

Development of Thermosensitive Ophthalmic *in situ* Gels of Bimatoprost for Glaucoma Therapy

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ABSTRACT

Background: In recent years, ophthalmic *in situ* gels have gained wide importance for the sustained delivery of drugs into the eye by overcoming the demerits of conventional eye drops. **Objectives:** The present investigation was undertaken to formulate and evaluate Bimatoprost loaded thermosensitive ophthalmic *in situ* gels for providing prolonged drug release pattern with good patient acceptance. **Methods:** Bimatoprost thermosensitive ophthalmic *in situ* gels were prepared by the cold method using temperature dependent polymers, Poloxamer 188/poloxamer 407 in combination with HPMC K4M as viscosifier used in three different concentrations. The prepared *in situ* gels were evaluated for appearance, clarity, pH, gelling capacity, gelation temperature, drug content and drug release study. The optimized batch of the formulation was subjected to drug release kinetics, *ex vivo* drug permeation, sterility, isotonicity, *in vitro* ocular irritancy test and short-term stability studies for 3 months. **Results:** From the drug release study, it was found that formulation; BT-5 had the highest drug release with Higuchi release kinetic mechanism. The formulation (BT-5) was found to be sterile and the HET- CAM test confirmed that there was no ocular irritation and the formulation was stable for a period of 3 months without any significant changes in the evaluation parameters. **Conclusion:** Bimatoprost thermosensitive ophthalmic *in situ* gels can be a better alternative approach to provide sustained delivery of the drug by reducing the frequent drug instillation for the treatment of glaucoma.

Key words: Bimatoprost, Poloxamer 407, *In situ* gels, Viscosity, Isotonicity.

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INTRODUCTION

Glaucoma is one of the complex eye disorders, which is characterized by a rise in Intra Ocular Pressure (IOP) than a normal eye can tolerate. Glaucoma is commonly known as a silent killer of vision loss and the second leading cause of blindness worldwide. Glaucoma is of two types- open angle and closed angle glaucoma. Open angle glaucoma is the chronic one affecting most of the people.¹⁻⁴

Bimatoprost, which is a prostaglandin analogue, reduces the higher IOP by two mechanisms, pressure dependent and pressure independent. Recent studies in clinical trials prove that it is an effective drug remedy to control open angle glaucoma and has FDA scientific approval.⁵ Bimatoprost

is now available as conventional eye drops, but eye drops have some demerits such as no sustained action, corneal elimination, less ocular contact time and lachrymal drainage.

In order to resolve the above said demerits of Bimatoprost eye drops, an attempt has been made to develop a stable, sustainable drug delivery system called ophthalmic *in situ* gels.⁶ These are the gelling liquids that convert the drug solutions into a gelling system, once the formulation is administered in to the eye. These undergo physico-chemical phase changes in the cul-de-sac of the eye and get convert into gels that retain in the corneal tissue with good contact time and release the drug for prolonged time periods for sustained action.

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Ophthalmic *in situ* gels can be prepared by three different approaches, change in pH, change in the ionic system and change in temperature.⁷

In situ gels prepared by change or rise in temperature are referred to as thermosensitive *in situ* gels, which undergo sol-gel transition by an increase in temperature from 20-25°C to 35-37°C. The increase in temperature is characterized by cross linked polypropylenes called poloxamers which are hydrophilic in nature and give colorless transparent gel.⁸ Poloxamers are also called as pluronics which are responsible to form a micellar network to increase the temperature resulting into viscous transparent gelling liquids. Poloxamer 188 and poloxamer 407 were the two most commonly used thermosensitive polymers and their use in combination is found to be significant for effective *in situ* gelling systems.⁹

MATERIALS AND METHODS

Bimatoprost pure drug of the pharmaceutical grade was purchased from Dr. Pradeep Reddy's laboratory, Hyderabad. HPMC K4 M, Poloxamer 188 and Poloxamer 407 were procured from Hi media laboratory, Mumbai. Sodium chloride, Dibasic sodium phosphate, Sodium hydroxide and Benzalkonium chloride were procured from Lobachem suppliers, Mumbai.

