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Development and Optimization of a Dual-Responsive Levofloxacin Ophthalmic ιν Situ Gel Using a 2^3 Full Factorial Design for Sustained Ocular Drug Delivery

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ABSTRACT:
Poor ocular bioavailability associated with conventional levofloxacin eye drops, resulting from rapid precorneal drainage and nasolacrimal clearance, limits therapeutic efficacy and necessitates frequent administration. The present study aimed to develop and optimize a dual-responsive ophthalmic in situ gelling system of levofloxacin using Poloxamer 407, Carbopol 940, and HPMC E50LV to prolong precorneal residence time and achieve sustained drug release. A 2^3 full factorial design was employed to evaluate the influence of polymer concentrations on viscosity and drug release behavior. Eight formulations (F1–F8) were prepared and evaluated for physicochemical properties, gelation characteristics, rheological behavior, drug content, in vitro drug release, release kinetics, factorial optimization, and antimicrobial activity. The formulations exhibited acceptable physicochemical characteristics with a pH ranging from 5.0 to 5.6 and drug content between 98.23 and 101.66%. Rheological studies confirmed pseudoplastic flow behavior with a marked increase in viscosity under physiological conditions, indicating rapid sol-to-gel transition after administration. Among all formulations, F4 exhibited optimum performance, showing immediate gelation with prolonged gel residence and 100.65% cumulative drug release over 8 h, whereas the marketed formulation released the drug completely within approximately 2 h. Drug release predominantly followed the Higuchi model (R^2 = 0.9806) with a Korsmeyer–Peppas diffusion exponent (n = 0.4972), indicating diffusion-controlled release. The optimized formulation also retained potent antibacterial activity, demonstrating 96.51% efficacy against Staphylococcus aureus and 85.06% efficacy against Pseudomonas aeruginosa. The factorial model exhibited excellent predictive capability (R^2 = 0.9997 for viscosity and 0.9980 for T_{90}). Overall, the optimized levofloxacin in situ gel demonstrated superior rheological characteristics, sustained drug release, preserved antimicrobial activity, indicating its potential to enhance ocular residence time, improve therapeutic efficacy, and reduce dosing frequency compared with conventional ophthalmic formulations.

1. INTRODUCTION:

Ocular bacterial infections, including conjunctivitis, keratitis, and corneal ulcers, are among the most common eye disorders requiring topical antibiotic therapy. Topical ophthalmic administration is the preferred treatment because it provides direct drug delivery to the site of infection while minimizing systemic exposure [1]. However, conventional eye drops exhibit poor ocular bioavailability due to rapid tear turnover, blinking, nasolacrimal drainage, and the corneal epithelial barrier, resulting in the retention of less than 5% of the administered dose on the ocular surface. These limitations necessitate frequent dosing and may reduce therapeutic efficacy and patient compliance [2]. In situ

gelling systems have emerged as an effective strategy to overcome these challenges. Such formulations are administered as low-viscosity solutions and undergo rapid sol-to-gel transformation in response to physiological stimuli, thereby increasing precorneal residence time, reducing drug loss, and providing sustained drug release. Among the available polymers, Poloxamer 407 is widely used as a thermoresponsive polymer because of its reversible temperature-dependent gelation, whereas Carbopol 940 exhibits pH-responsive swelling upon contact with tear fluid. Their combination produces a dual-responsive gel system capable of rapid gel formation under ocular conditions [3]. HPMC E50LV further enhances viscosity, improves gel stability, and contributes to controlled drug release. Levofloxacin, a broad-spectrum third-generation fluoroquinolone antibiotic, is widely prescribed for the treatment of bacterial conjunctivitis, keratitis, and other ocular infections because of its excellent activity against both Gram-positive and Gram-negative pathogens. Nevertheless, commercially available levofloxacin eye drops require frequent administration owing to their short precorneal residence time, which may compromise patient adherence and therapeutic effectiveness [4].

Although several ophthalmic in situ gels have been reported, limited studies have systematically optimized a dual-responsive levofloxacin in situ gel using statistical experimental design while simultaneously evaluating rheological properties, sustained drug release, and antimicrobial efficacy [5]. Therefore, the present study aimed to develop and optimize a dual-responsive levofloxacin ophthalmic in situ gel using Poloxamer 407, Carbopol 940, and HPMC E50LV employing a 2^3 full factorial design. The developed formulations were evaluated for physicochemical characteristics, gelation behavior, rheological properties, in vitro drug release, release kinetics, factorial optimization, and antimicrobial activity to identify an optimized formulation for sustained ocular drug delivery.

