In-Situ Gel for Ophthalmic Delivery of Dorzolamide Hcl and Netarsudil Dimesylate: Formulation, Development, and Evaluation

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Abstract:

A gel-forming ocular drug delivery system incorporating Dorzolamide Hydrochloride and Netarsudil Dimesylate was developed and evaluated for its efficacy. Conventional ophthalmic dosage forms often face limitations such as inefficient drug transport to the posterior segment of the eye, low ocular bioavailability, rapid drug elimination via nasolacrimal drainage, and pulse-drug release following topical administration. In this study, Dorzolamide Hydrochloride and Netarsudil Dimesylate were procured from Micro Labs Pvt. Ltd., Bangalore, India. The polymeric agents HPMC K4M and Chitosan were sourced from G.R.Y Institute of Pharmacy, Borawan, Khargone, Madhya Pradesh. The formulated in situ gel demonstrated a sustained drug release profile lasting up to 480 minutes, significantly outperforming a marketed eye drop which released its drug content within 80 minutes. Comprehensive evaluation of the formulation was conducted based on parameters including clarity, sol pH, gelling pH, sol and gel viscosity, drug content, and percentage cumulative drug release. The results indicated excellent formulation clarity, optimal gelling pH, and robust gelling ability at physiological conditions. The in situ gel systems of Dorzolamide Hydrochloride and Netarsudil Dimesylate were successfully formulated, showing prolonged ocular residence time and controlled drug release behavior.

Keywords: Ophthalmic drug delivery; Dorzolamide hydrochloride; Chitosan; HPMC; In-situ gel; HET; CAM Test; Draize test

1. Introduction

Ocular drug delivery is one of the most fascinating and difficult fields for pharmaceutical experts to engage in. The eye is extremely resilient to the impacts of environmental contaminants due to its anatomy, physiology, and biochemistry. Overcoming the eye's defenses without permanently damaging the tissue is the formulator's difficulty. The traditional ocular dosage forms of ocular solutions, suspensions, and ointments are ineffective for treating some of the most severe conditions of our day [1]. Conventional ocular dosage forms present several limitations, including suboptimal bioavailability, pulsatile drug release following topical administration, systemic absorption via nasolacrimal drainage, and insufficient delivery to posterior ocular tissues. Despite these challenges, topical ophthalmic formulations remain the primary therapeutic approach for both anterior and posterior segment disorders. Conditions such as glaucoma and uveitis, as well as external infections like conjunctivitis, blepharitis, and keratitis, are commonly managed through topical application. Among these, ophthalmic solutions—particularly eye drops—are the most widely utilized due to their cost-effectiveness, ease of formulation and manufacturing, and favorable patient compliance, notwithstanding transient visual blurring. However, rapid precorneal drug loss resulting from tear turnover and nasolacrimal drainage significantly compromises therapeutic efficacy. It is estimated that less than 5% of the administered dose penetrates intraocular tissues, with the majority undergoing systemic absorption through the conjunctiva and nasolacrimal duct. To overcome these limitations, extensive research is being directed toward the development of advanced ocular drug delivery systems. Recent innovations aim to enhance corneal drug permeation, prolong precorneal residence time, and reduce systemic exposure. A promising strategy involves the integration of multiple delivery mechanisms to optimize therapeutic outcomes [2-4]. In this context, in-situ gelling systems have garnered considerable attention. Derived from the Latin term *in situ*, meaning "in its original place," these systems undergo a sol-to-gel phase transition upon exposure to specific physiological stimuli such as temperature, pH, ionic strength, or UV irradiation on the ocular surface. This transition facilitates prolonged retention and sustained drug release, thereby improving bioavailability and therapeutic efficacy [5].

2. Materials and Methods

Micro Labs Ltd. in India was kind enough to provide a sample of their Dorzolamide Hydrochloride and Netarsudil dimesylate combination product for us to test. G.R.Y Institute of Pharmacy, Borawan, Khargone, Madhya Pradesh was the source for the Bezalkonium Chloride, HPMC K4M, K 1OOM, Hyroxyethyl cellulose and Chitosan. Mannitol and Sodium chloride were acquired from Loba Chemie Pvt. Ltd, Mumbai.

