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FORMULATION DEVELOPMENT AND EVALUATION OF pH TRIGGERED IN SITU OPHTHALMIC GEL OF BESIFLOXACIN HYDROCHLORIDE

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ABSTRACT

The aim of the present work was to formulation and evaluation of pH Triggered in-situ Ophthalmic Gel of Besifloxacin Hydrochloride to overcome the drawbacks obtained by conventional eye drop. There are two independent variables were used i.e. Carbopol 934 and HPMC K100. Carbopol 934 were used as gelling agent and HPMC K100 were used as bioadhesive polymer. Besifloxacin Hydrochloride shows activity against a wide range of Gram-positive and negative ocular pathogens: examples are *Corynebacterium pseudodiphtheriticum, Moraxella lacunata, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pneumoniae and Streptococcus salivarius*. The in situ gelling system involves sol-to-gel transition in the cul-de-sac upon instillation to avoid pre corneal elimination. The formulations were prepared by 3^2 factorial design. The prepared formulations were evaluated for clarity, pH, viscosity, Bioadhesive strength of gel, gel strength gel, Drug Content, In-vitro Drug Release Study, Antibacterial Activity, Isotonicity Evaluation, HET-CAM Test and stability studies. The drug content was in the range of 97-99.57 %. Formulation F5 selected as optimized on the basis of evaluation. It shows highest drug release upto 8hrs. It shows good antibiotic activity against Staphylococcus aureus. The optimized formulation was isotonic with blood cells. It passes sterility test. The optimized formulation passes the ocular irritancy test i.e. HET-CAM Test. The formulation kept for the stability study for 3 months. Short term stability study indicates that room temperature $40^0 \pm 2^0$ was appropriate storage condition for formulations.

Keywords: pH Triggered, bioadhesive polymer, Carbopol 934, HPMC K100, HET-CAM Test, Antibacterial Activity.

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INTRODUCTION

One of the most challenging and interesting drug delivery is ophthalmic drug delivery for the pharmaceutical scientist. The anatomy, biochemistry and physiology of the eye render this organ delicately impermeable to foreign substances. To evade the protective barriers of the eye, the challenge to the formulator is to circumvent the protective barriers of the eye without causing permanent tissue damage. The development of newer, more sensitive diagnostic techniques and therapeutic agents render urgency to the development of more successful ocular delivery system[1]. Several new preparations have been developed for ophthalmic use, not only prolong the contact time of the vehicle at ocular surface, but also to slow down the elimination of the drugs. This problem can be overcome by using *In-situ* gel forming ophthalmic drug delivery systems, prepared from polymers that exhibit reversible phase transition and pseudo-plastic behavior to minimize interference with blinking. In situ gel forming drug

delivery is a type of mucoadhesive drug delivery system. Such system can be formulated as liquid dosage form suitable for administration by instillation in to the eye, which upon exposure to the eye, shift to the gel phase depends upon physiological pH condition of eye [2,3]. pH sensitive polymers contain pendant acidic or basic groups that can either accept or release protons in response to changes in environmental pH. In case of weakly acidic group, swelling of hydrogel increases as the external pH increases, while decreases in case of weakly basic groups. Gelling of the solution is triggered by a change in pH[4]. In situ gelling system becomes very popular nowadays because of their several advantages over conventional drug delivery systems like sustained and prolonged release of drug, reduced frequency of administration, improved patient compliance and comfort[5]. In this study, In situ gelling system of Besifloxacin Hydrochloride were prepared using polymers carbopol 934 and HPMC K100. Carbopol 934 used in concentration 0.1-0.3 % w/v and HPMC K100 was in concentration 0.6-1% w/v.

MATERIAL AND METHOD

Besifloxacin Hydrochloride was obtained from Ajanta Pharma Ltd. Kandiwali West, Mumbai, India as a gift sample. Carbopol 934 and HPMC K100 were purchased from Research-Lab Fine Chem. Industry –Mumbai.

Development of Besifloxacin Hydrochloride Ophthalmic Gel

Composition of formulation batches as per 3^2 factorial design shown in Table 1.