2. MATERIALS AND METHODS

2.1 Materials

Levofloxacin hemihydrate was received as a gift sample from Mepro Pharmaceuticals, India. Poloxamer 407 (Pluronic F127) was procured from BASF Corporation, Germany. Carbopol 934 and Carbopol 940 were obtained from Corel Pharma Chem, Ahmedabad, India. Hydroxypropyl methylcellulose (HPMC E50LV, HPMC K4M, and HPMC K100M) were supplied by Colorcon India Pvt. Ltd., India. Benzalkonium chloride was used as a preservative, while boric acid was used as an isotonicity-adjusting agent. Citro-phosphate buffer (pH 5.0) served as the vehicle for formulation preparation. All other chemicals and reagents used were of analytical reagent (AR) grade, and double-distilled water was used throughout the study.

2.2 Characterization of Levofloxacin

2.2.1 Organoleptic Properties

Levofloxacin was evaluated for its colour, appearance, odour, and physical characteristics by visual inspection [6].

2.2.2 Determination of Melting Point

The melting point of levofloxacin was determined using the capillary tube method with a digital melting point apparatus. Finely powdered drug was filled into a capillary tube and heated gradually until complete melting occurred. The observed melting point was recorded [7].

2.2.3 Solubility Study

The solubility of levofloxacin was determined in distilled water, artificial tear fluid (ATF), methanol, phosphate buffer (pH 7.4), and citro-phosphate buffer (pH 5.0). Excess drug was added to 10 mL of each solvent and shaken continuously for 24 h at room temperature. The solutions were filtered through a 0.45 μm membrane filter, appropriately diluted, and analyzed spectrophotometrically at 287 nm [8].

2.2.4 UV Spectrophotometric Analysis

The absorption spectrum of levofloxacin was recorded between 200 and 400 nm using a UV–Visible spectrophotometer to determine the wavelength of maximum absorbance (λmax). The λmax was found to be 287 nm [9].

2.2.5 Preparation of Calibration Curve

A standard stock solution of levofloxacin (100 μg/mL) was prepared separately in artificial tear fluid and citro-

phosphate buffer (pH 5.0). Appropriate dilutions were made to obtain concentrations ranging from 2–14 µg/mL. The absorbance of each solution was measured at 287 nm, and calibration curves were constructed by plotting absorbance against concentration [9].

2.3 Preliminary Screening and Selection of Polymers

Preliminary experiments were performed to identify suitable polymers for the development of an ophthalmic in situ gelling system. Poloxamer 407 was investigated as a thermoresponsive polymer at concentrations ranging from 5–24% (w/v), while Carbopol 934 and Carbopol 940 were evaluated as pH-responsive polymers at concentrations between 0.2 and 2.0% (w/v). Different grades of hydroxypropyl methylcellulose (HPMC E50LV, HPMC K4M, and HPMC K100M) were investigated as viscosity-enhancing agents in combination with Carbopol. Each polymeric solution was evaluated for clarity, pH, viscosity, phase transition temperature, and gelation behaviour under physiological conditions. Based on clarity, gelling characteristics, and ease of formulation, Carbopol 940, Poloxamer 407, and HPMC E50LV were selected for the preparation of the final formulations [10, 11].

2.4 Experimental Design

A 2^3 full factorial design was employed to optimize the formulation variables. Three independent formulation variables were selected and studied at two levels (−1 and +1). X_1: Poloxamer 407 concentration (5 and 10% w/v), X_2: Carbopol 940 concentration (0.2 and 0.4% w/v) and X_3: HPMC E50LV concentration (0.4 and 0.8% w/v). Eight experimental formulations (F1–F8) were prepared according to the factorial design matrix shown in Table 1. The effects of these independent variables on gelation behaviour, viscosity, and drug release characteristics were evaluated [12].

