# Formulation and Evaluation of Norfloxacin-Loaded in Situ Ophthalmic Gel for Sustained Antibacterial Action

Mr. Amit Anil Jawadwar[1], Dr. Kavaljit Satish Birajdar[2], Mr. Dasrao Ashok Patil[3]
[1,2,3]*Department of Pharmaceutics, Tatyaraoji More College of Pharmacy, Omerga, Maharashtra, India*
doi.org/10.64643/IJIRTV13I1-205236-459

***Abstract*—Conventional ophthalmic solutions suffer from poor bioavailability because the instilled dose is rapidly lost from the precorneal area through tear turnover and nasolacrimal drainage, necessitating frequent dosing. In situ gelling systems overcome this limitation by being instilled as a free-flowing liquid that undergoes a sol-to-gel transition in the conjunctival cul-de-sac, thereby prolonging ocular residence time. In this work, a pH-triggered in situ gel of norfloxacin (0.3% w/v), a broad-spectrum fluoroquinolone, was developed for the treatment of bacterial conjunctivitis, corneal ulcer and blepharitis using Carbopol 940 as the gelling polymer and hydroxypropyl methylcellulose (HPMC K15M) as a viscosity enhancer, with mannitol and benzalkonium chloride as the tonicity-adjusting agent and preservative, respectively. Five formulations (F1–F5) were prepared and evaluated for clarity, pH, gelling capacity, viscosity, drug content, in vitro diffusion, antibacterial activity and accelerated stability; drug–excipient compatibility was confirmed by FTIR and DSC. All formulations showed an ocular-compatible pH (5.9–6.9), instantaneous gelation on contact with simulated tear fluid and a drug content of 79.56–85.23%. The optimized formulation, F5, exhibited the highest viscosity (1456 cP) and gelling capacity, released 81.43% of norfloxacin over 8 h following a non-Fickian (Korsmeyer–Peppas) mechanism, retained antibacterial activity against Staphylococcus aureus and Pseudomonas aeruginosa comparable to the pure drug, and remained stable under accelerated storage conditions. The developed in situ gel therefore represents a promising, patient-compliant alternative to conventional norfloxacin eye drops.**

***Index Terms*—Carbopol 940; HPMC K15M; in situ gel; norfloxacin; ocular drug delivery; sustained release.**

## I. INTRODUCTION

The eye is anatomically and physiologically suited to local, non-invasive drug administration, and topical instillation therefore remains the most widely used route for treating anterior-segment disorders such as conjunctivitis, keratitis and blepharitis. However, conventional ophthalmic solutions are cleared from the precorneal area within minutes of instillation. A standard eye drop delivers about 50–75 µL, whereas the conjunctival sac can normally retain only 7–10 µL; the excess volume is lost by reflex blinking, tear dilution and nasolacrimal drainage, so that less than 5% (often only 1–3%) of the instilled dose reaches the intraocular tissues [1], [2]. Frequent dosing is therefore required, which compromises patient compliance, while prolonged use of ointments causes blurred vision and ocular irritation.

To overcome these precorneal constraints, considerable attention has been directed toward in situ gelling systems, which are instilled as low-viscosity solutions and undergo a sol-to-gel phase transition in the cul-de-sac in response to a physiological stimulus such as a change in pH, temperature or ionic strength [3]. The resulting gel adheres to the ocular surface, increases precorneal residence time, permits accurate and reproducible dosing, and provides sustained drug release with reduced systemic absorption and dosing frequency [4]. Polymer-based in situ gelling approaches for ocular delivery, employing Carbopol, cellulose ethers, Poloxamers and ion-sensitive polysaccharides such as gellan gum, sodium alginate and xanthan gum, have been extensively reviewed [5]–[9].