Methods

Preparation of in-situ gelling systems

The clarity, gelling capacity, and viscosity of aqueous solutions containing various grades of Hydroxyethyl Cellulose (HEC: 250 L Pharm, 250 G Pharm, 250 M Pharm, 250 H Pharm, 250 HX Pharm, 250 HHX Pharm), Hydroxypropyl Methylcellulose (HPMC: K4M, K15M, K100M, E5, E15, E50), and Chitosan were evaluated to identify the most suitable polymers for in situ gelling systems. For the preparation of in situ gel-forming formulations containing Dorzolamide Hydrochloride and Netarsudil Dimesylate, a two-part process was employed:

Part A: In one container, 40 mL of distilled water was taken, and the selected polymer (excluding Chitosan) was gradually added under continuous stirring at 40–60 °C to obtain various concentrations. Separately, Chitosan was dissolved in a 1% acetic acid solution and then incorporated into the polymer solution prepared above.

Part B: In a separate container, 45 mL of distilled water was combined with boric acid under stirring. Mannitol, Dorzolamide Hydrochloride, and Netarsudil Dimesylate were then added and stirred until a clear solution was achieved. The pH of this mixture was adjusted to 5.2–5.5 using 1 N sodium hydroxide solution. Subsequently, the solution from Part B was added to Part A and stirred continuously for 15 minutes. The final volume was adjusted to 100% of the intended batch size. Gentle, consistent stirring ensured the formulation was homogeneous. The resulting solution was filtered through a 0.2 μm filter paper and aseptically transferred to sterile vials for storage [3].

Evaluation of in-situ gelling formulations

Test for appearance/ clarity

Clarity test was done by visual inspection of each container after pouring the solution in to a glass beaker and to observe it under a good light, and viewed against a black and white background, with the contents set in motion with a swirling action. [6, 7]. The pH of Fix dose formulations (H-1 to H-10) was measured using a calibrated digital pH meter (LABTECH, DPH) at room temperature. The pH measurements were made in triplicate.

Determination of viscosity of preformed ophthalmic gels in ATF

The Rheology of the developed formulations was determined before and after gelation by Brookfield Viscometer using SC18. The viscometer's spindle was fastened to its lower shaft. Twenty millilitres of solution and premade gels were placed in a container, and the motor was activated to rotate the spindle inside of the container at different rpm at 20°C. Constant readings of torque and viscosity (cP) were recorded at 25, 50, 75 and 100 (rpm) rotational speed [8].

Drug content determination

Estimation of Dorzolamide Hydrochloride and Netarsudil Dimesylate in an Ophthalmic In Situ Gelling Solution Using stability indicating RP-HPLC method for estimation of Dorzolamide and Natarsudil. The procedure involves HPLC method; 1 ml of each formulation was diluted. Diluent used was acetonitrile: methanol: 0.01N Ammonium acetate buffer in ratio of 45:35:20 v/v and the solutions were scanned at 242 λmax of drugs. [10, 11].

In-vitro drug release study

In vitro release study was carried out by Franz diffusion cell. The formulation was placed in the donor compartment and freshly prepared simulated tear fluid in the receptor compartment. Between donor and receptor compartments, dialysis membrane was placed which was previously soaked with simulated tear fluid for overnight. It was tied to one end of the diffusion cell, and rotation speed was set at 50 rpm and the temperature of the medium was maintained at 37 ± 0.5°C. Aliquots of 1ml were withdrawn at different time intervals of 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 h and 1 ml of fresh medium was replaced. The withdrawn samples were diluted to 10 ml in volumetric flask with simulated tear fluid and were analyzed by UV spectrophotometer at λmax. [12–13].

Fig 1: In-Vitro dissolution study in Franz Diffusion cell.

