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Formulation, In-vitro Evaluation, and Animal Study of Levofloxacin/Tinidazole loaded pH-dependent In-Situ Gel for Ophthalmic Drug Delivery

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ABSTRACT

Conventional ocular drug delivery forms (eye drops, eye ointments, and eye gels) possess poor bioavailability, less retention, and rapid precorneal discharge. In veterinary medicine, treating eye infections in animals like rabbits, dogs, and cats poses similar challenges. An in-situ gel drug delivery system (ISG-DDS) provides sustained action with a low formulation cost, which can be advantageous for veterinary applications. This study developed and characterised a pH-responsive ophthalmic ISG of levofloxacin and tinidazole with the potential to be used in both human and veterinary medicine. Carboxypol 980 (CBL-980) was used to make the ISG gel, and hydroxypropyl cellulose (HPC) changed the viscosity. Nine formulas of ISG were prepared. To find out about the drug content, clarity, gelling time, pH, viscosity, and stability of the product we got, we did a release study, as well as a DSC and FTIR visual evaluation. Albino rabbits (*Oryctolagus cuniculus*) were utilized to check for safety and ocular irritation. In Fourier-transform infrared spectroscopy (FTIR) and differential scanning calorimetry (DSC), there was no significant interaction between the drug and the additives. This showed that the drug changed into an amorphous form. The results show that ISG was translucent, with a pH ranging from 5.4 ± 0.22 to 7.8 ± 0.26. The ISG formulas 1, 2, and 3 required 39, 36, and 34 minutes, respectively, for gelling and showed approximately 4-5 times more viscosity than all batches. The optimum formula (formula 1) showed the highest drug content, prolonged drug release up to 6 h, stability for 3 months, and safety to use for animals (no indication of inflammation). Tests done in the lab and on animals show that making a pH-dependent ISG that is loaded with Levofloxacin/Tinidazole and used to deliver drugs to the eye has a lot of potential. The in-vitro trials demonstrated a gradual release of the drug over time, a crucial factor in maintaining its presence on the eye's surface for an extended duration. This is critical for effectively treating eye infections. Animal studies further corroborated the findings, demonstrating their safety. Nevertheless, additional clinical trials are necessary to confirm these findings and evaluate the effectiveness of ISG in real-life situations.

Keywords: novel delivery, ophthalmic, pH-dependent, in-situ gel, levofloxacin, tinidazole

INTRODUCTION

Conjunctivitis refers to an extensive spectrum of conditions characterized by inflammation of the conjunctiva. Inflamed eyes are most frequently caused by conjunctivitis, which are frequently linked to a viral or bacterial infection (1). *Pseudomonas aeruginosa*, *Chlamydia trachomatis*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, and the most common infections that exacerbate bacterial conjunctivitis are Haemophilus species (2). In veterinary medicine, ocular infections in animals, particularly in pets like dogs and cats, present similar challenges (3). These infections can significantly

affect the quality of life and may lead to severe complications if not properly treated. Conventional ocular treatments in veterinary patients face issues such as poor bioavailability and rapid elimination due to blinking and tear flow, similar to the challenges seen in human medicine (4).

One benefit of using ocular in situ gel for illnesses that require multiple drug therapies is the ability to combine different medications with the same formula. Levofloxacin (LEV) is a fluoroquinolone antibiotic that has a wide range of action against Gram-positive and Gram-negative aerobic and atypical bacteria, as well as minimal activity against most anaerobic bacteria. LEV is readily available in eye drops and ointment forms (5). Tinidazole is a structural analogy of metronidazole. Europe and developing countries commonly use tinidazole (TNZ) to treat amoebic and parasitic infections (6). In the case of a serious infection, the patient should apply topical antibiotic treatments (7). Applying ophthalmic drops directly to the conjunctival cul de sac is the recommended method for treating eye diseases (8). The main issues with eye drops are their rapid and extensive elimination due to eye blinking, tear circulation, and preparation drainage. As a result, the drops stay on the surface of the eye for less time, which reduces their effectiveness in reaching the eye tissues (9). Over the past ten years, researchers have developed DDS that rely on ISG technology. ISG-based DDSs are available in sol form before administration and are responsible for developing gels in reaction to a variety of endogenous stimuli, including pH elevation, temperature elevation, and ion incidence (10). Polymers that contain ocular DDS undergo a phase transition (sol-to-gel). It caused changes in the eye under specific physiological conditions (11). The phase transition on the eye's surface classifies three types of systems: a) pH-responsive systems, such as carbopol. b) Temperature-sensitive approaches such as pluronic. c) Ion-triggered systems consist of sodium alginate and gellan gum (12). Compared to the traditional DDS, the sol-gel transition of this system that responds to stimuli offers a practical approach for achieving sustained and prolonged drug release, drug delivery, improved stability, and biocompatibility. All pH-dependent systems exhibit a sol-gel transition as soon as the pH reaches 7.4 due to ocular fluid. Acidic polymers are polyanions with pH dependency. Still, as the body pH is elevated, the polymer's ionisation occurs and enlarges in the presence of water (11).

In-situ gel formulation systems overcome the challenges of poor flowability and non-uniform dosing associated with conventional eye drops. Non-irritant: They are less likely to irritate compared to conventional eye drops. Stability: pH-dependent gels remain stable when stored and administered.