pH-triggered systems based on Carbopol 940 have been used to sustain the release of ofloxacin for up to 8 h [10], while Carbopol–methylcellulose combinations have provided sustained release of pefloxacin mesylate [11]. Ion-activated (Gelrite-based) and temperature-sensitive (Poloxamer-based) in situ gels have similarly been reported for tropicamide [12] and timolol maleate [13], respectively, illustrating the versatility of the in-situ

gelling approach across drug classes. Among ocular surface infections, bacterial conjunctivitis, corneal ulcer and blepharitis caused by Staphylococcus aureus and Pseudomonas aeruginosa are particularly common, while fungal keratitis remains an important cause of corneal blindness, especially in tropical regions [14]. Norfloxacin is a first-generation, broad-spectrum fluoroquinolone that acts by inhibiting bacterial DNA gyrase (topoisomerase II) and topoisomerase IV, thereby blocking bacterial DNA replication, transcription and repair, and is active against the organisms most frequently implicated in these infections. Conventional norfloxacin eye drops, however, require frequent instillation because of rapid precorneal elimination.

The present work describes the formulation and evaluation of a pH-triggered in situ ophthalmic gel of norfloxacin using Carbopol 940 as the gelling polymer and HPMC K15M as a viscosity-enhancing agent, with the objective of prolonging ocular residence time, achieving sustained drug release and retaining antibacterial efficacy against ocular pathogens.

## II. MATERIALS AND METHODS

A. Materials: Norfloxacin, Carbopol 940, HPMC K15M, mannitol and benzalkonium chloride (BKC) were procured as analytical-grade samples from the sources listed in Table I. Carbopol 940 is a high-molecular-weight, lightly cross-linked polyacrylic acid that remains in solution at acidic pH but forms a gel at neutral/alkaline pH, whereas HPMC K15M is a non-ionic cellulose ether widely used as a viscosity-enhancing and mucoadhesive polymer in ophthalmic formulations [15]. All other chemicals and reagents used were of analytical grade.

Table I. Materials used and their sources

| Material | Source |
|---|---|
| Norfloxacin | Labware Chemicals, Mumbai |
| Carbopol 940 | Maher Chemicals, Mumbai |
| HPMC K15M | Kemphasol, Mumbai |
| Mannitol | Thomas Baker Pvt. Ltd., Mumbai |
| Benzalkonium chloride | Molychem Ltd., Mumbai |

B. Preformulation Studies: The procured norfloxacin sample was characterized prior to formulation. Organoleptic properties (colour, odour and nature) were recorded by visual and sensory examination, and the melting point was determined using a digital melting-point apparatus to confirm drug purity. The solubility of norfloxacin in chloroform, ethanol, methanol and water was assessed qualitatively.

For UV calibration, standard solutions of norfloxacin (2–10 µg/mL) in simulated tear fluid (STF, pH 7.4) were scanned between 200 and 400 nm against an STF blank using a UV-Visible spectrophotometer (Shimadzu UV-1800) to determine λmax, and absorbance was plotted against concentration to construct the calibration curve. Drug–excipient compatibility was assessed by FTIR (KBr pellet method, 400–4000 $cm^{-1}$, Perkin-Elmer spectrometer) on the pure drug and its physical mixtures with Carbopol 940 and HPMC K15M, and on the final gel formulation. Differential scanning calorimetry (DSC, Shimadzu DSC-60) of the pure drug was performed from 50 to 300°C at a heating rate of 20°C/min.

C. Preparation of In Situ Gel: A pH-triggered in situ gel was prepared by a cold dispersion method. Acetate buffer (pH 5.0) was prepared and HPMC K15M was dispersed in it under continuous stirring on a magnetic stirrer, followed by dispersion of Carbopol 940. Norfloxacin (0.3% w/v) was then incorporated and stirred until uniformly dispersed. Mannitol was added to render the formulation isotonic, and benzalkonium chloride (0.01% w/v) was incorporated as a preservative. Five formulations (F1–F5) were prepared by varying the Carbopol 940 and HPMC K15M concentrations, as summarized in Table II. The formulations were filled into vials, sealed with rubber stoppers and aluminium caps, sterilized by autoclaving (121°C, 15 psi, 20 min) and stored at 4–8°C until use.