Methodology

Preparation of Bimatoprost loaded ophthalmic *in situ* gels

Selection of dose of Bimatoprost

Dose of Bimatoprost in eye drops is 0.03% i.e each ml of formulation contains 0.3 mg of drug (0.3 mg/ml). A 9 mg of Bimatoprost was calculated for preparing 30 ml of *in situ* ophthalmic gels.

Preparation of Bimatoprost ophthalmic *in situ* gels

Thermosensitive ophthalmic *in situ* gels of Bimatoprost were prepared by the cold method. Poloxamer 188 was used in the concentration of 5% and poloxamer 407 was used in two different concentrations of 15 and 16% as temperature triggering polymers. HPMC K4 M was added in the different concentrations of 0.10, 0.15 and 0.20 g as viscosifying agent to enhance the viscosity. Dibasic sodium phosphate (80 mg) was used as a buffering agent. Sodium chloride was used in the concentration of 0.9% for making the formulations isotonic and 0.01% of benzalkonium chloride was used as antimicrobial agent.¹⁰

All the above mentioned ingredients including Bimatoprost were added in 20 ml of purified water and dissolved by a magnetic stirrer for sufficient time till the solutions become clear. To adjust pH in the ophthalmic range, 0.1 N sodium hydroxide was added dropwise as quantity sufficient and the final volume of *in situ* gels was made up to 30 ml with purified water. All the formulated *in situ* gels were filtered through 0.22 µ sterile syringe filters and transferred to 20 ml sterile vials. The formulation composition of Bimatoprost loaded *in situ* gels is shown in Table 1.

Evaluation of Bimatoprost thermosensitive ophthalmic *in situ* gels

Appearance and flow nature: All the formulations were visually inspected for their general appearance and flow nature.¹¹

Clarity: Clarity of all the formulations was done checked under a fluorescent light against white and dark background.¹¹

pH: pH of the formulations was determined using a digital pH meter. An average of 3 trials was taken.¹²

Table 1: Formulation of Bimatoprost ophthalmic *in situ* gels.

Ingredients	Formulation code					
	BT-1	BT-2	BT-3	BT-4	BT-5	BT-6
Bimatoprost (mg)	9	9	9	9	9	9
HPMC K4M (g)	0.10	0.15	0.20	0.10	0.15	0.20
Poloxamer 188 (%)	5	5	5	5	5	5
Poloxamer 407 (%)	15	15	15	16	16	16
Sodium chloride (%)	0.9	0.9	0.9	0.9	0.9	0.9
Dibasic sodium phosphate (mg)	80	80	80	80	80	80
Benzalkonium chloride (%)	0.01	0.01	0.01	0.01	0.01	0.01
0.1N NaoH (ml)	q.s to adjust pH	q.s to adjust pH	q.s to adjust pH	q.s to adjust pH	q.s to adjust pH	q.s to adjust pH
Purified water q.s (ml)	30	30	30	30	30	30

Gelling capacity

Gelling capacity is one of the important criteria for ophthalmic *in situ* gels. It is the ability of the formulation to undergo sol to gel transition when the formulation is administered to cul de sac of eye. It was evaluated by placing a drop (27 μ l) of formulation in a vial containing 2 ml of freshly prepared simulated tear fluid (composition- sodium chloride-0.67g, sodium bicarbonate-0.2g and Calcium chloride - 0.008g) which was equilibrated at 37 \pm 2 $^{\circ}$ C and the gelling capacity was visually evaluated by observing the time taken for gelation and time taken to dissolve the gel.¹³

The grading of the gelling capacity was shown below.

Grading:

- No gelation

+ Gelation within few seconds and remained for a few minutes.

++ Gelation occurs immediately and remained for 6-8 hr.

+++ Gelation occurs immediately and remained for an extended period.

