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Development and evaluation of a pH triggered *in situ* ocular gel of brimonidine tartrate

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ABSTRACT: The aim of the present research work was to prepare and evaluate *in situ* gel forming ophthalmic drug delivery system loaded with brimonidine tartarate (BT). In this work, carbopol and hydroxy propyl methyl cellulose (HPMC), ophthalmic gel-forming mucoadhesive polymers, which gets converted to gel in the lachrymal fluid were used as in the preparation of pH t riggered *in situ* gel formulation. The formulations were then autoclaved at 121°C for 15 min and evaluated for pH, clarity, gelling capacity, drug content, viscosity, and *in vitro* release. The developed formulations exhibited extended release of drug over a period of 8 hours in *in vitro* studies and therefore it could increase the residence time in eye. The optimized formulations were finally tested for its ability to cause irritation in male albino rabbits. The results indicated that the formulations did not irritate or damage the cornea, iris and conjunctiva.

KEYWORDS: Brimonidine tartarate; *in situ* gel; ophthalmic drug delivery; viscosity enhancement; *in vitro* release.

1. INTRODUCTION

Glaucoma is the second most common cause of blindness worldwide, after cataract and there were 60.5 million people with open angle glaucoma and angle closure glaucoma in 2010, increasing to 79.6 million by 2020. Glaucoma is generally treated using traditional surgery, pills, eye drops, laser surgery, or a combination of any of these methods. One of the major challenges in ophthalmic drug delivery systems is to design new soluble ocular carriers without causing blurred vision and to get the drug into the target site to enhance the therapeutic effects [1]. More than 90% of the marketed ophthalmic formulations are available as eye drops. But, majority of the topically applied formulations do not remain in the eye for long time as they washed off from the eye by constant blinking of the eye, high tear turnover the impermeability of the drugs across corneal epithelial membrane, lachrymal drainage, and tear fluid dilution, which usually results in poor ocular bioavailability of the drugs [2]. As a result of these factors, the ocular bioavailability for the drugs administered is very poor.

Brimonidine tartrate (BT) is a selective alpha-2 adrenergic agonist, used to lower ocular pressure by decreasing production of aqueous humor and simultaneously by increasing uveoscleral outflow. Additionally it functions well in treating glaucoma in cardiopulmonary patients. But, patients taking BT continuously suffer from problems like ocular allergy and sub-clinical inflammation in conjunctiva [3]. As marketed form of BT exists only as solution, research works are necessary for the development of delivery systems that can prolong the release of BT for a long time in the eye to reduce the frequency of administration of BT.

Ophthalmic ointments offer high ocular bioavailability of the drugs by increasing the contact time at the cornea, resisting nasolacrimal drainage, and minimizing the dilution by tears. A major disadvantage of the ointment which restricts its usage is blurred vision. Application of ointments in ophthalmic delivery system relies on the fact that the drug particles may be present in the conjunctival sac for a longer time by

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reducing the precorneal drug loss [4]. The primitive ophthalmic solution, suspension, and ointment dosage forms are clearly no longer sufficient to combat ophthalmic diseases.

Novel ocular drug delivery systems aim to maintain effective drug concentration at the desired site in the eye for sufficient period. The aim of the current research is to develop in situ gel systems, which have received considerable attention over the past few years. In situ gels are liquids initially upon administration into the eye. Due to a change in the environment, it undergoes immediate gelation in the cul-de-sac of the eye to form viscoelastic gels and retain in the eye [5]. Some of the advantages offered by *in situ* forming polymeric delivery systems include reduced wastage of drug, reduced frequency of administration, ease of administration, comfort, and improved patient compliance. Conversion of liquid to gel in situ occurs due to different stimuli like change in temperature, pH and solvent exchange. *In situ* gels are usually administered either by vaginal or ocular or rectal or oral or intravenous or intraperitoneal routes. Numerous natural and synthetic polymers are generally used in the formulation and development of *in situ* gels. The use of carbopol for the preparation of *in situ* gelling systems is due to its ability of the aqueous solutions to transform into a stiff gel at elevated pH [6]. But, the amount of carbopol necessary to form a gel produces formulations with high pH, which are difficult to be neutralized by the buffering action of the ocular tear fluid [7, 8]. Hence, a reduction in the amount of carbopol, without compromising the gelling capacity and rheological properties of the *in situ* gel formulation will be useful for achieving the desired objectives.

