

DEVELOPMENT AND EVALUATION OF NEPAFENAC OCULAR *IN SITU* GEL FOR THE TREATMENT OF PAIN AND INFLAMMATION

Sakshi Singh Chouhan^{1*}, Ruchi Khare Shrivastava²

^{1*}Institute of Pharmaceutical Sciences, SAGE University, Indore, India-472001, sakshisinghchouhan.1997@gmail.com, Orcid: <https://orcid.org/0009-0002-0734-5610>

²Institute of Pharmaceutical Sciences, SAGE University, Indore, India-472001

ABSTRACT

Introduction: Ocular pain and inflammation are common complications associated with postoperative conditions and ocular disorders, requiring effective and sustained drug delivery systems. Conventional eye drops suffer from rapid precorneal elimination, resulting in poor bioavailability and reduced therapeutic efficacy. Nepafenac, a poorly water-soluble nonsteroidal anti-inflammatory drug (NSAID), presents additional formulation challenges. In this context, the present study, with potential for patentable innovation, aimed to develop, optimize, and evaluate a pH-triggered ocular *In situ* gelling system of Nepafenac. The designed formulation seeks to enhance drug solubility, prolong precorneal residence time, and improve overall therapeutic performance.

Materials and Methods: Nepafenac was complexed with 2-hydroxypropyl- β -cyclodextrin (HP- β -CD) to improve aqueous solubility, and the optimized inclusion complex (1:2 ratio, CD3) was selected based on solubility and drug content. The *In situ* gel was formulated using Carbopol 940 as a pH-sensitive polymer and HPMC E4M as a viscosity enhancer. The formulation was evaluated for physicochemical properties, including clarity, pH, gelation capacity, and viscosity. Rheological behavior was assessed to determine flow characteristics. *In vitro* drug release, ex vivo corneal permeation studies, sterility testing, isotonicity, ocular irritation (Draize test), hemocompatibility, and *In vivo* pharmacokinetic and anti-inflammatory studies in rabbits were conducted.

Results: The optimized inclusion complex showed significantly enhanced solubility with high drug content ($98.9 \pm 0.5\%$). The developed *In situ* gel exhibited appropriate clarity, physiological pH, and immediate gelation upon contact with simulated tear fluid. Rheological analysis confirmed pseudoplastic flow behavior with optimal viscosity (420 ± 12 cP), ensuring ease of administration and prolonged retention. *In vitro* release studies demonstrated sustained drug release ($\sim 95.2\%$ over 24 hours), while ex vivo permeation studies revealed enhanced drug permeation (86.9% in 12 hours) with a steady-state flux of approximately $11.2 \mu\text{g}/\text{cm}^2/\text{h}$. The formulation was found to be sterile, isotonic, and non-irritant. *In vivo* studies indicated significantly improved ocular bioavailability and superior anti-inflammatory efficacy compared to conventional formulations.

Discussion: The combination of HP- β -CD complexation and pH-sensitive *In situ* gelling system effectively addressed the limitations of Nepafenac, including poor solubility and rapid ocular clearance. The optimized formulation demonstrated favorable physicochemical and rheological properties, ensuring sustained drug release and enhanced corneal permeation. Improved pharmacokinetic and pharmacodynamic performance confirmed the potential of the system to enhance therapeutic efficacy and patient compliance.

Conclusion: The developed pH-triggered ocular *In situ* gel of Nepafenac represents a safe, stable, and effective drug delivery system for the management of ocular pain and inflammation. This approach significantly improves drug solubility, ocular bioavailability, and therapeutic outcomes, offering a promising alternative to conventional ophthalmic formulations.

KEYWORDS: Nepafenac, *In situ* gel, ocular drug delivery, cyclodextrin, Carbopol, sustained release, anti-inflammatory.

INTRODUCTION

The eye is a highly specialized organ protected by several anatomical and physiological barriers that limit drug absorption [1,2]. Conventional ophthalmic formulations such as eye drops suffer from rapid drainage, tear turnover, and nasolacrimal elimination, leading to poor bioavailability of less than 5% [3]. This necessitates frequent dosing, which can reduce patient compliance and therapeutic effectiveness.

Ocular inflammation and pain are commonly associated with various conditions such as conjunctivitis, uveitis, postoperative inflammation, and corneal injuries. Effective management requires sustained drug delivery to maintain therapeutic concentrations at the site of action [4].

In situ gel systems have emerged as a promising alternative to conventional dosage forms. These systems are instilled as drops and undergo phase transition into gels upon exposure to physiological conditions such as temperature, pH, or ionic strength. This transformation prolongs precorneal residence time, enhances drug absorption, and reduces dosing frequency [5].

Nepafenac is a NSAID commonly used to manage postoperative ocular inflammation and pain, particularly after cataract surgery[6]. The controlled and localized delivery of nepafenac via the *In situ* gel minimizes systemic exposure to the drug, reducing the risk of systemic side effects that can occur with systemic NSAID use. *In situ* gels can enhance the bioavailability of poorly water-soluble drugs by solubilizing them and increasing their absorption rate [7]. This can lead to better drug efficacy. *In situ* gels are often administered in a liquid form and then transform into a gel at the application site. This makes them easy to administer, especially in cases where intravenous or invasive routes are not practical[9-12]. Current developments focus on the incorporation of novel polymers, mucoadhesive agents, and solubility enhancers such as cyclodextrins to overcome challenges associated with poorly soluble drugs like nepafenac. Furthermore, integration of nanotechnology-based carriers, including nanoparticles, liposomes, and dendrimers, into *In situ* gels has shown significant potential in improving drug penetration and targeted delivery within ocular tissues.

Future perspectives in this field are directed towards the design of smart, stimuli-responsive systems that can respond to environmental triggers such as pH, temperature, and ionic strength for controlled and site-specific drug release. Biodegradable and biocompatible hydrogels are also being explored to enhance patient comfort and eliminate the need for frequent dosing. In addition, emerging technologies such as microneedle-based delivery, drug-eluting contact lenses, and gene-based therapies are expected to further revolutionize ocular therapeutics by enabling precise, sustained, and minimally invasive drug administration. These advancements indicate a strong potential for the development of next-generation ophthalmic formulations with improved therapeutic efficacy and patient compliance.

