RESEARCH ARTICLE

Formulation and Evaluation of Dual Responsive In situ Gel of Gentamycin for Ocular Delivery

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Abstract

Background/Objective: Conventional ophthalmic solutions often exhibit limited therapeutic efficacy due to rapid precorneal elimination and nasolacrimal drainage. In situ gelling systems overcome these limitations by transitioning from liquid drops to gels under physiological conditions, prolonging ocular residence time. This study aimed to develop and evaluate a dual-responsive gentamicin in situ ophthalmic gel.

Methods: Formulations were prepared using the cold method and evaluated for clarity, pH, gelling capacity, flowability, gelation temperature, drug content, viscosity, and sterility. The optimized formulation was designed to remain liquid during administration and undergo sol–gel transition upon exposure to physiological tear pH (7.4) and precorneal temperature. Its in vitro drug release and ex vivo corneal permeation were compared with those of a marketed formulation. Antimicrobial activity, histopathological characteristics, in vivo ocular irritation, and stability were also assessed.

Results: The optimized formulation containing 0.03% Carbopol and 18% Pluronic F127 exhibited suitable dropability at room temperature and effective gelation under precorneal conditions. Compared with the marketed eye drops, the formulation demonstrated 1.59-fold greater corneal penetration and sustained drug release for up to 10 hours. Antimicrobial evaluation confirmed that gentamicin retained its antibacterial activity after incorporation into the gel. Histopathological examination revealed no significant alterations in corneal microscopic architecture, while in vivo ocular irritation studies confirmed its non-irritant nature. Stability studies further demonstrated that the formulation remained stable under the investigated conditions.

Conclusion: The developed dual-responsive gentamicin in situ gel demonstrated improved corneal penetration, prolonged drug release, preserved antibacterial activity, and good ocular tolerability. Therefore, it represents a promising alternative to conventional ophthalmic solutions, potentially improving ocular drug delivery and therapeutic efficacy.

Keywords: Gentamycin sulfate, In situ gel, Carbopol, Pluronic, ocular delivery

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1. Introduction

The unique anatomic structure and effective eye-protective systems are challenging for formulation chemists to research. A direct ophthalmic delivery system is an attractive option for treating various eye diseases[1]. Although different ophthalmic delivery systems such as ointment, suspension, and inserts have been developed, they have various drawbacks, such as blurred vision and a sticky sensation when using ointment and gel, difficulty administering inserts, and dosage heterogeneity when using suspension. [2, 3]. About 90% of ophthalmic formulations are available in the market as eye drops due to the ease of administering appropriate amounts. Due to fast precorneal clearance, only 5% of given drugs penetrate intraocular tissue and are lost, necessitating repeated instillation to achieve therapeutic efficacy[4, 5, 6]. Repeated instillation, on the other hand, may have negative consequences. *In situ* gel-forming solutions, which consist of polymers undergoing sol-gel phase transition by induction of

ambient variables such as pH [7,8], temperature [9,10,11], and ionic composition[5], are created to address these issues. The viscoelastic characterization of *in situ* gels assumed adequate mechanical strength to resist clearance due to blinking, decreased dose frequency, enhanced patient acceptability, and increased local drug bioavailability [12].

Pluronic (Poloxamer), a thermoresponsive polymer, is widely used in an ocular delivery system that consists of polyethylene oxide-polypropylene oxide-polyethylene oxide (PEO-PPO-PEO), the triblock copolymers, where changes in temperature and concentration cause the PEO and PPO domains to have different physical properties. Both PEO and PPO remain hydrated at temperatures below the critical micellar concentration. However, when the temperature rises, PPO chains become dehydrated and less soluble than PEO, resulting in the production of gels [13]. Pluronic is encountered with disadvantages like low mucoadhesive property, which has been addressed by adding the pH-sensitive polymer Carbopol. A physical admixture of these two polymers forms an aqueous composition of gels in response to simultaneous changes in pH and temperature. It was possible to generate a pH and thermoreversible gel at pH and temperature close to the eye by formulating a Pluronic/Carbopol based *in situ* gel at a particular concentration. The combination of these polymers improves gel strength and mucoadhesive properties[6].