Low bioavailability of drugs is observed if they are administered as conventional liquid ophthalmic formulations due to high tear turnover rate, low residence time and constant lacrimal drainage in the eye, which results in the residence time of 5 min in the eye. Thus, ophthalmic solutions cannot provide and maintain an adequate concentration of drug in the precorneal area. More than 75% of the applied ophthalmic solutions are lost either through the nasolachrymal drainage or either absorbed systemically through the conjunctiva, which lowers the ocular drug availability. To overcome these problems, researchers have widely investigated and designed systems such as electrolyte triggered in situ gel, niosomal *in situ* nasal gel, sustained release mucoadhesive *in situ* gel and ocular *in situ* gel [9-12].

In the present study, *in situ gel* systems have been designed as an alternative drug delivery platform, which can reside for a longer time and prolong the release of the drug in the eye, *in situ* gel formulation of BT using carbopol 940, and HPMC via pH triggered gelling system was investigated and evaluated for the gel parameters, *in vitro* and *in vivo* evaluation.

2. RESULTS AND DISCUSSION

2.1. Preparation of Formulations

In situ gels prepared using carbopol have excellent property to transform into a gel from aqueous solutions in the physiological conditions of the eye. HPMC is additionally added along with carbopol to enhance the gelling capacity, viscosity and to maintain the pH of the *in situ* gel. In this study BT loaded *in situ* gels were prepared and characterized for the management of glaucoma.

Clarity is one among the major important characteristic features of ophthalmic preparations to avoid potential irritation and damage to the eye. All the developed *in situ* gel formulations containing BT were evaluated for clarity by visual observation against a black and white background and the results indicated that the formulations were clear without any gritty particles.

2.2. FT-IR drug-polymer compatibility study

The IR spectra obtained was examined for important functional groups. The IR spectral interpretation from the IGF 6 formulation was shown in Figure 1 D and matches with the original spectra of the drug as shown in Figure 1C.

Characteristic peaks for methyl group (CH_3) was observed at 2969 cm^{-1}, methylene (CH_2) at 736 cm^{-1}, cyclohaxane (C_6H_6) at 975 cm^{-1}, and methoxy group (CH_3O), which is evident from its characteristic two peaks, confirm the BT molecule. Similarly, IR spectrum of the carbopol polymer is shown in Figure 1B and shows characteristic peaks for dimethyl group $(CH_2)_2$ at 1346 cm^{-1}, methylene group CH_2 at 2928 cm^{-1} and C=C at 1713 cm^{-1}. HPMC characteristic peaks are shown in Figure 1 A for dimethyl group $(CH_2)_2$ at 1356 cm^{-1}, methylene group CH_2 at 2935 cm^{-1}, and C=C at 1729 cm^{-1}. It was then compared with the formulation

spectrum. Results confirmed absence of chemical interaction between the drug and the excipients and the drug was found to be stable.

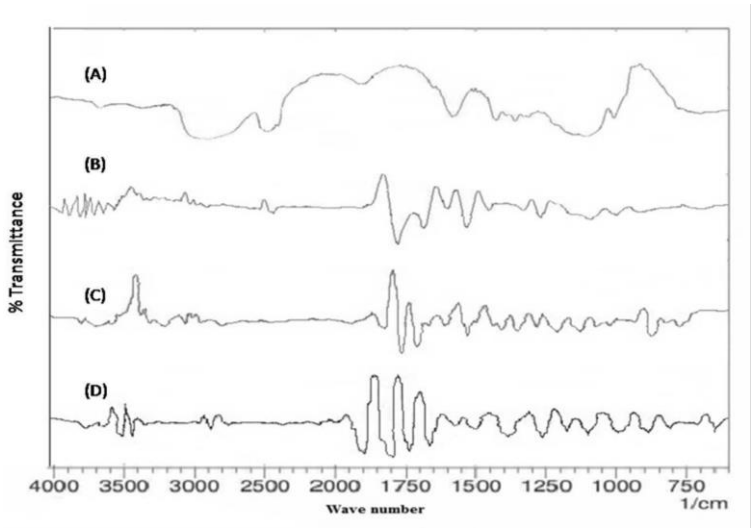

Figure 1. FTIR spectrum (A): HPMC, (B): carbopol, (C): BT, and (D): IGF6

2.3. Determination of pH

Generally, the sol–gel transition pH for *in situ* gels should be significantly lower than the pH of eye. The normal pH of the tears ranges from 6.5 to 7.6 and lack a strong buffering system. Therefore, the pH of the administered *in situ* gel formulation will determine the eye's current pH. The pH of all the *in situ* gel formulations was found to be 4.2. The formulation containing HPMC and carbopol, in the ratio of 3:2 was in liquid state at room temperature and underwent rapid transition into viscous gel at the pH of the tear lacrimal fluid (pH 6.8).