++++ Stiff gels

Viscosity measurement

The viscosity of ocular *in situ* gels is important to determine the contact time between the drug and ocular tissue. The viscosity measurement was carried out by using Brookfield viscometer DVII + pro model. From the literature, it was evident that the formulation at 25 \pm 2 $^{\circ}$ C should have a viscosity of 5–1000 cps and at 37 \pm 2 $^{\circ}$ C should have a viscosity of about 50–50,000 cps. The samples were analyzed both before and after gelation using spindle no. 61. The angular velocity of the spindle was increased from 5, 10, 20, 30, 50 and 100 and the viscosity of all the formulations was recorded.¹⁴

Gelation temperature

Transfer 10 ml of the formulation in to a 50 ml beaker and it is kept on a magnetic stirrer with a thermostatically controlled heater. The temperature of the stirrer was increased with the increment of 1 $^{\circ}$ C and the temperature was checked using a thermometer. The rotation of the bead gradually becomes slow as gelation starts and the temperature at which the rotation of magnetic bead was stopped is considered as gelation temperature.¹⁵

Drug Content Estimation

1ml of formulation equivalent to 100 mg was taken and diluted with 100 ml of simulated tear fluid to make a concentration of 10 μ g/ml. The final dilutions were made with simulated tear fluid according to the beer's range. The drug content was analyzed and estimated

by taking the absorbance at 294 nm against the blank reagent using UV-Visible spectrophotometer.¹⁶

In vitro drug release study

In vitro drug release study was carried out by Franz diffusion cell consisting of donar and receptor compartments. The donar compartment was filled with 25 ml of STF by placing a magnetic bead inside and the dialysis membrane which was soaked in STF for an overnight was mounted on the receptor compartment in such a way that dialysis membrane was in contact with STF in receptor compartment. Franz diffusion cell assembly containing STF was maintained at a temperature of 37 \pm 0.5 $^{\circ}$ C and was rotated at a speed of 50 rpm. 1ml of *in situ* gel was placed on the dialysis membrane. Aliquots of 1ml were withdrawn at different time intervals of 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 hrs and 10 ml of fresh STF was replaced. The withdrawn samples were diluted to 10 ml with STF and were analyzed by UV spectrophotometer at 294 nm. The percentage cumulative drug release (% CDR) was calculated.¹⁷

Drug release kinetics

The data obtained for *in vitro* release were fitted into the equation for the zero order, first order, Higuchi and Korsmeyer-peppas release models. The best fitting model was selected on the basis of higher regression coefficient value (R^2).¹⁸

Ex vivo drug permeation study

Ex vivo drug permeation study was carried out by using the modified Franz diffusion chamber and the goat corneal membrane was used for drug permeation study. The whole eyeball of the goat was procured from a slaughter house and carried to laboratory in the cold condition in normal saline maintained at 4 $^{\circ}$ C. From the eye ball, the cornea was carefully removed along with 5-6 mm of surrounding sclera tissue and the obtained cornea was washed with cold saline. Washed corneas were kept and soaked overnight in simulated tear fluid. The study was performed in the same manner as that of *in vitro* drug release procedure and the release study was done for a period of 12 hr.¹⁹

Sterility test

The sterility test was performed using soyabean casein digest media for fungi and fluid thioglycolate medium for bacteria. This test was performed according to the procedure of the direct inoculation method. The inoculated media were kept for incubation of 7 days and thereafter observed for the presence or absence

of the microbial growth by comparing the optimized formulation with positive and negative controls.²⁰

Isotonicity test

It was tested by mixing a few drops of blood with the optimized formulation and was observed under high resolution Biovis particle size analyzer microscope at 45X magnification. The procedure was repeated for marketed ophthalmic formulation for the comparison of RBCs. The RBCs were observed to check shrinkage, bulging of cells by comparing the shape of RBCs with optimized formulation and marketed formulation of Bimatoprost eye drops.²¹

In vitro ocular irritancy evaluation by HET-CAM (Hen's egg test choriallantonic membrane) test

Since the Draize Rabbit test has been banned, an OECD recommended *in vitro* ocular irritation test was conducted known as the HET-CAM test. In this test, freshly collected white leghorn chicken eggs (not older than 7 days) weighing between 50-60 g were used and those with physical damage, cracks were rejected. Three groups were made each containing 3 eggs.

Negative control: Here the eggs were treated with 0.3 ml of 0.9% NaCl as a standard.

Test group: In this group eggs were tested with 0.3 ml of the optimized formulation.

Positive control: In this, eggs were treated with 0.3 ml of 1 % SDS (Sodium dodecyl sulfate) as an irritant for comparison with negative control and test.