Table II. Composition of norfloxacin in situ gel formulations (f1–f5)

| Code | NFX (%) | HPMC (g) | CP940 (g) | MAN (g) | BKC (mL) | AB (mL) |
|---|---|---|---|---|---|---|
| F1 | 0.3 | 0.08 | 0.08 | 2.0 | 0.004 | 40 |
| F2 | 0.3 | 0.16 | 0.08 | 2.0 | 0.004 | 40 |
| F3 | 0.3 | 0.08 | 0.16 | 2.0 | 0.004 | 40 |
| F4 | 0.3 | 0.16 | 0.24 | 2.0 | 0.004 | 40 |
| F5 | 0.3 | 0.08 | 0.24 | 2.0 | 0.004 | 40 |

NFX = norfloxacin; HPMC = hydroxypropyl methylcellulose K15M; CP940 = Carbopol 940; MAN = mannitol; BKC = benzalkonium chloride; AB = acetate buffer (pH 5.0).

D. Evaluation of In Situ Gel: Clarity was assessed visually against black-and-white backgrounds. pH was measured with a calibrated digital pH meter. Drug content was determined by diluting 1 mL of formulation to 100 mL with distilled water, further diluting a 5 mL aliquot to 25 mL, and measuring the absorbance at 272 nm.

Gelling capacity was determined by adding a drop of formulation to 2 mL of freshly prepared STF (pH 7.4) equilibrated at 37°C and noting the time for gelation and subsequent gel erosion. Rheology (viscosity) was measured using a Brookfield DV-III+ rheometer (spindle no. 4) over a shear-rate cycle of 1–60 rpm, and spreadability was determined from the spread diameter of the gel under an applied load.

In vitro drug diffusion studies were carried out using a cellophane-membrane diffusion cell, with STF (pH 7.4, 50 mL) as the receptor medium maintained at 37±1°C and stirred at 50 rpm. Samples (1 mL) were withdrawn hourly for 8 h, replaced with fresh medium, and analyzed at 285 nm.

Antibacterial activity was evaluated by the agar-cup diffusion method against S. aureus and P. aeruginosa. Wells (0.5 cm) bored in seeded nutrient agar were filled with either pure drug solution or the optimized formulation (F5, 0.3% w/v), and plates were incubated at 35°C for 24 h before measuring the zone of inhibition.

Accelerated stability of the optimized formulation (F5) was studied at 40±2°C/75±5% RH as per ICH Q1A guidelines, with appearance, pH, spreadability, viscosity and drug release monitored for 3 months.

## III. RESULTS AND DISCUSSION

### A. Preformulation Studies

Norfloxacin was obtained as a white-to-pale-yellow, odourless, free-flowing powder with a melting point of 221°C, consistent with the reported value and confirming the purity of the procured sample (Table III). The drug was soluble in chloroform, slightly soluble in ethanol, sparingly soluble in methanol and only partially soluble in water (Table III), in agreement with its reported BCS classification.

Table III. Preformulation characteristics of norfloxacin

| Parameter | Observation |
|---|---|
| Colour | White to pale yellow |
| Odour | Odourless |
| Nature | Free-flowing powder |
| Melting point | 221°C |
| Solubility – chloroform | Soluble |
| Solubility – ethanol | Slightly soluble |
| Solubility – methanol | Sparingly soluble |
| Solubility – water | Partially insoluble |

The UV absorption spectrum of norfloxacin in simulated tear fluid showed a characteristic absorption maximum at 272 nm. The calibration curve constructed over the range 2–10 µg/mL (Fig. 1) was linear, with the regression equation $y = 0.0744x - 0.0238$ and a correlation coefficient ($R^2$) of 0.9985, confirming that the method obeys Beer–Lambert's law over the studied concentration range and is suitable for drug-content and release estimations.

