxifloxacin hydrochloride eye drops.[19]

Antibacterial activity

In comparison to other created formulations, Formulation F2 exhibited the highest zone of inhibition values against S. aureus (28.66 mm) and E. coli (30.99 mm), respectively. Therefore, F6 formulation was chosen for additional research. A study on the antimicrobial activity of F6 formulation utilizing Gram-positive S. aureus and Gram-negative E. coli organisms was conducted. Gram-positive S. aureus and Gram-negative E. coli were shown to have a zone of inhibition of 28.66 and 30.99 mm, respectively, for the F2 ophthalmic formulation. Table 5 displays the antibacterial activity findings. According to the study, when manufactured as a ophthalmic system, moxifloxacin hydrochloride preserved its antibacterial effectiveness against certain strains of E. coli and S. aureus.

Table 7: Zone of inhibition for formulation F2 and pure drug against Escherichia coli and Staphylococcus aureus at a concentration of 2µg/ml.

Microorganism	Concentration (mcg/ml)	Zone of inhibition (mm)	
		Standard (Pure Drug)	F2
Gram-positive Staphylococcus aureus	2µg/ml	29.78	29
Gram negative Escherichia Coli	2µg/ml	30.89	30.33

Optimization of eye drop

The DOE version software 11 suggested nine experimental runs from 13 factorial designs, which were performed. The values obtained for viscosity (R1), particle size (R2), in vitro drug release (R3) and pH (R4) were reported in table 3.

The study's responses were analysed using a variety of mathematical models, such as 2FI, linear, cubic, and quadratic. Factors' effects on the dependent response were evaluated using regression polynomial equations. ANOVA was used to evaluate the gathered data at a significance level of 0.05 percent. The analysis made use of statistical factors like Fischer's value, the degrees of freedom, the sum of squares, and the mean square. A model is considered statistically significant if its p-value is less than 0.05, and not statistically significant if its p-value is more than 0.05. Below are the polynomial equations that were produced by the software.

For viscosity

$R1 = +154.00+46.61A+45.46B+48.32C+44.72AB-37.00AC-35.14BC+15.46A^2-72.18B^2+24.54C^2$

For particle size

$R2 = +263.09+5.05A+23.03B-1.02C$

For pH

$R3 = +6.66+0.1020A-0.0373B+0.4196C$

For In vitro drug release

$R4 = +71.49-11.25A-0.6360B+1.08C$

First-order main effects, intercept coefficients, interaction effects, and higher-order effects with both positive and negative signs that show antagonistic and synergistic effects of A, B and C on R1, R2, R3 and R4 are all included in the polynomial equations. Table 8 displays the factor effects and p-values derived from the replies.

To investigate the additional correlation between the independent and response variables, 3D plots of the contour and response surface were utilized. Equation (R1)'s major components had a synergistic effect on response R1 (table 8). The viscosity of the formulation, which is directly correlated with its polymer concentration, is crucial for determining its ocular residence time. The intrinsic viscosity-building ability of HPMC-K4M is enhanced by the solvent sheath layer that envelops each individual particle. A significant rise in viscosity was also shown by the data and fig. 1A when the amounts of A, B, and C were increased. Non-Newtonian flow was seen in the rheological study of all formulations. For easy instillation into the eye, where it will usually improve eye contact time, the formulation must have the ideal viscosity in a solution

form. When administered, ocular formulations should have no effect on the precorneal tear film's pseudoplastic properties.

The effect of cyclodextrin concentration on particle size is depicted in fig. 1B. Equation (R2) indicates that the linear contributions of A and B had a synergistic effect on R2. The quadratic contributions of B2 and the interaction effects of A and B were not considered as the p-value was greater than 0.05 (table 8), and the particle size of the prepared formulations decreased as the polymer concentration increased. A more viscous medium tends to result in smaller particle sizes by slowing down particle movement, reducing the tendency to form large aggregates.

