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Original Article

Nepafenac Loaded Emulgel for Controlled Ocular Delivery: *in vitro* and *ex vivo* Characterization

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ABSTRACT

Objectives: Emulgel, known for their unique combination of properties from both emulsions and gels, have attracted significant interest from the pharmaceutical and cosmetic industries for their versatility and usefulness. This research aims to design and assess a novel emulgel containing nepafenac for ocular drug delivery. **Materials and Methods:** Extensive literature research guided the formulation. Novel emulgel formulations (F1 to F9) blended O/W emulsion with castor oil, oleic acid, linseed oil and a self-emulsifying agent i.e. poloxamer 188. The gel phase was prepared by employing pluronic F-127, HPMC K 15M alone, or in their combinations. The O/W phase was integrated into the gel phase to form the emulgel. **Results:** 9 nepafenac emulgel batches were formulated, with oil phase and polymer concentration variations. All batches were evaluated for emulsion and gelling properties. Batch F6 was selected as the optimized formulation as its characteristics exhibited a pH of 7.2, a refractive index of 1.356, 75.12% transmittance, a viscosity of 1892 centipoise (cP), a globule size of 224 nm and zeta potential of -24 mV that are more suitable for ocular applications. *In vitro* and *ex vivo* drug release showed controlled release for 8 hr, with no histological changes. HET CAM and rabbit ocular irritation tests indicated no irritation/toxicity upon administration. Sterility testing confirmed the absence of contamination and stability studies uphold consistent characteristics over time. **Conclusion:** The newly developed nepafenac emulgel holds promise for efficient ocular drug delivery with favourable physicochemical properties, sustained drug release, tissue compatibility and a robust safety profile. It presents a strong candidate for future developments in ocular therapies, potentially expanding the treatment options for ocular conditions.

Keywords: *Ex vivo* permeation, Nepafenac, Novel Emulgel, Ocular Drug Delivery, Ocular irritancy/ toxicity.

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INTRODUCTION

Ocular drug delivery is a specialized field of pharmaceutical research and development focused on the distribution of therapeutic agents to the eye to treat a wide range of ocular diseases and conditions. The eye poses special difficulties in administering medications because of its intricate structure, inherent obstacles and the necessity for accurate dosing to attain the desired therapeutic results while reducing adverse reactions. During cataract surgery, various ocular tissues, including retina, sclera, aqueous and vitreous humour, iris, cornea, ciliary body, choroid and conjunctiva, are manipulated and incised. This medical procedure activates the creation of prostaglandins through enzymes such as phospholipase, COX 1 and COX 2. As a result, the most frequent and possibly severe side effect of this procedure, known as cystoid macular inflammation, might happen, causing signs like discomfort, photophobia and miosis.[1,2] To tackle these problems, topical corticosteroids and Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) have been utilized. Studies show that using these drugs together is successful in lowering inflammation after surgery.[3,4] Nonetheless, finding the correct equilibrium between how well patients can tolerate the drugs and their safety for the cornea remains difficult for drug manufacturers, due to the specific concerns related to the eye's structure and function.

Nepafenac (2-amino-3-benzoylbenzeneacetamide) is a strong NSAID drug that is explicitly designed for usage in the eyes and is used to ease the discomfort and swelling that occurs after cataract surgery.[5,6] It is important in managing the pain and swelling experienced after cataract surgery. Its efficacy in reducing eye inflammation and pain has made it a key element in eye medication. This drug is generally given as eye drops or mixed into special ocular medications. Presently, it is available as an eye drop solution in a concentration of 0.1% (Amplinak Opthalmic Suspension 5 mL). Nepafenac's discrete features, such

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as its low systemic absorption and a safe profile, position it as an excellent option for addressing different eye diseases, including inflammation after surgery, uveitis and ocular discomfort. Its specific mechanism of action and broad applicability have solidified its status as an effective drug for eye treatment, leading to better results and patient comfort. Nonetheless, nepafenac is categorized as a class IV substance in the Biopharmaceutical Classification System,[7,8] encounters obstacles due to limited tissue penetration and poor solubility in water.[9,10] This has kindled interest for creating new nepafenac ocular formulations with better solubility and absorption rates, a challenging endeavour for ocular-topical medications.[11,12] Techniques like penetration enhancers, cyclodextrin based systems,[8] viscosity adjusters,[13] nano fibres based delivery systems,[14] and even external forces such as electrical charges or ultrasound waves have been investigated to boost its absorption.[15]

The preferred mode of topical treatment for ocular conditions is often an eye drop solution, especially when localized action is required, such as in the cornea or anterior chamber. However, natural mechanisms like lacrimal secretion and blinking reflex, coupled with corneal impermeability, limit drug retention and necessitate frequent administration.[16,17] Furthermore, ocular drug delivery faces various challenges, including limited drug bioavailability in crucial ocular tissues, short duration of drug action necessitating frequent dosing, natural barriers hindering drug distribution, patient discomfort during administration,[18] and the need for precise dosing control. Furthermore, delivering drugs to the posterior section of the eye poses exceptional challenges. Ensuring drug stability, safety and cost-effectiveness while pursuing customized therapies for individual patients are ongoing concerns in this field. Addressing these challenges is essential for improving the effectiveness, patient experience and accessibility of ocular drug treatment.[19] So, to overcome this problematic situation and formulation difficulties researchers and pharmaceutical companies have developed innovative approaches and formulations to enhance drug bioavailability, prolong drug release and improve patient compliance.[20,21]

To overcome rapid dilution, formulations with increased viscosity have been investigated, as they extend drug residence time and improve absorption in the pre-corneal area. Equally Oil-in-Water (O/W) and Water-in-Oil (W/O) emulsions are extensively employed as vehicles for delivering drugs to the cornea due to their therapeutic properties.[22] These emulsions offer a balance of elegance and ease of removal, crucial in ophthalmic preparations.[23] Gels for ophthalmic have several favourable properties such as being thixotropic, easily removable, emollient and compatible with excipients.[24] Emulgels, a combination of emulsions and gels, have gained popularity as vehicles for ocular drug delivery, providing high patient acceptability and the advantages of both forms.[25] They are often referred to as gelled emulsions or emulgels, offering a promising avenue for delivering various drugs to the eye.[26] Emulgel has high patient acceptability since they possess the advantages of both emulsion and gels.[27,28]

Therefore, current work has been undertaken to develop an emulgel formulation containing nepafenac for efficient control of ocular inflammatory conditions.

MATERIALS AND METHODS

Materials

Nepafenac graciously donated by Flax Laboratories Pvt. Ltd., Mira Bhayander, Mumbai, Castor oil and HPMC (Research-Lab Fine Chem Industry, Mumbai), Linseed oil Oleic acid and Pluronic F-127 (Loba Chemie Pvt. Ltd., Mumbai), Poloxamer 188 (BASF, Mumbai). All the solvents and chemicals were of the highest quality and purity for analysis; and utilized exactly as directed.

Methods

Emulgel Development and Characterization

The preparation of an emulgel dosage form involves three key steps: the preparation of the emulsion phase, the preparation of the gel phase and the merging of these two phases to form the emulgel.[29] Nepafenac was initially dissolved in oil to produce the emulsion phase, constituting the oil phase, at a concentration of 300 mg within 1.5 g of oil. Three oils castor oil, oleic acid and linseed oil were used in the study. Subsequently, Oil-in-Water (O/W) emulsion phase was generated by using this nepafenac-infused oil. The aqueous phase was established by dissolving poloxamer 188 (an emulsifying agent) and benzalkonium chloride (acting as a preservative) in distilled water. A homogeneous emulsion was achieved through continuous mixing and agitation of the oil phase into the aqueous phase at 1500 rpm.[30] The gel phase was created by dissolving the gelling ingredient in an iso-osmotic solution that contained 3% glycerin. Various gelling agents were utilized at different concentrations during the preparation of the gel phase. Finally, the emulgel was formulated by mixing emulsion and gel phases in a 1:1 weight ratio for 15 min at 1500 rpm, resulting in a uniform and smooth emulgel (Figure 1). The composition details of all the developed formulations are given in (Table 1).

Evaluation of Emulgel

pH determination

The pH was determined using a pH meter (EQ-610, Equiptronics, India) by positioning the electrode tip into emulgel for 2 min and the outcome was documented.

Refractive Index

The refractive index was determined by placing one drop of formulation on the slide of the refractometer and observing under Abbe's Refractometer (Rolex, India).

% transmittance

It reflects the degree of transparency of the formulation. % Transmittance was measured by using UV visible spectrophotometer (Shimadzu 1800) at 650 nm after diluting formulations with distilled water.