2.4. Drug content determination

Drug content was in between 64-112%. Least percentage of drug content was observed in IGF 7 formulation where the amount of carbopol was high. This higher concentration of carbopol along with HPMC prevents the drug loading, but the higher concentration of HPMC along with carbopol in IGF 3 & IGF 6 showed higher amounts of drug loading (Table 3).

2.5. Rheological Studies

In this study, formulations with (HPMC:carbopol) in the ratio 3:2 (IGF 6- 754±0.01 Cps), 1:3 (IGF 7- 545±0.06), 2:3 (IGF 8- 556±0.03), 1:1 (IGF 9- 845±0.10) had suitable viscosity in comparison with the other formulations.

2.6. Gelling capacity study

The gelling efficiency was reported in Table 2. The ocular shear rate of eye is very high, ranging from 0.03 s^{-1} during inter-blinking periods to 4250–28500 s^{-1} during blinking. Therefore, viscoelastic fluids with a viscosity that is high under the low shear rate conditions and low under the high shear rate conditions are often preferred for ophthalmic applications. Generally, an optimum *in situ* gel formulation should be a free flowing solution (with low viscosity) which can be easily applied into the conjunctival sac. The solution will then transform into a gel at the pH of the eye to increase the precorneal residence time. These enhanced residence time of the gel will increase the contact time of the BT at eye and passage of higher concentrations across the cornea to enhance the ocular bioavailability. The results of gelling studies indicated that the formulation containing HPMC and carbopol in the ratio of 3:2 was found to be more efficient in retaining drug and preventing premature drug release as compared with marketed eye drop solution.

The developed gel demonstrated adequate strength when it was pressed with a pair of fine forceps, indicating that it could withstand the low shear forces likely to be encountered in the cul-de-sac of the eye. In IGF 2 formulation comprising HPMC:carbopol (2:1), when HPMC concentration increases, the viscosity increases to maximum but no gelation is observed. But when the carbopol concentration is increased in IGF 4

HPMC:carbopol (1:2), viscosity decreases and the gel is formed but it dissolves rapidly. In IGF 6 formulation HPMC:carbopol (3:2), when the HPMC is increased and in IGF 7 formulation HPMC:carbopol (1:3) when the carbopol is increased, optimum viscosity suitable for ophthalmic application is formed with immediate gelation. Formulation IGF 6 showed an optimum viscosity which favors easy instillation of the drops into the eye and to rapidly transform to a gel upon instillation.

Table 2. Determination of physicochemical properties of gel.

Formulation	Gelling capacity	Viscosity (cp)	% Drug content
IGF1	-	644±0.10	96±0.01
IGF2	-	1069±0.05	112±0.05
IGF3	+	447±0.23	100±0.12
IGF4	+	860±0.05	88±0.11
IGF5	++	532±0.06	84±0.02
IGF6	+++	754±0.01	88±0.03
IGF7	+++	545±0.06	64±0.12
IGF8	+++	556±0.03	100±0.02
IGF9	+++	845±0.10	80±0.08

- No gelation; + Gels after few minutes, dissolve rapidly; ++ Gelation immediate, remains for few hours; +++ Gelation immediate.

2.7. *In vitro* drug release studies

The *in vitro* drug release profile in Figure 2 for the gelled samples is characteristic for hydrophilic matrices. The plot of cumulative percent of BT released against time up to 8 hours was linear as expected suggesting that drug was initially released by diffusion and not through dissolution of the hydrogel. For the marketed eye drops, the drug release was similar to the release from IGF6. Marketed formulation release was completed at the end of 5th hour and the amount of drug released was 94.5±0.51, whereas the IGF 6 formulation release was quick and similar to marketed formulation and the release extended upto 8 hours 87.46±0.15%. Drug release was rapid (35-50%) in the beginning 1 hour indicating a burst release and then declined with time in formulation IGF 6 but not in formulation IGF7. But, the *in vitro* release from IGF8, IGF9 shows slow release from the beginning. For IGF 6, more than 54.3±1.15% of the drug was released into the medium within 120 min and for IGF7 it was about 47.6±0.57%. In the case of IGF8 and IGF9, about 43.46±0.35% and 34.4±0.264% of BT, respectively, was released into the medium in 120 min and is shown in Figure 2. Correlation between viscosity and *in vitro* release was not observed in our study. As the normal residence volume of the lacrimal fluid in the human eye is only 7.5–10 µl, dissolution of BT from the *in situ* gel formulation in the cul-de-sac will occur at a comparatively slower rate than observed in the *in vitro* experiments. Hence the formulations IGF8 and IGF9 were not taken for further studies. Even though the formulation IGF8 and IGF9 shows good gelation and drug content, the formulation failed to provide extended drug release with higher concentration of HPMC:carbopol (Figure 2).