Table 1. Experimental design of the 2^3 full factorial formulations

Batch	X_1 (Poloxamer 407, % w/v)	X_2 (Carbopol 940, % w/v)	X_3 (HPMC E50LV, % w/v)
F1	5	0.2	0.4
F2	10	0.2	0.4
F3	5	0.4	0.4
F4	10	0.4	0.4
F5	5	0.2	0.8
F6	10	0.2	0.8
F7	5	0.4	0.8
F8	10	0.4	0.8

2.5 Formulation of Levofloxacin In Situ Ophthalmic Gel

Eight formulations (F1–F8) were prepared according to the composition presented in Table 2. The concentration of levofloxacin was maintained at 0.5% (w/v) in all formulations, whereas the concentrations of Poloxamer 407, Carbopol 940, and HPMC E50LV were varied according to the factorial design. Benzalkonium chloride (0.01% w/v) was incorporated as the preservative, boric acid (1.64% w/v) was used as the isotonicity-adjusting agent, and citro-phosphate buffer (pH 5.0) was used as the vehicle [13].

Table 2. Composition of Levofloxacin in situ ophthalmic gel formulations

Ingredients (% w/v)	F1	F2	F3	F4	F5	F6	F7	F8
Levofloxacin	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Poloxamer 407	5	10	5	10	5	10	5	10
Carbopol 940	0.2	0.2	0.4	0.4	0.2	0.2	0.4	0.4
HPMC E50LV	0.4	0.4	0.4	0.4	0.8	0.8	0.8	0.8
Benzalkonium chloride	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Boric acid	1.64	1.64	1.64	1.64	1.64	1.64	1.64	1.64
Citro-phosphate buffer (pH 5.0)	q.s. to 100 mL	q.s. to 100 mL	q.s. to 100 mL	q.s. to 100 mL	q.s. to 100 mL	q.s. to 100 mL	q.s. to 100 mL	q.s. to 100 mL

2.6 Preparation of In Situ Ophthalmic Gel

Citro-phosphate buffer (pH 5.0) was prepared according to the standard procedure. The required quantity of HPMC E50LV was dispersed in the buffer under continuous magnetic stirring until a clear solution was obtained. Carbopol 940 was gradually added to the HPMC solution with continuous stirring until complete hydration of the polymer

was achieved. The required amount of Poloxamer 407 was then slowly incorporated into the hydrated polymeric solution while stirring continuously for approximately 1 h [14]. The partially dissolved dispersion was refrigerated at 4 ± 1°C for 24 h to ensure complete dissolution of Poloxamer 407 by the cold method. Separately, levofloxacin and benzalkonium chloride were dissolved in citro-phosphate buffer (pH 5.0) and slowly added to the polymeric dispersion under gentle stirring until a homogeneous solution was obtained. Boric acid was subsequently added to adjust isotonicity, and the final volume was made up to 100 mL with citro-phosphate buffer (pH 5.0). The prepared formulations were filled into sterilized ophthalmic containers and subjected to terminal sterilization before further evaluation [15].

2.7 Evaluation of Formulations

2.7.1 Appearance and Clarity

All formulations were visually examined against black and white backgrounds for colour, clarity, transparency, and the presence of particulate matter.

2.7.2 pH Measurement

The pH of each formulation was determined using a calibrated digital pH meter at room temperature. Measurements were performed in triplicate, and the mean values were recorded.

2.7.3 Drug Content

An accurately measured quantity of formulation equivalent to 5 mg of levofloxacin was diluted with artificial tear fluid, and the absorbance was measured at 287 nm using a UV–Visible spectrophotometer. Drug content was calculated from the calibration curve [16].

2.7.4 Gelling Capacity

The gelling capacity was evaluated by adding one drop of formulation into 2 mL of freshly prepared artificial tear fluid maintained at 37 ± 0.5°C. The gelation behaviour and gel residence time were visually observed and graded as follows: **(−):** No gel formation, **(+):** Gelation after a few minutes and remained for a short duration, **(++):** Immediate gelation with moderate gel residence and **(+++):** Immediate gelation with prolonged gel residence.

2.7.5 Rheological Evaluation

The viscosity of all formulations was measured using a Brookfield digital viscometer equipped with a suitable spindle. Measurements were carried out at rotational speeds ranging from 0.5 to 12 rpm under non-physiological conditions (25 ± 2°C, pH 5.0) and physiological conditions (37 ± 0.5°C, pH 7.4). The rheological behaviour was assessed by comparing viscosity profiles under both conditions [17].