Prior research has created multiple ISG formulations that have been used to treat different eye conditions. Literature uses various polymers such as chitosan, sodium alginate, HPC, CBL-980, and poloxamer to create diverse formulations (13). The two drugs chosen were LEV and TNZ. LEV is widely used as eye drops in bacterial infections, while TNZ is used as a model drug representing ocular parasite infection treatment. Additionally, an intelligent delivery system can combine LEV and TNZ to minimize toxicities and improve overall bioavailability at an infection site (14). This study is all about making a gel using a pH-dependent polymer called CBL-980 and HPC to deliver LEV and TNZ directly into the eye. This could help improve the release of drugs and make more medicine available in the eye. It could also be used to treat eye infections in animals.

MATERIALS AND METHODS

Alembic Pharma Pvt. Ltd., India is the source of levofloxacin and tinidazole. Hydroxypropyl cellulose was purchased from Celotech, Suzhou, China. We procured Carbopol 980 from Oryn Healthcare LLP, Gujrat, India. Sodium chloride, benzalkonium chloride, simulated lacrimal fluid, and distilled water were gifted from Thomas Baker, India.

Creation and advancement of ophthalmic pH-dependent ISG

The rationale behind the selection of different formulation batches was to ensure product quality control, which varies based on concentration. Thus, nine pH-dependent ISG formulations were created using varying amounts of CBL-980, HPC, NaCl, and BKC, as indicated in Table 1. We carefully introduced the hydroxypropyl cellulose (HPC) into 70 mL of filtered water, maintaining the water temperature at 50°C, to achieve a clear and see-through mixture. The mixture was stirred continuously over the hot plate manufactured by Stuart in the United Kingdom. We then refrigerated the mixture overnight. The magnetic stirrer continuously swirled the mixture at around 70°C, distributing CBL-980 evenly throughout the HPC dispersion. We used a sonicator to dissolve 0.6% of both LEV and TNZ in approximately 20 mL of filtered water, resulting in a transparent pharmacological solution. We combined the polymer dispersion with the solution and added water to reach a total volume of 100 mL. A sterilized membrane filter with 0.22 µm pore size filtered the mixture. The final vessels, previously sterilized and sealed to prevent germ entry, received the filtered liquid. We added NaCl to lower the gelation temperature and used BKC as a preservative to shield the ocular membrane from irritation and stop germ growth (15).

Table 1. The formulas for pH-dependent ophthalmic ISG

Ingredients (%)	F1	F2	F3	F4	F5	F6	F7	F8	F9
LEV	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
TNZ	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
CBL-980	0.20	0.25	0.30	0.30	0.50	0.70	0.50	0.75	1.00
HPC	0.30	0.25	0.20	0.70	0.50	0.30	1.00	0.75	0.50
NaCl	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
BKC	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02

LEV; levofloxacin, TNZ; tinidazole, CBL-980; Carbopol 980p, HPC; hydroxypropyl cellulose, NaCl; sodium chloride, and BKC; benzalkonium chloride

Drug-excipient Reaction Study using DSC and FTIR

Differential Scanning Calorimetry (DSC) examined the selected ISG formula, pure medicines, polymers, and

physical mixtures for alterations in their thermal behavior. We heated an airtight aluminum container containing a 5 mg sample of powdered substance between 25 and 250°C at a scanning rate of 10°C/min (16).

Additionally, we studied the compatibility between the drugs and the polymers in the optimum formula. We performed Fourier-transform infrared spectroscopy analysis (FTIR) for the pure drugs, polymers, physical mixture, and the optimum formula. We mixed a 2 mg sample of each tested component with dry potassium bromide before pressing it into a disc. We analyzed the disc using FTIR spectroscopy to ensure compatibility at a range of 400–4000 cm^{-1} wavenumber (17).

ISG Evaluations

ISG formulations were designed and assessed for several evaluation parameters, including physical appearance, drug content percentage, pH, viscosity, clarity test, gelling property, in-vitro release profile, sterilization condition's effect on its viscosity, eye irritant test, and stability tests.

Physical Look, pH, and Clarity

We conducted clarity research to determine the presence of turbidity or dispersed particles. We analyzed all developed formulations with swirling motion against a white and black background. We used a digital pH meter to determine the pH of ISG formulations (18).

Gelling Property

The gelation experiment was conducted using cylindrical tubes loaded with 5 mL of phosphate buffer (PB) at pH 7.4 and synthetic, simulated lacrimal fluid (SLF, Thomas Baker, India). We designed the SLF to mimic the cation concentration. Using a standard dropper, 50 µL of each formulation were added to a tube containing SLF. We visually determined the gelation and gel dissolution times (19).

Viscosity

The Brookfield viscometer (LVT model) was used to examine the viscosity of the preparation at a different shear rate. Measurements of viscosity were taken before and after the sol was diluted with SLF to find out how much of a gel the mixture formed after being put in the eye. With wheel number 62 running at speeds between 3 and 30 rpm, the sample solution's viscosity was tested. It was measured by taking the mean of the two numbers on the dial (20).