Table 1: Composition of Formulation Batches As Per 3^2 Factorial Design

Formulation code → / Ingredients ↓	F1	F2	F3	F4	F5	F6	F7	F8	F9
Besifloxacin Hydrochloride (w/v)	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
Carbopol 934 (w/v)	0.1	0.2	0.3	0.1	0.2	0.3	0.1	0.2	0.3
HMMC K100 (w/v)	0.6	0.6	0.6	0.8	0.8	0.8	1	1	1
Disodium edentate (w/v)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Benzalkonium Chloride (w/v)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Purified water (ml)	100	100	100	100	100	100	100	100	100

Formulation of Ophthalmic in situ Gel:

The quantities of drug and other ingredients were weighed as per Table no.1 and formulations were prepared in following manner [6]:

Cleaning of glassware and container: All the glassware's were washed using distilled water. After washing glassware's dried at 160-165° for 1 hr in hot air oven for sterilization.

Preparation of solution 'A': Besifloxacin Hydrochloride was accurately weighed (0.6 g) and then dissolved in pH 6.8 phosphate buffer (50ml).

Preparation of polymer dispersion 'B': The Carbopol 934 and HPMC K100 was accurately weighed and then dissolved in distilled water (50ml) was allowed to hydrate for 24 h to produce a clear solution. The Benzalkonium chloride and Disodium edetate was added to the above polymer dispersion.

Mixing of ophthalmic formulation: The solution 'A' and solution 'B' was mixed with continued stirring and pH of formulation was maintained using 0.1N NaOH.

Sterilization of ophthalmic formulation: Prepared solutions were autoclaved at 121° for 15 min.

Aseptic filling to container: The formulation was aseptically transferred to previously sterilized glass bottles and sealed.

Evaluation of in-situ ophthalmic gel of besifloxacin hydrochloride

Physical parameter:

Clarity:

The formulations were visually checked for the clarity.

pH:

pH of each formulation was determined by using Digital pH meter (Sistronic Digital pH meter 335). This was previously calibrated by standard pH 4 and pH 7. The pH values were recorded immediately after preparation.

Rheological study:

Viscosity:

The rheological properties of gels were determined by the Brookfield viscometer; type DV-II + PRO using spindle no.61& 63. At two different pH the viscosity of formulations was taken at pH 6.8 and pH 7.4.

Measurement of the gel strength:

Measurement of the gel strength was carried out in 50 ml graduated cylinder. 25ml of sample was put in graduated cylinder. On the surface of gel a weight of 14.33g was placed. The gel strength was determined by the time in second required to penetrate the weight 5 cm into the gel at physiological temperature[12]. All measurements were performed in triplicate (n=3).

Bioadhesive Strength

"Detachment Stress is the force required to detach the two surfaces of mucosa when a formulation/gel is placed in between them". The detachment stress was measured by using a modified analytical balance (A). A fresh goat membrane was obtained from local slaughter house[7]. A section of fresh mucosa was cut from the goat eye and washed with saline solution.

Fabrication of equipment:

The equipment was fabricated by us in the laboratory as shown in figure 1. A double beam physical balance was taken, both the pans were removed. The left pan was replaced with a brass wire, to which was hanged a teflon disc (D), also locally fabricated. The dimensions are 2 cm height and include an expanded cap of diameter 3.8 cm and thickness 2 cm. Another teflon disc of 2 cm height and 1.5 cm diameter was placed right below the suspended disc upon the base of the balance. The right pan (B) was replaced with a lighter pan so that, the left pan weighs 5.25 gm more than the right pan. The lower Teflon block was intended to hold the mucosal tissue (E) of goat corneal membrane and to be placed in a beaker containing simulated tear fluid pH 7.4[7].

Measurement of adhesion force:

Goat corneal membrane was obtained commercially; the cornea was collected into a sterile container containing sterile buffer solution of pH 7.4. The corneal membrane brought was stored in a refrigerator until use.

The following procedure was used for all the test formulations using the above equipment. The goat corneal membrane was removed from refrigerator and allowed to attain equilibrium with ambient conditions in the laboratory. The goat corneal membrane was carefully excised, without removing connective and adipose tissue and washed with simulated tear fluid solution. The tissue was stored in fresh simulated tear fluid solution. Immediately afterwards the membrane was placed over the surface of lower teflon cylinder (E) and secured. This assembly was placed into beaker containing simulated nasal solution pH 7.4 at 37 ± 2°.From each batch, some quantity of gel was taken and applied on the lower surface of the upper teflon cylinder. The beaker containing mucosal tissue secured upon lower cylinder (E), was manipulated over the base of the balance so that, the mucosal tissue is exactly below the upper cylinder (D). The exposed part of the gel was wetted with a drop of simulated tear fluid solution, and then a weight of 10 gm was placed above the expanded cap, left for 10 minutes. After which the gel binds with mucin. The weight was removed. Then slowly and gradually weights were added on the right side pan till the gel separates from the mucosal surface/ membrane. The weight required for complete detachment is noted (W1) (W1-5.25G)) gives force required for detachment expressed in weight in grams. Procedure was repeated for two more times. Average was computed and recorded.