2.7.6 In Vitro Drug Release Study

The in vitro drug release study was performed using a diffusion cell containing artificial tear fluid (pH 7.4) maintained at 37 ± 0.5°C under continuous stirring. A predetermined quantity of formulation was placed in the donor compartment separated by a dialysis membrane. Samples were withdrawn at predetermined intervals and replaced with an equal volume of fresh dissolution medium to maintain sink conditions. The samples were analyzed spectrophotometrically at 287 nm, and the cumulative percentage drug release was calculated [18].

2.7.7 Drug Release Kinetics

The release data obtained from the in vitro drug release study were fitted to zero-order, first-order, Higuchi, and Korsmeyer–Peppas kinetic models. The coefficient of determination (R^2) was used to identify the best-fit kinetic model and elucidate the mechanism of drug release.

2.7.8 Optimization of Formulation

Optimization of the formulations was carried out using multiple linear regression analysis based on the 2^3 full factorial design. Polynomial equations were generated to evaluate the influence of formulation variables and their interaction terms on viscosity and the time required for 90% drug release (T_{90}). The optimized formulation was selected based on the desired rheological properties and sustained drug release profile [19].

2.7.9 Antimicrobial Efficacy Study

The antimicrobial activity of the optimized formulation was evaluated against *Staphylococcus aureus* and *Pseudomonas aeruginosa* by the agar cup diffusion method. Wells were prepared in inoculated nutrient agar plates, and the test and standard formulations were introduced into the wells. Following incubation at 37°C for 24 h, the zones of inhibition were measured, and the antimicrobial efficacy of the optimized formulation was compared with that of the standard preparation [20].

3. RESULTS AND DISCUSSION

3.1 Characterization of Levofloxacin

The physicochemical characteristics of levofloxacin are summarized in Table 3. The drug appeared as a buff yellow to almost yellow crystalline powder with a characteristic bitter taste and was odorless. The observed melting point ranged from 224 to 227°C, which is consistent with the reported pharmacopeial specification, indicating the purity and identity of the drug.

Table 3. Physicochemical characterization of levofloxacin

Parameter	Observation
Colour	Buff yellow to almost yellow
Appearance	Crystalline powder
Odour	Odorless
Taste	Bitter
Melting point (°C)	224–227
Solubility in water (mg/mL)	8–9
Solubility in artificial tear fluid (mg/mL)	11–14
Solubility in methanol (mg/mL)	>30
Solubility in phosphate buffer (pH 7.4) (mg/mL)	11–12
Solubility in citro-phosphate buffer (pH 5.0) (mg/mL)	10–11

3.1.2 UV Spectrophotometric Analysis

Levofloxacin exhibited a distinct absorption maximum (λmax) at 287 nm. The well-defined absorption peak confirmed that the selected wavelength was appropriate for quantitative analysis without apparent spectral interference.

3.1.3 Calibration Curve

Excellent linearity was observed over the concentration range of 2–14 µg/mL in both artificial tear fluid and citro-phosphate buffer (pH 5.0). The calibration curves are shown in Figures 1.

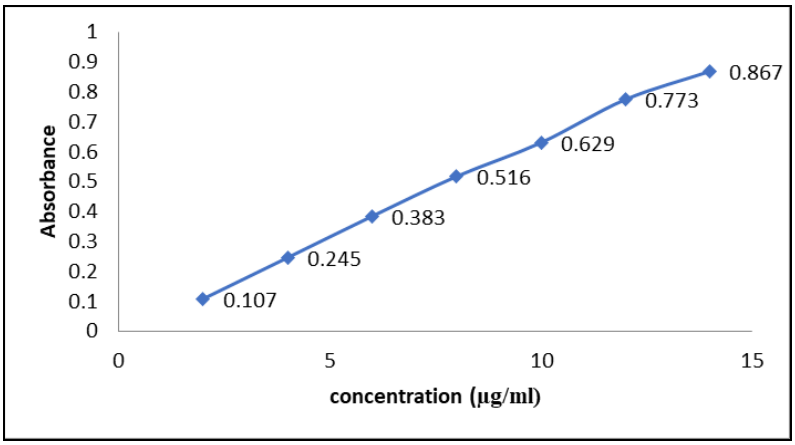

Figure 1. Calibration curve

3.2 Screening of Thermoresponsive and pH-Responsive Polymers

The screening results for Poloxamer 407 and Carbopol 940 are presented in Table 4. Increasing the concentration of Poloxamer 407 progressively decreased the phase transition temperature while improving gel strength. Weak or