Gentamicin eye drops are a common antibiotic used to treat eye infections. It inhibits bacterial protein synthesis by interfering with the binding of t-RNA to ribosomal RNA, causing the immature protein to develop. Conventional gentamicin eye drops are lost through nasolacrimal drainage and absorbed systemically through the conjunctiva, resulting in low therapeutic bioavailability[14]. This work aims to develop thermosensitive and mucoadhesive ophthalmic delivery systems containing Gentamicin using Poloxamer and Carbopol to overcome the disadvantages described earlier.

2. Materials and Methods

2.1 Materials

The chemicals used were: Gentamicin sulfate was a generous gift from Cipla Pharmaceuticals, Chennai. Pluronic F 127, carbopol 940 and Benzalkonium chloride (Loba Chemie Pvt. Ltd, Mumbai), sodium chloride (Spectrum, Kochi), Dialysis Bag (M.W. cut off 12,000–14,000) was purchased from Sigma Aldrich, Bangalore. Genticin eye drop (0.3%w/v gentamicin sulfate) purchased from medical shop. All other chemicals used were of analytical grade.

2.2 Methods

2.2.1 Preparation of Gentamicin in situ gel

In a citrophosphate buffer pH 6.0, dissolve the accuratel y weighedgentamicin sulfate. Pluronic F127 is disperse d and agitated in this drug solution at room temperature. In a citrophosphate buffer pH 6.0, prepare the desired c oncentration of carbopol 940. To the resulting solution, sodium chloride and benzalkonium chloride were added .

Add the gentamicin and Pluronic F127 dispersions to th e carbopol solution. The partially dissolved solutions w ere kept in the refrigerator until all
polymers had dissolved completely (24hours). These formulations were prepared aseptically, sterilized through a 0.2 µ membrane filter, and UV irradiated as terminal sterilization. The pH, gelling capacity, and gelation temperature of the formulations were investigated to select the optimal composition for *in situ* gelling systems [15]. Table 1 shows the details of the formulation.

2.3 Evaluation of *in situ* gel

2.3.1 Physicochemical parameters

The formulated *in situ* gels were examined for physical appearance and clarity[7], pH[7], gelling capacity[7,8], gelation temperature[16], flowability[17], drug content, and viscosity[18]

2.3.2 Morphological analysis [19,20]

The surface morphology of *in situ* gelling systems was examined at both pH 6.5 and pH 7.4 using Scanning Electron Microscopy (Joel JSM-6490la analytical SE). Samples were mounted on an aluminum stub with double-sided adhesive carbon tape, sputter-coated with gold using a vacuum evaporator, and examined with the Scanning Electron Microscope.

2.3.3 Sterility studies

Sterility test was performed by direct inoculation method for a period not less than 14 days

2.3.4 Drug release study and kinetic study [21]

The *in vitro* release of the formulations was studied using a modified diffusion testing apparatus with dialysis tubing (21 mm, Mw: 8000–14400 Da) using simulated tear fluid pH7.4. An aliquot amount of the *in situ* gel is placed in the dialysis tube was suspended in 30 mL of the simulated tear fluid. The medium was subjected to magnetic stirring at 50 rpm, and aliquots (1 mL each) of samples were withdrawn at predetermined time intervals and replaced with an equal volume of fresh dissolution medium. The samples were analyzed at 249 nm using a UV spectrophotometer. Similarly, *in vitro* release of 0.5ml of the marketed product(Gentoson) was studied.

The four kinetic models analyzed the *in vitro* release kinetics: zero-order, first-order, Higuchi, and Korsmeyer-Peppas using DD solver.

2.3.5 Ex vivo transcorneal permeation study[21]

Ex vivo permeation studies were carried out using cornea obtained from the goat eye. The permeation experiment was carried out in a Franz diffusion cell-like setup with the excised cornea between the donor and acceptor compartment with simulated fluid (pH 7.4). 0.5ml of drug g containing *in situ* gel (0.03%w/v) was placed on the top of the cornea. At defined time intervals, 3ml of the sample was withdrawn from the receptor compartment (simulated tear fluid pH 7.4) and replaced with fresh media. The absorbance of the drawn sample was noted at 249nm. Further, a comparative study of *in situ* gel with marketed eye drop was carried out.

2.3.6 Corneal hydration (HL%) [22]

After permeation study, corneal weight is noted, soaked in 1 ml of methanol, and dried at 90C overnight. The dried corneal weight is recorded, and percentage corneal hydration was calculated from the formula,

$$\text{Corneal hydration} = 1 - [(W_a/W_b)] * 100$$

2.3.7 Histopathological studies[23]

Histopathological study to evaluate the corneal change was carried out by treating cornea with normal saline and optimized *in situ* gel. The samples were dissected and preserved in buffered formalin solution and further stained and visualized under a microscope.