Drug content calculations

The drug content of the ISG formula was analyzed using the spectrophotometric technique. The analysis was performed by dispersing a one-gram sample of ISG in 100 ml of phosphate-saline buffer with a pH of 7.4 and exposing it to sonication for two hours. Subsequently, the resultant mixture was passed through a 0.45 µm Millipore filter and subjected to analysis by UV. We conducted spectrophotometric measurements of the absorbance at 288 and 318 nm for LEV and TNZ, respectively (21).

In-vitro drug release

The study aimed to optimize a selected formula using a Franz diffusion cell apparatus with an orifice diameter of 1 cm, as shown in Figure 1. Importantly, we affixed a synthetic membrane for in vitro experimentation to Franz cell open. The cylinder's internal diameter measured 3 cm^2. Before putting the membrane sample for in vitro experimentation together in cells, the rehydration process involved immersing the membrane sample in a 50-mL solution of PBS with a pH of 7.4. Drug diffusion research was conducted on a specific ISG formulation, chosen based on its drug content. Throughout the tests, the receptor chamber of the cell was kept at 32±1°C and was agitated by a magnetic bar at 500 rpm to guarantee sink conditions. To prevent the fluid from evaporating, we placed three ml of the sol ISG (equivalent to 36 mg LEV and TNZ) on the membranes and wrapped them in parafilm in the donor section. At different time intervals up to 6 hours, we removed a small amount (0.5 mL) of the sample from the release medium and replaced it with the same amount of fresh media. The concentrations of LEV and TNZ were assessed spectrophotometrically at 288 and 318 nm, respectively (22, 23).

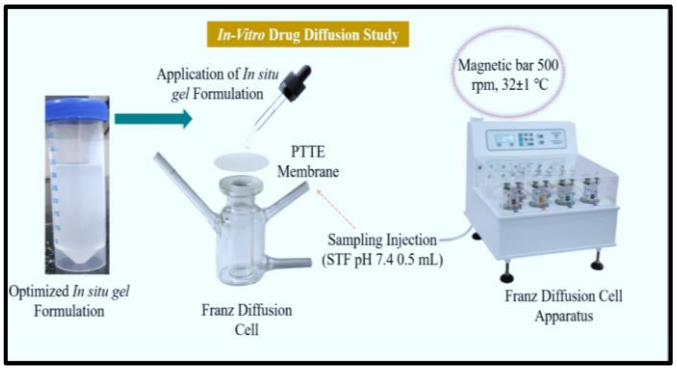

Figure 1. Schematic representation of Franz-diffusion cell experiment

Effect of sterilization test on viscosity

Sterility testing was performed on formulas F1-F3 to evaluate the impact of sterilization (using an autoclave) on the viscosity of the ISG before and after subjecting the formulation to autoclaving (24). To facilitate understanding, we divided the procedure into four parts for complete sterilization.

1. In the initial stage of the sterilization procedure, the medicine and BKC were meticulously combined in a distinct container and then passed through a 0.22µm membrane syringe filter (aseptic filtration).
2. After that, we thoroughly combined CBL-980 and HPC.
3. Before sterilization, we combined both mixtures (a and b) and determined their viscosity.
4. We exposed the resulting combination (c) to autoclaving (wet heat sterilization) for 1 hour and determined the viscosity.

Ocular irritation test

We conducted the test to evaluate the safety and non-irritating nature of the ISG optimum formula (F1). In this experiment, we used six 1.5-kg, 5-6-month-old, male white Albino rabbits. The experiment employed a modified Draize test. The animal care committee approved the study protocol in the Iraqi National Centre for Drugs Control and Research (approval number 205/2017). We considered the animal's right eye as a control and implanted the ISG into its left eye's conjunctival cul de sac. We adhered the eyelids together for approximately 10 sec to prevent the loss of the ISG preparations. Every animal was checked for ophthalmic reactions (e.g., redness, swollen discharge, iris, and corneal lesions) 5, 15, 30 min, and 1, 2, 3, 6, 12, and 24 hours after installation (23). The irritation was assessed using the following scores as shown in Table 2 (25).

Table 2. Assessment of irritation scores

Score	Evaluation
0	No inflammation indication (swelling, redness, or extreme tearing)
1	Minimal inflammation (mild tearing, redness)
2	Moderate redness (extreme tearing, moderate inflammation)
3	Extreme redness (extreme tearing, severe inflammation)

Accelerated stability study

We conducted accelerated stability studies, adhering to ICH Q1A standard (R2) standards, to assess the drug and formulation stability at four different times: (T0 for Day 1), (T1 for One Month from the Start Date), (T2 for Two Months from the Start Date), and (T3 for Three Months from the Start Date). The optimized formulas (F1-F3) were individually packaged in vials and stored for up to 3 months at a controlled temperature and humidity of 40°C±2°C and 75%±5% RH. The physical stability of the three formulations (F1-F3) was assessed by measuring several parameters including pH, viscosity, and the percentage assay of the formulations using the following equation (26):

$$\%Assay = \frac{conc.\,of\ the\ sample}{conc.\,of\ the\ standard} \times 100\%$$

Where, conc. of the sample=the sample concentration (LEV and TNZ) at time intervals using UV-spectroscopy, conc. of the standard concentration of LEV and TNZ from the calibration curve

Statistical Analysis

To compare the independent groups of irritant tests, we employed the Mann–Whitney U test.

