

Development and Optimization of a Thermoresponsive and pH sensitive Moxifloxacin Ophthalmic *In-Situ* Gel Using a 2³ Full Factorial Design for Sustained Ocular Drug Delivery

Bhavin Vasantry Bhimani^{1*}, Upendra L. Patel², Harsh Jitendrabhai Trivedi³

1. Research Scholar, Swarnim Startup and Innovation University; Associate Professor, Aarihant School of Pharmacy & BRI, Swarnim Startup and Innovation University, Gujarat, India. Email: bvb3636@gmail.com
2. Principal, Aarihant School of Pharmacy, Swarnim Startup and Innovation University, Gujarat, India. Email: upendralpatel02@gmail.com
3. Professor, Aarihant School of Pharmacy & BRI, Swarnim Startup and Innovation University, Gujarat, India. Email: harshtivedi28@gmail.com

Corresponding Author: Bhavin Vasantry Bhimani, Research Scholar, Swarnim Startup and Innovation University; Associate Professor, Aarihant School of Pharmacy & BRI, Swarnim Startup and Innovation University, Gujarat, India. bvb3636@gmail.com

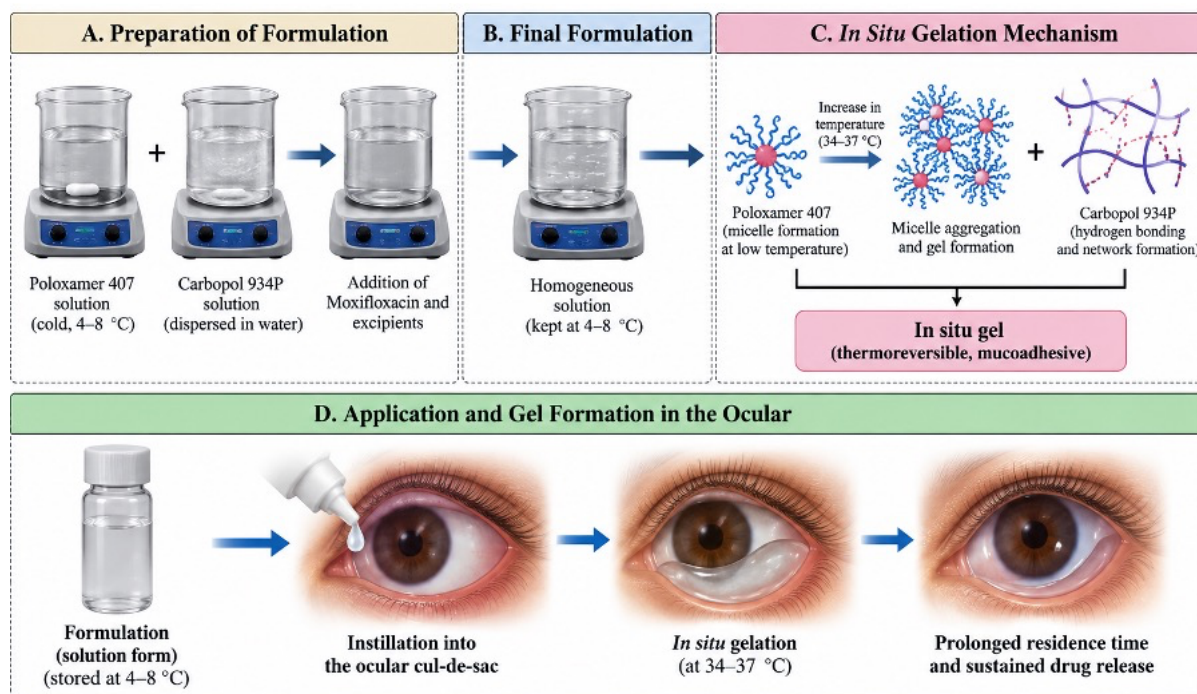
ABSTRACT

This study aimed to develop and optimize a thermoresponsive moxifloxacin ophthalmic *in situ* gel using a 2³ full factorial design for sustained ocular drug delivery. Poloxamer 407 (15-20% w/v), Carbopol 934P (0.5-1.0% w/v), and Chitosan (0.2-0.4% w/v) were selected as formulation variables, and their effects on gelation temperature, viscosity, pH, and cumulative drug release were investigated. The optimized formulation F5, containing 15% Poloxamer 407, 0.5% Carbopol 934P, and 0.4% Chitosan, exhibited a pH of 7.2 ± 0.1 , viscosity of 3400 ± 110 cP, and cumulative moxifloxacin release of $93.5 \pm 2.0\%$ over 8 h. The drug-release data showed the highest correlation with the Higuchi model ($R^2 = 0.987$), while the Korsmeyer-Peppas model yielded an n-value of 0.38, suggesting predominantly diffusion-controlled release under the applied model. The optimized formulation showed an HET-CAM irritancy score of 0.52 ± 0.03 and was classified as non-irritant according to the applied scoring criteria. During 3 months of accelerated stability testing at $40 \pm 2^\circ\text{C}$ and $75 \pm 5\%$ relative humidity, no appreciable changes in appearance, pH, drug content, or drug-release characteristics were observed. Overall, the developed moxifloxacin *in situ* gel demonstrated sustained *in vitro* drug release and acceptable physicochemical characteristics, while further *in vivo* studies are required to establish ocular residence time, pharmacokinetic performance, safety, and therapeutic efficacy.