The present study aims to develop an ocular *In situ* gel formulation for delivering anti-inflammatory and analgesic agents. The formulation is optimized to achieve appropriate gelation, sustained drug release, and patient acceptability.

MATERIALS AND METHODS

Materials

Nepafenac (NEP) was obtained as a gift sample from Dr. Reddy's Laboratories, Hyderabad, India. Carbopol 940 and hydroxypropyl methylcellulose (HPMC E4M) procured from SD Fine Chem., Mumbai. HP- β -CD was also obtained from SD Fine Chem., Mumbai. Disodium hydrogen phosphate, Citric acid and Benzalkonium chloride were procured from CDH, Mumbai. All materials used in the study were of analytical grade.

Formulation of Nepafenac / HP- β -CD Inclusion Complex

The inclusion complex of nepafenac with HP- β -CD was prepared in PBS pH 7.4. An excess amount of nepafenac (100 mg) was dissolved in an aqueous solution of HP- β -CD using a vortex mixer for 30 minutes to ensure complete solubilization [13]. For comparative studies, HP- β -CD was separately weighed in quantities of 50 mg, 100 mg, and 200 mg (Table 1) and dissolved in 10 mL of phosphate-buffered saline (PBS, pH 7.4) to prepare solutions of varying cyclodextrin concentrations suitable for complexation experiments[14,15].

Table 1: Formulation and Characterization of Solid Dispersion of Drug

Solid dispersion	Ration Drug: HP- β -CD
CD1	1:0.5
CD2	1:1
CD3	1:2

Characterization of Prepared Complex

The prepared nepafenac HP- β -CD inclusion complex was subjected to a series of physicochemical and analytical evaluations to confirm complex formation and assess its stability [16].

Visual and Physical Examination

The complex was inspected visually for changes in color, appearance, and texture compared to pure nepafenac. Any differences in homogeneity, crystallinity, or powder flow were noted to evaluate the physical integrity of the complex [13,16].

FTIR Spectroscopy

FTIR analysis was performed to identify potential molecular interactions between nepafenac and HP- β -CD. Samples of the inclusion complex were analyzed directly without KBr dilution, and spectra were recorded over the range of 4000–400 cm^{-1} . Shifts, disappearance, or broadening of characteristic peaks of nepafenac were monitored to confirm inclusion and detect hydrogen bonding or other interactions with HP- β -CD [17].

Solubility Studies

The solubility of nepafenac in the presence of varying concentrations of HP- β -CD was determined after heating and sonication, followed by equilibrium shaking for seven days. Solutions were filtered through 0.45 μ m membrane filters and analyzed in triplicate at 238nm. Enhanced solubility compared to the pure drug confirmed successful complexation and potential bioavailability improvement [18].

% Drug content

The concentration of nepafenac in the inclusion complex solutions was quantified using UV spectrophotometry. The method employed a C18 column, acetonitrile:water (40:60 v/v) mobile phase, and detection at 238 nm. The analysis confirmed the accurate incorporation of nepafenac into the HP- β -CD matrix and allowed calculation of encapsulation efficiency [13,19].

Formulation of Nepafenac Ocular *In situ* Gel

Nepafenac *In situ* gel was formulated using drug complex (CD3) to prepare a pH triggered system. Table 2 lists the formulation ingredients together with their corresponding proportions. The HPMC E4M was added and allowed to hydrate after the buffer salts were dissolved in 75 ml of filtered water. Carbapol 940 was then sprinkled over the mixture and left to hydrate for an entire night. Benzalkonium chloride (BKC) was added to the solution after Nepafenac was dissolved in a tiny amount of water and the drug solution was combined with the polymer solution using an overhead stirrer. Following the addition of purified water to get the volume up to 100 mL, the mixture was filtered through 0-2 mm filter paper [20,21]. The rapid precipitation of Carbapol happened when the drug and polymer solutions were combined because Carbapol causes a pH reduction [22]. To obtain a transparent drug and polymer solution, the drug was thus combined with a suitable amount of 0.1M NaOH before being added to the polymer solution [23].

Table 2: Composition of Nepafenac Ocular *In situ* gel

S. No.	Ingredients	Quantities (mg)
1	Nepafenac (CD3) equivalent to	100 mg
2	Carbapol 940	0.5 mg
3	HPMC E4M	1.25 mg
4	Benzalkonium chloride	0.002 mg
5	Citric acid	0.407 mg
6	Disodium hydrogen phosphate	1.125 mg
7	Sodium hydroxide	0.1 mg
8	Tween 80	0.5ml
9	Distilled water	100ml q.s.

Evaluation of Ophthalmic *In situ* Gel

Visual clarity and appearance

One of the most crucial aspects of ophthalmic preparations is their clarity. By visually observing each created formulation against a black and white backdrop, their clarity was assessed [24].

Determination of pH

pH is a critical parameter in the development of ophthalmic formulations, as it significantly influences both drug solubility and stability. An appropriate pH must be maintained to ensure the formulation remains stable while minimizing ocular irritation upon administration. Ideally, ophthalmic preparations should fall within a pH range of 5.0 to 7.4 to ensure compatibility with the eye. In this study, the pH of the prepared formulations was determined using a calibrated digital pH meter [25].

Determination of drug content

The drug content was determined by appropriately diluting 1 mL of the formulation to 50 mL with freshly prepared PBS, pH 7.4. From this solution, a 5 mL aliquot was further diluted to 50 mL using the same buffer. The concentration of nepafenac was then quantified by measuring the absorbance at 238 nm using a UV-Visible spectrophotometer [26].

Gelling capacity test (sol-to-gel transition/*In Vitro* gelation study)

To determine which compositions would work well as *In situ* gelling systems, the gelling capacity, time, and viscosity of each created formulation were assessed. By adding a drop of the system to a vial that contained two milliliters of recently generated, PBS pH 7.4 that had been equilibrated at 37 °C, and then visually evaluating the gel formation and recording the times it took for the gel to gel and dissolve against a black background, the gelling capacity was ascertained[27].