Procedure: The eggs were kept on a tray and placed in an incubator maintained at a temperature of $37 \pm 0.5^\circ\text{C}$ and relative humidity of $58 \pm 2^\circ\text{C}$. Manually the eggs were rotated 5 times per day for about 8 days. Candle the eggs on the 8th day of incubation to confirm the embryo growth. On confirmation, eggs were replaced into the incubator without rotation by keeping the large end upward and kept for one complete day. On the 9th day; mark the air cell on top and make a hole on the air sac of egg shell without injuring the membrane. All the eggs were treated with respective solutions and observed for the signs of hemorrhage, coagulation and lysis of blood vessels for a time period of 300 sec (5 min).^{22,23}

The irritation score (IS) formula is given below followed by irritation score value with inference in Table 2.

$$IS = \frac{(301 - H)}{300} \times 5 + \frac{(301 - L)}{300} \times 7 + \frac{(301 - C)}{300} \times 9$$

Where, H- Hemorrhage

L- Lysis of blood vessels

C- Coagulation

Table 2: Irritation score value with inference for HET-CAM Test.

Irritation Score	Inference
0-0.9	No irritation
1-4.9	Weak irritation
5-8.9	Moderate irritation
9-21	Severe irritation

Short term Stability studies for optimized formulation

The ICH has framed certain conditions and procedures to conduct short term stability studies to predict the physical and chemical stability of drug products. For ophthalmic preparations, the study was conducted for a time period of 3 months. The optimized formulation was stored in a glass vial and kept in the stability chamber at room temperature of $25 \pm 2^\circ\text{C}$ and $60 \pm 5\%$ Relative Humidity (RH) and at the accelerated temperature of $40 \pm 2^\circ\text{C}$ and $65 \pm 5\%$ Relative Humidity (RH). The parameters namely appearance, clarity, pH and drug content were evaluated for 1,2 and 3 months to verify the stability of optimized formulation.^{24,25}

RESULTS AND DISCUSSION

FTIR Spectroscopy

From the interpretation of the FTIR study, it was confirmed that the drug Bimatoprost was compatible with the polymers used for the formulation of ophthalmic *in situ* gels and there was no additional peak in the spectra's, confirming that there was no chemical interaction between the drug and polymers used.

The FTIR spectra of Bimatoprost, physical mixture of Bimatoprost with poloxamer 188 and poloxamer 407 are represented in Figure 1.

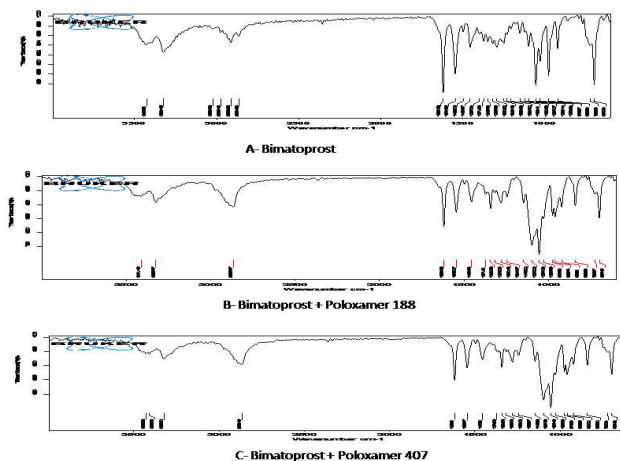


Figure 1: IR Spectrum of Pure Bimatoprost (A), Bimatoprost + Poloxamer 188 (B) and Bimatoprost + Poloxamer 407 (C).

Appearance

All the six formulations were found to be transparent clear liquids with free flowing nature. The results are shown in Table 3.

Clarity

All the prepared Bimatoprost ophthalmic *in situ* gels were found to be clear without the presence of any particles and particulate matter. The results are shown in Table 3.

pH

The pH of all the formulations was in the range of 6.8-7.3 and found to be within the physiological range of the human eye. The results are shown in Table 3.

Gelling capacity

All the formulations showed immediate gelation and remained for an extended period of time. The formulations with 15% of poloxamer 407 showed the gelling capacity for about 8 hr and the remaining three formulations with 16% of poloxamer 407 showed the gelling capacity for about 10 hr. The increase in gelling capacity was due to the increase in the concentration of poloxamer from 15-16% where the gelation time is exceeded due to the increased formation of micelles from polypropylene polymers. The results of the gelling capacity are shown in Table 3.