Figure 1: Modified Bioadhesion apparatus
A: Modified balance, B: Weighing pan, C: Weight
D: Upper taflon disc, E: Lower taflon disc
F: Corneal membrane G: Simulated tear fluid.

Calibration of test equipment:

Initially, a gel from the same batch was taken ten times and individual force required for complete detachment was noted and SD was calculated.

Force of adhesion (N):

$$\text{Bioadhesive strength} = \frac{\text{bioadhesive strength}}{1000} \times 9.81$$

$$\text{Bond strength (N/m2)} = \frac{\text{bioadheforce of adhesion (N)}}{\text{surface area of disk (m2)}}$$

Drug Content

For determination of drug content 1ml of formulation was taken and diluted with 100ml of phosphate buffer. Out of 100ml 5ml was withdrawn and again diluted with 25 ml of phosphate buffer[8]. The concentration of Besifloxacin Hydrochloride was determined by using UV Visible Spectrophotometer at 289nm.

In-vitro Drug Release Study

The laboratory designed diffusion cell was shown in figure 2. With the help of diffusion cell through egg membrane as a biological membrane the in-vitro drug release study of the formulation was carried out. Diffusion cell with inner diameter 1.4cm was used for the study. The formulation 1 ml were placed in donor compartment and in the receptor compartment freshly prepared 100 ml artificial tear fluid solution (sodium chloride 0.670g, sodium bicarbonate 0.200g, potassium chloride 0.248 g, calcium chloride dehydrated 0.008g, distilled water q.s. 100ml) was placed. Egg membranes were mounted in between donor and receptor compartment. The position of the donor compartment was adjusted so that egg membrane just touches the diffusion medium. The whole assembly was placed on the thermostatically controlled magnetic stirrer[12]. The temperature of the medium was maintained at 37° ± 0.5°. 2ml of sample is withdrawn from receiver compartment after 30 min, 1, 2, 3, 4, 5, 6, 7 & 8 hrs and same volume of withdrawn was replaced by fresh medium. The withdrawn samples was diluted to 10ml in

a volumetric flask with fresh artificial tear fluid and analyzed by UV spectrophotometer at 289 nm.

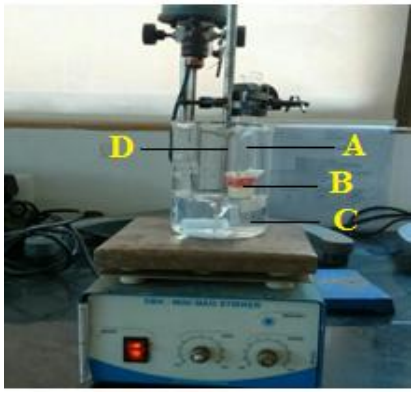


Figure 2: Laboratory designed diffusion cell.
A- Test tube containing formulation. B- Egg membrane.
C- beaker containing simulated tear fluid solution.
D- Magnetic stirrer.

Antibacterial Activity

The determination of antibacterial activity of formulations was carried out by using an agar medium. Standard petri dishes (9cm diameter) containing medium to a depth of 0.5cm were used for the study. The sterility of the lots was controlled before use. Suspension was prepared by suspending 1-2 colonies of Staphylococcus aureus (MH1714) from 24hr cultures in nutrient agar medium into tubes containing 10 ml of sterile saline. The tubes were diluted with saline. The inoculum (0.5mL) was spread over the surface of agar and the plates were dried at 35˚ for 15 min prior to placing the formulation. The bores of 0.5cm diameter were prepared and 2 drops of formulation (0.6%w/v) were added in the bores. After incubation at 35˚ for 24hrs, the zone of inhibition around the bores was measured[9,10,11,12].

Isotonicity Evaluation

For the study of isotonicity formulations were mixed with few drops of diluted blood on a slide. The diluted blood was prepared by using Grower's solution and Slide was observed under microscope at 45x magnification[12,13]. The shape of blood cells were compared with standard marketed ophthalmic formulation.