Viscosity measurement

Viscosity was measured using the Brookfield viscometer. The *In situ* gel compositions were stored in the sampling tube. The samples were evaluated in a circulating bath that was connected to the viscometer adaptor and kept at 37 $\pm$ 0.5°C before to each measurement. As the spindle no. 62 angular velocity was increased from one to four, the viscosity of the formulation was observed [28].

In Vitro drug release study

The *in vitro* release profile of nepafenac from the developed formulations was evaluated using a modified diffusion apparatus. Freshly prepared simulated tear fluid (pH 7.4) served as the diffusion medium. A semi-permeable membrane with an internal diameter of 3.4 cm was affixed to one end of a custom-fabricated glass cylinder open at both ends, which had been pre-soaked overnight in the diffusion medium[29]. An accurately measured volume of 2 mL of the formulation was introduced into the donor compartment (glass cylinder). The cylinder was positioned such that the membrane remained in slight contact with the surface of the diffusion medium contained in a beaker acting as the receptor compartment, filled with 100 mL of medium. The receptor medium was maintained at $37 \pm 2^\circ\text{C}$ and continuously stirred at 50 rpm using a magnetic stirrer. At predefined intervals, approximately 1 mL of the sample was withdrawn from the receptor compartment and replaced with an equal volume of fresh medium to maintain sink conditions. The collected samples were suitably diluted with the diffusion medium and analyzed at 238 nm using a UV spectrophotometer [30,31].

Sterility Testing

Sterility testing was conducted for the ocular formulations by the direct inoculation method, an essential evaluation for ophthalmic preparations. Two milliliters of the formulation were aseptically transferred into separate sterile flasks containing soybean-casein digest medium and fluid thioglycolate medium using a sterile pipette. The flasks were incubated for a minimum of 14 days at $20\text{--}25^\circ\text{C}$ and $30\text{--}35^\circ\text{C}$, respectively. At the end of the incubation period, the media were examined for any evidence of microbial growth [32].

Isotonicity Evaluation

The isotonicity of the formulation was assessed by mixing a few drops of the test preparation with freshly drawn blood and observing the red blood cells (RBCs) under a microscope. The integrity, shape, and morphology of the RBCs were compared with control samples treated with 0.9% NaCl (isotonic), hypotonic, hypertonic solutions, and a marketed formulation (Micronac of Micro labs ltd) to determine the isotonic compatibility [33].

Ex vivo Drug Permeation Studies across Cornea

Drug permeation studies were done by utilizing goat corneas. Entire eyeball of goat were obtained from a butcher place and stored in normal saline at 4°C . The cornea, along with approximately 5–6 mm of surrounding scleral tissue, was carefully excised and rinsed with chilled normal saline. The isolated tissue was then mounted onto a Franz diffusion cell, ensuring that the corneal surface remained in direct contact with the formulation present in the donor compartment. Subsequently, 1 mL of the formulation was placed onto the corneal surface for permeation studies. STF was added to the receptor compartment and maintained at $37 \pm 1^\circ\text{C}$ on a magnetic stirrer with a thermostatic hot plate [34]. 1 mL sample was withdrawn at predecided time intervals replacing with fresh dissolution medium each time and absorbance was recorded. The percentage drug permeated was determined and plotted against time and Permeation coefficient and Flux were calculated [35].

In vivo Studies

Animals

Male, healthy, New Zealand albino rabbits aged between 3–4 months, weighing 2.5 to 3.0 kg were used to investigate the ocular pharmacokinetics of Nepafenac from prepared *In situ* gel. The Institutional Animal Ethics Committee at Institute of Pharmaceutical sciences, Sage University, Indore, granted permission to conduct animal experiments. During the trial, the rabbits were kept in isolation in restraining cages and given access to food and drink at will. Allowable eye movement and lag times were unrestricted. Screening using external and slit-lamp revealed no ocular abnormalities before the study began.

Ocular irritation test

The Draize test is the most accurate method for determining the ocular irritancy of prepared formulations [36]. Six male New Zealand albino rabbits were administered the optimized Nepafenac loaded *in situ* gel formulation in order to measure ocular irritation. A measured volume of 50 μL of the optimized *in situ* gel formulation was carefully administered into the lower conjunctival sac of the right eye of each experimental animal. The untreated left eye served as the control. Ocular tolerance was assessed at specific time intervals of 1, 2, 4, and 8 hours following administration. At each observation point, detailed examination of the cornea, conjunctiva, and iris was performed to identify any signs of irritation such as redness, swelling (chemosis), excessive tearing, eyelid inflammation, or any morphological changes on the ocular surface. The extent of ocular irritation was evaluated using the established Draize scoring method, which grades irritation severity on a scale ranging from 0 (no observable irritation) to +3 (severe irritation with marked redness). Individual parameters including conjunctival redness, discharge, edema, and vascular congestion were scored separately. The total cumulative score was then calculated to assess the irritation potential as well as the reversibility of any observed effects associated with the *In situ* gel formulation [37,38].

Anti-Inflammatory Activity Study

The experimental protocol was duly submitted to and approved by the Institutional Animal Ethics Committee of the Institute of Pharmaceutical Sciences, Sage University, Indore. The rabbits were maintained under standard laboratory housing conditions in an air-conditioned environment at a controlled temperature of $22 \pm 0.5^\circ\text{C}$, with alternating light and dark cycles. They were provided with a regular diet and free access to water throughout the study. Acute ocular inflammation was induced in the right eye of each rabbit by instilling two drops of 5% w/v capsaicin extract at hourly

intervals for a duration of three hours. A total of six male rabbits, weighing between 2.5 and 3.0 kg, were randomly allocated into three experimental groups. (each group containing two rabbits):

Group I: Blank *In situ* gel (without drug)

Group II: Drug-loaded ocular *In situ* gel

Group III: Marketed ophthalmic formulation (Micronac)

Each rabbit was administered two drops of the assigned treatment into the right eye three times daily over a period of three days, whereas the left eye received two drops of normal saline and served as the control without inflammation induction. The progression of ocular inflammation was assessed visually and scored prior to treatment initiation, as well as on days 1, 2, and 3 following treatment. Scoring of ocular parameters was performed using defined scales: corneal opacity and ulceration were graded from 0 to 4; iris inflammation, including swelling and hyperemia, was scored from 0 to 2; conjunctival redness and visibility of blood vessels were evaluated on a scale of 0 to 3; and eyelid swelling and degree of closure were graded from 0 to 4 [39,40].