Rheological Study

Based on the results, it was found that formulations showed the viscosity ranging from 7.65 to 82.24 cps at $25 \pm 2^\circ\text{C}$ before gelation and 238.16- 2335.52 cps at $37 \pm 2^\circ\text{C}$ after gelation. The increase in viscosity was dependent on the concentration of HPMC K4M. The viscosity of the formulation was gradually increased with increase in the concentration of HPMC K4M. The viscosity of formulations was found to increasing with a decrease in rpm and the rheological graphs showed the Pseudoplastic flow nature of liquids. These shear rate properties of the *in-situ* gels can uniformly distribute the drug on the ocular surface and increase the ocular contact time for providing prolonged drug release. The results of viscosity for the *in-situ* gels before and after gelation were depicted in Figures 2 and 3.

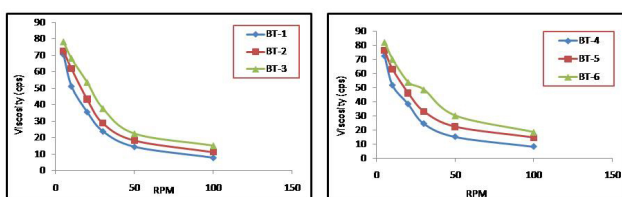


Figure 2: Viscosity of Bimatoprost *in situ* gels before gelation.

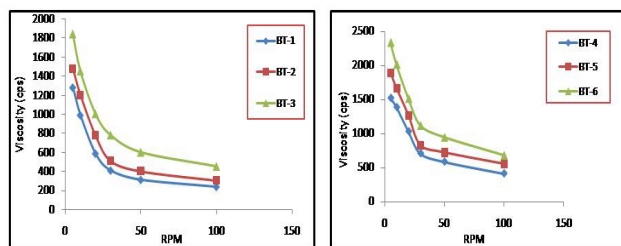


Figure 3: Viscosity of Bimatoprost *in situ* gels after gelation.

Gelation temperature

The gelation temperature of all the six formulations was found to be $36.8- 37.8^\circ\text{C}$. The formulations with 5% and 15% of poloxamer 188 and poloxamer 407 showed gelation temperature of 36.4 to 36.3°C whereas formulation BT-2 shown the temperature of 37.3°C which may be due to the optimum amount of HPMC K4M (0.15g) the formulation was not much viscous to influence the gelling capacity by micelle formation. A similar observation was seen in the formulation BT-5 which showed a higher temperature of 37.5°C in comparison to the remaining two formulations, BT-4 and BT-6 which showed a temperature of 37.2 and 36.6°C . From the study, it was revealed that gelation temperature was increased with higher gelling capacity and at the same time the higher concentration of HPMC K4M which increases the viscosity of the *in situ* gels and reduces the gelation temperature by reducing the formation of the micelles. The results for the gelation temperature of the formulations are shown in Table 3.

Table 3: Evaluation of appearance, clarity, pH, gelling capacity and gelation temperature of Bimatoprost *in situ* gels.

Formulation code	Appearance	Clarity	pH* (mean \pm SD)	Gelling capacity	Gelation temperature ($^\circ\text{C}$)
BT-1	Free flowing liquid	Clear	6.8 ± 0.13	++	36.4
BT-2	Free flowing liquid	Clear	7.3 ± 0.04	++	37.3
BT-3	Free flowing liquid	Clear	6.9 ± 0.36	++	36.3
BT-4	Free flowing liquid	Clear	7.2 ± 0.11	+++	37.2
BT-5	Free flowing liquid	Clear	7.3 ± 0.31	+++	37.5
BT-6	Free flowing liquid	Clear	7.0 ± 0.06	+++	36.6

Where, ++ indicates formulations retained as gels for 8 hr

+++ indicates formulations retained as gels for 10 hr

*Each value is the determination of 3 trials

Drug content estimation

The drug content of all the six formulations of Bimatoprost *in situ* gels was estimated by UV spectroscopy measuring the absorbance at 294 nm against blank and the drug content of all the formulations was in the range of 92.18-95.63%.