RESULTS

Creation and advancement of ophthalmic pH-dependent ISG

HPC has an intriguing characteristic known as the lower critical solution temperature (LCST). HPC rapidly dissolves in water at temperatures below approximately 45 °C. However, when the temperature above the lower critical solution temperature (LCST), it transforms into a state where it cannot be dissolved. Heating the solvent to around 50 °C aids in its dissolving without the risk of deterioration. Using HPC and CBL-980 polymers, ISG formulations were developed and evaluated according to the following criteria.

Drug-excipient reaction study using DSC and FTIR

Figure 2 shows the DSC of LEV, TNZ, carbopol, HPC, physical mixture, and ISG. When studied separately, TNZ showed a sharp endothermic peak at 125°C, and LEV showed a sharp endothermic peak at 105°C and 225°C, even if the thermal research of HPC shows an endothermic peak beyond 100°C. Finally, on the carbopol thermogram, there was an endotherm between 50 and 100°C. The FTIR of the physical mixture and the selected formula showed that peaks don't change significantly in comparison with pure drugs and polymers, as shown in Figure 3.

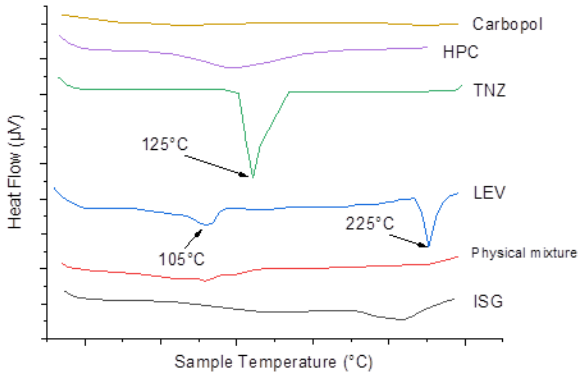

Figure 2. DSC of pure drugs, polymers, physical mixture, and ISG-F1

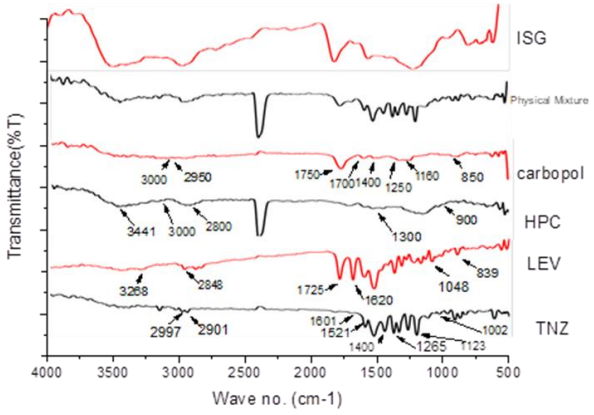

Figure 3. FTIR spectrum of pure drugs, polymers, physical mixture, and ISG-F1

Evaluations of ISG

The physical look, pH, and clarity

The prepared ISG formulations' pH, physical appearance, and transparency were assessed, and all

formulas (F1-F9) displayed a transparent and clear appearance (Figure 4). The pH of the F1-F3 batches ranged from 6.8 ± 0.12 to 7.4 ± 0.27. The pH of the F4-F8 batches ranged from 5.4 ± 0.22 to 6.1 ± 0.37, while the F9 batch had a pH of 7.8 ± 0.26.

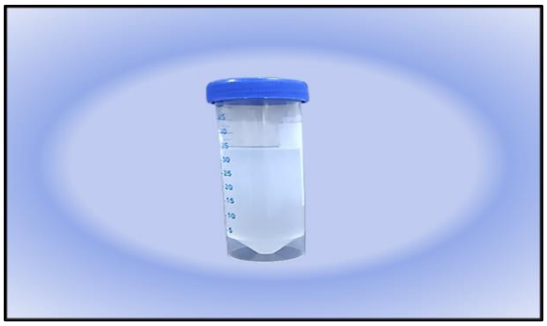

Figure 4. The physical look of a pH-dependant ISG formulation

Gelling property

To determine how long it took for each formulation (F1-F9) to gel, 50 µL of each was added to a cylindrical solution that already contained 5 mL of SLF. We then recorded the time it took to turn the mixture into gel in seconds. Upon the addition of a single droplet of SLF, a gel materialized within a time frame of 50–100 seconds and persisted for several minutes before dissolving. The testing results indicated that formulas F1, F2, and F3 took between 34 and 39 minutes for the gel to collapse. Formulas F4, F5, and F6 required a duration of 43 to 49 minutes for the gel to collapse. Formulas F7, F8, and F9 took between 38 and 53 minutes to collapse the gel.

Viscosity

Viscosity measurements were taken for the sol and gel phases of the formulations. The chosen formulas (F1-F3) exhibited a viscosity rise of roughly 4-5 times following gelation, as seen in Figure 5. On the other hand, some batches showed a roughly tenfold rise, comprising 0.5–1% CBL-980.

The F1, F2, and F3 formulas did not show rigid structure in the form of ISG, so the viscosity achieved was suitable for ocular drug delivery systems (DDS). Table 3 displays the comprehensive assessments.

Drug content calculations

The chosen batches (F1-F3), according to the assessments, were chosen for the estimation of drug content because they demonstrated relevant results for ocular drug delivery in the physical look, pH, clarity, viscosity, and gelling time. Table 4 displays the drug content (%) for all three formulas of LEV and TNZ. batches F1, F2, and F3. The F1 sample had the highest concentration of the drug, thus making it the chosen batch for in vitro experimentation.