***In-vivo* pharmacokinetics studies**

The drug concentration values obtained from the analysis were utilized to determine key pharmacokinetic parameters, including peak plasma concentration (C_{max}), time required to reach peak concentration (T_{max}), area under the concentration–time curve (AUC), elimination half-life ($t_{1/2}$), clearance (CL), and apparent volume of distribution (V_d) [41].

For this study, six New Zealand rabbits were randomly selected and allocated into two groups: a treatment group and a control group. In the treatment group, 50 μ L of the optimized *In situ* gel formulation was administered into the lower conjunctival sac of the right eye, whereas the control group received normal saline. Prior to administration, local anesthesia was achieved using two drops of tetracaine hydrochloride solution (1% w/v). Aqueous humor samples were collected from the anterior chamber at predetermined time intervals of 0.5, 1, 2, 4, 6, and 8 hours post-administration, using a 1.0 mL syringe fitted with a 29-gauge needle. The collected samples were subsequently analyzed using high-performance liquid chromatography (HPLC) [42,43]. Statistical evaluation of the data was carried out using SPSS software (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

Formulation and characterization of Nepafenac HP β CD Complex

The prepared solid dispersions (CD1–CD3) were visually inspected and compared with pure Nepafenac. The pure drug appeared as a crystalline, poorly flow able powder, whereas all inclusion complexes exhibited a more amorphous, free-flowing, and homogeneous appearance. A noticeable reduction in crystallinity was observed with increasing HP- β -CD concentration, particularly in CD3 (1:2 ratio), indicating improved dispersion of the drug within the carrier matrix. No discoloration or phase separation was detected, confirming physical stability and uniformity of the complexes.

FTIR spectroscopy was employed to verify the presence of both guest and host components within the inclusion complex. The FTIR spectra of pure nepafenac and HP β CD (Figure 1) were recorded and compared with that of the prepared complex. Characteristic absorption bands of the individual components were carefully analyzed against those of the complex. Any disappearance, shift, or alteration in peak intensity was considered indicative of successful inclusion complex formation.

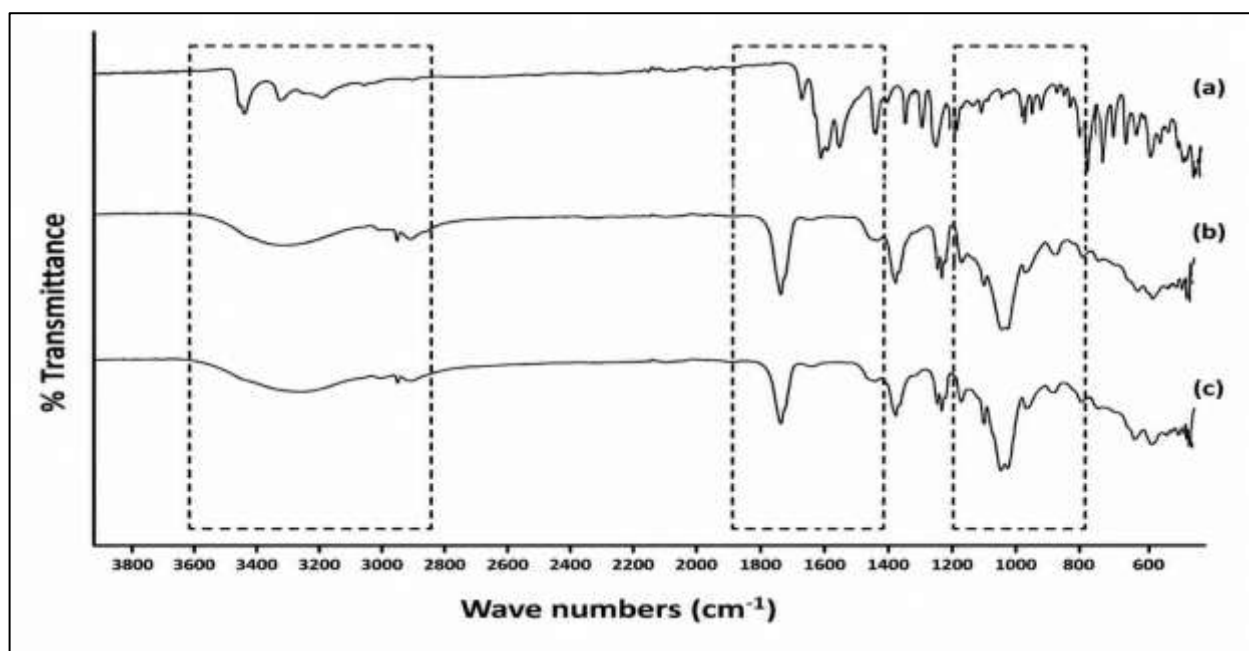


Figure 1: FTIR comparison (a) Pure NEP (b) HP β CD and (c) Drug complex

A marked increase in the aqueous solubility of Nepafenac was observed with increasing concentrations of HP- β -CD. The phase-solubility profile followed an AL-type curve, indicating a linear increase in drug solubility with cyclodextrin

concentration and suggesting the formation of a stable 1:1 inclusion complex. Among the formulations, CD3 (1:2 ratio) exhibited the highest solubility enhancement due to the availability of excess cyclodextrin for complexation. This improvement is expected to significantly enhance ocular bioavailability.

Drug content analysis revealed uniform incorporation of Nepafenac across all formulations, with values within acceptable limits (typically 95–99%). Encapsulation efficiency increased with higher HP- β -CD ratios, indicating improved entrapment of the drug within the cyclodextrin cavity. CD3 demonstrated the highest drug content and encapsulation efficiency, reflecting optimal complexation and minimal drug loss during preparation (Table 3).