Rheological study

Rheological data is crucial for assessing the flow properties and consistency of the prepared emulgel formulations. Further, it is significant in ophthalmic applications where product consistency, texture and viscosity became vital considerations in its performance. The viscosity of the prepared emulgel was determined using a Brookfield viscometer (model: DV-II+Pro) with 64-number spindles. The emulgel sample, formulated according to the specific formulation under investigation, was loaded into a glass container.[31,32] The viscometer was calibrated using standard reference fluids and the spindle was set to rotate at a fixed speed of 50 revolutions per minute (rpm). As the spindle rotated within the emulgel sample, the resistance to flow or shear force was measured and the resulting viscosity data, often recorded in centipoise (cP), were estimated. The procedure was typically repeated for consistency and to ensure the accuracy of the viscosity measurements.

Drug content

1 g sample of emulgel was dispersed into Simulated Tear Fluid (STF) 100 mL with a pH of 7.4 and subjected to sonication for 2 hr. After sonication, the resulting mixture was filtered through a 0.45 µm Millipore filter and subsequently analyzed using UV spectroscopy at a wavelength of 238 nm.[33]

In vitro drug release study

Franz Diffusion Cell apparatus (Orchid Scientific, India) was set up to grasp the dialysis membrane firmly in place throughout experiments of release studies. Dialysis membranes were prepared by soaking them into STF with a pH of 7.4 for duration of 30 min prior to the commencement of experiments. The receptor cell was filled with 5 mL of STF. Subsequently, the dialysis membrane was placed over the receptor cell and the donor cell was positioned on top, with both halves firmly fastened together. The compartment of the donor cell was loaded with emulgel formulation. Sampling from the receptor cell was conducted at time intervals of 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7 and 8 hr. To maintain sink conditions, the receptor cell was reloaded with STF pH 7.4. Samples that were previously withheld were collected for analysis using UV spectrophotometry and their absorbance was measured and analyzed at a wavelength of 238 nm.[34]

The *in vitro* release profiles of nepafenac emulgel were compared using similarity factor *f2* and difference factor *f1* with marketed formulation, as well-defined by the equation-

$$f2 = 50log\left\{\left[1 + (1/n)\sum_{t=1}^{n}(R_t - T_t)\right]^{-0.5} \times 100\right\}$$

$$f1 = \left\{\left[\sum_{t=1}^{n}|R_t - T_t|\right] \Big/ \left[\sum_{t=1}^{n} R_t\right]\right\} \times 100$$

Where "n" is the number of time points Tt and Rt are percentage releases at a time point (t) for the test and reference formulation, respectively.[35]

In vitro drug release data of all the emulgel batches was attempted to fit into mathematical models in order to determine drug release kinetics. The n and R^2 values for Zero order, First order, Higuchi equation, Hixson-Crowell equation and Korsmeyer-Peppas equation models were determined. The analysis was executed to determine the fit of most suitable model to the drug release from nepafenac emulgel.

Zeta potential, polydispersity index and globule size

Determining zeta potential using the particle size analyzer (Horiba SZ-100V2, Korea) is a crucial step in characterizing the emulgel formulation. The emulgel formulation was diluted with 10 mL of water. 2-3 mL of diluted solution was taken for measurements to obtain data about globule size and zeta potential of formulations.[36]

Zeta potential reflects the electrolytic mobility of particles and offers insights into the stability of the formulation. Additionally, the Polydispersity Index (PDI) assessment provides information about the uniformity of particle size distribution, emphasizing the importance of smaller particle sizes to prevent formulation instability.

Ex vivo Corneal Permeation

Ex vivo transcorneal permeation studies were conducted using freshly procured goat cornea placed within the Franz diffusion cell. The excised goat cornea was placed between the receptor and donor sections, with the inner epithelial surface facing the receptor compartment. STF (pH 7.4) was employed as the release medium, filling the receptor compartment. The Nepafenac emulgel mixture was spread over the donor cell, enveloping the cornea. The liquid in the receiving area was kept at a steady temperature of 37°C and continuously agitated at a speed of 50 rpm. At set times; small samples were taken from the receiving area over an 8-hr span and then examined at 238 nm.[37]

According to the following equation, the obvious corneal permeability coefficients (P_{app}, cms^{-1}) Were determined:[38,39]

$$P_{app} = \frac{\Delta Q}{\Delta t} \times \frac{1}{A.C_0.60}$$

In the given formula, the rate of drug flow through the corneal tissue is represented by 'ΔQ/Δt' (micrograms per minute). Here, 'A' denotes the area of the corneal surface that is exposed (square centimeters), 'C_o' represents the initial concentration of the drug in the donor's compartment (micrograms per cubic centimeter)

and '60' is used as a conversion factor to switch minutes into seconds. The rate of drug flow through the cornea is calculated from the slope of the line on the graph that shows the relationship between the amounts of drug that permeates (Q) and time.

Hydration of cornea

The corneal tissue excluding sclera was subjected to treatment with nepafenac emulgel to assess corneal hydration levels. The cornea was treated as a control with 0.9% normal saline solution. Each of the treated corneas was carefully weighed and then saturated with methanol for 5 min. After that cornea was allowed to dry, their weights were measured and percentage of hydration was subsequently calculated.[40]

HET-CAM Test

HET-CAM (Hen's Egg Test-Chorioallantoic Membrane test) was conducted as part of irritancy testing process. Eggs from newly laid hens from a poultry farm were gathered. These eggs were then kept in an incubator for three days at a temperature of 37°C. 12 hr after that, the eggs were manually turned. On day 3, a sterile procedure was employed to extract egg albumin, following which the hole was sealed with paraffin using a spatula. To allow Chorioallantoic Membrane (CAM) to develop away from eggshell, the eggs were placed at the equator. On day 10, a window was created at the equatorial region of the egg, through which 0.5 cc of test solution was introduced and left in contact with the CAM surface for 5 min. Throughout the entire experiment, 0.9% NaCl solution served as the control and it was observed to have minimal irritant effects. The evaluated results included the start of bleeding (from the blood vessels), the breakdown of blood vessels and clotting, evaluating both within and outside the blood vessels.

Histopathology Study

Histopathology studies were conducted to assess the irritancy of ocular formulation. The persistence of ocular discomfort was evaluated by comparing it to the control group, which involved using a goat cornea that had been excised. The degree of ocular irritation was found to be dependent on corneal anatomy. Freshly harvested goat corneas were immersed in an emulgel solution for 12 hr. Afterward, the corneas were promptly excised and fixed using an 8% formalin solution.[41,42] The tissue was subsequently embedded in molten paraffin, dehydrated with alcohol and allowed to solidify. Cross-sectional slices of the tissue were prepared and stained with hematoxylin. Histopathological examination was carried out using a digital Microscope, at a magnification of 100x. The results obtained from histopathological analysis were then compared to those of the control group.

Sterility Testing

Sterility is a critical requirement for ocular formulations as it has importance and necessitates sterility tests for emulgel formulation. Under aseptic conditions, sterility testing must encompass both aerobic as well as anaerobic bacteria, fungi, using both fluid thioglycollate medium as well as soybean-casein digest media. For the testing process, aseptic techniques were employed. Specifically, 100 mL of sterile water was used to dilute 1 mL of the optimized sterile formulation. To assess the presence of any microorganisms within the formulation 5 mL of the solution was introduced into respective medium. The cultures were maintained for minimum of 14 days at temperature range of 20-25°C in the incubator and were observed for development of turbidity corresponding to the growth of microorganisms.

Draize eye test

The study was completed at Marathwada Mitra Mandal's College of Pharmacy, Thergaon Pune, Maharashtra India according to the protocols permitted by the Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA), under the reference no. MMCOP/IAEC/01/2022 on the recommendations of the Institutional Animal Ethical Committee.

Rabbits are commonly favored over other animals for ocular irritancy studies due to their large eyes, which possess well-defined anatomy and physiology. Additionally, their ease of handling, cost-effectiveness and widespread availability make them a practical choice. It's worth noting that rabbit eyes tend to be more sensitive to irritating substances compared to human eyes.[43] Draize eye test, or *in vivo* eye irritation studies involved 3 rabbits; the procedure includes instilling 0.1 mL of optimized test sample into one eye and leaving the contralateral eye that was untreated as a reference.[44] Observations were made for one day and interpreted at three time points: 0, 6 and 24 hr following the application of test material. The method used an extensive grading scale to evaluate the intensity of eye abnormalities, such as those impacting the cornea (measuring transparency), iris (identifying the degree of inflammation, for example, erythema) and conjunctiva (checking for congestion, swelling and fluid, for instance, edema). Changes in these ocular structures were visually evaluated and the Draize score was calculated based on these observations. This methodology provided a thorough assessment of the potential irritant effects of emulgel formulation on the eyes.

The Mean Total Score (MTS) was calculated as follows:

$$MTS = \sum \frac{X1(n)}{5} + \sum \frac{X2(n)}{5} + \sum \frac{X3(n)}{5}$$

In the context of the study, X1(n), X2(n) and X3(n) represent the scores for the cornea, conjunctiva and iris, all of which are measured for each rabbit in the experiment.