2.3.8 In vitro antimicrobial activity [24]

In vitro antimicrobial activity was examined by agar diffusion assay with Staphylococcus aureus. The plates were inoculated with the microorganism and the various concentrations of marketed formulation(sample A) and optimized formulation(Sample B). At the defined temperature, the plates were incubated, and the zone of inhibition of microbial growth was measured.

2.3.9 In vivo ocular irritation study [25]

Acute eye irritation study was investigated according to OEDC guideline using New Zealand white rabbits, weighing 2-3 kg. Buprenorphine hydrochloride (0.01mg/kg, SC) and Proparacaine hydrochloride (0.5%) were applied to each eye. In addition, 0.1ml of the test substance is instilled into the lower conjunctival sac of the eye (right eye), and topical anesthetics (left eye) serve as the control. The cornea changes, conjunctiva, chemosis, iris were scored and compared to the control eye at 1, 24, 48, 72 hrs, 7, 14, and 21 days.

2.3.10 Accelerated stability studies [26]

Optimized formulation was subjected to stability study at different storage condition 4±2◦C, 30±2°C, 40±2◦C (75± 2 % RH) for 3 month. Samples were withdrawn after 15, 30, 60, and 90 days and evaluated for clarity, pH, drug content, gelation temperature, and *in vitro* release.

3. Results and Discussion

3.1. Preparation of formulations

Dual responsive *in situ* gel of gentamicin was developed by the cold method with Pluronic F127 and carbopol. The concentration of Pluronic F127 was *in situ* gel was selected to be 15% and 20%, based on the preliminary screening and literature. However, pluronic gels have drawbacks such as weak mechanical strength, rapid erosion, and non-biodegradability[27]. Thus, it was necessary to efficiently formulate other bioadhesive polymers like carbopol to formulate mucoadhesive and thermosensitive *in situ* gel. The formulated *in situ* gels were evaluated, and the results are tabulated in Table 2.

3.2. Evaluation of formulations

Gelation temperature is an important parameter for *in situ* gel formulation, as it is the temperature required to change from liquid to gel state. It is important to maintain the gelation temperature, thus, it remains liquid at room temperature and will be converted to gel under the physiological condition(32-36 °C). In the present study, all formulations show gelation temperature between the range of 29.41±1.23°C to 40.3±1.27°C. The results indicate that there exhibit an inverse relation between gelation temperature and the polymer concentration. The surface-active Pluronic F 127 consist of two polyoxyethylene chains and polyoxypropylene (water-insoluble). As the temperature increases, bulk viscosity increases due to the entanglement of micelles. At low temperatures, water act as a good solvent for both polyoxyethylene and polyoxypropylene. The inverse relation of polymer and temperature is due to the larger number and volume occupied by micelles[28].

All formulations are deemed satisfactory in terms of clarity, pH, and drug content. All formulations are transparent, and pH ranges from 6-6.5, which is comfortable for the eye. Also, the drug content ranges between 89.8±0.51 to 92.38±0.59 % showing the uniform drug distribution. All the results are tabulated in Table 2.

The sterility test confirmed that formulations are sterile at the end of 14 days at the specified temperature.

3.2.1. Viscosity study

The important parameters in the formulation of an *in situ* gel are viscosity and gelling capacity. The results are shown in Table 3. The formulation should have an optimal viscosity so that it is easy to administer and can rapidly convert to gel form in physiological conditions. The converted gel should retain its integrity for an extended period of time without dissolving or eroding,

allowing for sustained drug delivery to the site of action. According to the observations, viscosity and gelling capacity increase as polymer concentration increases. As a result, a delivery formulation is capable of forming a strong gel with excellent gelling capacity, as it will not drain from the administered site.

3.2.2. Morphological analysis (Scanning Electron Microscopy)

The formulation's gelling ability was confirmed by analysing the surface morphology at pH 6.5 and pH 7.4. The SEM images of *in situ* gel at pH 6.5 showed highly compact structure whereas at pH 7.4 it become loose surface. SEM is represented in Figure 4.