Keywords: Moxifloxacin, Dual-responsive, In situ gel, 2³ Full factorial design, Ocular drug delivery, Sustained release, Poloxamer 407, Carbopol 934P, Chitosan.

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Graphical Abstract:



INTRODUCTION

Ophthalmic drug delivery presents significant challenges because of the anatomical and physiological barriers of the eye. The precorneal tear film, blinking reflex, nasolacrimal drainage, and corneal epithelial barrier substantially limit the ocular bioavailability of topically administered drugs [1,2]. These limitations can necessitate frequent administration of conventional ophthalmic preparations, which may adversely affect patient adherence and therapeutic consistency [3]. Moxifloxacin hydrochloride is a fourth-generation fluoroquinolone antibiotic with broad-spectrum antibacterial activity against several Gram-positive and Gram-negative ocular pathogens, including *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Pseudomonas aeruginosa* [4]. It is used in the management of bacterial ocular infections, including bacterial conjunctivitis and other ocular infections. However, conventional moxifloxacin ophthalmic preparations are rapidly removed from the precorneal surface by tear turnover and nasolacrimal drainage, which can necessitate repeated administration to maintain adequate drug concentrations at the ocular surface. Consequently, approaches capable of increasing ocular residence time and providing prolonged drug release are of considerable interest [5]. In situ gelling systems have been investigated as an approach to overcome some of the limitations associated with conventional ophthalmic dosage forms. These systems are administered as liquids and undergo a sol-to-gel transition after administration in response to physiological stimuli such as temperature, pH, or ionic strength. Formation of a gel at the ocular

surface can increase formulation residence time and provide more prolonged drug release compared with conventional aqueous eye drops. In addition, administration in the form of a liquid before gel formation can facilitate instillation while allowing the formulation to form a gel after contact with the ocular environment [6]. Dual-responsive in situ gelling systems combine more than one stimulus-responsive mechanism to modulate gel formation and drug release. Poloxamer 407 is a temperature-responsive polymer that undergoes micellar aggregation and gel formation with increasing temperature, whereas Carbopol 934P can undergo swelling and network formation following neutralization and hydration. Chitosan is a natural polysaccharide with mucoadhesive properties and can interact with negatively charged mucosal components through electrostatic and hydrogen-bonding interactions. The combination of these polymers may therefore provide a formulation capable of exhibiting thermoresponsive gelation together with enhanced mucoadhesion and controlled drug release [7]. Experimental design approaches provide a systematic method for investigating the effects of multiple formulation variables and their interactions. Factorial designs allow simultaneous evaluation of formulation factors and their effects on selected quality attributes, thereby reducing the limitations associated with one-factor-at-a-time experimentation. A 2^3 full factorial design evaluates three factors at two levels each and generates eight experimental combinations, allowing assessment of the main effects and interaction effects of the selected variables. Such experimental-design approaches are consistent with systematic

formulation-development and Quality by Design principles [8]. Therefore, the present study aimed to develop and systematically evaluate a moxifloxacin ophthalmic in situ gel containing Poloxamer 407, Carbopol 934P, and Chitosan using a 2³ full factorial design. Poloxamer 407 concentration (X₁), Carbopol 934P concentration (X₂), and Chitosan concentration (X₃) were selected as the independent formulation variables, while gelation temperature, viscosity, pH, and cumulative drug release were evaluated as response variables. The optimized formulation was further investigated for physicochemical characteristics, in vitro drug-release kinetics, ocular irritation potential using the HET-CAM assay, and accelerated stability to assess its potential as a prolonged-release ophthalmic formulation.

MATERIALS AND METHODS

Chemicals and Reagents

Moxifloxacin hydrochloride was obtained as a gift sample from Cipla Ltd., Mumbai, India. Poloxamer 407 (CAS No. 9003-11-6) and Carbopol 934P (CAS No. 9003-01-4) were procured from Sigma-Aldrich Chemical Co., Mumbai, India. Chitosan (CAS No. 9012-76-4; molecular weight 50,000-150,000 Da; degree of deacetylation 85-95%) was obtained from HiMedia Laboratories, Mumbai, India. Benzalkonium chloride (CAS No. 63449-41-2), triethanolamine, sodium chloride, potassium chloride, sodium bicarbonate, and acetic acid were purchased from Loba Chemie, Mumbai, India. All other chemicals and reagents were of analytical grade and were used without further purification. Doubly distilled water was used throughout the study.