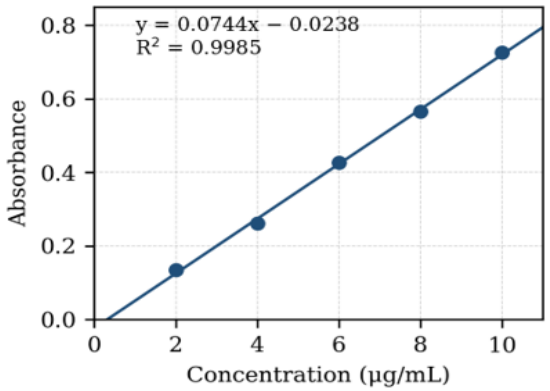


Fig. 1. Standard calibration curve of norfloxacin in simulated tear fluid (λmax = 272 nm).

### B. Drug–Excipient Compatibility

FTIR analysis of pure norfloxacin showed characteristic O–H, C–H, C=C and C–O stretching bands within the standard literature ranges (Table IV). The principal absorption bands of norfloxacin were also discernible, with only minor shifts, in the spectra of its physical mixtures with Carbopol 940 and HPMC K15M and in the final gel formulation, with no new or missing peaks. This indicates the absence of any major chemical interaction between norfloxacin and the selected polymeric excipients.

Table IV. FTIR spectral interpretation of pure norfloxacin

| Functional group | Standard range ($cm^{-1}$) | Observed ($cm^{-1}$) |
|---|---|---|
| O–H stretching | 3200–3600 | 3438.86 |
| C–H stretching | 2850–2960 | 2925.13 |
| C=C stretching | 1600–1680 | 1614.22 |
| C–O stretching | 1000–1300 | 1211.89 |

DSC analysis of norfloxacin showed a sharp endothermic peak at 221.30°C, corresponding closely to its reported melting point, followed by a decomposition exotherm at 316.81°C. The absence of any additional thermal event further supports the purity of the drug and its compatibility with the formulation excipients.

C. Physicochemical Evaluation of the In Situ Gel

All five formulations (F1–F5) were clear and free from visible particulate matter. The pH of the formulations ranged from 5.9 to 6.9 (Table V), within the range generally regarded as non-irritant for ocular tissues (5.0–7.4). All formulations underwent instantaneous gelation on contact with simulated tear fluid (pH 7.4) at 37°C and remained as a coherent gel for several hours, confirming the suitability of the Carbopol 940–HPMC K15M combination as a pH-triggered in situ gelling system.

Gelling capacity and viscosity increased progressively from F1 to F5 with increasing polymer concentration, with F5 showing the highest viscosity (1456 cP) and gelling capacity (0.178). Drug content of all batches ranged from 79.56% to 85.23%, indicating uniform drug distribution during preparation. Spreadability also increased from F1 to F5 (5.62 to 7.58 g·cm/s), reflecting the influence of polymer concentration on the consistency and applicability of the gel.

Table V. Physicochemical evaluation of formulations f1–f5

| Code | pH | GC | Visc. (cP) | DC (%) | Spread. (g·cm/s) |
|---|---|---|---|---|---|
| F1 | 6.6 | 0.057 | 991 | 81.52 | 5.62 |
| F2 | 6.8 | 0.115 | 1037 | 79.56 | 6.35 |
| F3 | 5.9 | 0.120 | 1160 | 82.86 | 6.72 |
| F4 | 6.1 | 0.125 | 1269 | 83.55 | 7.20 |
| F5 | 6.9 | 0.178 | 1456 | 85.23 | 7.58 |

GC = gelling capacity; Visc. = viscosity; DC = drug content; Spread. = spreadability.

D. In Vitro Drug Release

The cumulative drug release profiles of formulations F1–F5 over 8 h are shown in Fig. 2. All formulations exhibited a gradual, sustained release pattern without an initial burst, consistent with diffusion of the drug through the swollen polymer matrix. Formulation F5, containing the highest polymer concentration, released 81.43% of norfloxacin over 8 h — the highest among the batches studied — whereas F1–F4 released between about 71.5% and 75.4% over the same period.