3.2.3. In vitro drug release profile of all formulations and kinetic study

In vitro release studies of Gentamicin sulfate *in situ* gel formulations were performed using simulated tear fluid (STF) at pH 7.4 as the dissolution medium. The study found biphasic release, with an initial burst followed by a gradual release. Figure 3 shows the cumulative percent drug released in 600 minutes for formulations F1-F6: 65.43%, 70.58%, 75.68%, 83.65%, 89.39 %, and 79.748%, respectively. The initial burst release is important for maintaining therapeutic concentration, while the slow release is also important. In the aspects of antibacterial activity, an initial release pattern is advantageous because it helps achieve the therapeutic concentration of the drug in a minimal amount of time, followed by slow release to maintain and sustain the controlled release effect. Pluronic F 127 and carbopol 940 helps control the release rate of the drug over 10 hours. The F5 formulation, according to the in vitro release study, had a better drug release profile than the other formulations. In comparison, the formulated *in situ* gel the marketed eye drop released up to 4 hours This indicates the sustained release pattern of *in situ* gel and is graphically represented in figure 5.

Drug release data of all formulations were fitted to different kinetic models like Zero order, First order, Higuchi, and Korsmeyer Peppas equations to interpret kinetic modeling of drug release is tabulated in Table 4. All formulations follow the First order equation describes *in vitro* release is dependent on polymeric concentration. The regression coefficient value for the Higuchi equation indicates that the drug was released by diffusion mechanism through the gel matrix. From Korsmeyer Peppas equations ‘n’ value less than 0.45 suggest that drug release by Fickian diffusion mechanism, drug release rate is less than polymer relaxation (erosion). Therefore drug release is dependent on polymer diffusion.

Formulation F5 was chosen as an optimized one based on the results of physicochemical parameters, as it has a satisfactory visual appearance, pH, flowability, gelling capacity, gelation temperature, drug content, viscosity, and in vitro release study. As a result, formulation F5 was chosen for further evaluation.

3.3. Comparative study of *ex vivo* corneal permeation of optimized *in situ* gel with marketed eye drop

The corneal permeation characteristics of the *in situ* gel and eye drop are compared in *ex vivo* transcorneal permeation studies. The corneal flux of the optimized *in situ* gel was 37.25 (g/cm2), whereas the corneal flux of the eye drop was 25.46 (g/cm^2). As shown in Figure 6, the optimized formulation showed 1.5 times higher permeation across the freshly excised goat cornea (78.02 %± 1.3%) in 10 hours when compared to the marketed eye drop (Genticyn).

3.4. Corneal hydration

The optimized formulation's corneal hydration value was found to be 78.02±1.3, indicating that the formulation's drug-polymer concentration does not cause corneal damage. The normal corneal hydration level of mammalian cornea is between 75 to 80%[29].

3.5. Histopathological studies

The microscopic observations show that the optimized *in situ* gel does not affect the cornea's microscopic structure. When compared to a saline-treated cornea, the epithelium remained intact, and no significant differences in stromal thicknesses were observed. The observation is given in figure 7.

3.6. Evaluation *in vitro* Antibacterial Activity by Zone of Inhibition

The antibacterial activity of the optimized formulation was compared to that of the marketed formulation against Staphylococcus aureus. The optimized formulation had significantly higher antibacterial activity than the marketed formulation ($p<0.05$). This suggests that when gentamicin was formulated as an *in situ* gelling systems, it retained its antibacterial activity. In table 5, the zone of inhibition of various formulations is tabulated and graphically represented in figure 7 and 8.

3.7. *In vivo* ocular irritation study

Three New Zealand white rabbits were used in an *in vivo* eye irritation study, as recommended by the OECD in 2012 and given in Figure 9. Table 6 shows the results, which show that the optimised formulation did not irritate rabbit's eyes or cause any abnormal signs on the cornea, conjunctiva, or iris. As a result, the formulation has been accepted for ophthalmic use.

3.8. Stability study

The stability study of optimized formulation at 4± 2^{0}C, 30± 2^{0}C and 40± 5^{0}C were carried out for 3 months and represented in Table 7. The formulation retained clarity and no significant changes were observed in gelation temperature, drug content and *in vitro* release.