Test for sterility

The sterility test was carried out according to IP (2014). The method direct inoculation was used for sterility test. 5 ml of sterile fluid thioglycolate medium, artificial fluid thioglycolate and Soyabean casein digest medium were used. The three set were prepared each set containing three tubes of each medium. The first set, negative control was sterile media. The second set, positive control was the sterilized media incubated with staphylococcus aureus. And the third set was test set. The 1mL sterile optimized formulation was taken and this formulation was diluted with 100mL sterile water for injection, from this 5mL test solution were added in each medium. The incubation time was 14 days for detection of bacterial and fungal contamination at 20-25 ˚. The visual inspection of turbidity was used as method of detection[12,14,15].

HET-CAM Test

The Hen's Egg Test on the Chorioallantoic membrane (HET-CAM) is another alternative method to animal experimentation for assaying corrosives or sever ocular irritations, using Chorioallantoic membrane of embryonated hen's egg. This test assesses the damage to this membrane to determine the potential irritatation to the conjunctiva. Its well developed vascularization provides an ideal model for studies of ocular irritation[16,17].

9 Days fertilized hen's eggs
↓
The CAM (25%) is treated with the test item for 30 sec.
↓
Test item was wash off
↓
Effect assessed within 5 min.

End Point: Redness, Irritation

Stability studies

For the stability study the formulation was taken for 3 months. The test condition for stability study was temperature condition was at room temperature ($40^0 \pm 2^0$) a Relative humidity was 75± 5%. The formulations were evaluated mainly for their physical characteristics at the predetermined intervals of 30 days like appearance, clarity, pH, viscosity and drug content[18,19].

RESULT AND DISCUSSION

Physical parameter

Clarity

On careful visual inspection against dark and white background, all the prepared ophthalmic gel formulations were found to be free from any suspended particulate matter. All the formulations were found to be clear. The prepared formulations are as shown in Figure 3.

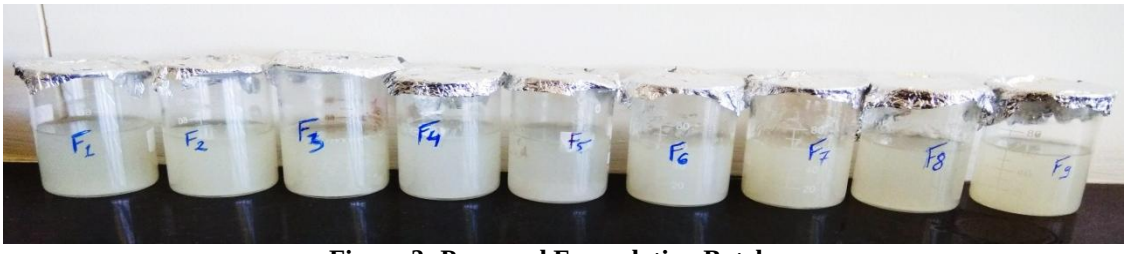


Figure 3: Prepared Formulation Batches

pH

The pH values of formulations were shown in Table 2. The pH of all the formulations from F1 to F9 was found to be in the range of 6.67 to 6.84. Ideally, the ophthalmic solutions should passes pH in the range of 6.5-8.5, so as to minimize discomfort or excessive tear flux causing faster drainage of the instilled dose due to corneal irritation.

Rheological study

Viscosity

The Viscosity profile of formulations at pH 6.8 and pH 7.4 is shown in Figure 4 and 5 respectively**.**

Viscosity v/s rpm plots for all formulations shows decrease in viscosity as shear rate (rpm) was increased which indicate that gel has the pseudo plastic flow. As pH was increased the increase in viscosity was observed. Concentration of Carbopol 934 and HPMC K-100 was a major factor affecting viscosity of formulations. In combination with Carbopol 934 and HPMC K-100 was shown considerable increase in viscosity when concentration of Carbopol 934 is 0.3% w/v & HPMC K-100 is 1% w/v.

Table 2: pH Values Of Formulations

S. N.	Formulation code	Observed pH (±S.D.)
1	F1	6.82 ± 0.001
2	F2	6.84 ± 0.001
3	F3	6.78 ± 0.002
4	F4	6.69± 0.004
5	F5	6.80± 0.001
6	F6	6.86 ± 0.001
7	F7	6.81 ± 0.01
8	F8	6.79 ± 0.004
9	F9	6.67 ± 0.001