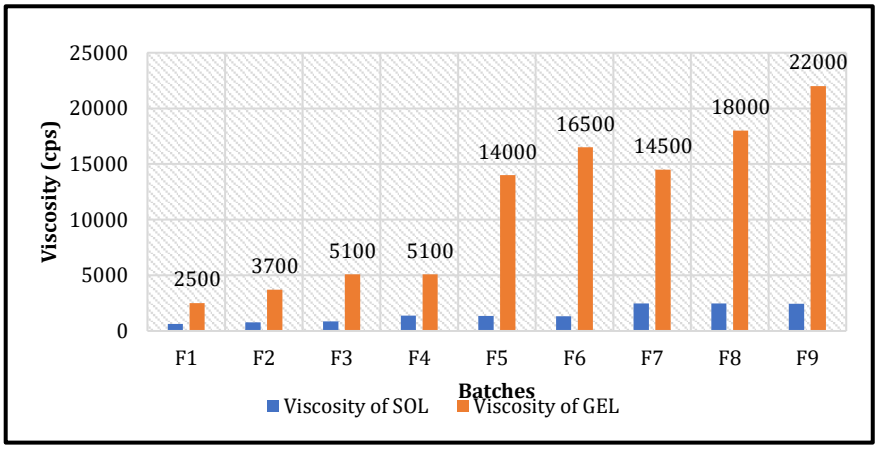

Figure 5. Viscosity graph for sol and pH-dependent ISG

Table 3. Overview of pH-dependent ISG formulas assessments (mean±SD)

Parameters	F1	F2	F3	F4	F5	F6	F7	F8	F9
Clarity	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear
sol viscosity (cps)	631±2.2	783±1.7	861±5.4	1389±3.54	1362±6.9	1331±9.25	2475±11.57	2460±21.08	2438±19.84
gel viscosity (cps)	2609±5.8	3803±4.6	5091±16.5	5100±1.5	14000±13.1	16500±10.0	14500±12.6	18000±11.9	22000±18.55
Gelling time (min)	39	36	34	55	43	49	38	48	53
pH of SOL	6.35	6.75	6.47	5.74	5.88	5.66	4.75	4.33	4.76

Table 4. Drug content calculations (mean±SD)

Formulas	% Drug content for LEV	% Drug content for TNZ
F1	99.54 ±0.42	99.82±0.06
F2	98.62 ±0.01	99.47±0.02
F3	99.46 ±0.05	98.77±0.04

In-vitro drug release studies

By using the Franz cells apparatus, testing was done to determine how well the medicine released from the gel that was created using the synthetic membrane. The in vitro study serves as a screening method during the development of ocular drug delivery formulations, evaluating the efficiency characteristics of different formulations. The F1 formula was chosen for the *in-vitro* drug release trial because, according to estimates of drug concentration, it contained the most drugs, 99.56±0.42 for LEV and 99.82±0.06 for TNZ, of the three selected batches. Figure 6 displays the temporal distribution of LEV and TNZ released through a synthetic membrane in the F1-ISG formula. The data analysis showed that the pH-dependent formulation had 1.80% and 1.72% log dual drug release, which is the same as 63% LEV and 53% TNZ cumulative release after 4 hours.

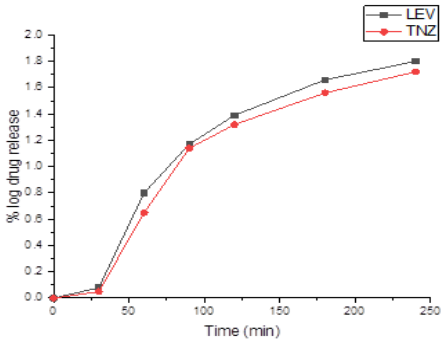

Figure 6. In-vitro drug release pattern of F1-ISG

Effect of sterilization test on viscosity

We investigated the impact of sterilization in an autoclave on the selected formulas (F1-F3). Table 5 contains data on formulation viscosity measurements before and after sterilization.

Table 5. Summary of sterilization effects on viscosity (mean±SD)

Formulas	Viscosity (cps)	
	Pre-sterilization	Post-sterilization
F1	2609 ± 5.8	2621 ± 17.2
F2	3803 ± 4.6	3821 ± 12.3
F3	5091 ± 16.5	5131 ± 47.3

Ocular irritation test

The absence of any inflammatory alterations in the scoring method across the independent groups precluded the use of the Mann-Whitney U test. Figure 7 below demonstrates that the right eye showed no signs of inflammation, such as redness, swelling, or tearing, for the next twelve hours following the insertion of the ISG, indicating that the ISG did not irritate the eye and resulted in a score of zero.

Accelerated stability study

As shown in Table 6, for three months, the chosen formula's stability was evaluated at 40±2°C and 75%±5% relative humidity. In each of the situations mentioned, the sample displayed exceptional physical qualities. Furthermore, none of the studied characteristics—pH, % assay, and viscosity—exhibited any significant changes.