Table 3: Characterization of Solid Dispersion of Drug

Solid Dispersion Code	Drug: HP- β -CD Ratio	Appearance	Solubility Enhancement	Drug Content (%)	Encapsulation Efficiency (%)
CD1	1:0.5	Slightly amorphous, less uniform	Moderate	95.2 $\pm$ 0.8	78.4 $\pm$ 1.2
CD2	1:1	Amorphous, homogeneous	High	97.6 $\pm$ 0.6	85.9 $\pm$ 1.0
CD3	1:2	Highly amorphous, free-flowing	Very high	98.9 $\pm$ 0.5	92.3 $\pm$ 0.9

Among the evaluated formulations, CD3 (Drug: HP- β -CD = 1:2) demonstrated superior performance in terms of solubility enhancement, drug content, encapsulation efficiency, and physical characteristics. The pronounced amorphization and improved molecular dispersion of Nepafenac in CD3 indicate effective inclusion complex formation. Therefore, CD3 was identified as the optimized formulation, suitable for further development in ophthalmic *In situ* gel systems due to its potential to significantly enhance drug solubility, stability, and bioavailability.

Evaluation of Optimized Ocular *In situ* Gel Formulations Containing Nepafenac

Visual clarity and appearance

Clarity is a critical quality attribute for ophthalmic preparations, as the presence of particulate matter or turbidity may cause ocular irritation and affect patient compliance [44,45]. Prepared formulation was visually inspected against both black and white backgrounds to assess transparency, presence of suspended particles, and overall homogeneity. The visual clarity and appearance of the formulation being completely transparent, homogeneous, and free from particulate matter confirm its suitability for ocular administration. This is particularly important because any turbidity or suspended particles can lead to irritation, blurred vision, or poor patient compliance. The clarity also indicates proper solubilization of Nepafenac, likely facilitated by complexation and uniform polymer dispersion (Table 4).

Determination of pH

The pH of ophthalmic formulation plays a vital role in ensuring drug solubility, chemical stability, and patient comfort. Ideally, the pH should be compatible with tear fluid (approximately 5.0–7.4) to avoid irritation upon instillation. The pH of optimized formulation was measured using a calibrated digital pH meter. The pH value (6.2 $\pm$ 0.04) falls comfortably within the physiological tolerance range of the eye (5.0–7.4), suggesting minimal risk of irritation upon instillation. Maintaining this pH is critical not only for patient comfort but also for drug stability and optimal performance of the pH-triggered gelation system (Table 4).

Determination of drug content

Drug content uniformity was evaluated to ensure accurate dosing of Nepafenac in each formulation. The drug content (98.6 $\pm$ 0.7%) indicates excellent uniformity and minimal drug loss during formulation. Such high content uniformity reflects efficient incorporation of Nepafenac within the polymeric matrix and ensures consistent therapeutic dosing (Table 4).

Gelling capacity test (sol-to-gel transition/*In Vitro* gelation study)

To determine which compositions would work well as *In situ* gelling systems, the gelling capacity, time, and viscosity of each created formulation were assessed. A key functional attribute, gelling capacity, was found to be excellent (+++), with instantaneous gelation upon contact with simulated tear fluid and prolonged gel integrity. This rapid sol-to-gel transition is essential to prevent drainage from the eye surface immediately after administration, thereby enhancing precorneal residence time. The sustained gel structure further supports prolonged drug retention (Table 4).

Viscosity measurement

The Brookfield viscometer III model was used to measure viscosity. The viscosity profile revealed desirable pseudoplastic (shear-thinning) behavior. At low shear rates, the formulation exhibited relatively higher viscosity, which aids in retention at the site of application. Under high shear conditions (such as blinking), viscosity decreases, allowing ease of spreading and minimizing discomfort. This rheological behavior is ideal for ophthalmic delivery systems as it balances administration convenience with therapeutic effectiveness (Table 4).

Table 4: Evaluation Parameters of Optimized Ophthalmic *In situ* Gel Formulation Containing Nepafenac

Parameter	Observation / Result
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Visual Clarity & Appearance	Clear, transparent, homogeneous, particle-free
pH	6.2 ± 0.04
Drug Content (%)	98.6 ± 0.7
Gelling Capacity	+++ (instant gelation, prolonged retention)
Gelation Time	Immediate (within seconds)
Viscosity (cP)	420 ± 12; decreases with shear
Rheological Behavior	Non-Newtonian, shear-thinning

***In Vitro* drug release study**

The *In Vitro* drug release profile (~95% over 24 hours) demonstrates a sustained release pattern, significantly improving upon conventional eye drops that are rapidly eliminated. The diffusion-controlled release mechanism suggests that the drug is gradually released from the gel matrix, maintaining therapeutic levels over an extended period. This can reduce dosing frequency and improve patient adherence (Table 5 and Figure 2).

Table 5: *In Vitro* Drug Release Profile of Optimized Ophthalmic *In situ* Gel Containing Nepafenac

Time (h)	Cumulative Drug Release (%)
0	0.0 ± 0.0
0.5	12.8 ± 1.1
1	25.4 ± 1.3
2	38.7 ± 1.5
4	50.9 ± 1.4
6	62.3 ± 1.6
12	79.4 ± 1.3
24	95.2 ± 1.5