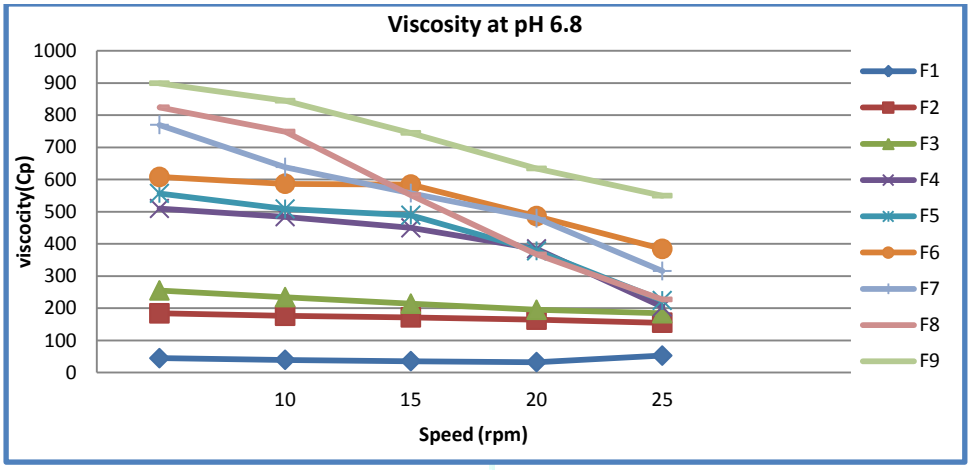


Figure 4: Viscosity profile of formulations at pH 6.8

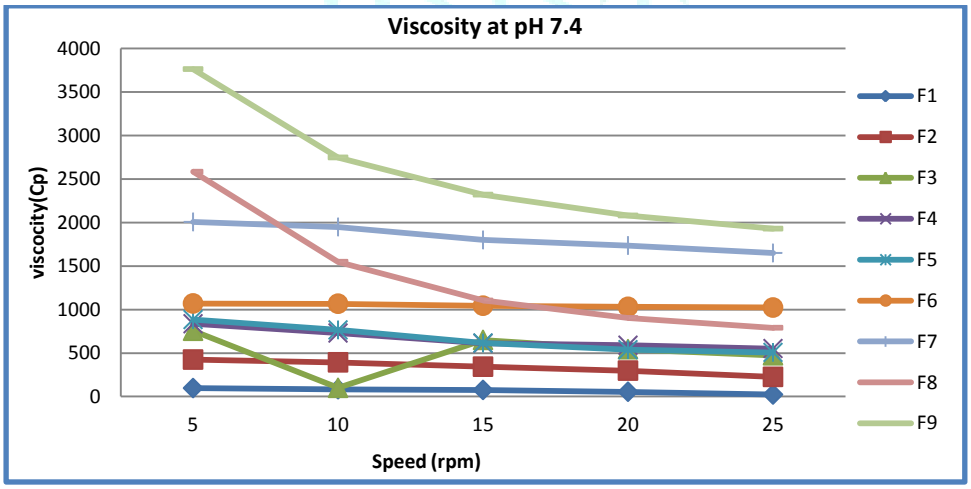


Figure 5: Viscosity profile of formulations at pH7.

Measurement of the Gel Strength

The gel strength of ophthalmic formulations is shown in Table 3.

The gel strength was found to be affected by concentrations of gelling agent, Bioadhesive polymers and also by the pH. Optimal bioadhesive gel must have suitable gel strength so as to be administered easily and can be retained Ocular region without leakage after administration. Gel strength of all formulations showed comparable results as that of viscosity results.

Table 3: Gel Strength of Formulations

S. N.	Formulation code	Gel strength (sec) ±S.D.)
1	F1	0.58±0.005
2	F2	0.61 ± 0.01
3	F3	0.84 ±0.07
4	F4	1.05 ± 0.05
5	F5	1.30 ± 0.08
6	F6	1.50 ± 0.43
7	F7	1.50 ± 0.41
8	F8	2.26 ± 0.12
9	F9	2.40 ± 0.04

Bioadhesive strength

The detachment stress of formulation is shown in Table 4.

Bioadhesive force means the force with which gels bind to ocular mucosa. Greater bioadhesion is indicative of prolonged residence time of a gel and thus prevents its drainage from cul-de-sac. The bioadhesion force increased significantly as the concentration of bioadhesion polymers increased. The Detachment Stress was determined for ophthalmic gels. Results of this test indicate that the variable Carbopol 964 and HPMC K100 both are having effect on bioadhesive strength. It shows that bioadhesive force was increased with the increasing concentration of the Carbopol 964 and HPMC K100.

Drug content

The percent drug content of ophthalmic gel formulations was shown in Table 5.

The percentage drug content of all prepared ophthalmic formulations was found to be in the range of 97-99.57 %. Therefore uniformity of content was maintained in all formulation.