delayed gelation was observed at concentrations between 5 and 12% (w/v), whereas concentrations of 18–24% (w/v) produced immediate gel formation with prolonged gel integrity. However, gelation temperatures between 28 and 35°C at higher polymer concentrations may reduce formulation stability during storage. Carbopol 940 exhibited a concentration-dependent decrease in pH accompanied by a marked improvement in gelation behavior. Concentrations of 0.2–0.4% (w/v) produced satisfactory gelation under physiological conditions while remaining fluid under non-physiological conditions. At concentrations above 0.5% (w/v), immediate gelation occurred under both conditions, indicating excessive gel strength that may adversely affect ocular instillation.

Table 4. Preliminary screening of Poloxamer 407 and Carbopol 940

Polymer	Concentration (% w/v)	pH	Phase transition temperature (°C)	Non-physiological gelation	Physiological gelation
Poloxamer 407	5	5.99	–	–	+
	10	6.10	>57	–	+
	12	6.22	>45	–	+
	14	6.31	42–44	–	++
	16	6.37	40–42	+	++
	18	6.48	38–39	++	+++
	20	6.54	34–35	++	+++
	22	6.70	30–32	++	+++
	24	6.78	28–29	+++	+++
Carbopol 940	0.2	4.26	—	–	+
	0.3	4.10	—	–	++
	0.4	3.92	—	–	++
	0.5	3.71	—	++	+++
	0.6	3.56	—	+++	+++
	1.0	3.02	—	+++	+++
	2.0	2.91	—	+++	+++

Note: *(–) No gelation; (+) Gelation after a few minutes; (++) Immediate gelation; (+++) Immediate gelation with prolonged gel integrity.*

3.3 Evaluation of Prepared In Situ Ophthalmic Gel Formulations

The prepared formulations (F1–F8) were clear, homogeneous, and free from visible particulate matter or phase separation. No precipitation was observed throughout the study, indicating satisfactory compatibility among the formulation components. The physicochemical properties of all formulations are summarized in Table 5. The pH of all formulations ranged from 5.0 to 5.6, which falls within the acceptable range for ophthalmic preparations and is expected to minimize ocular irritation while allowing rapid gel formation after contact with tear fluid. Only minor variations in pH were observed among the formulations, indicating that changes in polymer concentration had little influence on formulation acidity.

Drug content ranged from 98.23 to 101.66%, demonstrating excellent content uniformity among all batches. The results indicate uniform distribution of levofloxacin throughout the formulations without significant drug loss. The highest drug content was observed for F7 (101.66%), whereas F1 exhibited the lowest value (98.23%); however, all formulations complied with acceptable pharmacopeial limits.

A clear effect of polymer concentration on gelation behavior was observed. Formulations containing lower concentrations of Poloxamer 407 and Carbopol 940 (F1 and F5) showed either no gelation or weak gel formation, whereas increasing the concentration of these polymers markedly improved gel strength. Formulations F4, F7, and F8 exhibited immediate gel formation with prolonged gel integrity, indicating the synergistic contribution of the thermoresponsive and pH-responsive polymers.

The viscosity of the formulations also increased with increasing polymer concentration, particularly with higher levels of HPMC E50LV. Formulations containing 0.8% HPMC E50LV (F5–F8) exhibited substantially higher viscosities than those containing 0.4% HPMC E50LV (F1–F4). Among all formulations, F6 exhibited the highest apparent viscosity (1820 cP), whereas F3 showed the lowest viscosity (110 cP). The increase in viscosity is attributed to the greater polymer chain entanglement and formation of a more compact gel network at higher

polymer concentrations.

Table 5. Physicochemical evaluation of levofloxacin in situ ophthalmic gel formulations

Batch	pH	Drug content (%)	Gelling capacity	Viscosity at 12 rpm (cP)
F1	5.0	98.23	–	114
F2	5.4	98.32	++	170
F3	5.2	99.14	++	110
F4	5.3	101.12	+++	120
F5	5.0	99.56	–	1720
F6	5.6	100.34	++	1820
F7	5.2	101.66	+++	1220
F8	5.4	99.63	+++	1467

Note: *(–) No gelation; (++) Immediate gelation with moderate gel residence; (+++) Immediate gelation with prolonged gel residence.*

3.4 Rheological Evaluation

The comparative viscosity of the optimized formulation and the marketed product is summarized in Table 6 and Figure 2. All formulations exhibited pseudoplastic flow behaviour, with viscosity decreasing as the shear rate increased. A marked increase in viscosity was observed under physiological conditions, confirming rapid in situ gel formation. Formulations containing higher polymer concentrations showed greater viscosity because of stronger gel network formation. Among all batches, F4 exhibited rheological characteristics comparable to the marketed formulation while maintaining appropriate gel strength and ease of instillation, indicating its suitability for ophthalmic application.