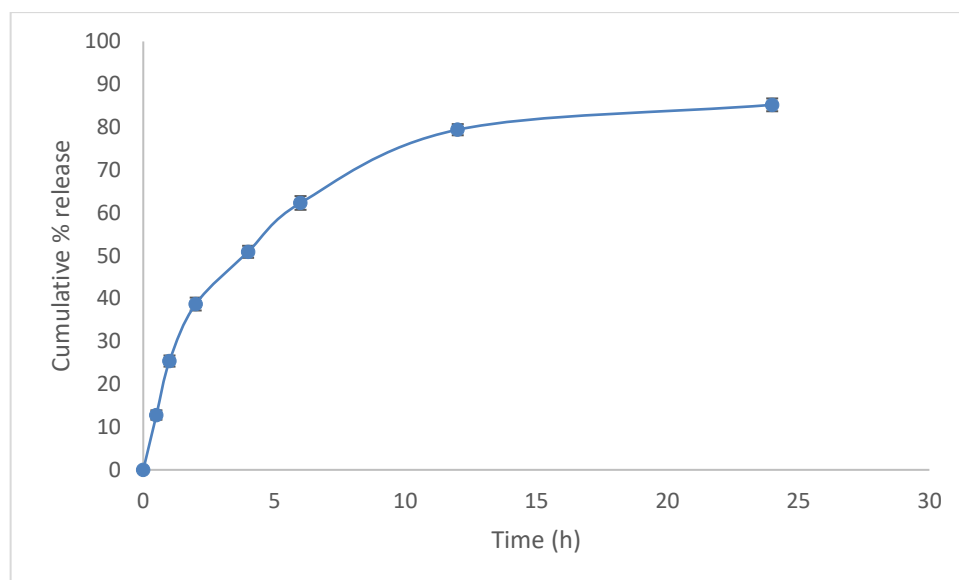


Figure 2: Cumulative % release of NEP from ocular *In situ* gel

Sterility Testing

Sterility testing of the ophthalmic *In situ* gel formulation containing Nepafenac confirmed the absence of microbial contamination in both aerobic and anaerobic culture conditions. The formulation showed no visible signs of turbidity or microbial growth in either soybean-casein digest medium or fluid thioglycolate medium after 14 days of incubation. This clearly indicates that the formulation was effectively sterile and free from viable microorganisms.

The use of two different culture media and incubation temperatures ensured comprehensive detection of both aerobic and anaerobic bacteria, which is essential for ophthalmic preparations due to their direct application to sensitive ocular tissues. The absence of microbial growth in both media demonstrates that aseptic manufacturing conditions were successfully maintained during formulation preparation and filling. These results confirm that the optimized formulation meets the sterility requirements for ophthalmic use, ensuring safety and minimizing the risk of ocular infections upon administration. The findings support the suitability of the developed *In situ* gel system for further preclinical and clinical evaluation (Table 6).

Table 6: Sterility Test Results of Optimized Ophthalmic *In situ* Gel Containing Nepafenac

Medium Used	Incubation Temperature	Incubation Period	Observation (Initial)	Observation (After 14 Days)	Interpretation
Soybean-Casein Digest Medium	20-25 °C	14 days	Clear medium	No turbidity / no microbial growth	Sterile

Fluid Thioglycolate Medium	30-35 °C	14 days	Clear medium	No turbidity / no microbial growth	Sterile
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Isotonicity Evaluation

Microscopic examination revealed that RBCs treated with the optimized formulation retained their normal biconcave disc shape, similar to the isotonic control (0.9% NaCl solution). There was no evidence of hemolysis, swelling, or crenation, indicating that the formulation does not disrupt cellular osmotic balance. In contrast, RBCs exposed to hypotonic solution showed swelling and hemolysis due to water influx, while those in hypertonic conditions exhibited shrinkage and crenation due to water efflux. These control comparisons clearly validate the sensitivity of the method and confirm that the test system is reliable. The behavior of the marketed formulation also demonstrated isotonic compatibility, providing a benchmark for comparison. The optimized formulation showed comparable results, indicating that its osmolarity is well adjusted for ocular administration (Table 7).

Table 7: Isotonicity Evaluation of Optimized Ophthalmic *In situ* Gel Containing Nepafenac

Sample	RBC Morphology	Cell Integrity	Hemolysis/Crenation	Interpretation
0.9% NaCl (Isotonic control)	Normal biconcave disc shape	Intact	None	Isotonic reference standard
Hypotonic solution (control)	Swollen RBCs	Distorted	Hemolysis observed	Hypotonic effect
Hypertonic solution (control)	Shrunked, crenated RBCs	Compromised	Cell shrinkage observed	Hypertonic effect
Marketed formulation (Micronac)	Normal morphology	Intact	No hemolysis	Isotonic
Optimized formulation	Normal biconcave disc shape	Intact	No hemolysis or crenation	Isotonic and compatible

Ex vivo Studies

Drug Permeation across Cornea

The cumulative permeation profile showed a gradual and controlled increase in drug diffusion over time, reaching approximately 86.9% permeation after 12 hours. The initial phase exhibited a moderate burst release, likely due to drug present near the gel surface, followed by a sustained diffusion phase governed by the polymeric matrix of the *In situ* gel system.

The calculated flux values indicated a relatively stable permeation rate during the mid-phase (2-6 hours), suggesting consistent drug diffusion across the corneal barrier. The slight decline in flux observed at later time points may be attributed to depletion of drug concentration in the donor compartment and increased diffusion resistance of the corneal tissue over time.

The enhanced permeation can be attributed to several factors, including prolonged precorneal residence time due to gelation, controlled drug release from the polymeric network, and improved solubilization of Nepafenac within the formulation. These factors collectively contribute to maintaining a sustained concentration gradient, which drives passive diffusion across the cornea (Table 8 and Figure 3).

Steady-state Flux: ~11.2 µg/cm²/h

Permeability Coefficient (Papp): 1.1×10^{-5} cm/s (approx.).

Table 8: Ex vivo Corneal Permeation Study of Optimized Ophthalmic *In situ* Gel Containing Nepafenac Using Goat Cornea

Time (h)	Cumulative Drug Permeation (%)	Flux (µg/cm ² /h)
0	0.0 ± 0.0	0
1	10.5 ± 0.8	10.5
2	22.8 ± 1.1	11.4
4	48.2 ± 1.5	12.0
6	65.7 ± 1.7	10.9
12	86.9 ± 1.4	8.6