Conclusion

The current study demonstrated that using an ocular delivery system to deliver gentamycin for eye infection can overcome the ocular disadvantage. The optimization studies clearly showed that a gel capable of forming a strong gel with good gelling capacity is suitable for delivery because it will not drain from the administered site. As a result, the study shows that the formulation improves drug penetration through the cornea, and the optimized formulation did not show any irritation on the animal model and did not have any formulation-related problem. The gel also passes short-term stability tests, indicating that it is physically and chemically stable.

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Table 1: Representing the Composition of Formulation

INGREDIENTS	FORMULATION CODE					
	F1	**F2**	**F3**	**F4**	**F5**	**F6**
Gentamicin sulphate (mg)	0.03	0.03	0.03	0.03	0.03	0.03
Pluronic F127 (mg)	16	18	20	16	18	20
Carbopol (mg)	0.02	0.02	0.02	0.03	0.03	0.03
Sodium chloride (mg)	0.009	0.009	0.009	0.009	0.009	0.009
Benzalkonium chloride (mg)	0.001	0.001	0.001	0.001	0.001	0.001

Citrophosphate buffer q.s	10ml	10ml	10ml	10ml	10ml	10ml

Table 2 Evaluation of Physicochemical Parameters

Formulation	Transparency at temperatures (°C)			pH	Flow ability at temperatures (°C)			Gelation temperature (°C)	Drug content (%w/v)
	2± 1°C	27 ± 1°C	36± 1°C		2± 1°C	27 ± 1°C	36± 1°C		
F1	+++	+++	+++	6.53±0.57	+++	+++	++	40.3±1.27	89.8±0.5197
F2	+++	+++	++	6.56±0.15	+++	++	++	38.23±0.21	87.91±1.509
F3	+++	++	++	6.66±0.15	+++	++	-	38.47±0.48	87.91±1.509
F4	+++	++	+++	6.6±0.01	+++	++	-	34.53±0.20	85.14±1.058
F5	+++	+++	+++	6.63±0.15	+++	+++	-	37.56±1.03	92.38±0.597
F6	+++	++	++	6.5±0.1	+++	+	-	29.41±1.23	90.31±0.924

Transparency: +++ = Transparent; ++ = slightly translucent; - = Turbid

Flow ability: +++ = Very good; ++ = good; + = average; - = not flow

Table 3: Viscosity

Formulation Code	Viscosity in cps (spindleno.61), after gelling
F1	180±0.19
F2	354±0.23
F3	486±0.18
F4	943±0.38
F5	1410±0.28
F6	1438±0.32

Table 4: Kinetic study of all formualtions

Formulation code		r^2 ±SD		
	Zero order	First order	Higuchi	Korsmeyer peppas
F1	0.982	0.988	0.976	0.991
F2	0.974	0.986	0.978	0.99
F3	0.975	0.994	0.989	0.995
F4	0.924	0.992	0.991	0.99
F5	0.983	0.992	0.988	0.988
F6	0.956	0.991	0.988	0.99

Table 5: Evaluation *in vitro* Antibacterial Activity by Zone of Inhibition

Microorganism	Concentration (mg)	Zone of inhibition of Test samples (cm)

		Marketed eye drop (A)	Optimized *in situ* gel (B)
Staphylococus aureus	0.05	0	0
	0.1	0.3	0.4
	0.2	0.5	0.6
	03	0.7	0.9

The values are expressed as Mean ± SD; n = 2

Table 6: Score obtained from rabbit eye irritation assessment

Lesion	**Score obtained from assessment**						
	1hr	24hrs	48hrs	72 hrs	7 days	14 days	21 days
Cornea Grade	0	0	0	0	0	0	0
Iris	0	0	0	0	0	0	0
Conjunctivae	0	0	0	0	0	0	0
Chemosis	0	0	0	0	0	0	0

Table 7: Stability Study of Optimized formulation

Storage condition	***In vitro* release**				
	Freshly prepared	**15th day**	**30th day**	**60 th day**	**90th day**
Refrigerator condition (4± 2°C)	89.39±1.92	89.65±1.49	89.64±1.49	88.86±1.4	87.98±1.24
Room Temperature (30 ± 2°C)	89.92±1.49	89.53±1.95	88.27±1.49	87.21±1.42	86.41±1.92
40±2^{0}C	88.54±1.42	87.28±1.49	87.72±1.49	86.09±1.49	85.15±1.49