The scoring system for Draize eye test was followed as-Score 0 indicates no erythema and edema; Score 1 indicates very slight erythema and edema; Score 2 indicates well- defined erythema and edema; Score 3 indicates moderate to severe erythema and edema; Score 4 indicates severe erythema and edema.

Stability study

The stability studies were conducted by ICH (International Council for Harmonization) guidelines. The F6 formulation was placed inside stability chamber under controlled conditions of temperature and humidity, specifically at 25°C±2°C and 60%±5% RH, for duration of three months. The evaluation of stability included both chemical and physical aspects. Chemical stability was assessed by monitoring changes in drug content, while physical stability was assessed by examining alterations in the refractive index and pH of the formulation. This approach ensures the evaluation of the formulation's stability under specified conditions and in line with internationally recognized guidelines.

RESULTS

Emulgel Development and Characterization

As stated in the introduction, the objective of the current investigation was to develop nepafenac emulgel for ophthalmic delivery using different oils and different combination of polymers. The study evaluates the effect of oil (component of emulsion) and gelling agent (component of gel base) over the characteristics of emulgel. Three different oils such as castor oil, linseed oil and oleic acid and polymers like Pluronic F 127 and HPMC K15M as standalone and as combination polymers; total nine formulations were prepared by following two stage procedures. Firstly, preparation of drug containing o/w emulsion; and secondly incorporating emulsion to gelling base to form emulgel (Figure 1). A total of nine batches indicated as F1 to F9 were prepared (as mentioned in Table 1). These formulations were evaluated for the following properties of emulgel.

PH determination

pH test for ophthalmic formulations is essential to ensure patient comfort and ocular safety, as deviations can lead to discomfort, reduced therapeutic effect and potential tissue damage. The prepared ocular formulations exhibited a pH range of 6.2 to 7.4 which falls within the physiologically compatible pH range of 4.75 to 7.40 for eye products (Table 2).

Refractive Index

The refractive index is essential for ocular formulations to ensure compatibility with tear fluid, maintain optical clarity, minimize light scattering and enhance patient comfort. Matching the refractive index with ocular components during formulation is critical and crucial. The definitive average refractive index value for human tears was established at 1.33698±0.00110, signifying a precise and critical measurement in ocular research and ophthalmic formulation development.[45] The refractive index of all formulations was observed to fall within the range of 1.340 to 1.370, which closely resembles that of tear fluid (Table 2). Specifically, the refractive index of the optimized batch (F6) was determined to be 1.36±0.01. These values are well within the acceptable range, indicating similarity to tear fluid.

% transmittance

Generally, % transmittance should be more than 90% for ocular use. It exhibits the degree of transparency and clarity of the formulation. % transmittance of all the emulgel formulation is in the range of 67.12 %-93.32% (Table 2). Formulation F6 showed highest % transmittance as 93.32±1.8%, indicating its suitability for ocular use.

Rheological study

Viscosity of formulations is modified by the addition of HPMC K15. Formulations containing HPMC K15 exhibited higher

Table 1: Compositions of emulgel formulations in weight bases.

Batch	Nepafenac (mg)	Castor oil (g)	Oleic Acid (g)	Linseed oil (g)	Benzalkonium chloride (g)	Poloxamer 188 (mg)	PF127 %	HPMC K15M %	PF127+HPMC K15M %	Water (mL)
F1	300	1.5			0.01	650	25			15
F2	300	1.5			0.01	650		10		15
F3	300	1.5			0.01	650			20+7	15
F4	300		1.5		0.01	650	25			15
F5	300		1.5		0.01	650		10		15
F6	300		1.5		0.01	650			20+7	15
F7	300			1.5	0.01	650	25			15
F8	300			1.5	0.01	650		10		15
F9	300			1.5	0.01	650			20+7	15

* PF127-Pluronic F 127; HPMC-Hydroxypropyl methylcellulose.

viscosities (F5, F6, F8 and F9) than those without it (Table 2). The optimized batch, F6, showed particularly satisfactory results with a viscosity of 1892±4 cp at 50 rpm (Table 2).[46] From plot 'A' and plot 'B', it could be interpreted that as the shear increases the viscosity of emulgel formulation decreases (Figure 2).

Drug Content

The drug content is crucial for ensuring accurate dosing, therapeutic efficacy and patient safety in pharmaceutical formulations. It also plays a vital role in quality control, regulatory compliance and formulation stability, impacting the overall safety and effectiveness of the medication. Drug content measurements were rigorously conducted in triplicate, with calculated average values ensuring precision and accuracy. The drug content of all formulated emulgels falls under the range of 38.63±2.43% to 80.79±1.52% (Figure 3).

In vitro drug release study

In vitro drug release studies were conducted for all formulations F1 to F9 (Figure 4), including a comparison between the optimized batch F6 and a marketed formulation (Figure 4). Dialysis membranes were employed in this study, revealing sustained drug release over 8 hr. The *in vitro* drug release study categorized into two groups, A (F1-F5) and B (F6-F9), revealed distinct outcomes. Group A exhibited a drug release ranging from 65.4% to 85.1%, expressing the higher release than group B, where the release ranged from 39.8% to 63.4%.

In vitro drug permeation was observed to be increased over time for emulsion-loaded gel and 0.1% marketed formulation. The drug release rate from the emulgel was lower than that of the marketed formulation due to its higher viscosity. The drug release percentages were 99.39% for the marketed formulation and 72.84% for the emulgel after 8 hr of period (Figure 4).

For the optimized batch (F6), f1 (difference factor) value obtained between the formulations was 14.2, which is below the limit value of 15. This suggests that there is no significant dissimilarity between the drug release profiles. Additionally, f2 (similarity factor) values obtained between the formulations fell within the limit range of 50-100, with a value of 61.79.

The coefficient of determination (r^2) values were determined to pick up the best fitting model to the drug permeation from nepafenac loaded emulgel formulations. The n and R^2 values were calculated and reported for zero order, first order, Hixson Crowell, Higuchi and Peppas-Korsmeyer dissolution models. All the formulations exhibited different r^2 values for all the different models used in the current study. F1 followed first order release kinetics as the correlation coefficient (r^2) value was highest i.e. 0.999. F2, F3 and F6 followed zero order release with their correlation coefficient (r^2) values as 0.9923, 0.999 and 0.9984 respectively. It indicated linear but slower relation for the release of drug from emulgel formulations occurring mostly by diffusion. F4 formulation followed Higuchi kinetics with r^2 value of 0.9964 indicating release of drug from polymeric matrix with other excipients. F5, F7, F8, F9 followed Peppas-Korsmeyer kinetics as the correlation coefficient (r^2) values were 0.9932, 0.9983, 0.9951, 0.9917 and *n* values were 1.01, 1.14, 1.09, 1.13 respectively. It indicated Super case II anomalous type transport mechanism (Table 3).

Zeta potential, polydispersity index and globule size

The size of the globules, PDI and analysis of zeta potential of the optimized F6 nepafenac emulgel is crucial for its performance in ocular drug delivery. A smaller globule size was observed i.e. 224 nm (Figure 5), which will improve penetration and enhance the drug absorption due to a larger interfacial surface area. A low PDI value (<0.7) signifies a narrow and homogeneous droplet

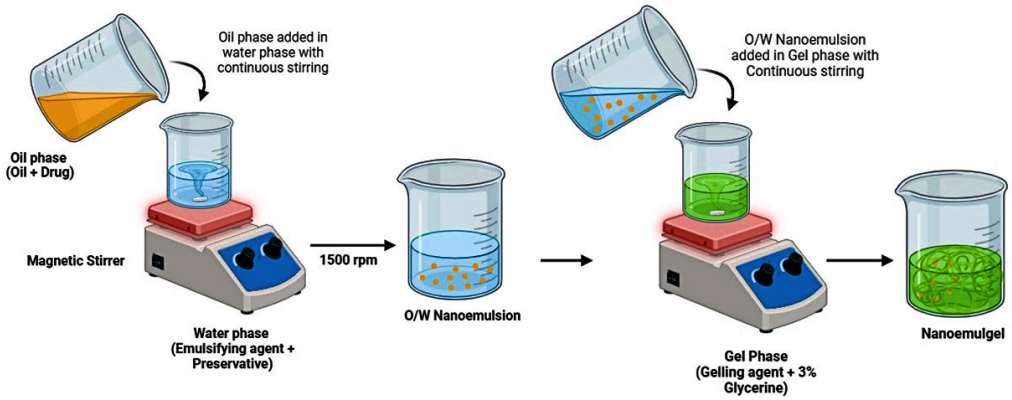


Figure 1: Graphical representation of emulgel preparation.