2³ Full Factorial Design

A 2³ full factorial design was employed to investigate the influence of three formulation variables using Design-Expert software (version 13.0, Stat-Ease Inc., Minneapolis, USA). Each factor was studied at two levels, coded as -1 and +1. The independent variables were Poloxamer 407 concentration (X₁: 15 and 20% w/v), Carbopol 934P concentration (X₂: 0.5 and 1.0% w/v), and Chitosan concentration (X₃: 0.2 and 0.4% w/v). The design consisted of eight experimental formulations (F₁-F₈), with each formulation prepared and evaluated in triplicate (n = 3). The response variables were gelation temperature (Y₁), viscosity at 37°C (Y₂), pH (Y₃), and cumulative drug release at 8 h (Y₄) [9]. The factor levels and formulation combinations are presented in Tables 1 and 2.

Table 1. 2³ Full Factorial Design: Factor Levels and Coded Values

Factor	Symbol	Low level (-1)	High level (+1)	Unit
Poloxamer 407	X ₁	15	20	% w/v
Carbopol 934P	X ₂	0.5	1.0	% w/v
Chitosan	X ₃	0.2	0.4	% w/v

Table 2. 2³ Full Factorial Design: Formulation Combinations

Formulation n	X ₁ : Poloxamer 407 (% w/v)	X ₂ : Carbopol 934P (% w/v)	X ₃ : Chitosan (% w/v)
F1	15	0.5	0.2
F2	20	0.5	0.2
F3	15	1.0	0.2
F4	20	1.0	0.2
F5	15	0.5	0.4
F6	20	1.0	0.4
F7	15	1.0	0.4
F8	20	0.5	0.4

Preparation of Moxifloxacin Ophthalmic *In Situ* Gel

The formulations were prepared using a modified cold method. Briefly, the required quantity of Poloxamer 407 was slowly dispersed in doubly distilled water and maintained at approximately 4°C with continuous magnetic stirring for 2 h until a clear solution was obtained. Carbopol 934P was separately dispersed in distilled water and allowed to hydrate for 1 h at room temperature. Chitosan was dissolved separately in 1% v/v acetic acid to obtain a clear viscous solution. The hydrated Carbopol 934P dispersion and Chitosan solution were gradually incorporated into the chilled Poloxamer 407 solution and mixed continuously at approximately 4°C for 1 h. Moxifloxacin hydrochloride equivalent to 5 mg was subsequently incorporated into the polymeric mixture and stirred until complete dissolution. Triethanolamine was added dropwise under continuous stirring to adjust the formulation pH to approximately 7.0-7.4. Benzalkonium chloride (0.01% w/v) was incorporated as a preservative. The final volume was adjusted to 50 mL with doubly distilled water. The prepared formulations were designated F₁-F₈ according to the factorial design and stored in tightly closed amber glass containers at 4°C until further evaluation [10-11].

Table 3. Composition of Moxifloxacin Ophthalmic In Situ Gel Formulations (F₁-F₈)

Ingredient / Variable	F ₁	F ₂	F ₃	F ₄	F ₅	F ₆	F ₇	F ₈
Moxifloxacin HCl (mg)	5	5	5	5	5	5	5	5
Poloxamer 407 (% w/v)	15	20	15	20	15	20	15	20
Carbopol 934P (% w/v)	0.5	0.5	1.0	1.0	0.5	1.0	1.0	0.5
Chitosan (% w/v)	0.2	0.2	0.2	0.2	0.4	0.4	0.4	0.4
Benzalkonium chloride (% w/v)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Triethanolamine	q.s. to pH 7.0-7.4	q.s. to pH 7.0-7.4	q.s. to pH 7.0-7.4	q.s. to pH 7.0-7.4	q.s. to pH 7.0-7.4	q.s. to pH 7.0-7.4	q.s. to pH 7.0-7.4	q.s. to pH 7.0-7.4
Doubly distilled water	q.s. to 50 mL	q.s. to 50 mL	q.s. to 50 mL	q.s. to 50 mL	q.s. to 50 mL	q.s. to 50 mL	q.s. to 50 mL	q.s. to 50 mL

Evaluation of Formulations

Visual Inspection and Drug Content Uniformity

The formulations were visually examined for clarity, color, homogeneity, particulate matter, and phase separation. Drug content was determined by diluting 1 mL of formulation with methanol followed by sonication for 10 min. The solution was subsequently diluted with distilled water to obtain a final concentration of 10 µg/mL. Absorbance was measured at 288 nm using a UV-Visible double-beam spectrophotometer (Systronics UV-2202, Systronics India Ltd., Ahmedabad, India). Drug content was calculated using a previously established calibration curve for moxifloxacin hydrochloride over the concentration range of 2-10 µg/mL [12].