HET CAM test

Fertilized chicken eggs weighing between 50–60 g and within 7 days of embryonic development were selected for experimentation. Eggs were carefully examined using candling to eliminate any that exhibited developmental anomalies, such as deformations, thin or cracked shells, or signs of nonviability. During incubation, the eggs were maintained at 35 ± 0.3 °C with $58 \pm 2\%$ relative humidity. They were manually rotated five times daily until day 8. Candling was repeated on day 8 to exclude any further damaged or nonviable eggs. On day 9, eggs were positioned with the large end upward and left in the incubator without manual turning for 24 hours. After this, a final candling was performed to screen out defective samples. The air cell of each viable egg was marked and aseptically drained, taking care not to rupture the inner membrane during shell removal. Once the CAM surface was exposed, a 0.9% NaCl solution was applied as the negative control. For the positive control, 0.1 N NaOH was used, and the solution was preheated to 35 °C for 30 minutes. The test substance was then applied to the CAM, and the tissue was monitored for up to 8 hours for signs of irritation or vascular response [14].

Evaluation of test results

The study was approved by the Animal Ethics Committee under protocol number RCPCR/2025/IAEC/27. Experimental procedures were conducted using rabbits following the Draize eye irritation test methodology. A 10 µL volume of the test liquid (or 10 mg of the test solid) was instilled into the lower conjunctival sac, followed by irrigation with saline solution to minimize residual exposure. Clinical signs including ocular redness, irritation, and lacrimation were assessed using a semi-quantitative scoring scale ranging from 0 (normal) to 10 (severe) at predefined time points: 1 hour, 24 hours, 48 hours, 72 hours, 7 days, and 21 days post-administration. Rabbits were selected as the model species due to their relatively large ocular surface area, well-characterized anatomical and physiological features, ease of handling, and cost-effectiveness, making them a standard choice for ophthalmic irritation assessments [15-16].

Test for sterility

Sterility testing in pharmaceutical product is a critical process to ensure the absence of the viable microorganism, which is essential for the safety and efficacy of drugs and medical devices. The testing is conducted using USP -<71> sterility tests method. The test samples were extracted from the accelerated stability chamber and subsequently subjected to sterility testing. A sterile pippte was used to directly inoculate the samples into the culture media. The inoculated samples were then incubated at temperatures of 20-25 ℃ (soybean casein digest) and 30-35 ℃ (fluid thioglycolate) for a duration of 14 days. [17–20].

Stability studies (Effect of temperature and humidity)

The in-situ gel formulations were kept out of the light for three months at accelerated condition 40C±2ºC/75%±5%RH in high-density polyethylene preservative free bottles. Drug content was assessed in the formulations. The samples were tested at monthly intervals. [21–25].

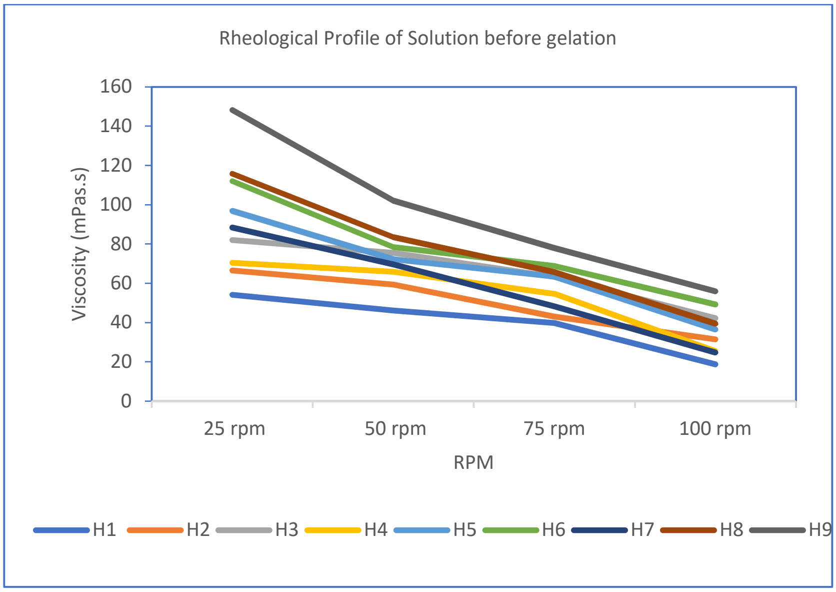

Fig. 2. Brookefield LVDVIII + Programmable Viscometer-Spindle no. SC-18.