Table 2: Characterization of nepafenac-loaded emulgel (pH, RI, % transmittance, viscosity).

Batch	PH	Refractive Index	% Transmittance	Viscosity (cp)
F1	6.7±0.05	1.346±0.005	67.12±1.6	1567±2
F2	6.3±0.1	1.366±0.005	68.82±0.6	1237±4
F3	7.1±0.05	1.376±0.005	80.62±0.5	1187±3
F4	7.2±0.11	1.356±0.005	85.12±0.7	1347±5
F5	6.9±0.05	1.37±0.01	83.32±0.8	1463±6
F6	7.2±0.05	1.36±0.01	93.32±1.8	1892±4
F7	7.4±1.09	1.373±0.005	68.52±1.4	1098±12
F8	6.8±0	1.343±0.005	75.22±2.0	1678±3
F9	6.4±1.09	1.366±0.005	75.92±2.6	1674±5

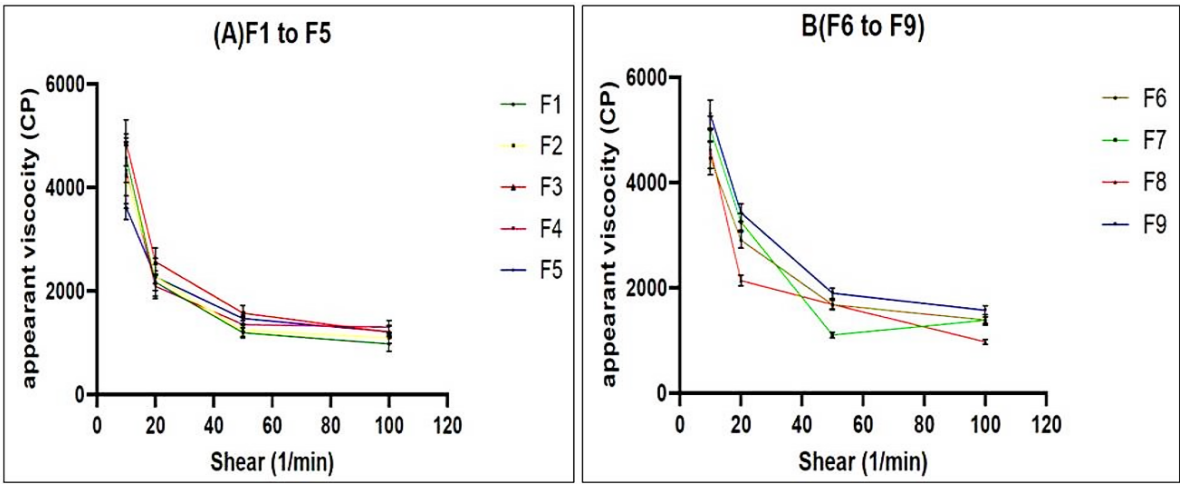


Figure 2: Graphical representation of rheological behaviour.

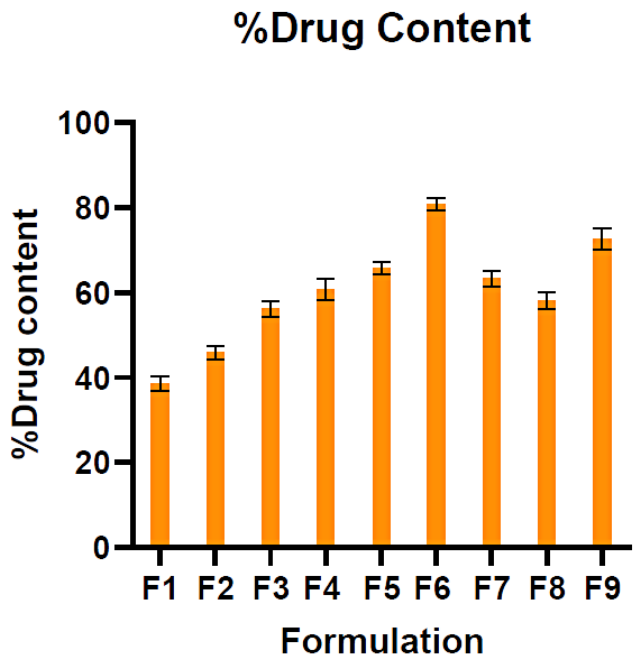


Figure 3: Graphical representation of % drug content

size distribution, ensuring greater stability, with the optimized formulation boasting a PDI of 0.232. Zeta potential, particularly a negative value like -24.0 mV in the optimized emulgel, (Figure 5) indicates repulsion between charged globules, contributing to system stability. These findings underscore the potential of emulgel for efficient drug delivery and stability in ocular applications.

Ex vivo transcorneal permeation

An *in vitro* transcorneal permeation study was conducted to compare an optimized emulsion formulation, F6 with a marketed one on how well they allowed a drug to pass through a goat's cornea. The experiment lasted for 8 hr and demonstrated better drug permeation through the cornea with the refined emulsion. The permeation rates of the drug were recorded at 50.96±4.52% for the marketed formulation and 79.54±2.00% for the optimized emulgel F6 after 8 hr (Figure 4), showing an improvement in drug permeability with the optimized emulsion throughout the study.

The results demonstrated that the apparent permeability coefficient 2.91±0.02 cm/sec × 10^6 and 0.69±0.02 cm/sec × 10^6 for optimized batch (F6) and marketed formulation respectively. The flux across corneal tissues was observed as 0.026±0.01 µg/mL for F6 and 0.023±0.01 µg/mL for marketed formulation respectively. Results showed an increase in the apparent corneal Permeability (P_{app}.) of the emulgel formulation compared to the marketed one, indicating improved drug permeation.[47]

Hydration of cornea study

A healthy, intact goat cornea typically maintains a hydration level within the range of 76% to 80%. Elevated hydration levels, ranging from 83% to 92%, are commonly associated with corneal damage.[48] The percent hydration of corneas treated with both the marketed formulation and optimized emulgel (F6) was observed to be consistent with the normal range of 78% to 80% respectively, which closely matches reported values.

HET-CAM Test

The HET-CAM examination serves as a different method for assessing the potential for irritation. The control group sets a standard, aiding in the comparison and understanding of any impacts noted in the experimental group subjected to the mixture. If the control group remains unchanged, any effects in the test group are likely attributed to the test formulation, aiding in assessing potential irritation or harm. This test reduces animal experimentation while ensuring the safety of products. With the optimized batch F6 of nepafenac emulgel serving as the test and a sodium chloride 0.9% solution as the control (Figure 6). The CAM, which comprises veins, arteries and capillaries, is responsive to inflammation,[49] making it suitable for this experiment. After instilling control and the test sample onto the egg membrane, no discoloration or hemorrhage of the membrane was observed. The ocular irritancy index score was found to be ≤0.9, which shows no irritancy of the HET-CAM membrane. This observation indicated that the nepafenac emulgel was non-irritating when compared to the control, suggesting its safety for use.[50]

Histopathological study

The histopathological evaluation of control using 0.9% sodium chloride performed and no any alteration or rupture of the corneal surface was observed (Figure 6). As a reference of the control; test performed for optimized batch F6 formulations of nepafenac ocular emulgel, conducted at 100X magnification and indicated no significant alterations in the tissue histopathology.[51]

Sterility testing

The optimized formulation F6 of nepafenac ocular emulgel was assessed for sterility by means of fluid thioglycollate and soybean casein digest medium.[52] The formulation was checked for microorganism growth in the individual mediums after its incubation period.[53] It was found that the formulation showed that there was no bacterial or fungal growth.

Draize Test

Draize eye test was conducted to assess the irritancy potential of the formulation. Here, the optimized batch F6 of nepafenac emulgel was tested on 3 rabbits. The right eye of each rabbit received the formulation (nepafenac emulgel), whereas the left eye was used as a control as shown in Figure 7. The evaluation for irritancy was conducted at multiple time points, specifically at 0, 6 and 24 hr. Importantly, even after 24 hr, no signs of irritation were observed. The Mean Total Score (MTS) was determined to

Table 3: Drug release kinetics study.

Formulation Code		F1	F2	F3	F4	F5	F6	F7	F8	F9
Model Parameters	R^2	0.9991	0.9923	0.9992	0.9964	0.9932	0.9984	0.9983	0.9951	0.9917
	K	-220.6	0.114	0.106	0.0267	0.78	0.122	0.77	0.87	0.89
	n					1.01		1.14	1.09	1.13
Model		First order	Zero order	Zero order	Higuchi	Peppas Korsmeyer	Zero order	Peppas Korsmeyer	Peppas Korsmeyer	Peppas Korsmeyer

Note: Zero order (Qt=Qo+Kot); First order (ln Qt=ln Qo+K_{1t}); Hixson-Crowell ($Qt^{1/3}$-$Qo^{1/3}$=KHCt); Higuchi (Qt=ln Qo+$KHt^{1/2}$); Peppas Korsmeyer (Mt/Mα=K_pt^n).