Table 4: Bioadhesive Strength of Formulations

Formulation code	Detachment Force (N) (±S.D)
F1	0.1898 ± 0.035
F2	0.2019±0.027
F3	0.2424 ±0.0005
F4	0.3507 ± 0.005
F5	0.4393 ± 0.005
F6	0.4869± 0.005
F7	0.5077± 0.005
F8	0.6407 ± 0.005
F9	0.6457± 0.0005

In-vitro drug release study

Out of nine formulations maximum release after 8 hrs was found for F5 formulation. This indicates release of 99.1 % drug availability.

In-vitro drug release profile of formulations was shown in Figure 6.

Table 5: Percent Drug Content of Ophthalmic Gel

Formulation Code	Drug content (%) (±S.D.)
F1	98.86± 0.12
F2	98.54± 0.17
F3	98.54±0.16
F4	98.97± 0.065
F5	99.57± 0.172
F6	97.67± 0.113
F7	98.97± 0.13
F8	98.34± 0.17
F9	97.58± 0.12

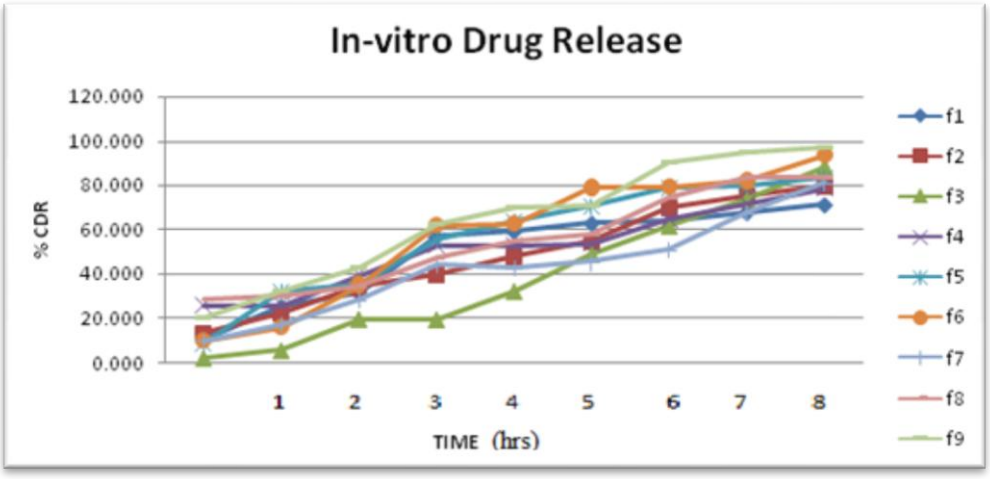


Figure 6: In-vitro drug release profile of formulations

Antibacterial activity

The standard value of Besifloxacin Hydrochloride against *Staphylococcus aureus* for maximum zone of inhibition is 30 mm. The study indicates that Besifloxacine Hydrochloride retained its antibacterial of efficiency when formulated as an ophthalmic *in-situ* gel and drug was active against selected strains of micro-organism. F5 formulation showed - 27.12 mm zone of inhibition and 93.73 % efficacy.

The zone of inhibition observed for selected micro-organism is shown in Figure 7.

Results obtained from antibacterial activity of F5 formulation resembles to release profile of drug which indicate the dependency of the antibacterial activity with the drug release from formulation.