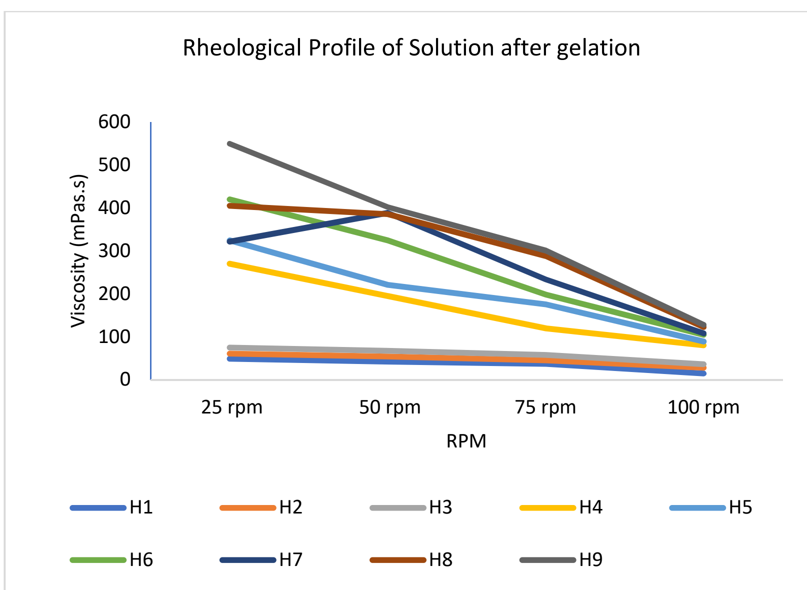

Fig.3. Rheogram of Preformed Ophthalmic Gels.

3. Result and Discussion

Dorzolamide Hydrochloride was found to be white crystalline powder. ND appeared to be off-white, odourless powder. The melting point of Dorzolamide Hydrochloride and ND was found to be 263-265 °C and 136.34-137.50 °C respectively which is complying with the melting point reported (264 °C for Dorzolamide HCL, No reference for ND) in the pharmacopeia. This confirms the provided drug sample was Dorzolamide Hydrochloride and ND. Dorzolamide Hydrochloride was found soluble in buffer pH 4.0, pH 7.0, pH 9.2, water and slightly soluble in methanol. Netarsudil dimesylate was found freely soluble in buffer pH 4.0, pH 7.0, pH 9.2 water and methanol, practically insoluble in dichloromethane. Standard solution of Dorzolamide Hydrochloride in artificial tear fluid (ATF) and water Scanned at UV–Visible Spectrophotometer to all wavelengths. The wavelength was found 253.2 nm for both ATF and water. The calibration Curve of Dorzolamide Hydrochloride in Water and ATF respectively (max-400 nm) was constructed. Beer-Lambert's law was followed in the concentration range of 5–25 µg/ml. The calibration curves of ND in ATF and water was constructed respectively. Beer Lambert's law was followed in the concentration range of 5–25 µg/ml. Dorzolamide Hydrochloride indicated in the treatment of elevated intraocular pressure in patients with ocular hypertension or open-angle glaucoma, with concentration of 22.3 mg/mL. According to the published works, the concentration of ND is **0.02%**. It was discovered that all the formulations were clear and translucent solution. Formulations containing HPMC and Chitosan share a similar pH value 5.0 to 6.0. Increasing the amounts of Chitosan (from 0.75 to 1.0%) and HPMC K4M (from 0.3 to 0.75%), raised the viscosity. The formulations exhibited shear thinning behavior. The viscosity of the formulations under physiological conditions (gel state in presence of ATF) was found to be higher as compared to the viscosity of formulations under non-physiological conditions (sol state). For the formulation (H6) in solution, form viscosity was found to be 112.1 cP at 25 rpm and for the same formulation in gel form, the viscosity was found to be increased to 420 cP at 25 rpm. This increase in viscosity suggested the occurrence of phase transition from sol to gel (see Figs. 2 and 3). There was consistent shear thinning behavior across all Chitosan preformed gel compositions (decreasing viscosity at increasing shear rate). Fig. 3 demonstrates that following gelation (at pH 7.4), the viscosity of Chitosan formulations is significantly greater than that of the solution form (at pH 5.5). The viscosity of Batch H1-H3 is low as there is no chitosan, while Batch H4-H9 are higher. When the concentrations of Chitosan and HPMC were raised from low to high, viscosity grew (see Figs. 2-3).