Table 4: Stability evaluation of optimized nepafenac emulgels batch F6.

Days	PH	Refractive Index	% Drug Content
0	6.7±0.05	1.346±0.005	78.24±1.16
30	6.3±0.1	1.366±0.005	76.36±1.98
60	7.1±0.05	1.376±0.005	77.70±2.68
90	7.2±0.11	1.356±0.005	78.29±2.31

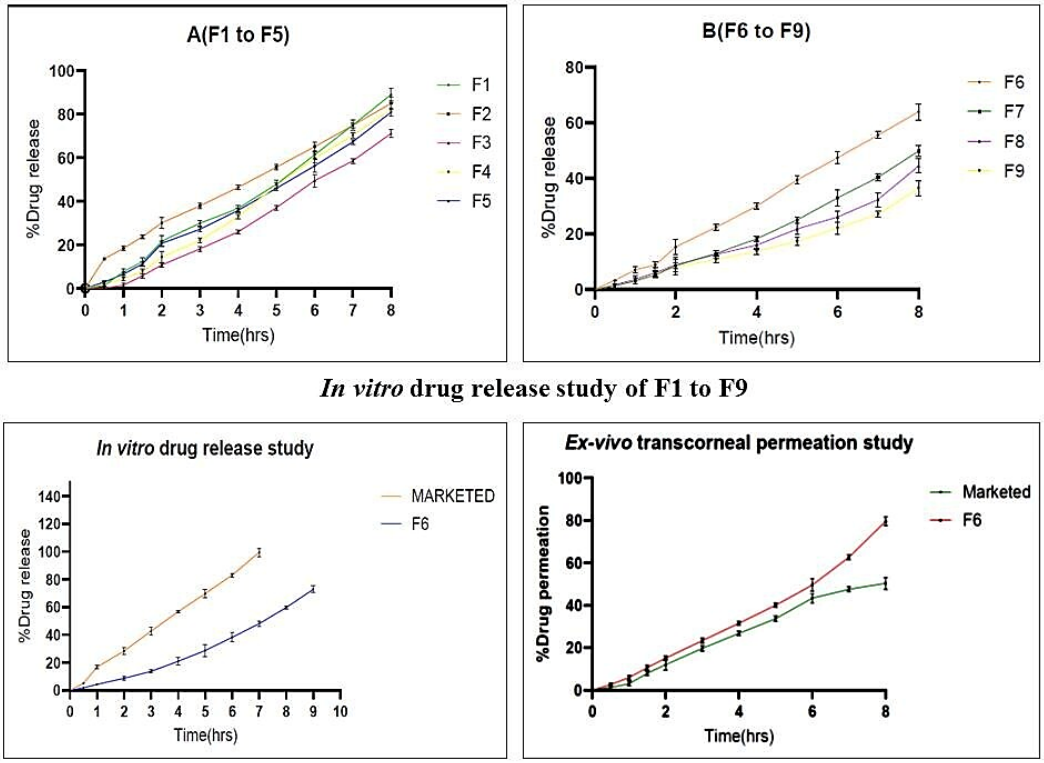


Figure 4: *In vitro* drug release study and *ex vivo* transcorneal permeation study.

be 0, which categorizes the formulation as non-irritating and safe for ophthalmic use.

Stability studies

A three-month stability study was conducted. It involved storing the optimized F6 formulation in a stability chamber under controlled conditions of temperature (25°C±2°C) and humidity (60%±5% relative humidity) for 3 months.[54] During this period, changes in drug content, pH and RI of the formulations were monitored (Table 4). The observations led to the inference that there were no significant changes in the formulation, indicating its stability over the three months.

DISCUSSION

The discussion of the findings from the current study gives essential insights into the development of nepafenac loaded emulgel formulation, its characteristics and their potential for treating the ocular inflammation. Emulgel development needs selection of suitable materials, appropriate manufacturing process. The study evaluated the impact of various formulation variables, especially type of oil and type of polymer and its combinations over the emulgel properties. It expressed the multifaceted relation between various formulation components and emulgel properties.

Numerous preliminary studies were carried out for the selection of suitable oil and polymers, their concentrations and combinations. The different oils were evaluated in the study so as to incorporate hydrophobic nepafenac to develop O/W emulsion. Castor oil, linseed oil, oleic acid were selected in the study to develop O/W emulsion. External aqueous phase of this emulsion was then gelled with different gelling agents like pluronic F 127, HPMC K 15M alone and its combinations. Pluronic containing emulgels were clear, less toxic, achieving controlled release and were suitable. But, we needed higher concentration of pluronic F 127 to obtain emulgel with these desired properties. Also, pluronic exhibits lower mucoadhesive properties. So, pluronic containing formulations were further modified by adding polymers like carbopol, HPMC to improve their mucoadhesive

characteristics and to modify release properties. Different grades of HPMC are useful due to their enhanced muco adhesion, safety, gelling characteristics and other physicochemical properties. In the current study, pluronic was selected alone and in combination of HPMC K15M to develop emulgel formulation to achieve controlled release of nepafenac and mucoadhesive characteristics.

All formulations exhibited desired pH, refractive index and % transmittance suitable for ocular applications (Table 2).

The viscosity of all emulgel formulations met the required standards; this is due to selected polymers, its combinations and their proportions in nepafenac emulgel. Such higher viscosity help in increasing the retention time of the formulation in the ocular cavity thus by giving the sufficient time for the corneal permeation and improving the ocular absorption. These formulations exhibited pseudoplastic rheology, characterized by shear thinning behaviour consistent with our initial screening data. This property allows the formulation to thin during application and thicken afterward, which is desirable for improving retention time and controlled release of the drug from topical ophthalmic products (Table 2, Figure 2). Such pseudo plastic performance allows withstanding varying shear rates that occur during blinking and inter-blinking periods. Similar results for rheological behaviour were reported in literature by Shen Y *et al*; 2014 for cyclosporine A,[31] Gadad A *et al*; 2016 for lomefloxacin,[55] Pawar P *et al*; 2013 for voriconazole.[56]

Optimized batch F6 exhibited the highest drug content of 80.79±1.52% signifying a consistent and uniform distribution of the drug within the developed formulations, where combination of PF 127 and HPMC K15M served as the primary gelling base. Further, drug content was observed to be improved for emulgels (F3, F6, F9) where PF 127 and HPMC K15M were used in combination as compared to emulgels (F1, F2, F4, F5, F7, F8) containing these polymers in solitude. This drug content of emulgel formulations underscores the promising role of PF-127 and HPMC K15M in the uniform distribution of drug in emulgels formulations. It could be also inferred that combination of these 2 polymers could be employed in order to improve various properties of emulgels.[57]

Formulations containing PF127 and HPMC K15M individually resulted in higher drug release within 8 hr, whereas formulations which combined these polymers showed comparatively lower release rates. This underscores the significant influence of polymer selection on drug release profiles (Figure 4).

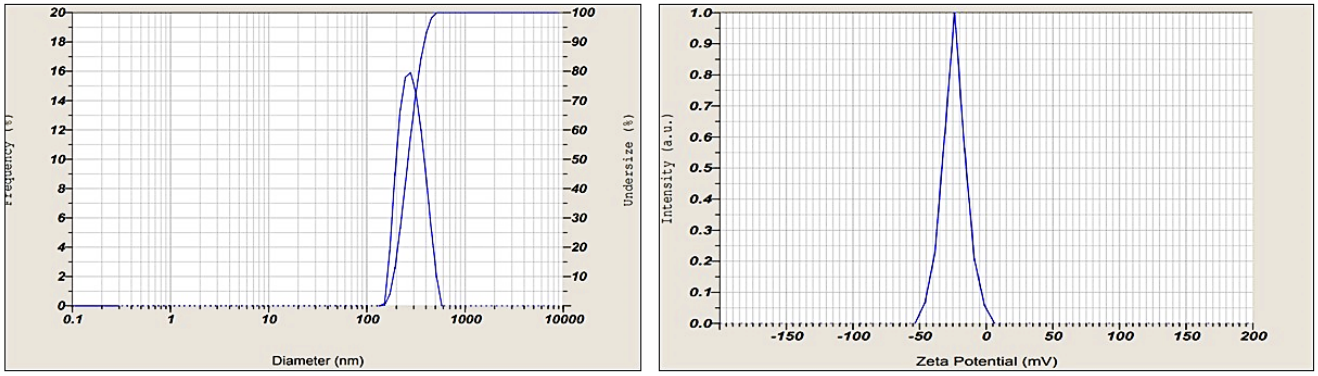
In vitro drug permeation of optimized F6 formulation was lower and sustained as compared to marketed preparation. The reason could be attributed to the higher viscosity, formulation design (choice of polymers) and dissolution kinetics.[58] Emulgel exhibited sustained drug release and its release rate was slowed down as it was incorporated into the gel, requiring more time for diffusion of drug from gel base as compared to the lower-viscosity marketed formulation.