FIGURES

Figure 1: Sol to gel transition

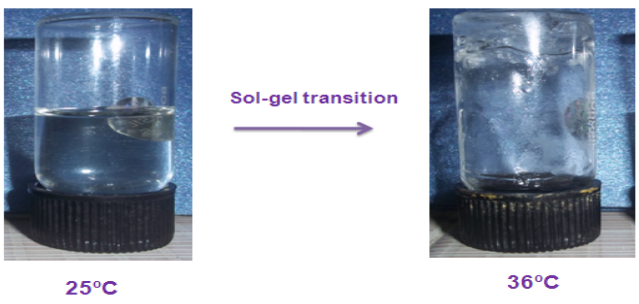


Figure 2: Morphological analysis of optimized of formulation F5 by SEM, at (A) pH 6.5 and (B) pH 7.4

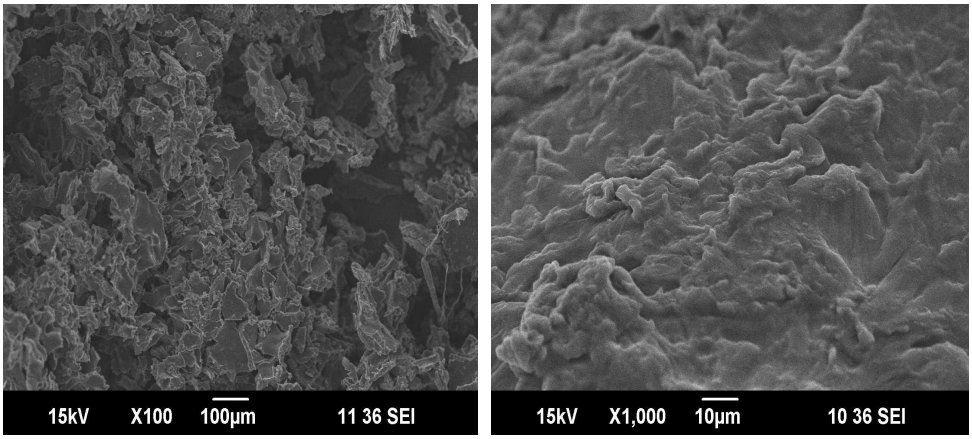


Figure 3: In vitro release study of formulation

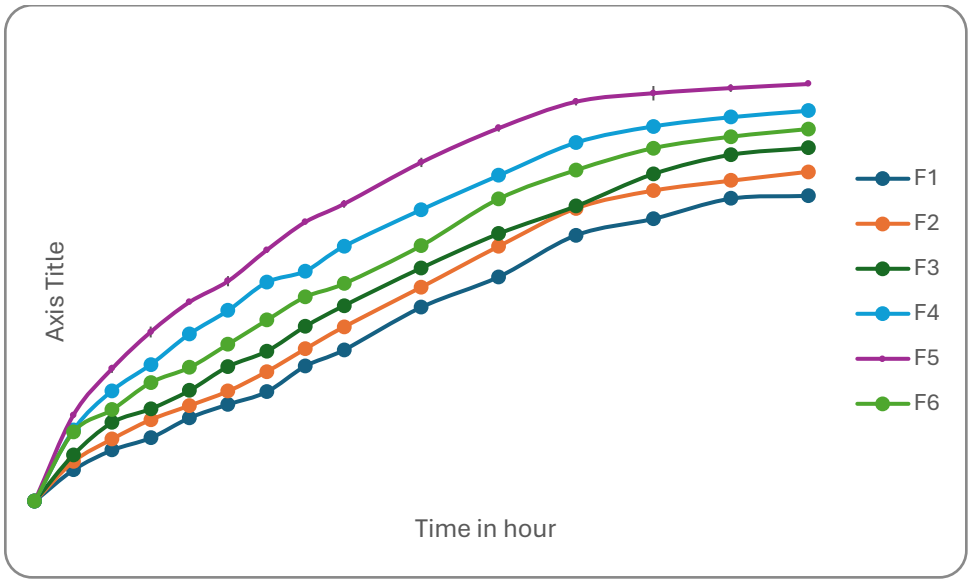


Figure 4: Comparative *in vitro* release study of optimized *in situ* gel and marketed eye drop