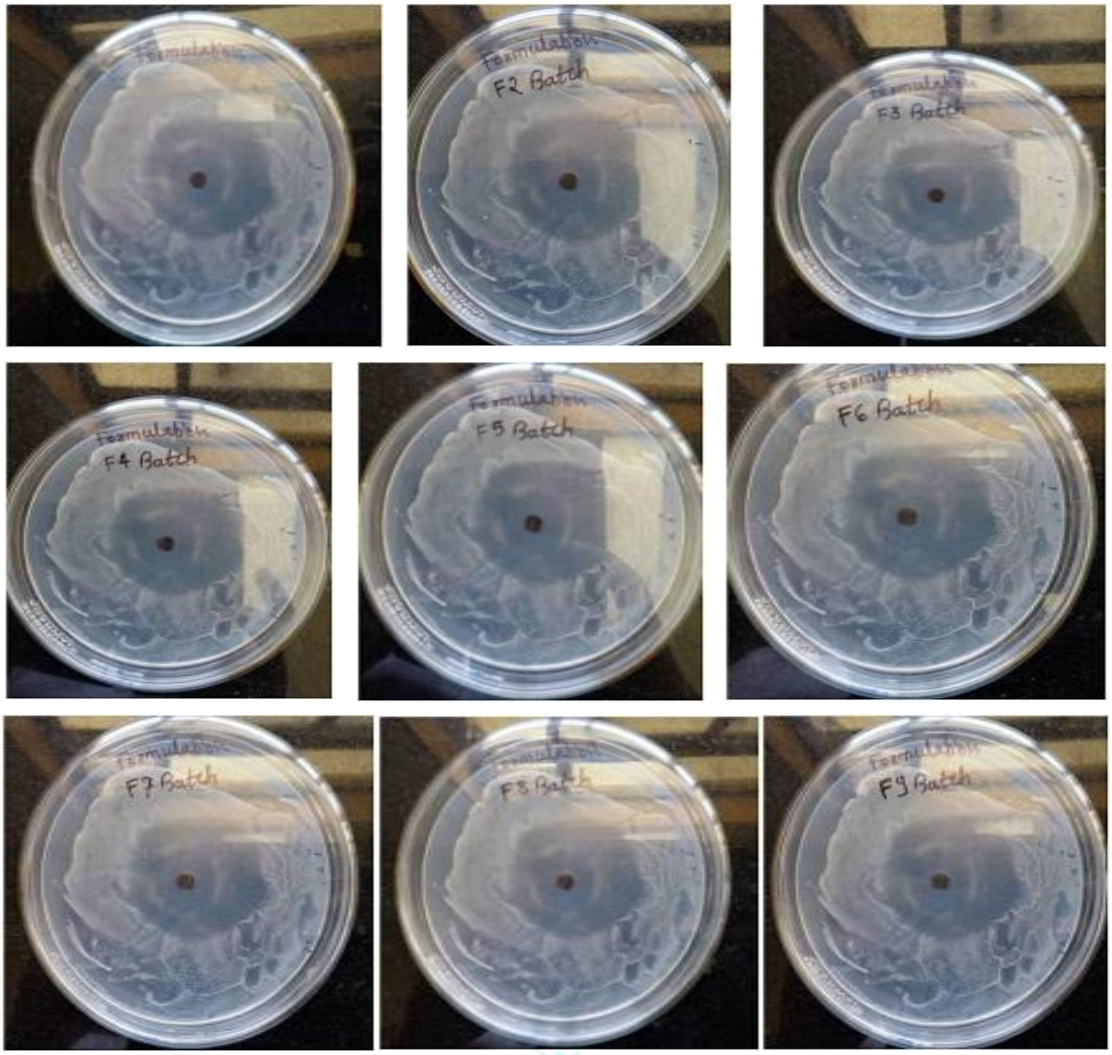


Figure 7: Zone of Inhibition of Prepared Formulations

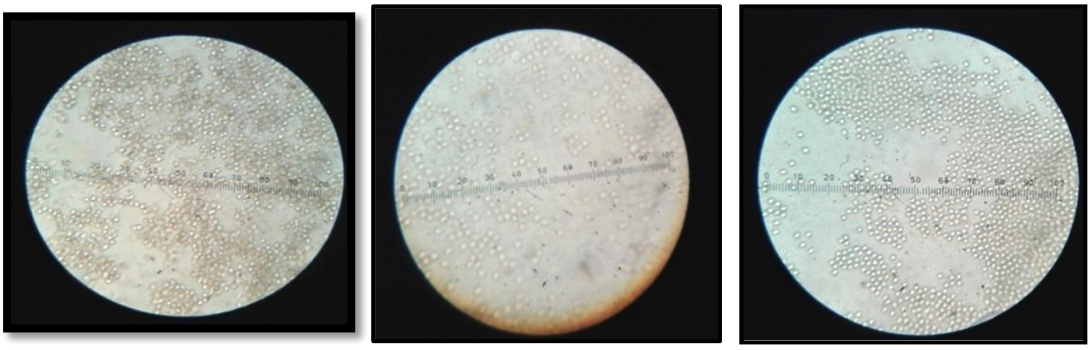
Test for sterility

There was no appearance of turbidity and hence no evidence of bacterial growth when optimized formulation was incubated for 14 days at 30-35˚c in case of fluid thioglycolate medium and at 20-25˚ in case of soyabean-casein digest medium. The preparations examined, therefore, passed the sterility test.

Isotonicity Evaluation

The shape of blood cells, blood cells with Besifloxacin Hydrochloride F5 and blood cells with Besivance as marketed formulation are shown in figure 8.