Determination of f1 (difference factor) and f2 (similarity factor) indicated a notable similarity between the optimized batch F6 and the marketed formulation in terms of their drug release behavior.

All 9 formulations exhibited different drug release kinetics depending upon the compositions used in their preparation. Such sort of results signifies use of combination of polymers in altering the release characteristics of emulgel (Table 3).

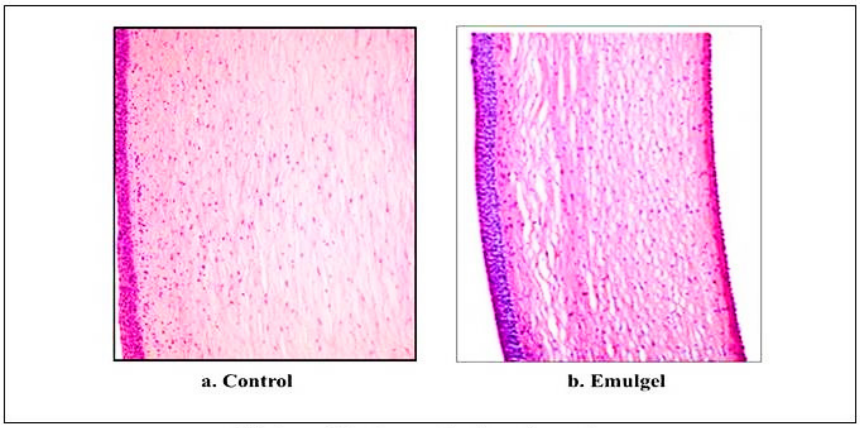
Uniform globule size, PDI and higher zeta potential value of F6 emulgel would help in uniform spreading of emulgel over corneal surface, better penetration of globules across the cornea and sustained release of the nepafenac from the formulation (Figure 5).

In vitro studies on transcorneal emulsion interactions are crucial for understanding how these formulations behave with the cornea, replicating conditions found in the human eye. These investigations examine how well drug passes through the gel emulsion, help refine the emulsion's composition and confirm the safety of the product. Also, optimized emulgel F6 demonstrated sustained drug permeation over the extended period of 8 hr (Figure 4). It may be attributed to the incorporation of an emulsion into



a. Particle size and PDI **b. Zeta potential**

Figure 5: PS, PDI and ZP of F6 emulgel formulation.



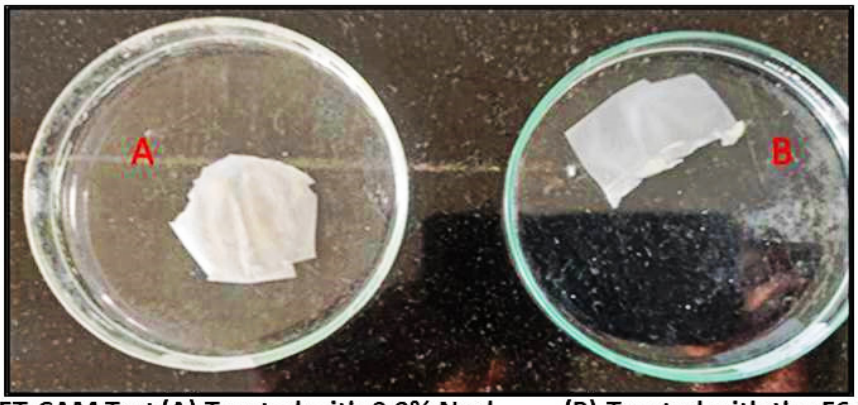


Figure 6: Histopathology study on goat cornea and HET-CAM test.

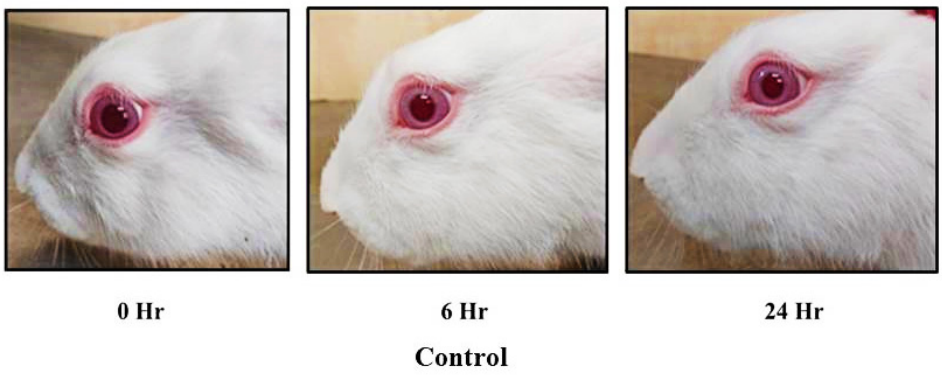


Figure 7: Ocular irritation study on rabbit eye (Draize eye test).

the gel matrix. In contrast, the marketed formulation permeated the drug directly from its lower-viscosity solution.

The capacity of a medication to cross the corneal protective layer relies on multiple elements, such as its chemical characteristics, dimensions, distribution coefficient, level of ionization and additional factors. Hydrophilic drugs face the primary barrier in the lipidic epithelium, while hydrophobic agents encounter resistance from the aqueous stroma. The predominant barrier in the cornea presents a comparison of the apparent corneal permeability (P_{app}.) between the marketed formulation and the emulgel. After 7 hr of study marketed formulation showed a decrease in drug release as compared to emulgel resulting into higher value of P_{app}. for F6 formulation as compared to the marketed one.

The hydration levels remained well within the normal range for optimized formulation F6. This suggested that it wouldn't cause any detrimental effects or damage to the corneas. It could be attributed to addition of hydrophilic HPMC K15M in the formulation. Such addition would have improved wetting and hydration properties resulting into providing the higher hydration effect which may further would not cause any harmful effect into the eyes.

The outcomes of HET-CAM test (Figure 6) offer strong evidence of the formulation's safe and non-irritating nature for ocular usage.

Histopathological studies for emulgel formulations are vital for assessing tissue compatibility, safety and regulatory compliance. They guide formulation optimization and ensure the safe use of these products. The finding underscores the formulation's safety, as it did not induce any ocular irritation or affect the cells of the corneal membrane. Furthermore, there were no observed ruptures in the corneal sections, as shown in (Figure 6). When the formulation was instilled into the eyes neither cell necrosis nor epithelium removal was seen.

Sterility testing is crucial for emulgel projected for ocular distribution as it approves the absence of detrimental microorganisms, confirming the product's safety and avoiding probable eye infections. It is a vital measure of quality control in pharmaceutical manufacturing. The optimized formulation F6 was sterile and free of microorganisms.

The extended observation period of 24 hr for Draize eye irritancy test further confirms the non-irritating nature and safety of the F6 emulgel formulation for ocular use. This outcome confirms the formulation's suitability for use in the eyes without causing any deleterious, irritant effects (Figure 7).[59]

Thus, outcomes of HET-CAM test, histopathological study and Draize test confirmed the suitability of F6 formulation for application into eyes without causing any irritation and detrimental effects.

Emulgel batch F6 was subjected to stability studies for 90 days period. Samples were periodically evaluated for stability parameters like pH, RI and drug content. No any significant change was observed in properties of F6 formulation during the study period confirming the stability over the three months (Table 4). Also, observed t test value of 0.6479 (t-test for two samples with equal variance) was below the t-critical value of 2.91, therefore, demonstrated no statistically significant difference in drug content of F6 formulation even after the period of three months that specifies the stability of product.

CONCLUSION

Ocular drug delivery is a dynamic field marked by its unique challenges and evolving solutions. Nepafenac, a potent NSAID, stands out for its effectiveness in addressing post-cataract surgery inflammation and pain. However, challenges such as limited tissue permeability and low water solubility persist, driving ongoing research into innovative approaches for enhanced delivery.

The emulgel formulation process is characterized by the creation of an oil-in-water emulsion phase and then incorporating it into a gel with gelling base, developing an effective delivery system for nepafenac. Among various batches designed for formulation, F6 was selected as the best option because it outperformed the other groups. This selection positions F6 as the preferred candidate for further studies.