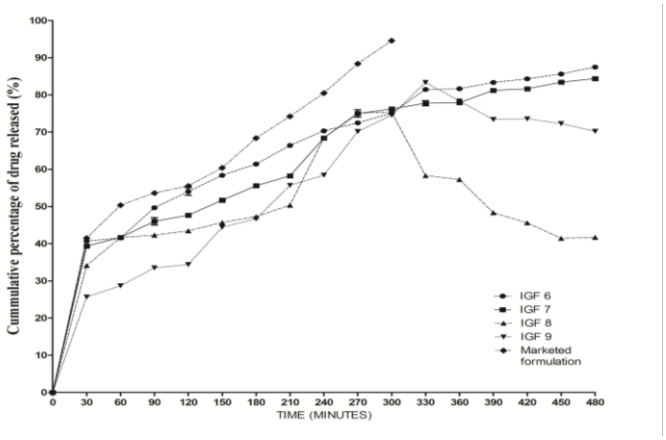

Figure 2. *In vitro* release profile of different formulations

Most of the ophthalmic vehicles are characterized by a high polymer concentration (25% poloxamer, 30% CAP), which is not well tolerated by the eye. In order to reduce the total polymer content and to enhance the gelling properties, a combination of polymers has been used in the study. Cellulose acetophthalate (CAP) latex coagulates when its native pH of 4.5 is raised by the tear fluid to pH 7.4 but IGF 6 formulation with HPMC:carbopol ratio (3:2) does not get coagulated [18].

2.8. Kinetic data model

Considering the viscosity, gelling capacity, drug content and *in vitro* release profile of the formulations, IGF6 was selected as an optimum formulation and selected for further analysis. In order to investigate the drug release mechanism, the *in vitro* release data (IGF 6) were fitted to various models. The drug release from IGF6 obeyed first order release kinetics (r^2=0.96). The release mechanism from the *in situ* gel containing dissolved drug was found to be non fickian or anomalous involving both diffusion and polymer relaxation (n=0.8). This indicates that release of BT from the *in situ* gel was dependent on two simultaneous process water migrations into the *in situ* gelling system and drug diffusion through continuously swelling gelling system. These results also state that incorporation of BT did not disrupt the formation of three dimensional network of gel.

2.9. Test for sterility

It also confirms that the gel formed *in situ* has the ability to preserve the integrity in the cul-de-sac without eroding or draining off from the eye for a prolonged period of time, which facilitates sustained release of BT. Sterility testing is an important parameter to be tested for all the formulations before administration to the animals into the eye, which unchecked may lead to false results of ocular irritation. The results of the sterility testing indicated absence of turbidity, which indicated absence of microbial growth. Thus, formulation IGF6 passed the test for sterility according to IP 1996. Terminal sterilization by autoclaving of polymeric solutions had no effect on the pH, gelling capacity, and viscosity of the formulations (data not shown).

2.10. *In vivo* ocular irritation studies

In vivo performance of formulation IGF6 *in situ* gel formulation HPMC:carbopol (3:2) showed tremendous ocular tolerance after the administration of to rabbit eyes three times a day for a week and subjected to ocular irritation study. The results indicated absence of redness, swelling or watering of eyes or ocular damage or abnormal clinical signs to the cornea or iris, or conjunctivae. Hence, the formulations were non irritant upon instillation to the eye. Considering the fact that the eye of rabbit is more susceptible to irritant substances than the eyes of human being, the results of this study indicated suitability of the formulation for administration to patients. Score evaluation of rabbit eye in modified draize score was performed. There was no reaction such as Conjunctival edema, Redness in conjunctiva, Secretion, Corneal opacity, and Iris involvement found.

2.11. Short term stability studies

Formulation IGF6 showed a drug content greater than 85% during 2 months storage at room temperature. Other test parameters: visual appearance, clarity, and pH have not shown any significant changes (Table 3). Thus, it can be interpreted that the prepared in situ gel can be stored at room temperature (24±1 ^{o}C). The pH of ophthalmic formulation was stable and hence it would not cause irritation upon administration. The viscosity of the formulation was almost same at througout the stability study, hence it is concider as stable.