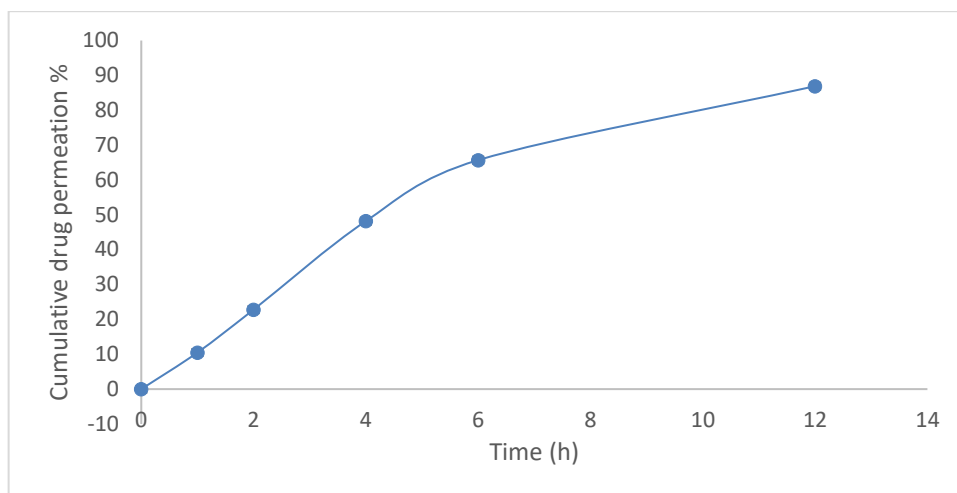


Figure 3: Cumulative drug permeation % of NEP from ocular *In situ* gel

***In vivo* studies**

Ocular irritation test

The ocular irritancy test conducted on the *In situ* gel formulation demonstrated an overall score of zero means the absence of any signs of inflammation, including redness, increased tear production, or edema. The ocular irritation potential of the drug loaded *In situ* gel against normal saline was investigated. Both the drug loaded *In situ* gel and normal saline demonstrated excellent ocular tolerability, with no observable alterations in the cornea, iris, or conjunctiva, and no evidence of ocular discharge (total irritation score = 0).

These findings indicate that the excipients used in the formulation are safe for topical application in the eye. The safety of the excipients can be attributed to several factors. Additionally, the perfect dispersion of the drug within the *In situ* gel may play a role in minimizing irritation. When the drug is efficiently dispersed within the gel structure, it is less likely to come into direct contact with the ocular tissues, reducing the potential for irritation. Overall, the absence of ocular inflammation and the safe response observed in the ocular irritancy test indicate that the *In situ* gel formulation is well-tolerated and suitable for topical application in the eye.

***In vivo* Anti-Inflammatory Study**

The anterior chamber is the ocular region most severely affected following 5% capsaicin-induced inflammation and is optimally managed using topical eye drop formulations. The anti-inflammatory efficacy of the optimized drug loaded *In situ* gel was therefore evaluated and compared with both the blank formulation and the marketed eye drop solution.

The findings demonstrated a significant reduction in the inflammation score ($p < 0.001$) from the first day of treatment in the group treated with nepafenac loaded *In situ* gel, with complete recovery achieved within three days. In contrast, the group treated with the conventional nepafenac solution required a longer duration to attain full recovery (Table 9).

Table 9: Clinical Examination Scoring of Inflamed Eyes Following Treatment with Nepafenac *In situ* gel and Micronac Eye Drop

Experimental Groups	Before the Start of Treatment	After One Day of Treatment	After Three Days of Treatment
Group I: Blank <i>In situ</i> gel (without drug)	8.2 ± 0.2	6.4 ± 0.6	2.8 ± 0.4
Group II: Nepafenac loaded ocular <i>In situ</i> gel	10.0 ± 1.0	4.1 ± 0.6	1.3 ± 0.1
Group III: Micronac Eye Drop	10.1 ± 0.9	7.4 ± 0.6	5.4 ± 0.8

The superior therapeutic performance of the Nepafenac *in situ* gel formulation may be attributed not only to the gel network acting as a sustained drug reservoir for continuous nepafenac release, but also to the mucoadhesive properties of carbopol, which enhance ocular residence time and prolong drug contact with the ocular surface. Conversely, the conventional Micronac eye drop solution was rapidly eliminated from the eye through tear turnover and nasolacrimal drainage, thereby limiting its anti-inflammatory efficacy.

In-vivo pharmacokinetics studies

Following topical administration of the nepafenac *in situ* gel formulation and conventional nepafenac eye drop (Micronac), the concentrations of nepafenac in the aqueous humor of rabbits were determined at different time intervals, as illustrated in Figure 4. The pharmacokinetic parameters, including maximum concentration (C_{max}), time required to reach C_{max} (T_{max}), area under the concentration–time curve (AUC), elimination rate constant (k), mean residence time (MRT), and half-life ($T_{1/2}$), were calculated for both formulations, and the results are summarized in Table 10.

The nepafenac *In situ* gel formulation exhibited a markedly higher C_{max} compared with the conventional nepafenac eye drop, indicating enhanced ocular penetration and improved drug availability within the aqueous humor. In addition, the

Tmax of the *In situ* gel formulation was prolonged, reflecting its sustained-release behavior and extended precorneal residence time. Nepafenac concentrations in the aqueous humor remained consistently higher over the studied time intervals following administration of the *In situ* gel compared with the marketed eye drop.

Moreover, the AUC values obtained for the nepafenac *In situ* gel were substantially greater than those of the marketed eye drop, demonstrating enhanced ocular bioavailability. The prolonged MRT and increased half-life further confirmed the ability of the *In situ* gel system to maintain therapeutic drug concentrations in the ocular tissues for an extended duration.

The improved pharmacokinetic performance of the nepafenac *In situ* gel may be attributed to the gel-forming and mucoadhesive properties of the formulation, which enhance corneal contact time and reduce rapid precorneal elimination through tear turnover and nasolacrimal drainage. Consequently, the developed *In situ* gel formulation represents a promising ocular drug delivery system capable of providing sustained drug release, improved ocular bioavailability, and prolonged therapeutic efficacy compared with the conventional nepafenac suspension.