Table 6. Comparative viscosity of optimized formulation (F4) and marketed formulation

Condition	F4 (cP)	Marketed (cP)
Non-physiological (12 rpm)	120	144
Physiological (0.5 rpm)	240000	186000
Physiological (12 rpm)	40900	42000

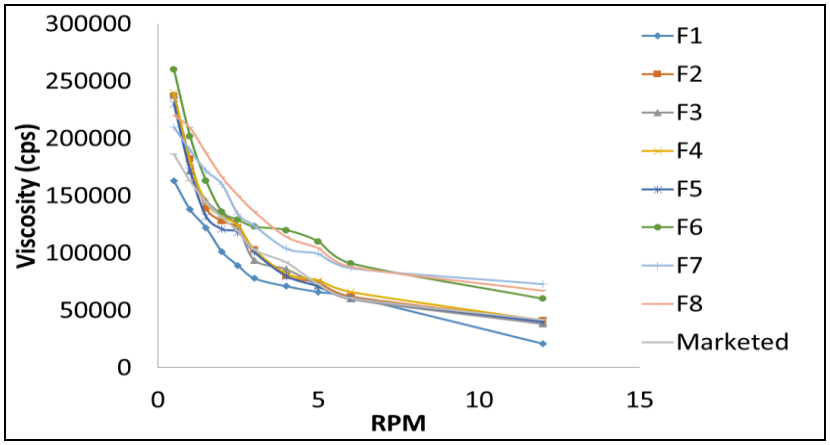

Figure 2. Comparative rheological profile of optimized formulation (F4) and marketed formulation.

3.5 In Vitro Drug Release Study

The in vitro release profiles are illustrated in Figure 3, while the release characteristics of the optimized formulation and marketed product are summarized in Table 7. The drug release profile was strongly influenced by polymer concentration. The marketed formulation exhibited rapid drug release within 2 h, whereas the developed formulations showed sustained release depending on polymer composition. Formulation F4 provided the most desirable release pattern, achieving 100.65% cumulative drug release over 8 h with controlled and sustained release. The prolonged release is attributed to the combined effect of Poloxamer 407, Carbopol 940, and HPMC E50LV in forming a stable gel matrix that effectively controlled drug diffusion

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Table 7. Comparative drug release profile of optimized formulation (F4) and marketed formulation

Time (h)	F4 (% release)	Marketed (% release)
0.5	25.29	63.74
1	43.87	82.15
2	59.92	101.63
4	79.69	-
6	93.86	-
8	100.65	-

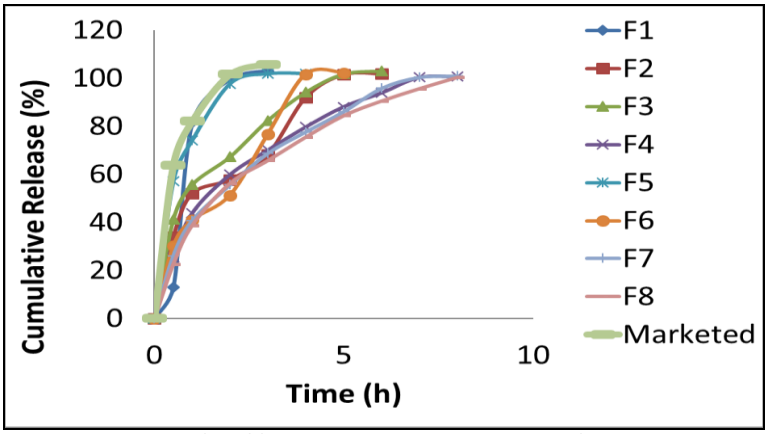

Figure 3. Comparative in vitro drug release profile of formulations F1–F8 and marketed formulation.