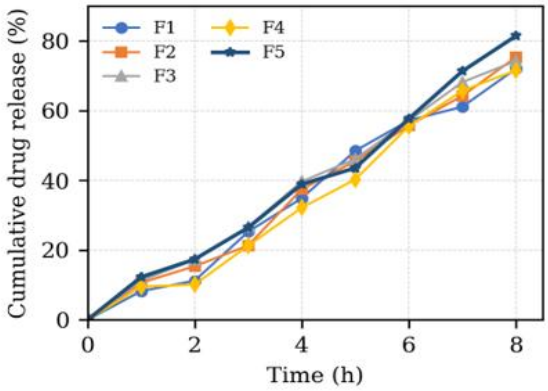


Fig. 2. In vitro cumulative drug release profiles of formulations F1–F5 in simulated tear fluid (pH 7.4, 37±1°C).

The release data fitted best to the Korsmeyer–Peppas model, suggesting a non-Fickian (anomalous) diffusion mechanism in which both drug diffusion and polymer chain relaxation/erosion govern release. On the basis of gelling capacity, viscosity, drug content and sustained release, F5 was selected as the optimized formulation for further antibacterial and stability evaluation.

E. Antibacterial Activity

The zones of inhibition produced by the optimized formulation (F5) and an equivalent concentration of pure norfloxacin against S. aureus and P. aeruginosa are summarized in Table VI. The gel formulation produced substantial zones of inhibition against both organisms (43 mm for S. aureus and 40 mm for P. aeruginosa), comparable to those produced by the pure drug solution (48 mm and 44 mm, respectively). This indicates that incorporation of norfloxacin into the in situ gelling matrix did not compromise its antibacterial activity, while the sustained-release behaviour of the gel can be expected to provide a more prolonged

antibacterial effect in vivo than an equivalent dose of the pure drug solution.

Table VI. Antibacterial activity (zone of inhibition)

| Test organism | Pure drug (mm) | Formulation F5 (mm) |
|---|---|---|
| Staphylococcus aureus | 48 | 43 |
| Pseudomonas aeruginosa | 44 | 40 |

F. Accelerated Stability

The optimized formulation F5 was subjected to accelerated stability testing at 40±2°C/75±5% RH for 3 months as per ICH Q1A guidelines (Table VII). No change in physical appearance was observed at any time point, and the pH, spreadability, viscosity and percentage drug release remained close to the initial values throughout the study period, confirming the physical and chemical stability of the optimized formulation under accelerated storage conditions.

Table VII. Accelerated stability data of optimized formulation f5

| Month | Appear-ance | pH | Spread. (g·cm/s) | Visc. (cP) | Release (%) |
|---|---|---|---|---|---|
| Initial | No change | 6.6 | 5.62 | 1037 | 81.52 |
| 1 | No change | 6.4 | 5.62 | 1120 | 82.20 |
| 2 | No change | 6.6 | 6.35 | 1160 | 82.57 |
| 3 | No change | 6.5 | 6.25 | 1260 | 81.26 |

## IV. CONCLUSION

A pH-triggered in situ ophthalmic gel of norfloxacin was successfully developed using Carbopol 940 as the gelling agent and HPMC K15M as a viscosity enhancer, with mannitol and benzalkonium chloride as the tonicity-adjusting agent and preservative, respectively. FTIR and DSC studies confirmed the absence of any significant drug–excipient interaction. The developed formulations exhibited an ocular-compatible pH, instantaneous in situ gelation, uniform drug content, and polymer-concentration-dependent viscosity, gelling capacity and spreadability. The optimized formulation, F5, provided sustained release of norfloxacin (81.43% over 8 h) following a non-Fickian diffusion mechanism, retained antibacterial activity against S. aureus and P. aeruginosa comparable to the pure drug, and remained stable under accelerated storage conditions. The pH-triggered in situ gel system therefore represents a simple, economically viable and patient-compliant alternative to conventional norfloxacin eye drops, offering prolonged precorneal residence and sustained antibacterial action for the management of ocular infections.