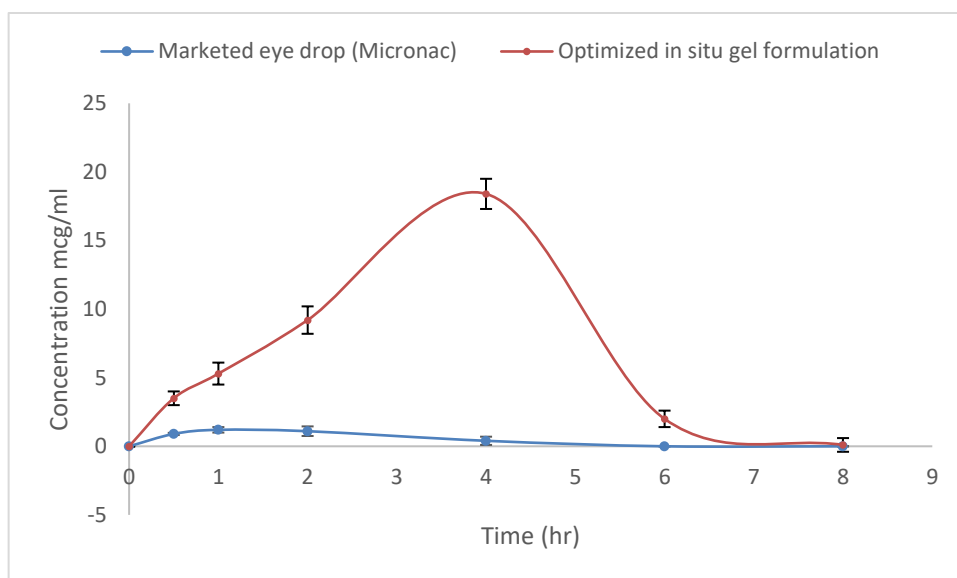


Figure 4: Concentration vs time curve of Nepafenac *In situ* gel formula and marketed eye drop (Micronac) in aqueous humor of albino rabbits.

Table 10: Pharmacokinetic parameters of proniosomal gel formulation and ACZ drug suspension

Pharmacokinetic parameters	Optimized <i>In situ</i> gel formulation	Marketed eye drop (Micronac)
Cmax (mcg/ml)	19.2 ± 1.32	0.908 ± 0.048
Tmax	4hr	2hr
AUC 0–8 (mcg.h/mL)	61.2 ± 2.86	3.04 ± 0.26
K (h ⁻¹)	0.628 ± 0.025	0.668 ± 0.215
T1/2 (h)	1.12 ± 0.08	0.901 ± 0.164
MRT (h)	3.68 ± 0.1	2.4 ± 0.05

Conclusions

The present study successfully developed a pH-triggered *In situ* gelling system for the ocular delivery of Nepafenac. The strategy of utilizing a 1:2 ratio of Nepafenac to HP-β-CD in the CD3 inclusion complex resulted in a "very high" solubility enhancement and an optimal drug content of 98.9 ± 0.5%. The optimized formulation, comprising Carbopol 940 and HPMC E4M, demonstrated ideal physicochemical attributes for ophthalmic application. It maintained a clear, transparent appearance with a pH of 6.2 ± 0.04 well within the physiological tolerance range and exhibited immediate, robust gelation (+++ capacity) upon contact with simulated tear fluid. Rheological analysis confirmed pseudoplastic behavior with a viscosity of 420 ± 12 cP, which ensures both ease of administration as a liquid and prolonged retention as a gel at rest. The formulation provided a controlled drug release of approximately 95.2% over 24 hours, significantly extending the duration of action compared to traditional drops. Ex vivo studies across goat cornea showed a high cumulative permeation of 86.9% after 12 hours, with a steady-state flux of ~11.2 μg/cm²/h, driven by the prolonged residence time and improved solubility.

The gel was confirmed to be sterile and isotonic, showing no irritation in the Draize test (score = 0) and no adverse effects on RBC morphology. Pharmacokinetic analysis in rabbits revealed significantly higher and values compared to the marketed formulation, confirming enhanced ocular bioavailability. Furthermore, the anti-inflammatory activity was significantly more effective, achieving a lower inflammation score (1.3 ± 0.1) after three days of treatment compared to the marketed eye drop (5.4 ± 0.8). In conclusion, the developed *In situ* gel system provides a stable, safe, and highly effective platform for the sustained delivery of Nepafenac. By reducing dosing frequency and maximizing drug absorption,

this formulation represents a promising therapeutic alternative for the management of postoperative ocular pain and inflammation.

Limitations of the Study

Despite the promising outcomes observed, the present study has certain limitations that should be acknowledged. Firstly, the *In vivo* evaluations were conducted on a limited number of animal models, which may not fully represent the variability observed in human ocular physiology. Although rabbits are widely accepted for ophthalmic studies, differences in tear composition, blinking rate, and corneal permeability may influence the translational applicability of the results.

Secondly, the study primarily focused on short-term pharmacokinetic and pharmacodynamic assessments. Long-term safety, stability, and efficacy studies were not performed, which are essential to establish the chronic use potential of the formulation. Additionally, the ocular irritation and biocompatibility assessments were limited to standard tests, and more advanced histopathological and molecular-level evaluations could provide deeper insights into tissue compatibility.

Furthermore, while the inclusion complex with HP- β -CD significantly improved drug solubility, the scalability and cost-effectiveness of the formulation for industrial production were not investigated. The study also did not explore the influence of varying environmental conditions, such as storage stability under different temperatures and humidity levels. Another limitation is the absence of direct clinical comparison with commercially available Nepafenac formulations in human subjects. Although the results indicate enhanced bioavailability and therapeutic efficacy, clinical trials are necessary to confirm these findings and ensure patient safety and compliance.

Lastly, the formulation was optimized using specific polymer concentrations, and a broader design space exploration could further refine the formulation parameters for improved performance.

Declarations

Ethics approval

Preclinical studies were performed as per the prior permission from the Institutional Animals Ethical Committee, Institute of Pharmaceutical sciences, Sage University, Indore, India.

Consent to participate

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

AI Disclosure

Grammarly was used to check grammar and spellings. Chatgpt free version was used to create graphical abstract.

Competing interests

The authors declare no conflict of interest.

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Authors' contributions

Conceptualization and methodology, Sakshi Singh; writing, review and editing, Ruchi Khare Shrivastava. Both authors have read the manuscript and given consent to publish the manuscript.

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