In vitro drug release study

The formulations BT-1, BT-2 and BT-3 containing 5% poloxamer 188 and 16% poloxamer 407 showed the drug release of 75.38-81.26 % up to 8 hr since the gelling capacity of these formulations was retained for 8 hr and the formulations BT-4, BT-5 and BT-6 containing 5% poloxamer 188 and 17% poloxamer 407 showed the release of drug from 80.37 - 86.63 % upto 10 hrs since the gelling capacity of these formulations was retained for an extended period of 10 hrs. It has been observed that drug release of all the formulations was increased with respect to the concentration of HPMC K4M in the concentration range of 0.10g-0.15 g and the drug release was reduced with increased concentration of 0.2 g of HPMC K4M because higher the viscosity of formulation lesser will be the permeation through the membrane and finally the release amount of the drug also will be lesser. Based on the results, it was confirmed that among all, formulation BT-5 shown the highest drug release of 86.63 % up to 10 hr and emerged as best.

This might be due to an increase in the concentration of poloxamer 407(17%) and the optimum concentration of HPMC K4M (0.15g) as a viscosifying agent. Hence formulation BT-5 was chosen as the optimized and was subjected to other evaluation parameters such as drug release kinetics study, *ex vivo* drug permeation study, sterility test and Isotonicity test. The drug release profile of formulated *in situ* gels was depicted in Figure 4.

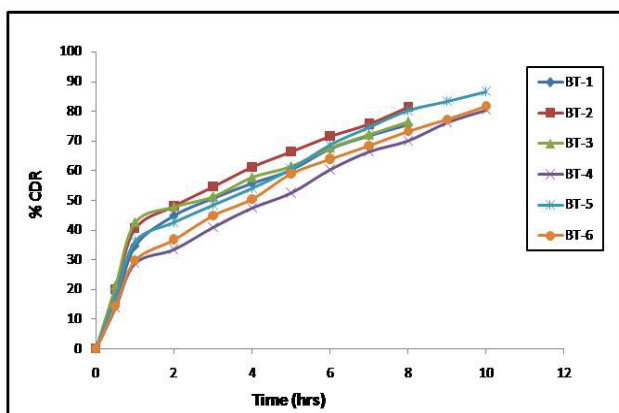


Figure 4: Drug release profile of Bimatoprost *in situ* gels.

Drug release kinetics

Based on the results, it was observed that Higuchi release kinetics showed the higher regression (R^2) of 0.988 indicating that the release of drug follows swelling of the polymeric system followed by diffusion system for sustaining the drug release. The Higuchi release kinetics profile for the optimized formulation, BT-5 was represented in Figure 5.

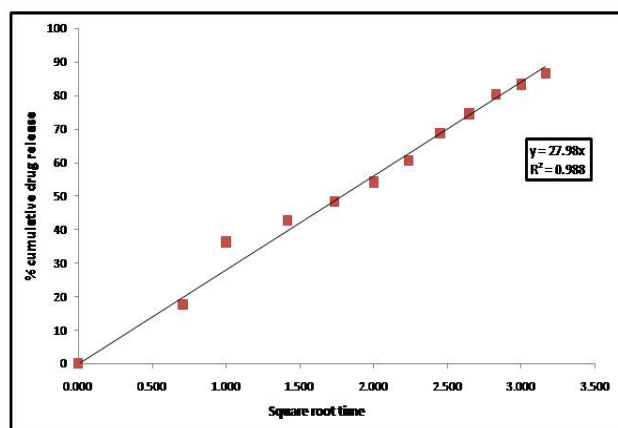


Figure 5: Higuchi drug release kinetics for BT-5.

Ex vivo drug permeation study

The drug permeation for the BT-5 *in situ* gel was found to be 67.45 % upto 12 hr. The slow release of the drug may be because of the fact that cornea is made up of many layers like epithelium, stroma and endothelium which are highly lipophilic in comparison with a dialysis membrane and the formulation may take more time to diffuse through these layers for the release of the drug. The *ex vivo* drug permeation along with *in vitro* release comparison for BT-5 is shown in Figure 6.