Gelation Temperature Determination

Gelation temperature was determined by the tube-inversion method. Approximately 2 mL of each formulation was transferred into a glass tube and placed in a temperature-controlled water bath. The temperature was gradually increased in 1°C increments, with equilibration at each temperature, and the tube was inverted to assess flow behavior. The gelation temperature was recorded as the temperature at which the formulation ceased to flow upon inversion. Measurements were performed in triplicate [13].

Viscosity Measurement

Viscosity measurements were performed at 37 ± 0.5°C using a Brookfield digital viscometer (DV3T, Brookfield Engineering Laboratories, Middleborough, MA, USA). Viscosity was measured at different spindle speeds to characterize the rheological behavior of the formulations.

The viscosity was determined before and after exposure to simulated tear fluid (STF) for 30 min to assess changes in viscosity under simulated ocular conditions. All measurements were performed in triplicate [14].

Preparation of Simulated Tear Fluid

Simulated tear fluid was prepared by dissolving 0.670 g sodium chloride, 0.200 g sodium bicarbonate, and 0.248 g potassium chloride in sufficient distilled water to obtain 1000 mL. The pH was adjusted to 7.4 [15].

pH Measurement

The pH of each formulation was measured at 25 ± 0.5°C using a calibrated digital pH meter (Systronics µ-PH-361, Systronics India Ltd., Ahmedabad, India). The instrument was calibrated using standard buffer solutions of pH 4.0, 7.0, and 9.0 before measurement. Measurements were performed in triplicate [16].

In Vitro Drug Release Study

In vitro drug release was evaluated using a dialysis membrane diffusion method. A cellulose acetate dialysis membrane with a molecular weight cut-off of 12,000 Da was pre-soaked in distilled water for 24 h. One gram of each formulation was accurately weighed and placed over the pre-equilibrated dialysis membrane mounted in a Franz diffusion cell. The receptor compartment contained 50 mL of simulated tear fluid (STF, pH 7.4) and was

maintained at $35 \pm 0.5^\circ\text{C}$ with continuous stirring at 100 rpm. Samples of 2 mL were withdrawn at predetermined intervals of 0.5, 1, 2, 3, 4, 5, 6, 7, and 8 h and immediately replaced with an equal volume of fresh STF maintained at the same temperature. The withdrawn samples were analyzed spectrophotometrically at 288 nm. All experiments were performed in triplicate. The cumulative percentage of drug released was calculated after correction for the volume of receptor medium withdrawn during sampling [17-18].

Drug Release Kinetic Modeling

The in vitro drug-release data were fitted to zero-order, first-order, Higuchi, and Korsmeyer-Peppas kinetic models to characterize the release behavior and mechanism. For the Korsmeyer-Peppas model, the release exponent (n) was determined from the slope of the plot of log cumulative drug release versus log time. For a planar matrix system, an n-value ≤ 0.45 indicates Fickian diffusion, $0.45 < n < 0.89$ indicates anomalous transport, and $n \geq 0.89$ indicates Case-II transport [19].

HET-CAM Irritation Test

The ocular irritation potential of the optimized formulation was evaluated using the hen's egg chorioallantoic membrane (HET-CAM) assay. Fertilized specific pathogen-free eggs weighing approximately 50-60 g were incubated at $37 \pm 1^\circ\text{C}$ with periodic rotation for 10 days. After incubation, the eggs were cleaned with 70% ethanol, and a 2×2 cm window was opened in the shell to expose the chorioallantoic membrane (CAM). The optimized formulation was applied to the exposed CAM. A 0.9% w/v sodium chloride solution was used as the negative control, whereas 1% w/v sodium hydroxide was used as the positive control. The CAM was observed for 5 min for hemorrhage, vascular lysis, and coagulation. The corresponding reaction times were recorded, and the irritancy score was calculated according to the HET-CAM scoring procedure. The formulation was subsequently classified according to the corresponding irritancy-score criteria [20].

Stability Study

The optimized formulation was subjected to accelerated stability testing. The formulation was stored in tightly closed amber glass containers at $40 \pm 2^\circ\text{C}$ and $75 \pm 5\%$ relative humidity for 3 months. Samples were evaluated at 0, 1, 2, and 3 months for appearance, pH, viscosity, drug content, and in vitro drug release. Changes in physical appearance, phase separation, drug content, rheological properties, and drug-release behavior were recorded and compared with the initial values [20].

Statistical Analysis

All experimental measurements were performed in triplicate and expressed as mean $\pm$ standard deviation (SD). Statistical analysis of the factorial design was performed using Design-Expert® software (version 13.0, Stat-Ease Inc., Minneapolis, USA). Analysis of variance (ANOVA) was used to evaluate the statistical significance of the main effects and interaction effects of the formulation variables. Model adequacy was assessed using the coefficient of determination (R^2), adjusted R^2 , predicted R^2 , F-value, p-value, and adequate precision. A p-value < 0.05 was considered statistically significant. Tukey's multiple-comparison test was used for pairwise comparison of formulation means where appropriate [21].