The Nepafenac emulgel preparations displayed a uniform, slight yellow transparent appearance and had a refractive index (1.356±0.005) similar to tear fluid. The optimized F6 nepafenac emulsion, featuring a particle diameter of 224 nm, a Particle Distribution Index (PDI) of 0.232 and a zeta potential of -24.0 mV, demonstrates enhanced spread ability over cornea, improved drug permeation through cornea, excellent stability and potential for effective ocular drug delivery. These formulations exhibited pseudoplastic rheology with shear thinning behaviour, showcasing the promising potential of PF-127 as a standalone component. The drug release rate from the emulgel was slower than that of the marketed formulation due to its higher viscosity indicating sustained effect over the period. For the optimized batch (F6), the f1 value (14.2) suggested no significant dissimilarity in release profiles and the f2 value (61.79) indicated notable similarity with the marketed formulation. The 8-hr drug permeation study showed improved drug permeation into the cornea, with values of 79.54±2.00% for the optimized emulgel. Optimized formulation (F6) maintained corneal hydration within the normal range, suggesting no detrimental effects. HET-CAM testing revealed non-irritating properties of the emulgel, further confirmed by ocular irritation assessments in rabbits using the Draize test. Sterility testing revealed the formulation was free from microorganisms. Stability studies over three months presented no substantial deviations in the invention, confirming its stability. In conclusion, we can obtain the modified release,

transparent, stable, effective, non-irritating emulsion loaded into gel i.e. emulgel type of formulation for the nepafenac. It can offer the alternate dosage form for nepafenac which helps in repositioning of the drug into the market and would help to extend the product life cycle.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

O/W: Oil in water; **HPMC:** Hydroxypropyl methylcellulose; **COX:** Cyclooxygenase; **PF:** 127-Pluronic F 127; **NSAID:** Non-steroidal inflammatory drug; **hr:** Hours; **STF:** Simulated tear fluid; **OII:** Ocular Irritation Index; **HET-CAM:** Hen's egg chorio allantoic membrane test; **MTS:** Mean Total Score.

ETHICS COMMITTEE APPROVAL

The protocols permitted by the Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA), under the reference no. MMCOP/IAEC/01/2022 on the recommendations of the Institutional Animal Ethical Committee.

SUMMARY

This study explored the potential of emulgel as an innovative vehicle for ocular drug delivery, with a particular emphasis on the drug nepafenac. The study demonstrated the formulation's non-irritant properties, stability and minimal impact on ocular histopathology, ensuring patient comfort and safety. In-depth *in vitro* and *ex vivo* investigations provided compelling evidence of the formulation's efficacy in achieving the controlled delivery of nepafenac. Overall, the research indicates the significance of emulgel as a promising approach for ocular medication delivery, offering improved patient care possibilities in the field of ophthalmology.

REFERENCES

1. Brian G, Taylor H. Cataract blindness-challenges for the 21st century. Bull World Health Organ. 2001;79(3):249-56.
2. Han X, Zou M, Liu Z, Sun Y, Young CA, *et al.* Time trends and heterogeneity in the disease burden of visual impairment due to cataract, 1990–2019: A global analysis. Front Public Health. 2023;11:1140533. DOI: 10.3389/fpubh.2023.1140533
3. Chastain JE, Sanders ME, Curtis MA, Chemuturi NV, Gadd ME, *et al.* Distribution of topical ocular nepafenac and its active metabolite amfenac to the posterior segment of the eye. Exp Eye Res. 2016;145:58-67. DOI: 10.1016/j.exer.2015.10.009.
4. Lane SS, Modi SS, Lehmann RP, Holland EJ. Nepafenac ophthalmic suspension 0.1% for the prevention and treatment of ocular inflammation associated with cataract surgery. J Cataract Refract Surg. 2007;33(1):53-8. doi:10.1016/j.jcrs.2006.08.043.
5. Urtti A. Challenges and obstacles of ocular pharmacokinetics and drug delivery. Adv Drug Deliv Rev. 2004;58(11):1131-5. DOI: 10.1016/j.addr.2006.07.027.
6. Wang Y, Xu X, Gu Y, Cheng Y, Cao F. Recent advance of nanoparticle-based topical drug delivery to the posterior segment of the eye. Expert Opin Drug Deliv 2018;15(7):687-701. DOI: 10.1080/17425247.2018.1496080.
7. Achouri D, Alhanout K, Piccerelle P andrieu V. Recent advances in ocular drug delivery. Drug Dev Ind Pharm. 2013;39(11):1599-17. DOI: 10.3109/03639045.2012.736515.
8. Lorenzo-Veiga B, Sigurdsson HH, Loftsson T. Nepafenac-Loaded cyclodextrin/polymer nanoaggregates: A new approach to eye drop formulation. Materials (Basel). 2019;12(2):229. DOI: 10.3390/ma12020229.
9. Shelley H, Babu RJ. Role of cyclodextrins in nanoparticle-based drug delivery systems. J Pharm Sci. 2018;107(7):1741- 1753. DOI: 10.1016/j.xphs.2018.03.021.
10. Wei D, Unalan HE, Han D, Zhang Q, Niu L, *et al.* A solid-state dye-sensitized solar cell based on a novel ionic liquid gel and ZnO nanoparticles on a flexible polymer substrate. Nanotechnology. 2008;19(42):424006. DOI: 10.1088/0957-4484/19/42/424006.
11. Ahmed S, Amin MM, Sayed S. Ocular Drug Delivery: A Comprehensive Review. AAPS PharmSciTech. 2023;24:66. https://doi.org/10.1208/s12249-023-02516-9.
12. Arora P, Mukherjee B. Design, development, physicochemical and *in vitro* and *in vivo* evaluation of transdermal patches containing diclofenac diethyl ammonium salt. J Pharm Sci. 2002;91(9):2076-89. doi: 10.1002/jps.10200.
13. Parsaee S, Sarbolouki MN, Parnianpour M. *In vitro* release of diclofenac diethylammonium from lipid-based formulations. Int J Pharm. 2002;241(1):185-90. doi: 10.1016/s0378-5173(02)00238-7.
14. Omer S, Nagy N, Szőcs E, Kádár S, Völgyi G, *et al.* Development of innovative electrospun nepafenac-loaded nanofibers-based ophthalmic inserts. Int J Pharm. 2023;647:12554. https://doi.org/10.1016/j.ijpharm.2023.123554.
15. Mohamed MI. Optimization of chlorphenesin emulgel formulation. AAPS J. 2004;6(3):81-7. DOI:10.1208/aapsj060326.
16. Panwar A, Upadhyay N, Bairagi M, Gujar S, Darwhekar G, *et al.* Emulgel: A review. Asian J Pharm Life Sci. 2011; 1(3); 333: 343.
17. Astrid S, Eva M Del A, Viktor KV, Tatiana T, Marika R, Arto U. Design principles of ocular drug delivery systems: importance of drug payload, release rate and material properties. Drug Discov Today. 2019;24(8):1446-57. DOI: 10.1016/j.drudis.2019.02.001.
18. Bhanu PV, Shanmugam V, Lakshmi PK. Development and optimization of novel diclofenac emulgel for topical drug delivery. Int J Comp Pharm. 2011;2(9):1-4.
19. Kostenbauder HB, Martin AN. A rheological study of some pharmaceutical semisolids. J Am Pharm Assoc Sci Ed. 1954;43(7):401-7.
20. Sintov AC, Botner S. Transdermal drug delivery using microemulsion and aqueous systems: influence of skin storage conditions on the *in vitro* permeability of diclofenac from aqueous vehicle systems. Int J Pharm. 2006;311(1-2):55-62. doi.org/10.1016/j.ijpharm.2005.12.019
21. Zhang Z. Zhifen H, Renlong L, Yi M, Wenjuan H, *et al.* Fabrication of a micellar supramolecular hydrogel for ocular drug delivery. Biomacromolecules.2016;17:798-807. DOI: 10.1021/acs.biomac.5b01526.
22. Gautam N, Kesavan K. Development of microemulsions for ocular delivery. Ther Deliv. 2017;8(5):313-30. DOI: 10.4155/tde-2016-0076.
23. Shah J, Nair AB, Jacob S, Patel RK, Shah H, et al. Nanoemulsion based vehicle for effective ocular delivery of moxifloxacin using experimental design and pharmacokinetic study in rabbits. Pharmaceutics. 2019;11(5):230. https://doi.org/10.3390/pharmaceutics11050230.
24. Wang M, Fang L. Percutaneous absorption of diclofenac acid and its salts from emulgel. Asian J Pharm Sci. 2008;3(3):131-41.
25. Yang X, Trinh HM, Agrahari V, Sheng Y, Pal D, *et al.* Nanoparticle-based topical ophthalmic gel formulation for sustained release of hydrocortisone butyrate. AAPS PharmSciTech. 2016;17:294-306. https://doi.org/10.1208/s12249-015-0354-5
26. Sabry HS, Al-Shohani AD, Mahmood SZ. Formulation and evaluation of levofloxacin and betamethasone ophthalmic emulgel. J Pharm Bioallied Sci. 2021;13(2):205-11. DOI:10.4103/jpbs.JPBS_338_20
27. Surini S, Leonyza A, Suh CW. Formulation and in vitro penetration study of recombinant human epidermal growth factor-loaded transfersomal emulgel. Adv Pharm Bull. 2020;10(4):586-94. DOI:10.34172/apb.2020.070
28. Khullar R, *et al.* Formulation and evaluation of mefenamic acid emulgel for topical delivery. Saudi Pharm J. 2012;20(1):63-7. DOI.org/10.1016/j.jsps.2011.08.001
29. Sabri LA, Sulayman HT, Khalil YI. An investigation release and rheological properties of miconazole nitrate from emulgel. Iraqi J Pharm Sci. 2009;8(2):26-31.
30. Sawant AA, Mohite SK. Formulation and evaluation of itraconazole emulgel for topical drug delivery. In Vitro. 2015;90(F9):84-6. DOI:10.5958/2231-5713.2015.00014.8
31. Shen Y, Ling X, Jiang W, Du S, Lu Y, *et al.* Formulation and evaluation of Cyclosporin A emulgel for ocular delivery. Drug Deliv. 2015;22(7):911-7. DOI.org/10.3109/10717544.2013.861883.
32. Pescina S, Govoni P, Potenza A, Padula C, Santi P, *et al.* Development of a convenient *ex vivo* model for the study of the transcorneal permeation of drugs: Histological and permeability evaluation. J Pharm Sci. 2015;104(1):63-71. DOI:10.1002/jps.24231.
33. Abdelbary GA, Amin MM, Zakaria MY. Ocular ketoconazole-loaded proniosomal gels: formulation, *ex vivo* corneal permeation and *in vivo* studies. Drug Deliv. 2017;24(1):309-19. DOI: 10.1080/10717544.2016.1247928.