Table 3. Stability data of IGF 6 stored at room temperature.

Formulation	Sampling day	Test parameters				
		Visual appearance	Clarity	pH	Drug content (%)	Viscosity
IGF 6	0	Transparent	Clear	4.2±0.01	88±0.03	545±0.06
	15	Transparent	Clear	4.14±0.03	87.6±0.01	542±0.03
	30	Transparent	Clear	4.1±0.03	86.3±0.05	538±0.02
	60	Transparent	Clear	3.88±0.10	85.2±0.03	530±0.01

3. CONCLUSION

In situ pH triggered gel formulations containing BT were successfully formulated using carbopol (a pH triggered gelling agent) and HPMC (a viscosity enhancing agent). The *in situ* gelling system can extend drug release over an 8 hours period and also enhance the penetration of the drug to the ocular regions. The formulation was stable for 60 days at room temperature and passed the sterility test. The results of the ocular irritation study did not show signs and symptoms of ocular damage and irritation to rabbit eyes. Thus, *in situ* ocular gels prepared using carbopol and HPMC loaded with BT can be less susceptible to drainage and it may produce longer residence time in the eye. These results support that BT *in situ* gel can be a promising drug delivery system alternative to conventional eye drops. But the study also warrants pharmacokinetic and *in vivo* studies. The novel approach can be used to enhance residence time of other drugs in the eye.

4. MATERIALS AND METHODS

4.1. Materials

BT was a kind gift from Centaur Pharmaceuticals Pvt. Ltd, India and was used without further purification. Hydroxy propyl methyl cellulose, carbopol, benzalkonium chloride was purchased from Hi media, Mumbai. Dialysis membrane (mol wt cut-off: 12000 Da; flat width 25 mm, diameter of 16 mm, capacity 60 ml ft) was purchased from Sigma Aldrich Chemicals, Saint Louis, MO. Artificial lacrimial fluid was prepared according to the official methods of Indian Pharmacopoeia.

4.2. Preparation of Formulations

Medicated pH triggered *in situ* gel formulations containing BT were prepared by gradually dissolving polymer HPMC in 50 ml of water by stirring. Separately, the drug was dissolved in the water. This solution was then mixed by gentle stirring with the HPMC solution and the preservative benzalkonium chloride was added. Then carbopol was sprinkled into the solution and gently stirred with a magnetic stirrer for 3 hours at 50 rpm until a homogeneous clear solution is obtained. The solutions were then equilibrated for 24 hours [13]. Totally nine formulations were prepared and their compositions are given in the Table 1.

Table 1. Composition of *in situ* gel formulation.

Formulation	Drug (mg)	HPMC (mg)	Carbapol (mg)	Preservative (µl)	Dist water (ml)
IGF1	7.5	100	100	0.1	50
IGF2	7.5	200	100	0.1	50
IGF3	7.5	300	100	0.1	50
IGF4	7.5	100	200	0.1	50
IGF5	7.5	200	200	0.1	50
IGF6	7.5	300	200	0.1	50
IGF7	7.5	100	300	0.1	50
IGF8	7.5	200	300	0.1	50
IGF9	7.5	300	300	0.1	50

4.3. FT-IR drug-polymer compatibility study

Drug-polymer interaction study was carried out to check the compatibility between drug and selected polymers. The sample of BT, HPMC, carbopol and IGF 6 of about 10 mg was individually mixed with 100 mg of potassium bromide to make the pellet and it was scanned under FT-IR spectroscopy from 400–4000 cm^{-1} using fourier transform infrared spectrophotometer (Jasco, Japan).

4.4. Gelling capacity study

Gelling capacity of the prepared formulations was determined by placing a drop of the sample into a watch glass containing 5 ml of pH 6.7 artificial lacrimial fluids (ALF) equilibrated at room temperature [14].

The visual assessment of gel formation and dissolution with time record was performed and the time recorded (Table 2).

4.5 Determination of pH

The pH of the formulations was measured by digital pH meter (Systronics System 361). The instrument was calibrated before each trial using standard buffers.

4.6. Rheological Studies

The viscosity of the formulations was measured using a Brookfield digital viscometer (RVT model) with the spindle No 63. The viscosity was measured at 100 rpm.