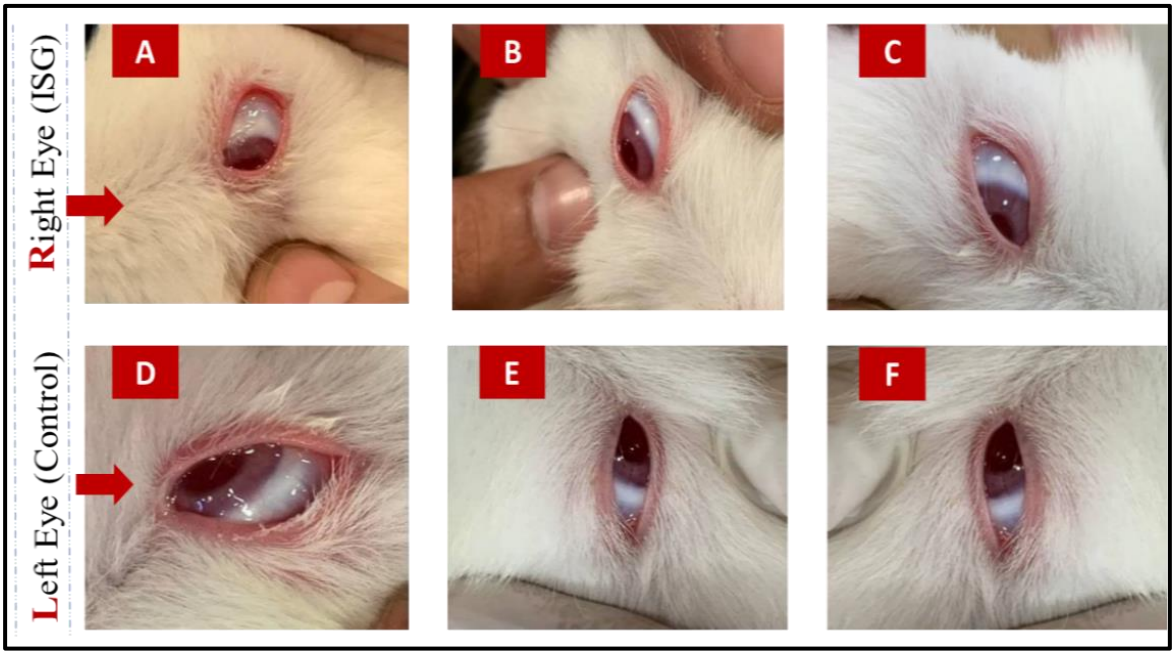

Figure 7. Ocular irritation test. After 1 hour for **(A)** and **(D)**, 6 hours for **(B)** and **(E)**, and 12 hours for **(C)** and **(F)**

Table 6. Stability study data for ophthalmic ISG formulations (mean±SD

	Parameters				
		Viscosity (cps)		% Assay	
Time	**pH**	**Sol**	**Gel**	**LEV**	**TNZ**
0 month (T0)					
F1	6.35	631±2.5	2609±5.8	99.54±0.42	99.82±0.06
F2	6.75	783±5.4	3803±4.6	98.62±0.01	99.47±0.02
F3	6.47	861±7.3	5091±16.5	99.46±0.05	98.77±0.04
1 month (T1)					
F1	6.49	644±3.3	2630±17	97.31±0.52	99.43±0.09
F2	6.88	749±2.1	3780±15	98.11±0.67	97.23±0.01
F3	6.91	852±5.4	5000±23	99.44±0.51	98.33±0.14
2 months (T2)					
F1	7.34	597±0.14	2704±0.32	97.87 ±0.67	98.85±0.02
F2	7.01	784±0.29	3715±0.22	99.01±0.51	97.02±0.05
F3	7.31	8177±0.58	5102±0.57	99.41±0.52	98.08±0.22
3 months (T3)					
F1	7.21	601±0.11	2617±2.6	97.90±0.11	98.13±0.19
F2	7	817±0.17	3745±2.4	99.15±0.25	96.90±0.03
F3	7.14	7915±0.25	5101±3.6	99.00±0.14	97.87±0.7

DISCUSSION

Creation and advancement of ophthalmic pH-dependent ISG

A pH-responsive in-situ gel (ISG) with levofloxacin (LEV) and tinidazole (TNZ) has been successfully developed. This offers a promising way to treat eye infections in both people and animals. Ocular infections in animals, such as rabbits, dogs, and cats, present significant treatment challenges, including poor drug retention and rapid clearance from the ocular surface. This study aimed to address these challenges by developing a pH-responsive ISG formulation that can prolong drug retention and enhance bioavailability. Carbopol and HPC played a crucial role in batch formulation. Carbopol is used as a thickener and pH-sensitive polymer. As the external pH elevates, the hydrogel swells and converts to gel form. It also controls the release of drugs. The HPC helps maintain plasticity and higher molecular mobility (27).

Drug-excipient reaction study using DSC and FTIR

The physical combination analysis revealed the presence of the drug peaks, although at a weaker intensity compared to the pure drug DSC curve. The interaction between the polymers (HPC and carbopol) and the medicines (TNZ and LEV) in the DSC pan during ramping could explain this. At temperatures below the drug's melting point, the polymer starts to melt, which could cause an interaction between the two. Once the temperature exceeds the medication's melting point, a small amount of the drug has already dissolved in the melted polymer, causing a decrease in peak intensity (28). The drug has completely changed from a crystalline to an amorphous state as a result of its peak disappearing from the ISG's DSC curve (29). The DSC thermograms of indomethacin and polyvinylpyrrolidone/vinyl acetate copolymers showed similar results (30).