RESULTS

Visual Inspection and Drug Content Analysis

All formulations (F₁-F₈) appeared as clear, colorless to pale-yellow viscous solutions at room temperature (25°C) and formed transparent gels upon heating. No phase separation, precipitation, or visible particulate matter was observed in any formulation. The drug content of the formulations ranged from 95.5% to 98.4%, indicating satisfactory drug content uniformity. The individual drug-content values and physical appearance of the formulations are presented in Table 4. The drug content ranged from 95.5% to 98.4%, indicating satisfactory drug content uniformity across the formulations and reproducibility of the formulation process.

Table 4. Drug Content and Physical Appearance of Moxifloxacin In Situ Gel Formulations (F₁-F₈)

Formulation	Appearance	Drug Content (%)	SD	RS D (%)
F ₁	Clear, colorless	96.2 $\pm$ 0.3	0.27	0.28
F ₂	Clear, colorless	97.8 $\pm$ 0.2	0.14	0.14
F ₃	Clear, pale yellow	95.5 $\pm$ 0.4	0.35	0.37
F ₄	Clear, pale yellow	98.4 $\pm$ 0.2	0.11	0.11
F ₅	Clear, colorless	96.8 $\pm$ 0.3	0.22	0.23
F ₆	Clear, pale yellow	97.5 $\pm$ 0.2	0.16	0.16
F ₇	Clear, pale yellow	95.9 $\pm$ 0.3	0.25	0.26
F ₈	Clear, colorless	98.1 $\pm$ 0.2	0.13	0.13

Values are expressed as mean $\pm$ SD (n=3).

Gelation Temperature Analysis

The gelation temperatures of the eight formulations ranged from $20.5 \pm 1.4^\circ\text{C}$ to $28.2 \pm 0.8^\circ\text{C}$, demonstrating the thermoresponsive behavior of the developed formulations. The variation in gelation temperature among the formulations indicated that the polymer composition influenced the sol-to-gel transition. Poloxamer 407 (X_1) exhibited a significant influence on gelation temperature ($p < 0.001$), with increasing Poloxamer 407 concentration associated with a reduction in gelation temperature. Carbopol 934P (X_2) showed a positive influence on gelation temperature ($p < 0.05$), whereas the interaction between Poloxamer 407 and Chitosan ($X_1 \times X_3$) was also significant ($p < 0.05$), indicating that the combined polymer concentrations influenced gelation behavior. The statistical analysis of the gelation-temperature response showed a significant model ($p < 0.0001$), with $R^2 = 0.9672$, adjusted $R^2 = 0.9224$, predicted $R^2 = 0.7983$, and adequate precision of 12.453, indicating an adequate relationship between the formulation variables and gelation-temperature response.

Viscosity Analysis

The viscosity of the formulations at 37°C ranged from 2100 ± 85 to 5200 ± 200 cP before exposure to simulated tear fluid (STF), as measured at 50 rpm. Following exposure to STF, the apparent viscosity decreased with increasing spindle speed. Poloxamer 407 (X_1) showed a significant positive effect on viscosity ($p < 0.001$), while Carbopol 934P (X_2) also significantly increased viscosity ($p < 0.01$). Chitosan (X_3) showed a moderate positive influence on viscosity ($p < 0.05$). The interaction between Poloxamer 407 and Carbopol 934P ($X_1 \times X_2$) was highly significant ($p < 0.001$), indicating an interaction between these variables in determining the rheological behavior of the formulations. The decrease in viscosity with increasing spindle speed indicated shear-thinning behavior, which may facilitate administration during instillation while maintaining the formulation at the ocular surface after administration.

Table 5. Viscosity of Moxifloxacin In Situ Gel Formulations Before and After STF Exposure

Formulation	Before STF, 50 rpm (cP)	After STF, 75 rpm (cP)	After STF, 100 rpm (cP)
F ₁	2100 ± 85	1100 ± 45	720 ± 30

F ₂	3200 ± 120	1650 ± 65	1080 ± 45
F ₃	2800 ± 95	1450 ± 55	950 ± 40
F ₄	4100 ± 150	2100 ± 80	1380 ± 55
F ₅	3400 ± 110	1750 ± 70	1150 ± 48
F ₆	4800 ± 180	2480 ± 95	1620 ± 65
F ₇	3800 ± 130	1950 ± 75	1280 ± 52
F ₈	5200 ± 200	2680 ± 105	1750 ± 70

Values are expressed as mean $\pm$ SD (n=3).

pH Analysis

The pH values of the formulations ranged from 6.8 ± 0.1 to 7.3 ± 0.1 . The observed pH values were within the range considered acceptable for ophthalmic formulations. The statistical analysis of the pH response showed a significant model ($p < 0.001$), with $R^2 = 0.9587$, adjusted $R^2 = 0.9046$, predicted $R^2 = 0.8245$, and adequate precision of 9.847. Carbopol 934P (X_2) showed a significant effect on the pH response ($p < 0.001$), while Chitosan (X_3) showed a moderate effect ($p < 0.05$). No significant interaction effects were observed between the factors for the pH response ($p > 0.05$).