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4a. H6 Before gelation **4b.** H6 after gelation

Fig 4: Before and after gelling of the H6 optimized formulation.

Batches with a high concentration of Chitosan and HPMC have good gelling ability but less clarity, so batch H6 is optimal in both regards. Dorzolamide Hydrochloride and Netarsudil dimesylate (ND) were tested in a cellophane membrane diffusion assay (ATF) utilising a modified Franz diffu- apparatus to measure their cumulative percent release. All formulations tested for both drugs showed in vitro release of Dorzolamide Hydrochloride and ND for up to 10 h.

Table 1: 32 Factorial design was used for optimization.

Independent variables	Dependent variables
A = Chitosan B = HPMC K4M	Y1 = pH of sol Y2 = Gelling pH Y3 = Viscosity of sol Y4 = Viscosity of gel Y5= % Drug Release Drug D Y6= % Drug Release Drug ND

Researchers found that the percentage of cumulative drug release decreased with increasing chitosan and HPMC concentrations in an in vitro drug release investigation. Due to the high concentrations of both polymers in Batch H7-H9, the release of both Dorzolamide Hydrochloride and ND is very low (72.2-85.30% and 81.57%-94.69% respectively, up to 10 h). Since there is only HPMC in Batch H1-H3, the drug is released at a faster rate for up to 10 h [26], with more than values of 80 % for Dorzolamide Hydrochloride and ND within 6 Hrs. Formulation H6 shown to have the drug release at a steady rate for up to 10 h (98.62 % and 98.32% respectively) (see Figs. 3-4).

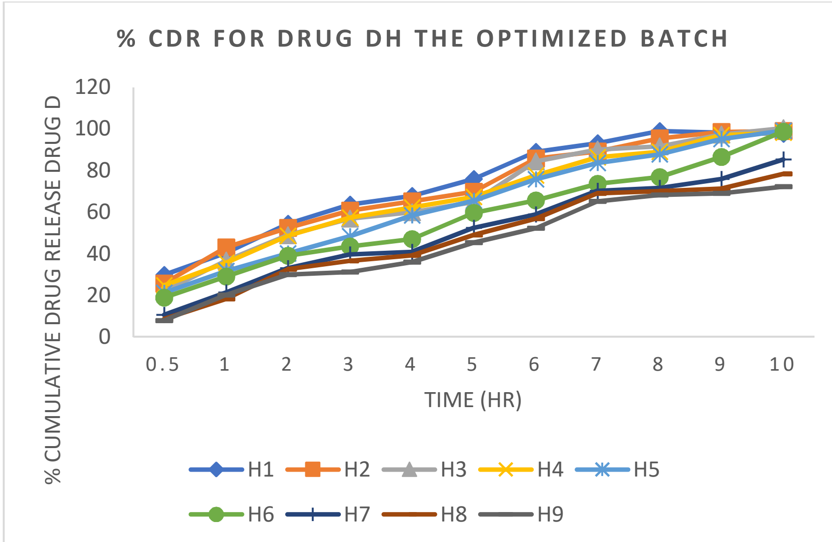


Fig.5. Percent Cumulative Release of Drorzolamide HCl (D).