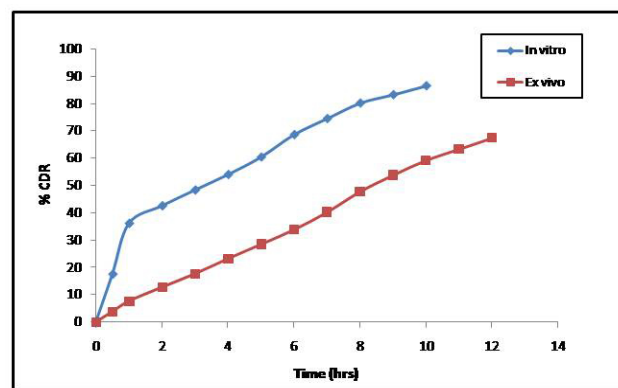


Figure 6: Comparison of *in vitro* and *ex vivo* drug release of BT-5.

Sterility test

In positive control (Growth promotion test) it was observed that both medias (Fluid thioglycolate and soyabean casein digest agar) promoted the growth of microorganisms after the incubation of 7 days indicated by the appearance of turbidity, whereas there was no microbial growth in negative control and in the optimized formulation (BT-5), confirming that the formulation was found to be sterile in nature.

Isotonicity test

The Isotonicity test revealed that there was no shrinkage or bulging of RBCs with optimized formulation (BT-5) when compared with the marketed formulation. It was confirmed that the formulation was found to be isotonic with RBCs.

In vitro ocular irritancy test by HET-CAM Method for BT-5

The eggs of positive control treated with 1% SDS showed irritation mean score of 14.07 indicating severe irritation with blood vessels lysis and hemorrhage in the chick embryo, the negative control group treated with 0.9% Nacl showed mean score of 0.04 and the mean score of optimized formulation was 0.05 which confirms that there was no irritation in the chick embryo and the formulation was found to be non-irritant and non-toxic when compared with positive and negative control groups.

The results of mean irritation score for the negative control, positive control and optimized formulation were discussed in Table 4 and the HET-CAM test images are depicted in Figure 7.

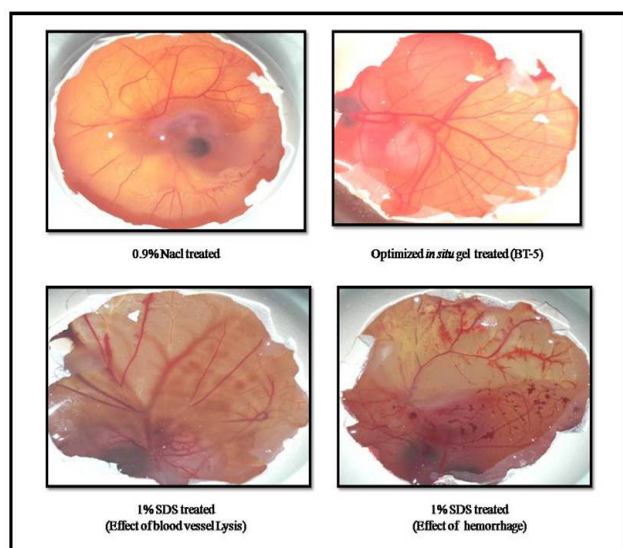


Figure 7: HET- CAM test for optimized formulation (BT-5).

Table 4: *In vitro* ocular irritancy for BT-5 by HET-CAM test.

Test compound	Mean Irritation Score	Inference
0.9 % Nacl (Negative control)	0.04	No irritation
1% SDS (Positive control)	14.07	Severe irritation
Optimized formulation (BT-5)	0.05	No irritation

Stability studies for BT-5 formulation

The optimized formulation of Bimatoprost (BT-5) was evaluated for appearance, clarity, pH and drug content for their stability for a short term of 3 months. From the results, it was found that optimized formulations did not show any significant changes in the parameters evaluated and the formulation was found to be stable for a period of 3 months. The results of stability studies for optimized formulations were discussed in Table 5.

Table 5: Results of appearance, clarity, pH and drug content for BT-5 at $25 \pm 2^\circ\text{C}/60 \pm 5\% \text{RH}$ and at $40 \pm 2^\circ\text{C}/65 \pm 5\% \text{RH}$.