The FTIR spectrum showed that peaks in Figure 3 don't change significantly, indicating no significant interaction between the drug and additives. The FTIR spectra of Tazarotene binary ethosomes and their excipients showed comparable results (31).

Evaluations of ISG

The physical look, pH, and clarity

All nine batches exhibited a translucent and clear look, which guarantees adherence by patients. Furthermore, the formulation was reportedly free of any particle materials that could potentially cause injury to the eye area. The pH range of ISG formulas F1-F3 was acceptable, consistent with the pH of the lacrimal fluid (32). The pH range of F9 surpasses the pH of lacrimal fluid, making it unsuitable for ocular delivery. The Lithuanian Royal Jelly's ocular in situ gel showed similar results (33).

Gelling property

The selected formulations (F1-F3) demonstrated a consistently acceptable gelling time for ocular injection; a longer gelling time reduces the formulation's effectiveness (34). One of the significant findings of this study was that the ISG formulations had a longer gelling time and increased viscosity upon exposure to the ocular pH. The gelling time ranged from 34 to 39 minutes for the optimal formulations, indicating sufficient residence time on the ocular surface. This prolonged retention can be particularly beneficial in veterinary applications, where administering eye drops to animals can be challenging and frequent dosing is often impractical. In veterinary medicine, common eye conditions include bacterial conjunctivitis, keratitis, and parasitic infections. For instance, bacteria like Staphylococcus aureus and Streptococcus species can cause conjunctivitis in dogs. Cats frequently suffer from conjunctivitis caused by Chlamydia felis and Mycoplasma felis. Additionally, ocular parasitic infections, such as those caused by Thelazia callipaeda, a nematode parasite, can affect both dogs and cats. The ISG formulation, which contains LEV and TNZ, can provide an effective treatment for these conditions by ensuring prolonged contact time

with the ocular surface, enhancing drug absorption, and reducing the frequency of administration. Bimatoprost is administered through thermosensitive ophthalmic in situ gels for the treatment of glaucoma. The results of the Sparfloxacin emulsome-loaded thermosensitive in situ gel for ophthalmic delivery were similar (35).

Viscosity

The viscosity of batches F1, F2, and F3 was considerably greater than the desired level for ocular preparation. Therefore, we found that a concentration of 0.3% CBL-980 and HPC was appropriate for the ISG formulation (36). The instilled formulation's viscosity plays a significant role in dictating the drug's duration of residence in the eye. We used shear thinning formulas, and as angular velocity increased, so did shear stress. The administration of ophthalmic preparations should not significantly impact the pseudoplastic nature of the precorneal tear film. Ocular shear rates are quite high, ranging from 0.03s-1 during interblinking intervals to 4250–28,500 s-1 while blinking. Therefore, viscoelastic fluids with a high viscosity in low shear rates and a low viscosity in high shear conditions are suitable. The formulations (F1-F9) showed reduced viscosity and were in a liquid state at pH 6.0. Raising the pH to 7.4, which is the pH of the tear fluid, the solutions turned into viscous gels (27). This is evident in the use of a pH-responsive in situ gel for the ophthalmic delivery of dorzolamide hydrochloride, utilizing Carbopol (37).

Drug content calculations

The prepared formulation exhibits an evenly distributed drug due to the smallest variance in drug content. We observed the same results with moxifloxacin ion-activated in situ gel using gellan gum, HPMC, and sodium alginate (38).

In-vitro drug release studies

The chosen ISG formula F1 showed longer drug release for up to 6 hours because it had a very high viscosity after quickly gelling. F1's gelling ability also lasts for up to 6 hours because of the polymers' inherent properties, which include hydroxyl and carboxyl groups. These groups undergo cross-linking processes, which strengthen the intermolecular contacts within the polymer matrix and create strong bridges. These bridges are impacted by the gelling strength because they help create a stiff matrix. The drug release studies demonstrated that the ISG formulations provided a sustained release of both LEV and TNZ. The cumulative release of 63% LEV and 53% TNZ within 240 minutes suggests that the ISG formulation can maintain therapeutic drug levels in the ocular tissue for an extended period. This sustained release is crucial for treating chronic ocular infections in animals, reducing the need for frequent administration, and improving compliance. For instance, a single application of the ISG formulation in a dog with bacterial conjunctivitis could provide continuous antimicrobial activity, reducing the need for multiple daily eye drops, which can be stressful for both the animal and the owner. We observed the same result with ion-triggered in situ gel for ocular delivery of ciprofloxacin HCl and olopatadine HCl (39).

Effect of sterilization test on viscosity

There was no discernible change in the viscosity of an ocular drug delivery system containing anionic and nonionic polymers compared to that of ofloxacin in situ gelling (40). Consequently, it demonstrates that the product can undergo sterilization before filling during manufacture, all while maintaining the formula's viscosity.

Ocular irritation test

The ocular irritation test on albino rabbits revealed that the propolis and balsam poplar buds phenolic compounds in situ poloxamer-based gels in the ISG formulations were non-irritating and safe for ocular application (41). This finding is significant for veterinary applications, as it suggests that the formulation can be well-tolerated by animals, minimizing the risk of adverse reactions. In practice, this means that veterinarians can confidently prescribe this ISG formulation for treating ocular infections in pets without worrying about causing discomfort or irritation.