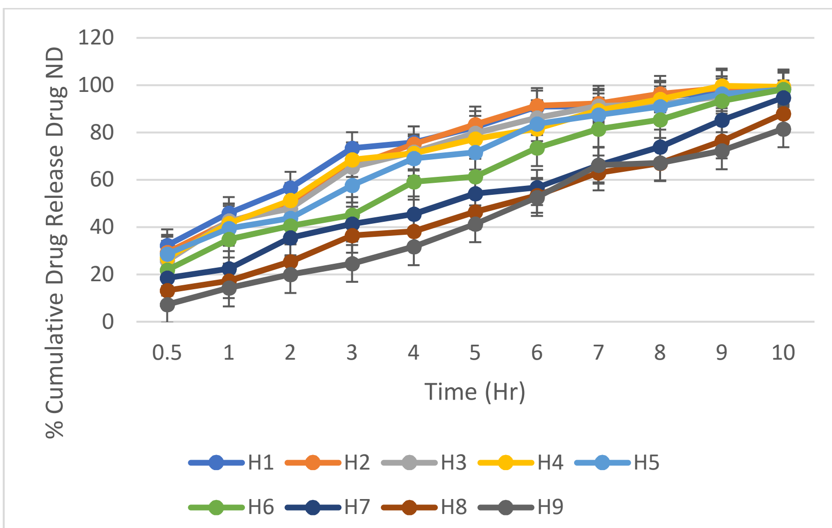


Fig.6. Percent Cumulative Release of Netarsudil Dimesylate (ND).

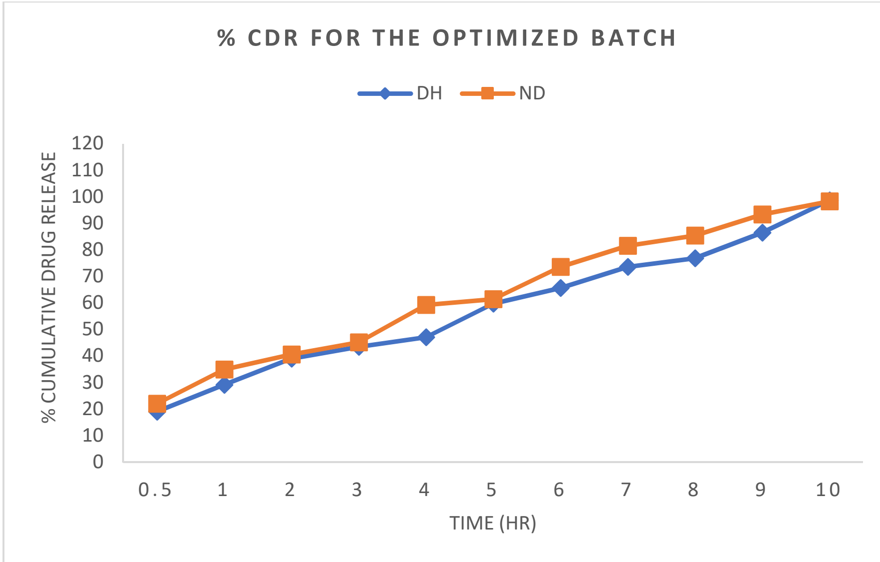


Fig.7. Cumulative Drug Release Rate of the Optimal Batch H6 in %.

Table 2: Experimental matrix for chitosan and HPMC K4M based in situ gelling systems.

Ingredients	H1	H2	H3	H4	H5	H6	H7	H8	H9
Drug D	2.2 g	2.2 g	2.2 g	2.2 g	2.2 g	2.2 g	2.2 g	2.2 g	2.2 g
Drug N	0.28g	0.28g	0.28g	0.28g	0.28g	0.28g	0.28g	0.28g	0.28g
Boric acid	0.475g	0.475 g	0.475 g	0.475 g	0.475 g	0.475 g	0.475 g	0.475 g	0.475g
Mannitol	0.05g	0.05g	0.05g	0.05g	0.05g	0.05g	0.05g	0.05g	0.05g
HPMC K4M	0.3 g	0.5 g	0.75g	0.3g	0.5g	0.75g	0.3g	0.5g	0.75g
Chitosan	-	-		0.75 g	0.75 g	0.75 g	1.0	1.0 g	1.0g
0.1N NaOH (ml)	Qs to pH 5.3								
DW (ml) qs	100	100	100	100	100	100	100	100	100