In Vitro Drug Release Studies

The cumulative drug-release profiles of all eight formulations over 8 h are presented in Table 6 and Figure 1. All formulations exhibited progressive drug release throughout the study period, with cumulative drug release at 8 h ranging from $88.5 \pm 3.2\%$ to $97.8 \pm 1.2\%$. The release profiles showed an initial relatively rapid-release phase followed by a slower release phase. The initial release may be associated with drug located near the surface of the hydrated polymeric matrix, whereas the subsequent release may be governed predominantly by diffusion through the hydrated polymeric network. Among the formulations, F₈ exhibited the highest drug release ($97.8 \pm 1.2\%$), whereas F₁ showed the lowest drug release ($88.5 \pm 3.2\%$) at 8 h. Formulation F₅ exhibited $93.5 \pm 2.0\%$ drug release at 8 h. The differences in drug-release behavior among the formulations may be associated with differences in polymer concentration and the resulting polymeric network structure. Formulation F₅, containing 15% Poloxamer 407, 0.5% Carbopol 934P, and 0.4% Chitosan, was selected as the optimized formulation based on the reported desirability-function analysis.

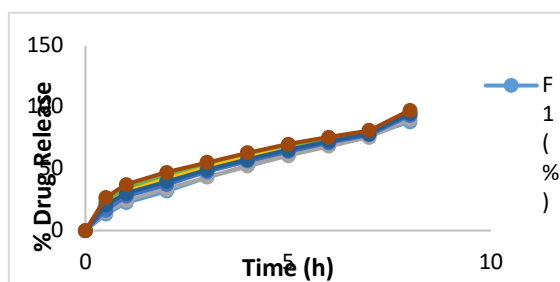


Figure 1. In vitro cumulative drug-release profile of moxifloxacin from F1-F8 formulations over 8 h.

Table 6. In Vitro Drug Release Profiles of Moxifloxacin In Situ Gel Formulations (F1-F8)

Time (h)	F1 (%)	F2 (%)	F3 (%)	F4 (%)	F5 (%)	F6 (%)	F7 (%)	F8 (%)
0.5	13.2 ± 1.2	18.5 ± 1.5	14.8 ± 1.1	22.3 ± 1.8	16.5 ± 1.3	24.1 ± 1.6	20.5 ± 1.4	26.8 ± 1.5
1	22.5 ± 1.8	28.3 ± 2.0	24.1 ± 1.5	32.7 ± 2.2	27.6 ± 1.9	35.2 ± 2.1	30.4 ± 1.7	37.5 ± 2.0
2	32.1 ± 2.1	38.5 ± 2.3	33.8 ± 1.8	42.9 ± 2.5	37.4 ± 2.2	45.6 ± 2.4	39.7 ± 2.0	47.1 ± 2.3
3	43.5 ± 2.5	48.2 ± 2.6	43.0 ± 2.0	52.1 ± 2.8	47.8 ± 2.4	54.3 ± 2.6	49.1 ± 2.2	55.5 ± 2.5
4	52.8 ± 2.3	56.1 ± 2.4	52.2 ± 1.9	60.5 ± 3.0	56.3 ± 2.5	62.7 ± 2.7	57.4 ± 2.3	63.2 ± 2.6
5	61.2 ± 2.0	63.5 ± 2.1	60.8 ± 1.7	67.4 ± 2.7	64.1 ± 2.3	69.5 ± 2.5	65.2 ± 2.1	70.1 ± 2.4
6	69.5 ± 2.1	69.8 ± 2.2	68.1 ± 1.8	73.2 ± 2.9	71.5 ± 2.4	75.1 ± 2.6	72.3 ± 2.2	76.4 ± 2.5
7	77.2 ± 2.2	76.0 ± 2.3	75.8 ± 1.9	79.1 ± 3.1	77.8 ± 2.5	79.4 ± 2.7	78.5 ± 2.3	81.2 ± 2.6

	1.9	2.0	1.8	2.0	1.8	2.0	1.7	1.8
8	88.5 ± 3.2	92.3 ± 2.1	90.2 ± 2.8	94.8 ± 1.9	93.5 ± 2.0	96.2 ± 1.5	95.1 ± 1.8	97.8 ± 1.2

Values are expressed as mean ± SD (n=3).

Drug Release Kinetic Analysis

The drug-release data were fitted to zero-order, first-order, Higuchi, and Korsmeyer-Peppas models. Among the evaluated models, the Higuchi model showed the highest coefficient of determination for all formulations, with R^2 values ranging from 0.986 to 0.993. The Korsmeyer-Peppas release exponent (n) ranged from 0.35 to 0.43. For the optimized formulation F5, the Higuchi model showed an R^2 value of 0.987, while the Korsmeyer-Peppas model gave an n-value of 0.38. The observed n-values were below 0.45, indicating a predominantly diffusion-controlled release mechanism under the applied model. The complete kinetic parameters are presented in Table 7.