3.6 Drug Release Kinetics:

The release kinetics indicated that the developed formulations predominantly followed the Higuchi diffusion model. The optimized formulation (F4) exhibited controlled drug release with an N value close to 0.5, suggesting diffusion-controlled release. The kinetic parameters of the optimized and representative formulations are in Table 8. The Higuchi model provided the best fit for most formulations, confirming diffusion as the predominant drug release mechanism. Formulation F4 exhibited a Higuchi correlation coefficient of 0.9806 and an n value of 0.4972, indicating controlled diffusion from the hydrated gel matrix.

Table 8. Drug release kinetic parameters of representative formulations

Formulation	Best-fit kinetic model	Higuchi (R^2)	Korsmeyer–Peppas (n)	Release mechanism
F2	Higuchi	0.9566	0.4389	Fickian diffusion
F3	Higuchi	0.9858	0.3780	Fickian diffusion
F4	Higuchi	0.9806	0.4972	Diffusion-controlled
F6	Higuchi	0.9897	0.5560	Non-Fickian diffusion
F7	First-order	0.9513	0.5025	Anomalous diffusion
F8	Higuchi	0.8863	0.5291	Non-Fickian diffusion

3.7 Optimization by 2^3 Full Factorial Design

The factorial design demonstrated that polymer concentration significantly influenced both viscosity and sustained drug release. The responses of the optimized and representative formulations are presented in Table 9. Increasing the concentrations of Poloxamer 407 and Carbopol 940 improved gel strength and prolonged drug release, whereas HPMC E50LV predominantly increased viscosity. Formulation F4 exhibited the most desirable balance between rheological properties and sustained drug release and was therefore selected as the optimized formulation.

Table 9. Factorial optimization responses

Formulation	Viscosity (cP)	T90 (min)	Performance
F1	20900	149.17	Low viscosity and rapid release
F4	40900	345.78	Optimum balance of viscosity and sustained release
F6	60000	231.36	High viscosity
F7	73000	348.41	Very high viscosity
F8	67000	362.59	Maximum sustained release
Check point	55700	249.30	Experimental values close to predicted responses

3.8 Antimicrobial Efficacy Study

The antimicrobial activity of the optimized formulation (F4) is summarized in Table 10. The optimized formulation exhibited excellent antibacterial activity against both test microorganisms. The mean efficacy against Staphylococcus aureus (96.51%) was higher than that against Pseudomonas aeruginosa (85.06%), although both organisms showed distinct zones of inhibition. These findings indicate that incorporation of levofloxacin into the in situ gel system did not compromise its antibacterial activity and supports the suitability of formulation F4 for sustained ocular drug delivery.

Table 10. Antimicrobial efficacy of optimized formulation (F4)

Microorganism	Mean zone of inhibition (cm)	Mean efficacy (%)
STAPHYLOCOCCUS AUREUS	2.08 ± 0.69	96.51
PSEUDOMONAS AERUGINOSA	1.25 ± 0.33	85.06

4. CONCLUSION:

The present study successfully developed and optimized a dual-responsive levofloxacin ophthalmic in situ gel using Poloxamer 407, Carbopol 940, and HPMC E50LV through a 2^3 full factorial design. The optimized formulation exhibited desirable physicochemical properties, including acceptable pH (5.3), excellent drug content (101.12%), rapid sol-to-gel transition under physiological conditions, and pseudoplastic rheological behavior favorable for ocular administration. The formulation provided sustained drug release, achieving 100.65% cumulative release over 8 h, whereas the marketed ophthalmic formulation released the drug completely within approximately 2 h. Drug release predominantly followed the Higuchi diffusion model ($R^2 = 0.9806$), indicating diffusion-controlled release from the polymeric gel matrix. Furthermore, the optimized formulation retained excellent antibacterial activity against Staphylococcus aureus and Pseudomonas aeruginosa, confirming that the polymeric system did not compromise the antimicrobial efficacy of levofloxacin. The factorial optimization model demonstrated excellent predictive ability, supporting its usefulness in formulation development. Overall, the developed in situ gel offers significant advantages over conventional eye drops by prolonging precorneal residence time, providing sustained drug release, and potentially reducing dosing frequency while improving patient compliance. These findings suggest that the optimized formulation is a promising alternative for the effective topical management of bacterial ocular infections.

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