## ACKNOWLEDGMENT

The authors gratefully acknowledge the Department of Pharmaceutics, Tatyaraoji More College of Pharmacy, Omerga, for providing the laboratory facilities and instrumentation support required to carry out this work.

## REFERENCES

[1] K. S. Rathore and R. K. Nema, “An insight into ophthalmic drug delivery system,” International Journal of Pharmaceutical Sciences and Drug Research, vol. 1, no. 1, pp. 1–5, 2009.

[2] A. Kumar, R. Malviya, and P. K. Sharma, “Recent trends in ocular drug delivery: A short review,” European Journal of Applied Sciences, vol. 3, no. 3, pp. 86–92, 2011.

[3] Y. Sultana, R. Jain, M. Aqil, and A. Ali, “Review of ocular drug delivery,” Current Drug Delivery, vol. 3, no. 2, pp. 207–217, 2006.

[4] B. K. Nanjawade, F. V. Manvi, and A. S. Manjappa, “In situ-forming hydrogels for sustained ophthalmic drug delivery,” Journal of Controlled Release, vol. 122, no. 2, pp. 119–134, 2007.

[5] N. K. Sahane, S. K. Banarjee, D. D. Gaikwad, S. L. Jadhav, and R. M. Thorat, “Ocular inserts: A review,” Drug Invention Today, vol. 2, no. 1, pp. 57–64, 2010.

[6] G. Rajoria and A. Gupta, “In-situ gelling system: A novel approach for ocular drug delivery,” American Journal of Pharmacy Research, vol. 2, no. 4, pp. 1–9, 2012.

[7] T. S. Nerkar, N. A. Gujarathi, B. R. Rane, S. R. Bakliwal, and S. P. Pawar, “In-situ gel: Novel approach in sustained and controlled drug delivery system,” Pharma Science Monitor, vol. 4, no. 4, pp. 1–18, 2013.

[8] P. R. Kute, S. B. Gondkar, and R. B. Saudagar, “Ophthalmic in-situ gel: An overview,” World Journal of Pharmacy and Pharmaceutical Sciences, vol. 4, no. 4, pp. 549–568, 2015.

[9] A. Ludwig, “The use of mucoadhesive polymers in ocular drug delivery,” Advanced Drug Delivery Reviews, vol. 57, no. 11, pp. 1595–1639, 2005.
[10] B. Srividya, R. M. Cardoza, and P. D. Amin, “Sustained ophthalmic delivery of ofloxacin from a pH-triggered in situ gelling system,” Journal of Controlled Release, vol. 73, nos. 2–3, pp. 205–211, 2001.
[11] Y. Sultana, M. Aqil, A. Ali, and S. Zafar, “Evaluation of carbopol-methyl cellulose based sustained release ocular delivery system for pefloxacin mesylate using rabbit eye model,” Pharmaceutical Development and Technology, vol. 11, no. 3, pp. 313–319, 2006.
[12] M. R. Gokulgandhi, J. R. Parikh, and M. M. Dharmesh, “A pH-triggered in-situ gel forming ophthalmic drug delivery system for tropicamide,” Drug Delivery Technology, vol. 5, pp. 44–49, 2007.
[13] A. H. El-Kamel, “In vitro and in vivo evaluation of Pluronic F127-based ocular delivery system for timolol maleate,” International Journal of Pharmaceutics, vol. 241, no. 1, pp. 47–55, 2002.
[14] M. Srinivasan, “Fungal keratitis,” Current Opinion in Ophthalmology, vol. 15, no. 4, pp. 321–327, 2004.
[15] R. C. Rowe, P. J. Sheskey, and S. C. Owen, Handbook of Pharmaceutical Excipients, 5th ed. London, U.K.: Pharmaceutical Press, 2006.