Table 7: Drug Release Kinetic Parameters of All Formulations (F1-F8)

Formulation	Zero-Order		First-Order		Higuchi		Korsmeyer-Peppas		
	R^2	Slope	R^2	Slope	R^2	Slope	n	R^2	Slope
F1	0.8922	1.25	0.9155	-0.185	0.9936	3.52	0.46	0.96	0.38
F2	0.8988	1.85	0.9192	-0.192	0.9938	5.25	0.44	0.96	0.36
F3	0.8855	2.98	0.9138	-0.178	0.9930	6.12	0.43	0.96	0.39
F4	0.9020	1.05	0.9192	-0.165	0.9931	4.85	0.43	0.97	0.35
F5	0.8990	2.15	0.9132	-0.172	0.9877	4.25	0.38	0.97	0.37
F6	0.909	1.0	0.919	-0.188	0.9936	3.6	0.43	0.99	0.34

	0	9	4		9	8	3	7	
	5	5	0		2	5	6	2	
F7	0.	1	0.	-0	0.	3	0	0.	0.
	8	2.	9	.1	9	5.	.	9	3
	9	3	3	75	9	6	4	6	8
	5	5	5		1	5	1	8	
F8	0.	1	0.	-0	0.	3	0	0.	0.
	9	1.	9	.1	9	7.	.	9	3
	0	5	4	90	9	5	3	7	3
	8	5	2		3	0	5	4	

HET-CAM Irritation Test

The HET-CAM results for the optimized formulation F5 and the controls are presented in Table 8 and Figure 2. The negative control (0.9% NaCl) showed no observable hemorrhage, vascular lysis, or coagulation during the observation period and was classified as non-irritant. The optimized formulation F5 also showed no evidence of hemorrhage, vessel lysis, or coagulation on the CAM. The reported irritancy score was 0.52 ± 0.03 , corresponding to the non-irritant classification according to the scoring criteria used in the study.

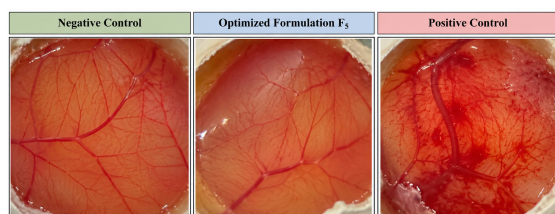


Figure 2. Representative HET-CAM images of the negative control, optimized formulation F5, and positive control.

The positive control (1% NaOH) produced rapid hemorrhage, vascular lysis, and coagulation, with an irritancy score of 8.52 ± 0.05 . These findings demonstrated the responsiveness of the HET-CAM assay under the experimental conditions.

Table 8. HET-CAM Irritancy Assessment of Optimized Formulation and Controls

Sample	Hemorrhage Time (sec)	Lysis Time (sec)	Coagulation Time (sec)	Irritancy Score	Classification
0.9% NaCl (Negative)	$>300 \pm 1.53$	$>300 \pm 1.00$	$>300 \pm 0.57$	0.48 ± 0.02	Non-irritant

Control)					
F5 (Optimized Formulation)	$>300 \pm 1.85$	$>290 \pm 1.53$	$>290 \pm 1.53$	0.52 ± 0.03	Non-irritant
1% NaOH (Positive Control)	185 ± 2.50	175 ± 3.00	160 ± 2.80	8.52 ± 0.05	Moderate irritant

Values are expressed as mean $\pm$ SD (n=3).

Stability Studies

The stability results for the optimized formulation F5 stored at $40 \pm 2^\circ\text{C}$ and $75 \pm 5\%$ relative humidity for 3 months are presented in Table 9. No phase separation, precipitation, or color change was observed during the study period. The pH remained within the range of 7.1-7.3, while drug content remained above 96%. The 8-h drug release remained above 92% throughout the study. Only minor variation in viscosity was observed during storage. Overall, the evaluated physicochemical characteristics remained relatively consistent throughout the 3-month accelerated stability study.

Table 9. Stability Parameters of Optimized Formulation (F5) Under Accelerated Conditions ($40^\circ\text{C}/75\% \text{ RH}$)

Parameter	Month 0 (Initial)	Month 1	Month 2	Month 3
Appearance	Clear, colorless	Clear, colorless	Clear, colorless	Clear, colorless
pH	7.2 ± 0.1	7.1 ± 0.1	7.2 ± 0.1	7.3 ± 0.1
Viscosity at 37°C , 50 rpm (cP)	3400 ± 110	3520 ± 120	3480 ± 115	3550 ± 125
Drug Content (%)	96.8 ± 0.3	96.5 ± 0.4	96.2 ± 0.3	96.0 ± 0.4
Drug Release at 8 h (%)	93.5 ± 2.0	93.2 ± 2.1	92.8 ± 1.9	93.0 ± 2.0
RSD (%)	-	<8	<8	<8

Values are expressed as mean $\pm$ SD (n = 3).