Stability studies at $25 \pm 2^\circ\text{C}/60 \pm 5\% \text{RH}$ for optimized Bimatoprost <i>in situ</i> gel (BT-5)				
Months	Appearance	Clarity	pH	Drug content
1	Free flowing liquid	Clear	7.1 ± 0.14	95.11 ± 1.13
2	Free flowing liquid	Clear	7.3 ± 0.52	94.90 ± 0.68
3	Free flowing liquid	Clear	7.2 ± 0.37	95.53 ± 0.73
Stability studies at $40 \pm 2^\circ\text{C}/65 \pm 5\% \text{RH}$ for optimized Bimatoprost <i>in situ</i> gel (BT-5)				
Months	Appearance	Clarity	pH	Drug content
1	Free flowing liquid	Clear	7.0 ± 0.18	95.02 ± 0.58
2	Free flowing liquid	Clear	7.1 ± 0.38	95.51 ± 0.66
3	Free flowing liquid	Clear	7.2 ± 0.27	94.88 ± 0.37

CONCLUSION

The current research study was an attempt to formulate thermosensitive ophthalmic *in situ* gels for Bimatoprost for the management of glaucoma. Bimatoprost ophthalmic *in situ* gels were prepared by the cold method using two temperature dependent polymers poloxamer 188 and poloxamer 407, HPMC K4M was used in three different concentration to enhance the viscosity of formulations.

Over all six formulations were made by varying concentrations of poloxamer 407 and HPMC K4M. Drug release study showed that *in situ* gels prepared with 16% poloxamer 407 were remained for release of drug up to 10 hr and formulation BT-5 was optimized as best among all showed highest drug release with Higuchi release kinetics and was found to be sterile, isotonic and non-irritant in nature. The optimized formulation (BT-5) was stable for a period of 3 months without any changes in the evaluation parameters. Hence from the results of the present investigation, it can be concluded that Bimatoprost thermosensitive ophthalmic *in situ* gels can be successfully be formulated as an effective and better alternative drug delivery approach for the glaucoma.

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CONFLICT OF INTEREST

The authors declare that there was no conflict of interest.

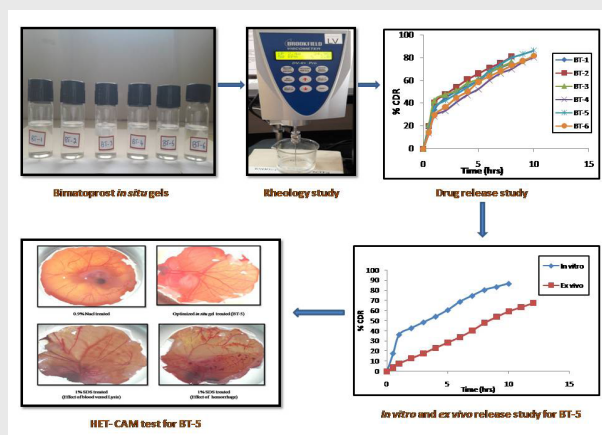
ABBREVIATIONS

IOP: Intra ocular pressure; **FDA:** Food and drug administration; **STF:** Simulated tear fluid, **q.s:** Quantity sufficient; **HET-CAM:** Hen's egg test choriocallantonic membrane; **mg:** Milligram; **ml:** Millilitre; **µg:** Microgram; **min:** Minutes; **hr:** Hours; **°C:** Degree Celsius; **RH:** Relative humidity; **NaCl:** Sodium chloride; **SDS:** Sodium dodecyl sulfate; **RBCs:** Red blood cells; **nm:** Nanometer; **g:** Gram; **rpm:** Revolutions per minute; **cps:** Centipoise; **FTIR:** Fourier transform infra red; **IS:** Irritation score; **OECD:** Organization for economic co-operation and development

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PICTORIAL ABSTRACT



SUMMARY

- The present work was an attempt to prepare thermoensitive ophthalmic *in situ* gels for Bimatoprost for the glaucoma therapy. Poloxamer188/407 were used in combination as temperature triggering polymers and HPMC K4M was used as viscosity enhancer.
- The formulated Bimatoprost ophthalmic *in situ* gels were evaluated for appearance, clarity, pH, gelling capacity, viscosity, gelation temperature, *in vitro* drug release, *ex vivo* drug permeation, sterility test, isotonicity test and *in vitro* ocular irritancy test.
- Bimatoprost thermosensitive ophthalmic *in situ* gel showed sustained drug release with isotonic and non-irritant properties and the formulation was found to be stable for a period of three months.

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