In equation (R3), the linear and quadratic contributions of A and C had a synergistic effect on the responses R3. The interaction effects of A, B, and quadratic effects of C were considered statistically non-significant, as indicated by their p-values exceeding the threshold of 0.05. Therefore, these effects were excluded from the analysis (table 8). As illustrated in fig. 1C

The effect of HPMC-K4M concentration on in-vitro drug release considering A and C had a synergistic effect and B have an antagonistic effect on R4. Therefore, these effects were excluded from the analysis (table 4). As illustrated in fig. 1C, the release profile of the drug was found to increase with lower polymer concentration and vice versa. The order of drug release from the formulation, as determined through in vitro analysis, was found to be as follows, F5>F8>F1>F2>F6>F9>F3>F7>F4 (fig. 2). This might be due to the increased viscosity because of higher polymer levels resulting in enhanced thickness, hence causing a reduction in the surface area and retardation in the drug release rate from the formulation.

Table 8: Summary of quadratic models with factor effect and corresponding p-value.

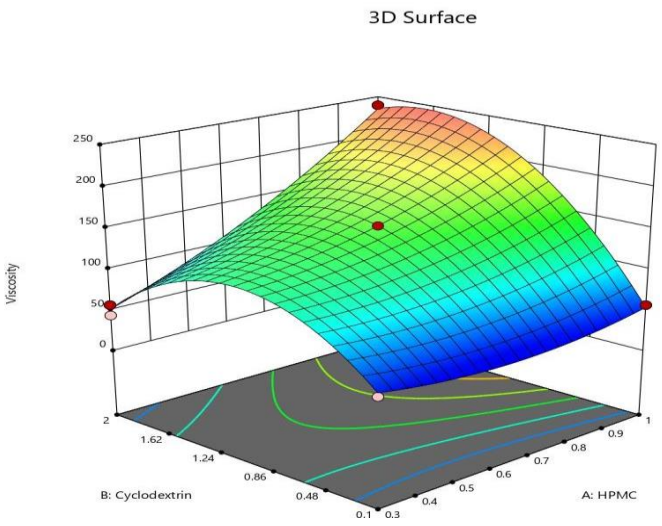
Factor	R1: Viscosity Factor Effect	p-value	R2: Particle size	p-value	R3: pH	p-value	R4: In vitro drug release factor effect	p-value
A	46.61		5.05		+0.102		-11.25	
B	45.46		23.03		-0373		-0.6360	
C	48.32		-1.02		+0.4196		+1.08	
AB	15.96		--		--		--	
AC	-37.00		--		--		--	
BC	-35.14		--		--		--	