HET-CAM test

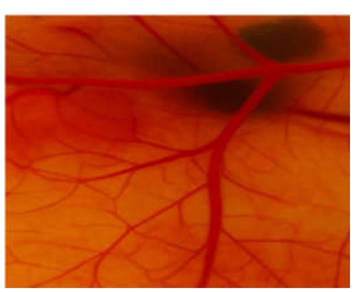
The HET-CAM test was conducted to evaluate the irritancy potential of the optimal formulation compared with standard positive and negative controls. Observations were recorded at 2, 4, 6, and 8-hours post-application on fertilized hen's eggs maintained under incubation conditions. Vascular reactions specifically coagulation and hemorrhage were observed as early as a few minutes following exposure to the positive control (0.1 N NaOH), with severity increasing over time. The negative control (0.9% NaCl) and the optimal test formulation exhibited no visible signs of irritation, hemorrhaging, or other vascular abnormalities throughout the observation period.

Table 3: HET CAM Test Observation.

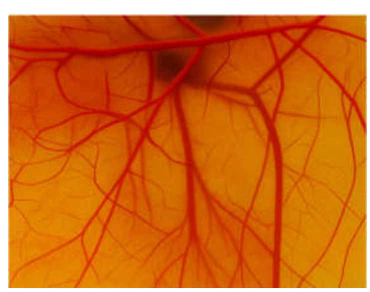
Treatment	Coagulation	Hemorrhage	Onset	Reaction Duration	Score	Classification
0.1 N NaOH (Positive)	Present	Severe	< 5 min	Persistent up to 8 h	3	Severe irritant
0.9% NaCl (Negative)	Absent	None	-	-	0	Non-irritant
H6 (Optimized Formulation)	Absent	None	-	-	0	Non-irritant

Table 4: HET CAM Test Criteria

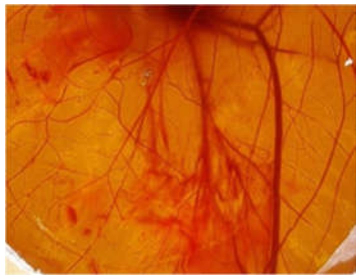
Effects	Score	Inference
No visible hemorrhage and coagulation	0	Nonirritant
Simple membrane discoloration	1	Mild irritant
Due to membrane bleeding or discoloration, structures are partially concealed.	2	Moderately irritant
Structures are completely covered as a result of membrane hemorrhages or discoloration.	3	Severe irritant



8a. 0.9% NaCl (Negative)



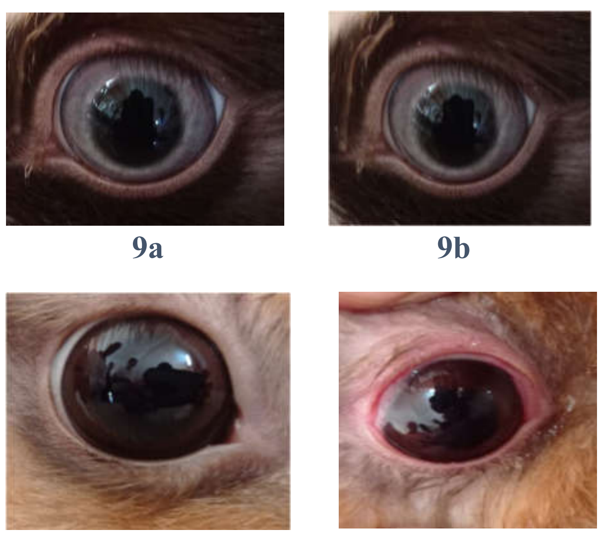
8b.Optimal Formulation (H6)



8C. 0.1 N NaOH (Positive)

Fig 8: HET CAM study on egg after a. Instillation of 0.9% Saline solution b. Formulation H6, c. 0.1N NaOH.