Accelerated stability study

Furthermore, the accelerated stability study demonstrated that the ISG formulations maintained their physicochemical properties over three months, indicating excellent stability under storage conditions. We observed similar results with thermoresponsive ophthalmic in situ gel loaded with dexamethasone sodium phosphate (DSP), tobramycin sulphate (TS), poloxamer 407, and hydroxopropyl methyl cellulose (HPMC) K4M (42). Furthermore, the accelerated stability study demonstrated that the ISG formulations maintained their physicochemical properties over three months. This stability is essential for veterinary products, ensuring that the formulation remains effective throughout its shelf life. Veterinary clinics and pet owners can store the product without concern for rapid degradation, making it a practical solution for long-term treatment plans.

In this study, LEV and TNZ containing ISG was successfully prepared by incorporation into pH-responsive ISG polymers CBL-980 and HPC. The pH-responsive ISG formulation developed in this study offers a novel and effective approach for delivering ocular drugs to treat infections in both humans and animals. The prolonged drug retention, sustained release, and excellent stability of the ISG formulation make it a suitable candidate for veterinary applications. Future studies should focus on clinical trials involving veterinary patients to validate the efficacy and safety of the formulation in real-world settings. This novel drug delivery system in veterinary ophthalmology could significantly improve the management of ocular infections in animals, enhancing their quality of life and overall health

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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صياغة وتقييم في المختبر ودراسة على الحيوانات لجل موضعي محمل بليفوفلوكساسين/تينيدازول المعتمد على الدالة الحامضية لتوصيل أدوية العيون

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الخلاصة

في التوصيل الدوائي للعين، تمتلك الأشكال التقليدية توافرًا حيويًا ضعيفًا، واحتباسًا أقل، وإفرازًا سريعًا للدواء من العين. في الطب البيطري، يشكل علاج التهابات العين في الحيوانات مثل الأرانب والكلاب والقطط تحديات مماثلة. والتي يمكن أن تكون مفيدة للتطبيقات البيطرية. يوفر نظام توصيل الدواء الهلامي في الموقع مدة طويلة من العمل وتكلفة صياغة منخفضة. قام هذا البحث بتطوير وتمييز نظام توصيل الدواء الهلامي في الموقع المستجيب الدالة الحامضية من الليفوفلوكساسين والتينيدازول للاستخدام المحتمل في كل من الطب البشري والبيطري. تم في هذا البحث تطوير وتمييز جل هلامي للعين لأدوية الليفوفلوكساسين والتينيدازول استجابة للرقم الهيدروجيني. تم تحضير تسع عينات من الجل الهلامي باستخدام كاربوبول كعامل تبلور وهيدروكسي بروبيل السليلوز كمعدل لزوجة. تم تمييز المنتج الذي تم الحصول عليه من خلال الاشعة تحت الحمراء, مسعر المسح التباني, التقييم البصري والوضوح ودرجة الحموضة وزمن التبلور واللزوجة ومحتوى الدواء والثبات ودراسة الإطلاق. تم استخدام أرانب ألبينو للتحقق من السلامة وتهيج العين. لا يوجد تفاعل كبير بين الدواء والمواد المضافة في الاشعة تحت الحمراء, مسعر المسح التباني دل على تحول الدواء إلى الصفة غير المتبلورة. تظهر النتائج أن الجل الهلامي كان شفافًا مع درجة حموضة تتراوح من ٠,٢٢ الى ٠,٢٦. تطلبت عينة ١, عينة ٢, عينة ٣ وقت ٣٩, ٣٦, و ٣٤ دقيقة الى التوالي للتبلور وأظهرت لزوجة أكبر بحوالي ٤-٥ مرات من جميع الدفعات. أظهرت العينة المثالية ١ أعلى محتوى دوائي، وأطول إطلاق دوائي يصل إلى ٦ ساعات، وثبات لمدة ٣ أشهر، وأمن للاستخدام على الحيوانات (لا يوجد مؤشر على الالتهاب). إن تطوير جل موضعي يعتمد على الدالة الحامضية ومحمل بالليفوفلوكساسين/تينيدازول لتوصيل الأدوية العينية يُظهر إمكانات كبيرة، كما هو موضح في ضوء نتائج التقييم في المختبر والتحقيقات على الحيوانات. وأظهرت التجارب المختبرية أن الدواء يتم إطلاقه تدريجيًا مع مرور الوقت، وهو أمر مهم للحفاظ على الدواء على سطح العين لفترة طويلة. وهذا أمر بالغ الأهمية لعلاج التهابات العين بشكل فعال. وقد تم تأكيد النتائج بشكل أكبر خلال دراسة على الحيوانات، والتي أظهرت السلامة. ومع ذلك، فإن التجارب السريرية الإضافية ضرورية لتأكيد هذه النتائج وتقييم فعالية الجل الموضعي المعتمد على الدالة الحامضية في حالات الحياة الحقيقية.

الكلمات المفاحية: : التوصيل الحديث، طب العيون، تعتمد على الدالة الحامضية، الجل المتحول موضعي، الليفوفلوكساسين، التينيدازول