DISCUSSION

The (2³) full factorial design was employed to investigate the influence of Poloxamer 407, Carbopol 934P, and Chitosan on the major formulation characteristics of the moxifloxacin in situ gel. The results demonstrated that variation in polymer concentration affected gelation temperature, viscosity, pH, and drug-release behavior.

The gelation-temperature results demonstrated that Poloxamer 407 concentration was an important factor influencing the thermoresponsive behavior of the formulations. Increasing Poloxamer 407 concentration was associated with a reduction in gelation temperature. This behavior may be attributed to increased micellar aggregation and polymeric network formation at higher Poloxamer concentrations. Carbopol 934P and Chitosan may additionally modify the polymeric environment and consequently influence the sol-to-gel transition.

The viscosity results demonstrated that increasing concentrations of Poloxamer 407 and Carbopol 934P increased the viscosity of the formulations. Poloxamer 407 contributes to viscosity through the formation of micellar structures, whereas neutralized Carbopol 934P contributes through formation of a hydrated polymeric network. Chitosan may further contribute through intermolecular interactions with the other polymers. The decrease in viscosity with increasing spindle speed indicates pseudoplastic or shear-thinning behavior, which can facilitate administration while maintaining the formulation within the ocular region after instillation.

The pH values of the formulations remained within the range reported as suitable for ophthalmic administration. The optimized formulation F5 exhibited a pH of 7.2 ± 0.1 , which was close to the reported physiological pH of the tear film. This relatively close pH may help minimize discomfort associated with substantial pH differences following ocular administration.

The drug-release profiles demonstrated progressive release of moxifloxacin over 8 h. Formulation F8 showed the highest cumulative drug release, whereas F1 showed the lowest. The differences among formulations may be related to the concentration and combination of the polymers, which influence hydration, network structure, and diffusion pathways within the formulation.

The optimized formulation F5 exhibited $93.5 \pm 2.0\%$ drug release at 8 h. The highest R^2 values were obtained with the Higuchi model, suggesting that

diffusion contributed substantially to drug release. The Korsmeyer-Peppas n -value for F5 was 0.38, supporting a predominantly diffusion-controlled mechanism under the applied model.

The HET-CAM study demonstrated that the optimized formulation F5 produced no observable hemorrhage, vascular lysis, or coagulation during the observation period and was classified as non-irritant according to the reported scoring criteria. The positive control produced pronounced vascular reactions, whereas the negative control showed no significant irritation response. These findings provide preliminary evidence of ocular compatibility under the experimental conditions.

The accelerated stability study demonstrated that the optimized formulation maintained relatively consistent physicochemical characteristics during 3 months of storage at $40 \pm 2^\circ\text{C}$ and $75 \pm 5\%$ relative humidity. The pH remained within the observed range, drug content remained above 96%, and drug release remained above 92% at 8 h. These results indicate satisfactory stability of the formulation under the investigated accelerated-storage conditions.

Comparison with previously published moxifloxacin in situ gel systems provides contextual support for the observed drug-release and gelation characteristics. However, direct comparison between formulations should be interpreted cautiously because formulation composition and experimental conditions may differ among studies.

The factorial-design approach provided a systematic framework for evaluating the influence of the selected formulation variables. Based on the reported desirability-function analysis, F5 was selected as the optimized formulation, providing a balance among the investigated formulation characteristics.

CONCLUSION

The present study successfully developed a moxifloxacin ophthalmic in situ gel containing Poloxamer 407, Carbopol 934P, and Chitosan using a (2³) factorial design. The formulation variables influenced the gelation behavior, viscosity, pH, and drug-release characteristics of the prepared formulations.

Among the investigated formulations, F5 containing 15% Poloxamer 407, 0.5% Carbopol 934P, and 0.4% Chitosan was selected as the optimized formulation according to the reported desirability-function analysis. F5 exhibited a pH of 7.2 ± 0.1 and released $93.5 \pm 2.0\%$ of moxifloxacin over 8 h. The

drug-release data showed the highest fit to the Higuchi model ($R^2 = 0.987$), while the Korsmeyer-Peppas release exponent was 0.38, indicating a predominantly diffusion-controlled release pattern under the applied model.

The optimized formulation was classified as non-irritant in the HET-CAM assay and maintained its evaluated physicochemical characteristics during 3 months of accelerated storage. These findings support the potential of the developed formulation as a sustained-release ophthalmic in situ gel. Further in vivo ocular residence, pharmacokinetic, safety, efficacy, and clinical studies are required to establish its therapeutic performance and suitability for prolonged ocular administration.

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