Isotonicity testing of Optimized formulation (F5) exhibited no change in the shape of blood cells. The blood cell size was found in 6-7µm range which reveals the isotonic nature of the formulation as compare with standard ophthalmic marketed preparation; this indicates the maintenance of tonicity in prepared formulations.



a **b** **c**

Figure 8: Shape of Blood Cells

a, b and c are the image of blood cells, blood cells with besifloxacin hydrochloride in situ ophthalmic gel, blood cells with marketed formulation

HET-CAM Test

The results of ocular study indicate that the formulation F5 was non irritant and no ocular damage or abnormal clinical signs were visible.

The ocular irritation study on Chorioallantoic membrane of Hen's Egg's shown in Figure 9.

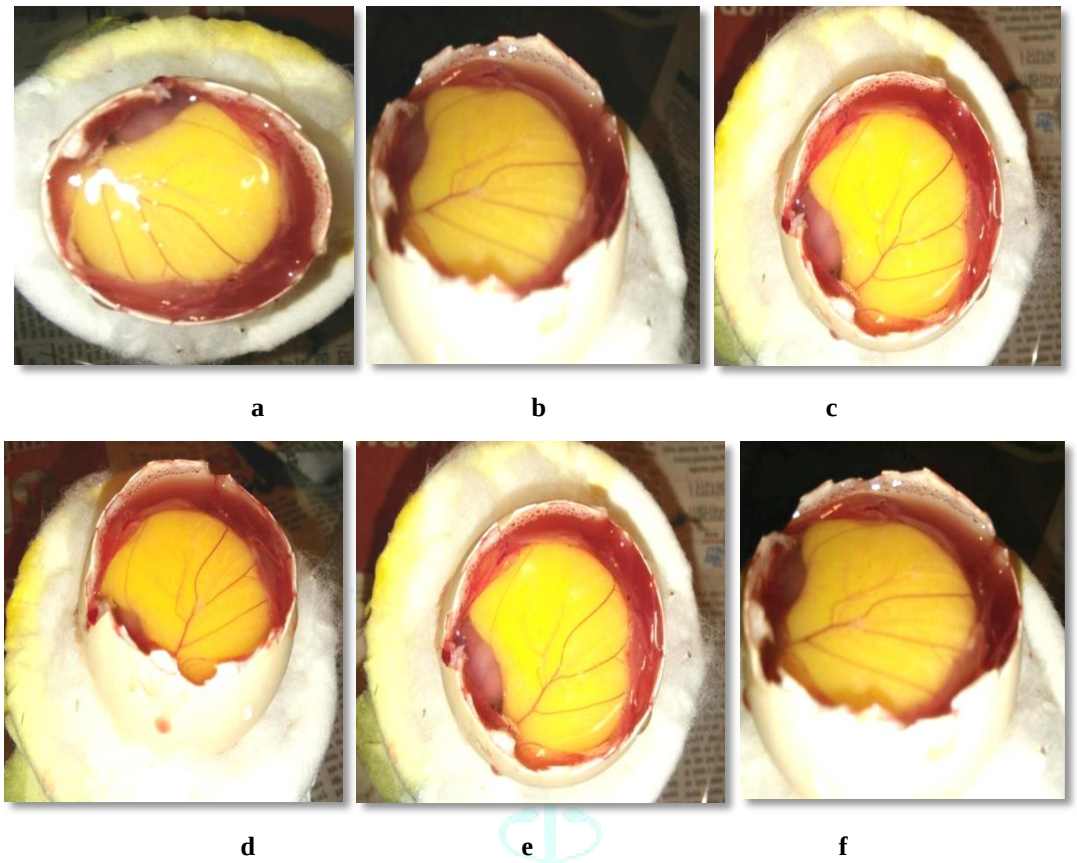


Fig. 9: The ocular irritation study on Chorioallantoic membrane of Hen's Egg's.

A, b, c, d, e, f are images of the ocular irritation study on Chorioallontoic membrane of Hen's Egg at the time of instillation, after 1min, 2 min, 3 min, 4 min, 5 min respectively.

Stability study

Stability study of optimized F5 formulation at room temperature was shown in Table 6. Formulations at room temperature were found to be stable upto3 months. There is no change in drug content, pH, clarity.

Table 6: Stability Study Data for F5 Batch

S. N.	Observation	Before Stability Testing	During Study		
			30 Days	60 Days	90 Days
1	Clarity	Clear	Clear	Clear	Clear
2	Visual appearance	Transparent	Transparent	Transparent	Transparent
3	pH	6.8	6.8	6.8	6.82
4	Drug Content	99.1%	99%	99%	98.97%

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