Factor Coding: Actual

Viscosity
Design Points:
Above Surface
Below Surface
44 248

X1 = A
X2 = B

Actual Factor
C = 0.3

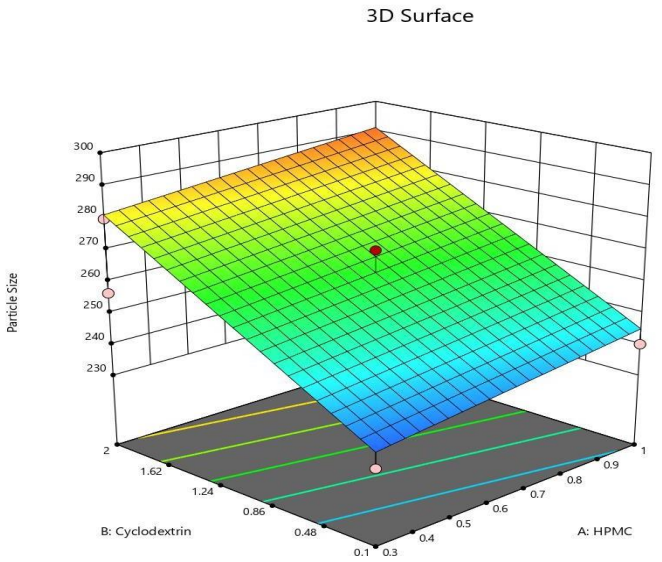


Factor Coding: Actual

Particle Size
Design Points:
Above Surface
Below Surface
230.1 296.7

X1 = A
X2 = B

Actual Factor
C = 0.3

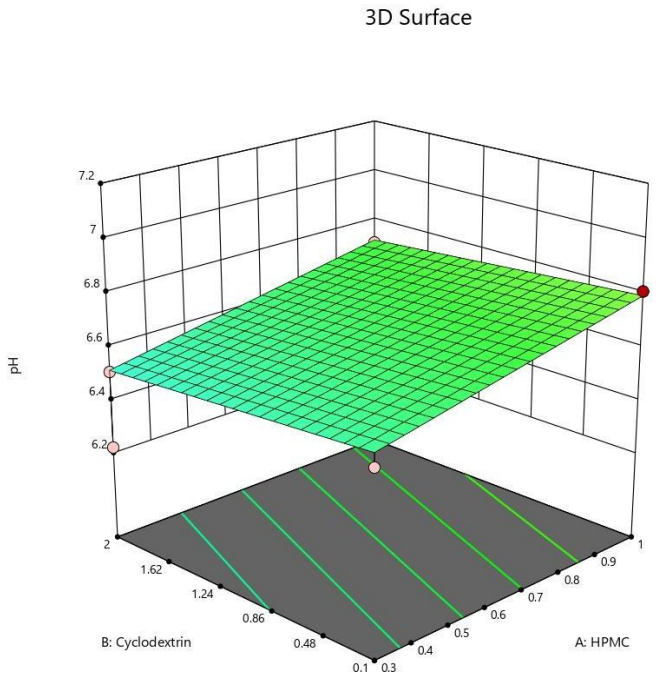


Factor Coding: Actual

pH
Design Points:
Above Surface
Below Surface
6.22 7.19

X1 = A
X2 = B

Actual Factor
C = 0.3

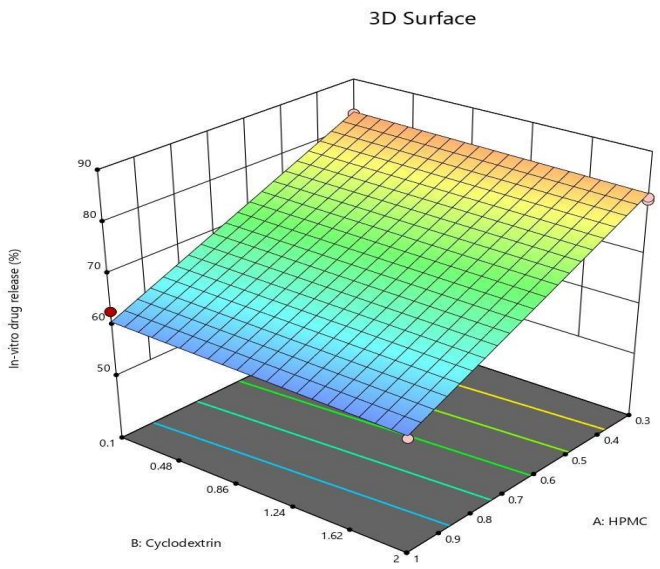


Factor Coding: Actual

In-vitro drug release (%)
Design Points:
Above Surface
Below Surface
57.97 85.9

X1 = A
X2 = B

Actual Factor
C = 0.3

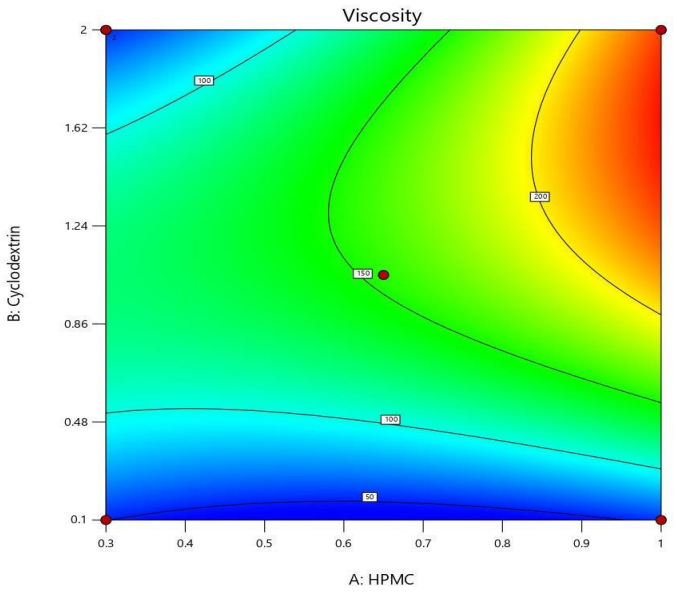


Factor Coding: Actual

Viscosity
Design Points

44 248
X1 = A
X2 = B

Actual Factor
C = 0.3

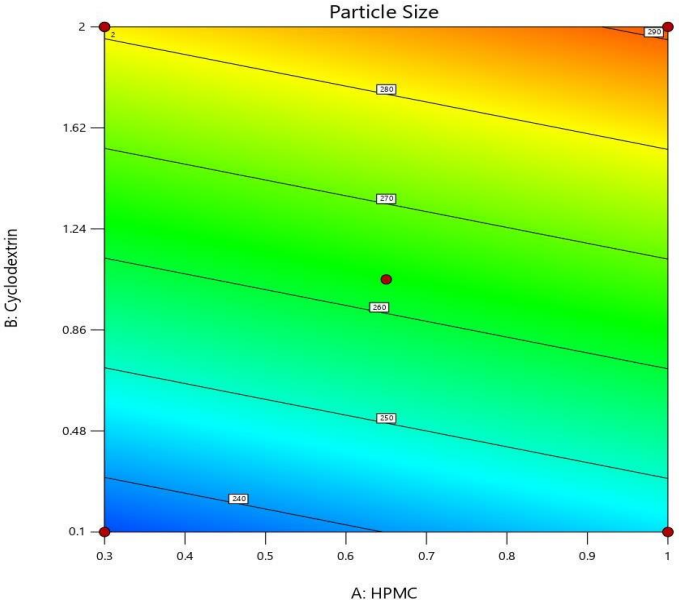


Factor Coding: Actual

Particle Size
Design Points

230.1 296.7
X1 = A
X2 = B

Actual Factor
C = 0.3



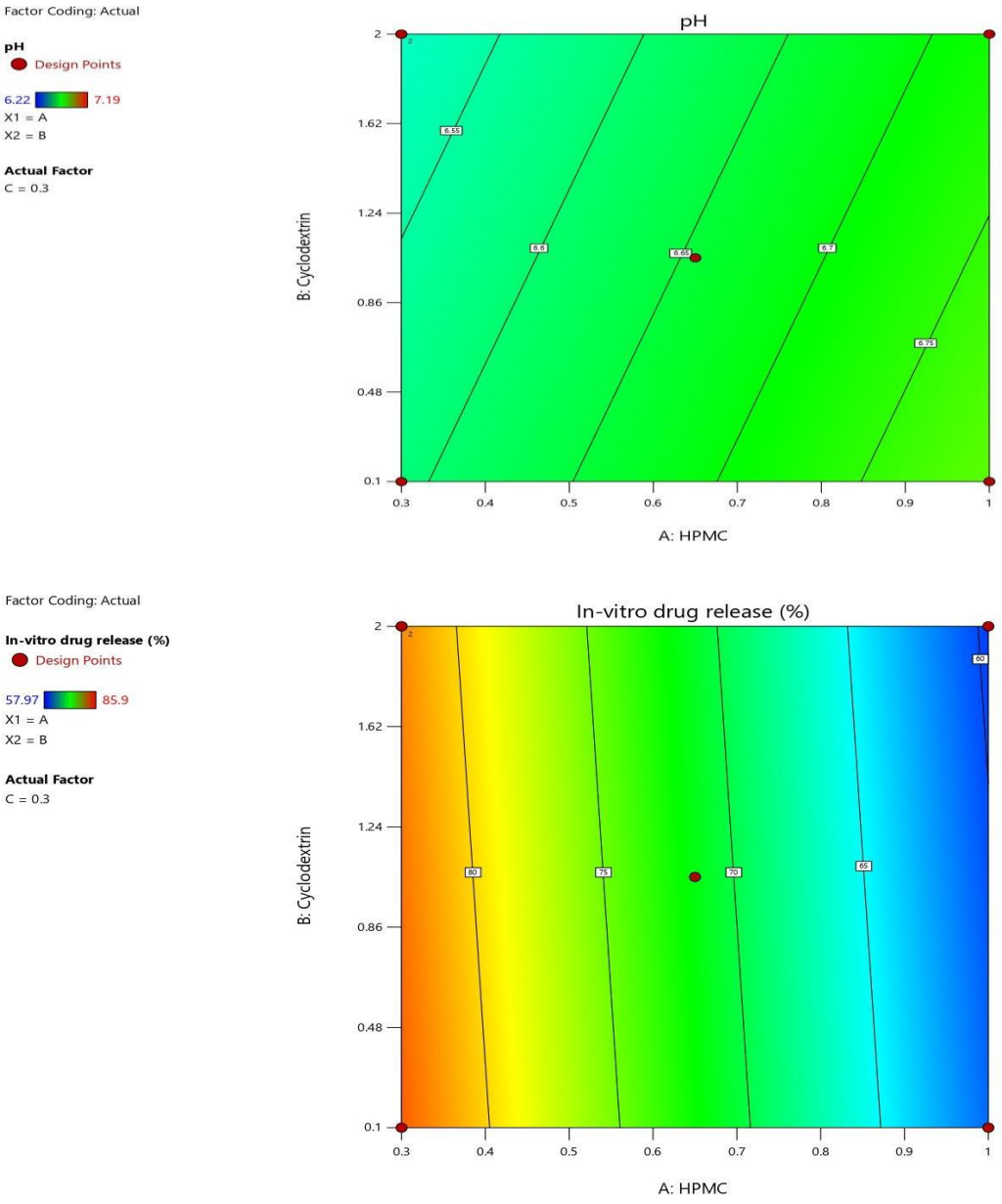