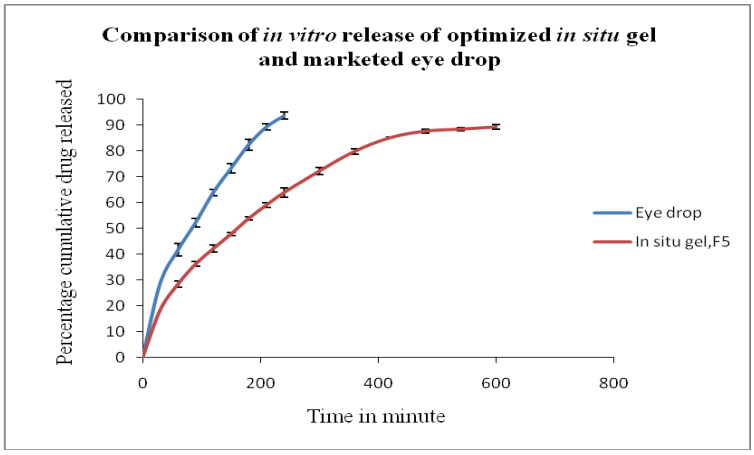


Figure 5: Comparative *ex vivo* permeation studies of optimized *in situ* gel and marketed eye drop

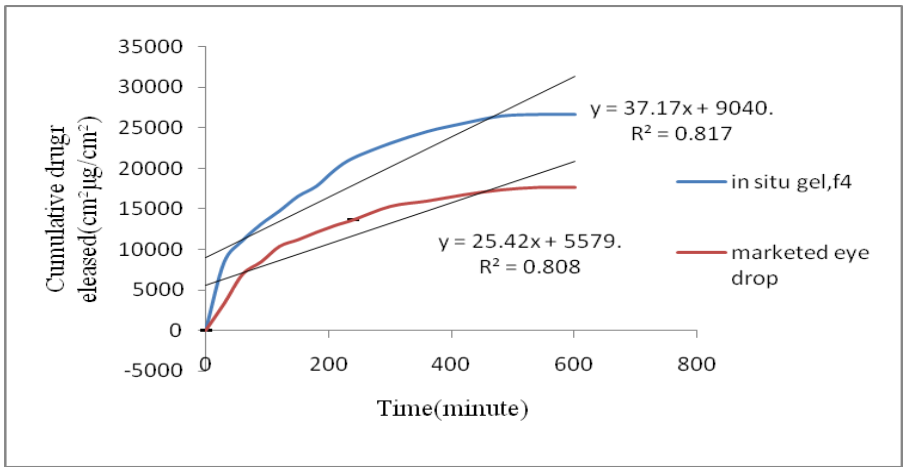


Figure 6: (a) Photographs of histology of cornea treated with normal saline (b)Photographs of histology of cornea treated with *in situ* gel