34. Gade S, Patel KK, Gupta C, Anjum MM, Deepika D, *et al.* An *ex vivo* evaluation of moxifloxacin nanostructured lipid carrier enriched *in situ* gel for transcorneal permeation on goat cornea. J Pharm Sci. 2019;108(9):2905-16. DOI.ORG/10.1016/J.XPHS.2019.04.005
35. Abbas MN, Khan SA, Sadozai SK, Khalil IA, Anter A, *et al.* Nanoparticles loaded thermoresponsive *in situ* gel for ocular antibiotic delivery against bacterial keratitis. Polymers. 2022;14:1135. doi:10.3390/polym14061135.
36. Shelley H, Rodriguez-Galarza RM, Duran SH, Abarca EM, Babu RJ. *In situ* gel formulation for enhanced ocular delivery of nepafenac. J Pharm Sci. 2018;107(12):3089-97. DOI.ORG/10.1016/J.XPHS.2018.08.013
37. Jain P, Sharma RK, Gupta A, Singh S, Thakur R, *et al.* Preparation of levofloxacin loaded *in situ* gel for sustained ocular delivery: *in vitro* and *ex vivo* evaluations. Drug Dev Ind Pharm. 2020;46(1):50-6. DOI.org/10.1080/03639045.2019.1698598
38. Wilhelmus KR. The Draize Eye Test. Ophthalmology. 2001;108(6):1050-1055. DOI:10.1016/s0039-6257(01)00211-9.
39. Abraham MH, Hassanisadi M, Jalali-Heravi M, Ghafourian T, Cain WS, *et al.* Draize rabbit eye test compatibility with eye irritation thresholds in humans: a quantitative structure-activity relationship analysis. Toxicological Sciences. 2003;76:2384-391. https://doi.org/10.1093/toxsci/kfg242
40. Cormier EM, Parker RD, Henson C. Determination of the intra- and inter laboratory reproducibility of the low volume eye test and its statistical relationship to the Draize Eye Test. Regul Toxicol Pharmacol. 1996;23(2):156-61. DOI:10.1006/rtph.1996.0037.
41. Craig JP, Simmons PA, Patel S, Tomlinson A. Refractive index and osmolality of human tears. Optom Vis Sci. 1995;72(10):718-24.
42. Mohamed MI. Optimization of chlorphenesin emulgel formulation. AAPS J. 2004;6(3):26. DOI:10.1208/aapsj060326.
43. Dludla SBK, Mashabela LT, Ng'andwe B, Makoni PA, Witika BA. Current advances in nano-based and polymeric stimuli-responsive drug delivery targeting the ocular microenvironment: a review and envisaged future perspectives. Polymers. 2022;14:3580. DOI:10.3390/polym14173580.
44. Al-Hussaniy HA, Almajidi YQ, Oraibi AI, Alkarawi AH. Nanoemulsions as medicinal components in insoluble medicines. Pharmacia. 2023;70(3):537-47. DOI:10.3897/pharmacia.70.e107131.
45. Alhowyan AA, Kalam MA, Iqbal M, Raish M, El-Toni AM, *et al.* Mesoporous silica nanoparticles coated with carboxymethyl chitosan for 5-fluorouracil ocular delivery: characterization, *in vitro* and *in vivo* studies. Molecules. 2023;28:1260. DOI:10.3390/molecules28031260.
46. Bin-Jumah M, Gilani SJ, Jahangir MA, Zafar A, Alsheri S, *et al.* Clarithromycin-loaded ocular chitosan nanoparticle: formulation, optimization, characterization, ocular irritation and antimicrobial activity. Int J Nanomedicine. 2020;15:7861–7875. DOI:10.2147/ijn. s269004.
47. Satyanarayana SD, Abu Lila AS, Moin A, Moglad EH, Khafagy E-S, *et al.* Ocular delivery of bimatoprost-loaded solid lipid nanoparticles for effective management of glaucoma. Pharmaceuticals. 2023;16:1001. DOI:10.3390/ph16071001.
48. Velya M, Ruban O. Research on the selection of emulgel preservative with thick extract of feverfew. Ann Mechnikov's Inst. 2022;2:68-72. DOI: 10.5281/zenodo.6634854.
49. Deepthi S, Jose J. Novel hydrogel-based ocular drug delivery system for the treatment of conjunctivitis. Int Ophthalmol. 2019;39:1355-66. DOI:10.1007/s10792-018-0955-6.
50. Hemmati M, Ghasemzadeh A, Haji Malek-kheili M, Khoshnevisan K, Koohi MK. Investigation of acute dermal irritation/corrosion, acute inhalation toxicity and cytotoxicity tests for Nanobiocide®. Nanomed Res J. 2016;1(1):23. DOI:10.7508/nmrj.2016.01.004.
51. Mahboobian MM, Mohammadi M, Mansouri Z. Development of thermosensitive *in situ* gel nanoemulsions for ocular delivery of acyclovir. J Drug Deliv Sci Technol. 2020;55:101400. DOI: 10.1016/j.jddst.2019.101400.
52. Narayana S, Ahmed MG. Design and evaluation of ocular hydrogel containing combination of ofloxacin and dexamethasone for the treatment of conjunctivitis. Braz J Pharm Sci. 2022;58:e20180. DOI.org/10.1590/s2175-97902022e20180
53. Patil AV, Mahajan HS. Modified pea starch based ocular films of azelastine hydrochloride:Development and characterization. Carbohydr Polym Technol Appl. 2021;2:100078. doi.org/10.1016/j.carpta.2021.100078.
54. Djayet C, Bremond-Gignac D, Touchard J, Secretan P-H, Vidal F, *et al.* Formulation and stability of ataluren eye drop oily solution for aniridia. Pharmaceutics. 2021;13(1):7. https://doi.org/10.3390/pharmaceutics13010007
55. Gadad AP, Wadklar PD, Dandghi P, Patil A. Thermosensitive *in situ* gel for ocular delivery of lomefloxacin. Indian J Pharm Educ Res. 2016;50(2):S96-105.
56. Pawar P, Kashyap H, Malhotra S, Sindhu R. Formulation design of thermo sensitive voriconazole *in situ* gelling system for ocular drug delivery: *In vitro*, stability and antifungal activities assessment. BioMed Research International. 2013;1-8.
57. McKenzie B, Kay G, Matthews KH, Knott RM, Cairns D. The Hen's egg chorioallantoic membrane (HET-CAM) test to predict the ophthalmic irritation potential of a cysteamine-containing gel: quantification using Photoshop® and Image J. Int J Pharm. 2015;490(1-2):1-8. DOI: 10.1016/j.ijpharm.2015.05.023.
58. Sahu RK, Singh B, Saraf SA, Kaithwas G, Kishor K. Photochemical toxicity of drugs intended for ocular use. Arch Ind Hyg Toxicol. 2014;65(2):157-67. DOI:10.2478/10004-1254-65-2014-2461.
59. Samimi MS, Mahboobian MM, Mohammadi M. Ocular toxicity assessment of nanoemulsion *in-situ* gel formulation of fluconazole. Human & Experimental Toxicology. 2021;40(12):2039-47.

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