4.7. Drug content determination

For determination of drug content, 1 ml of different *in situ* gelling formulations was placed in a dialysis membrane containing 5 ml of ALF freshly prepared and equilibrated at room temperature and the gel formation was assessed visually. Then the tubes were kept in a beaker containing 50 ml of ALF and formulations were dialyzed for 30 min at 50 rpm and the dialysis medium was replaced with fresh quantities of STF. Samples were analyzed spectrophotometrically at 248 nm using a blank [15].

4.8. *In vitro* drug release studies

The *in vitro* release study of BT from the prepared *in situ* gelling system was studied using dialysis tube method. Two milliliter of formulation was filled into a dialysis bag (molecular weight cutoff 12,000-14,000 daltons) which was placed in a beaker containing 50 ml of ALF. The beaker was placed with a magnetic stirrer, maintained at 50 rpm at 37°C±0.5°C temperature. Over a period of 8 hours, 2 ml of buffer solution was withdrawn and replaced by an equal volume at constant intervals and the amount of BT was estimated using UV spectrophotometer. The data obtained from *in vitro* drug release studies were fitted to various release kinetic models like zero order, first order, Higuchi model, Korsmeyer–Peppas and the best fit model was selected to understand the mechanism of drug release of BT from the prepared *in situ* gel.

4.9. Test for sterility

Test for sterility was carried under sterile conditions to avoid accidental contamination of the product during the test. A direct inoculation method was employed as per Indian Pharmacopoeia (IP 1996). Soybean casein digest medium and alternate fluid thioglycollate medium were used as a culture media during the test for sterility. The reconstituted mixture (30 gm) was suspended in distilled water (1000 ml). The mixture was sterilized by autoclaving at 121°C and 15 lbs pressure for 15 min. After cooling, 100 ml of the media was transferred to the test tube and sterile *in situ* gel was removed from the bottle using syringe. This was diluted with fluid from solution under laminar airflow. This solution was finally passed though the 0.45µm filter membrane by applying vacuum. The filter paper was removed and placed in the two media under incubation for seven days at 37°C. Daily both the media were observed for the presence of any microbial contamination and compared with *S.Aureus* and *C.Albicans* [16].

4.10. *In vivo* ocular irritation studies

Male New Zealand albino rabbits weighing 2.5-3.0 kg were used to evaluate the potential of the formulations to cause acute eye irritation / corrosion. The Institutional Animals Ethical Committee of PSG Institute of Medical Science and Research approved the study and the study was performed within the guidelines of Council for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India (Approval No 348/2017/IAEC). Totally six animals, divided into three groups were used for the study. Animals were housed in standard cage in a ventilated room under room temperature of 24 ± 1°C with 12 hours light/dark cycle. Animals were acclimatized for minimum of 5 days to the laboratory conditions prior to experimentation and were fed with standard food pellets and water ad libtum. The right eye of each rabbit served as control while the left eye was used for testing the ability of the formulation to cause ocular irritation. The formulation (50 µl of ISGF6) was instilled into the conjunctival sac of the test eye in each of the rabbits by gently pulling the lower lid away from the eye ball. After dosing the formulation into the eye, the eye lids of the rabbits were held together for 1 s to avoid loss of the dosage form.

The potential ocular irritancy and damaging effects of the selected ophthalmic gel forming solution were evaluated in male New Zealand albino rabbits as per modified Draize test. For this purpose, six rabbits

(two rabbits per group) were selected for the study. In the first group, 50 µl of test formulation with preservative was administered to the left eye three times a day for a week, and 0.9% NaCl solutions were used for control eyes. Second group of animals was administered *in situ* gel without drug and third group was administered with the medicated *in situ* gel containing BT without preservative. The eyes were evaluated for signs of irritation to conjunctiva, iris and cornea at 1, 24, 48 and 72 hours.

4.11. Short term stability studies

Stability studies were performed for the optimized formulation ISGF6 after subjecting it to sterilization. Sterile gel forming ophthalmic solutions were filled in glass vials and closed with a rubber stopper. The formulations were maintained at room temperature (24±1°C) for 60 days. The samples were withdrawn periodically and estimated for drug content, pH, visual appearance, and gelation [17].

4.12. Statistical analysis

All data analyses were performed with statistical package for social science (SPSS version 20.0, Chicago, IL, USA); a significance level of 0.05 was considered as statistical significance. Data were expressed as mean ± SD, median (inter quartile range) or number (%), as appropriate. Chi-square test was used to test the association for categorical variables. The Student's t-test was done for group comparisons.

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