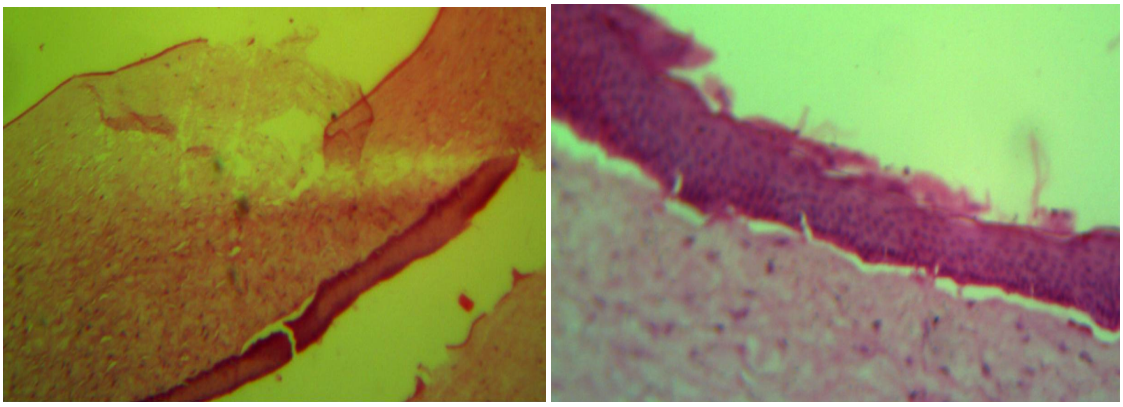


Figure 7. Representing zone of inhibition of *in vitro* Antibacterial activity

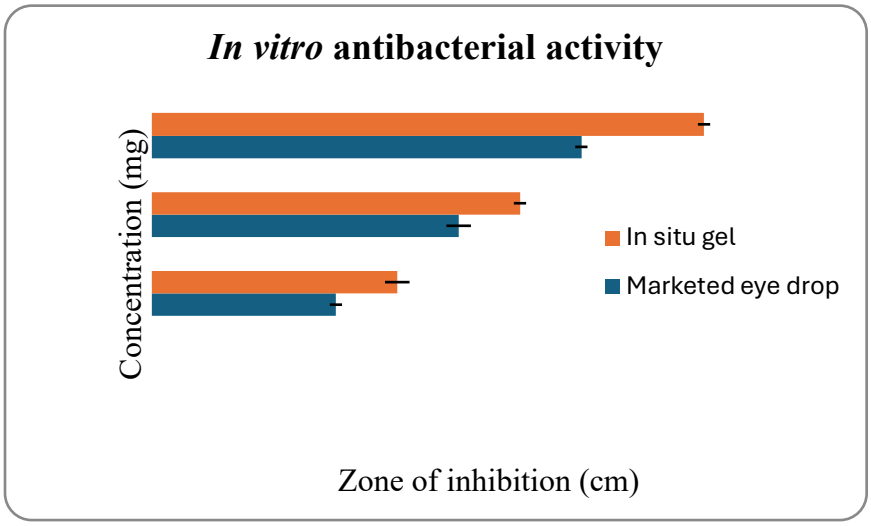


Figure 8: *In vitro* Antibacterial activity of optimized *in situ* gel with marketed eye drop

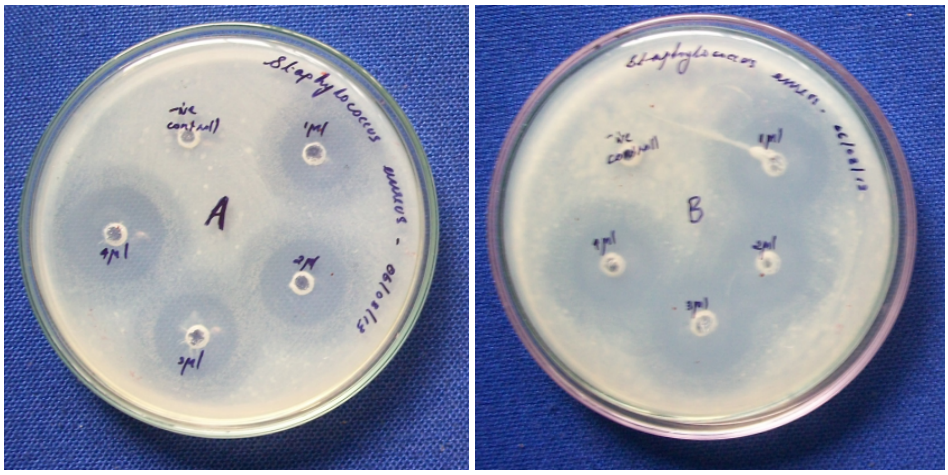


Figure 9: Ocular irritation test

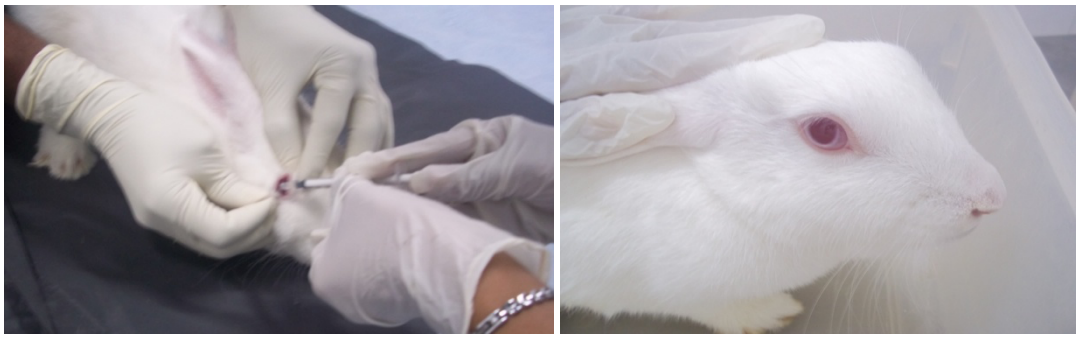


Figure 9 : Stability Study of Optimized formulation