Fig. 10: Contour and 3D response surface plots of (A) Effect of HPMC K4M and cyclodextrin on viscosity (B) Effect of HPMC K4M and cyclodextrin on particle size (C) Effect of HPMC K4M and cyclodextrin on in pH. (D) Effect of HPMC K4M and cyclodextrin on in-vitro drug release. HPMC K4M: Hydroxypropyl methylcellulose.

Validation of optimized formulation

Using the DOE software (version 11) and considering the above findings, an optimized formulation was generated with desirability. This optimized formulation was then prepared and utilized for subsequent evaluation studies. In the following equation (R5), the obtained actual and predicted values from the software were substituted to determine the residual error. The residual percentage error was nearer to the predicted values within±15%, as shown in table 5.

R5: % Residuals = Predicted-Actual (× 100)

Predicted

Table 9: Validation of the Moxifloxacin hydrochloride eye drop optimized formulation.

	Independent variables			Responses				Desirability
	Concentration							
	HPMC K4M	Cyclodextrin	Sodium citrate	Viscosity	Particle size	pH	In vitro drug release	
Software suggested composition	**0.3**	**0.1**	**0.366**	**53.079**	**234.677**	**6.73**	**83.735**	**0.765**
Practically performed	**0.3**	**0.1**	**0.3**	**46.78**	**230.1**	**6.71**	**82.98**	
Residual error (%)				**6.3**	**4.577**	**0.02**		

CONCLUSION

The optimized formulation (F2) contained 0.3%w/v HPMC K4M, 0.1%w/v of Cyclodextrin and 0.3%w/v sodium citrate wherein HPMC imparted sustained release property and cyclodextrin is increasing stability and bioavailability of the formulation. The eye drops will get good patient acceptance because it is easy to instal and gradually erodes by gel, avoiding the need for removal. Hence, it can be concluded that eye drops with viscosity agent are a viable alternative to conventional eye drops by providing sustained release of medicament resulting in decreased frequency of administration leading to better patient acceptance.

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Conflicts of interest

There are no conflicts of interest.

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