When final preservative free formulation and formulation with 0.02% Benzalkonium chloride (BKC) were instilled into the eyes of rabbits for twice a day for 7 days, the test eye was monitored for signs of irritation such redness, edoema, or excessive watering. The final formulation caused no irritation, redness or sign of oedema, while the Benzalonium chloride solution shows redness in eye after instillation for two time a day after 7 days. All of these things being absent proves that the final ophthalmic formulation is safe for use around the eyes. (Tables 1-10).



9a **9b**

9C **9d**

Fig 9: Draize Eye test on rabbit a. Day 1 of Instillation of H6, b. after 7 days of instillation of H6, c. Day 1 of Instillation of formulation with 0.02% BKC, d. after 7 days of instillation of formulation with 0.02% BKC.

Table 5 : Gelling capacity of the formulations

B. No	Gelling capacity
H1	+
H2	+
H3	++
H4	++
H5	++
H6	+++
H7	+++
H8	+++
H9	+++

*(n = 3).

Table 6: Estimation of Drug Contents for Drug D and Drug ND.

B. No	Drug D (%)	Drug ND (%)
H1	97.79±1.35	99.23±1.21
H2	98.98±1.26	98.23±1.02
H3	98.45±1.78	96.38±1.58
H4	99.30±1.34	98.36±1.46
H5	99.90±1.58	96.62±1.28
H6	99.84±1.65	100.4±1.08
H7	95.97±1.46	97.85±1.07
H8	96.45±1.25	96.98±1.37
H9	97.86±1.87	96.68±1.22

*(n = 3).

Table 7: Optimization of the Formulation

Name	H6
Conc. Of Chitosan	0.75%
Conc. Of HPMC	0.75%
pH of sol	5.25
pH of gel	7.03

Table 8: Evaluation of Optimized Formulation.

Sr. No.	Evaluation Parameter (mean ± SD)*	Result
1	Clarity	Clear colorless solution free from any visible particle
2	Gelling ability	+++
3	pH of sol	5.25 ± 0.06
4	pH of gel	7.03 ± 0.05
5	Viscosity of sol	112.1 ± 1.78
6	Viscosity Gel	420 ± 1.25
7	Drug Content Dorzolamide Hydrochloride	99.84 ± 1.65
8	Drug Content ND	100.4 ± 1.08

*(n = 3).

Table 9: Percentage Cumulative Release of Optimized Formulation (H6)

% Cumulative Release (Hrs)	DH	ND
0	0	0
0.5	18.98±1.34	21.98±1.35
1	29.08±1.69	34.91±1.28
2	38.98±1.42	40.54±1.22
3	43.47±1.94	45.12±1.36
4	47.04±1.28	59.28±1.67
5	59.67±1.38	61.34±1.64
6	65.61±1.02	73.54±1.67
7	73.54±1.56	81.50±1.47
8	76.78±1.02	85.43±1.10
9	86.51±0.48	93.34±1.37
10	98.62±1.26	98.32±1.22

*(n = 3).

Table 10: Scoring Chart for HET-CAM Test.

Time (hrs.)	**Positive Control (0.1 N NaOH)**	**Negative Control (0.9% NaCl)**	**Test Substance (Optimized Batch)**
2	3	0	0
24	3	0	0
48	3	0	0
72	3	0	0

4. Conclusion

Using polymers like Chitosan and HPMC K4M, which are stable, Isotonic, non-irritant, and have good antibacterial activity, an in situ gelling system for Dorzolamide Hydrochloride and Netarsudil dimesylate was developed, demonstrating enhanced residence time with good gelling capacity at physiological pH and sustained drug release for a period of 8 h. The new approach has the potential to replace the use of traditional eye drops in the treatment of ocular illnesses.

Data availability

Data will be made available on request.

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