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Development and Characterization of In Situ Ophthalmic Gel of Bepotastine Besilate

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ABSTRACT

The aim of the present work was development and characterization of in-situ Ophthalmic Gel of Bepotastine Besilate 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. 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 , Drug Content, In-vitro Drug Release Study, 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 8 hours. It shows good antihistaminic 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 400±20 was appropriate storage condition for formulations.

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

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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) Ocular disposition and elimination of a therapeutic agent is dependent upon physicochemical, microbiological, pharmaceutical properties and ophthalmic irritancy properties of ocular dosage forms as well as the relevant ocular anatomy and physiology. Generally topical application of drugs is the method of choice under most circumstances because of its convenience and safety for ophthalmic chemotherapy.(2) Ophthalmic delivery system is a challenging area for the formulation chemist due to unique anatomy and physiology of the eye. The anatomy and physiology of the eye render this organ delicately impervious to foreign substances. The challenge to the formulator is to avoid the protective barriers of the eye without causing permanent tissue damage.(3) 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 behaviour 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. The various approaches that have been attempted to increase the bioavailability and the duration of the therapeutic action of ocular drugs can be divided into two categories. The first one is based on the use of sustained drug delivery systems which provide the controlled and continuous delivery of ophthalmic drugs.(4)

MATERIALS AND METHODS

Bepotastine Besilate was obtained from Precise Chemi pharma pvt ltd. Ghatkoper West, Mumbai, India as a gift sample. Carbopol 934 and HPMC K100 were purchased from Research-Lab Fine Chem. Industry –Mumbai.

Development of Bepotastine Besilate Ophthalmic Gel:

Composition of formulation batches as per 3^2 factorial designs 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
Bepotastine Besilate (w/v)	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
HPMC K100 (w/v)	0.6	0.8	1	0.6	0.8	1	0.6	0.8	1
Carbopol 934 (w/v)	0.1	0.1	0.1	0.2	0.2	0.2	0.3	0.3	0.3
Monobasic sodium phosphate (w/v)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Disodium edetate (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 :[5]

- **Cleaning of glassware and container:** All the glassware's were washed with distilled water and then sterilized by drying at 160-165°C for 1 hour in hot air oven.
- **Preparation of solution 'A':** Accurately weighed quantity (1.5gm) of the Bepotastine besilate was dissolved in 50ml phosphate buffer (pH6.8).
- **Preparation of polymer dispersion 'B':** The Carbopol 934 and HPMC K100 was dissolved in distilled water was allowed to hydrate for 24 hours 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 121̊ C for 15 min.
- **Aseptic filling to container:** The formulation was aseptically transferred to previously sterilized glass bottles and sealed.

Evaluation of Ophthalmic In-situ Gel Formulation:

Evaluation of in-situ ophthalmic gel of Bepotastine Besilate

1. 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 pH 4 and pH 7. The pH values were recorded immediately after preparation.

2. Rheological study:

Viscosity:

The rheological properties of gels were determined by the Brookfield viscometer; type DV-II + PRO using spindle no.61& 63.Viscosity of the formulations were taken at two different pH 6.8 and at pH7.4.

3. Measurement of the gel strength:

A sample of 25 mL of the gel was put in a 50 mL graduated cylinder. A weight of 14.33 g was placed on the gel surface. The gel strength which is an indication for the ophthalmic gel at physiological temperature was determined by the time in seconds required by the weight to penetrate 5 cm into the gel.[6]All measurements were performed in triplicate (n=3). The apparatus used for measuring gel strength is shown in Fig.1

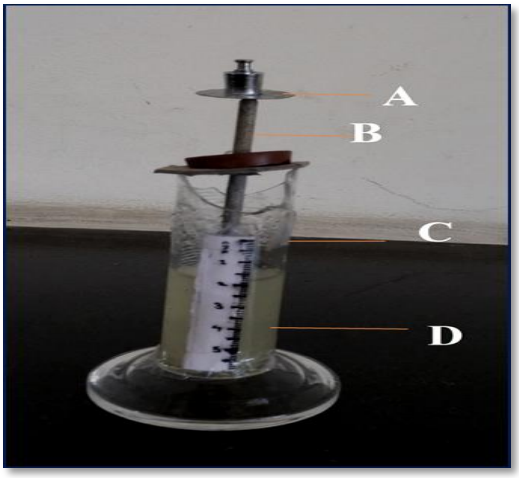


Fig.1: Gel strength measuring device

(A): Weights (B): Device (C): Graduated cylinder (D): Gel.

4. 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. A section of fresh mucosa was cut from the goat eye and washed with saline solution.[7]

i) Fabrication of equipment:

The equipment was fabricated by us in the laboratory as shown in figure 3. 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]

ii) 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°C. 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.

iii) 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. [8]

iv) Force of adhesion (N):

Bioadhesive strength = (bioadhesive strength/1000) × 9.81

Bond strength (N/m^2) = force of adhesion (N)/surface area of disk (m^2)

Fig. 2: 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.

5. Drug Content

The drug content was determined by taking 1ml of the formulation and diluting it to 100ml with phosphate buffer. Aliquot of 5 ml was withdrawn and further diluted to 25 ml with phosphate buffer. Bepotastine besilate concentration was determined at 261nm by using UV-Visible spectrophotometer.[9]

6. In-vitro Drug Release Study

In-vitro release study of the formulated ophthalmic in-situ gel was carried out by using diffusion cell through egg membrane as a biological membrane. Diffusion cell with inner diameter 1.4cm was used for the study. The formulation 1 ml were placed in donor compartment and 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 in receptor compartment. 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.[10] The temperature of the medium was maintained at 37°C ± 0.5°C. 2ml of sample is withdrawn from receiver compartment after 30 min, 1, 2, 3, 4, 5, 6, 7 & 8 h and same volume of fresh medium is replaced. The withdrawn samples was diluted to 10ml in a volumetric flask with phosphate buffer and analyzed by UV spectrophotometer at 261 nm.[11]

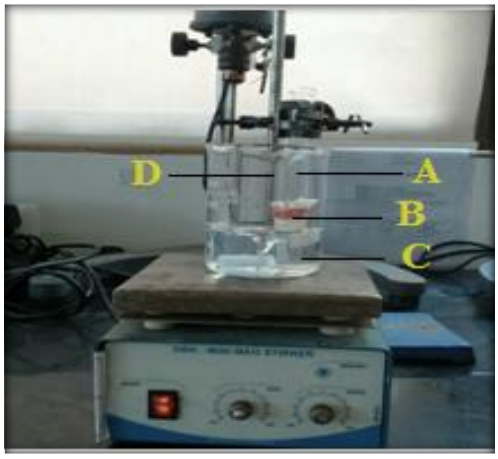


Fig. 3: Laboratory designed diffusion cell.

(A): Test tube containing formulation (B): Egg membrane (C): Beaker containing simulated tear fluid solution (D): Magnetic stirrer.

7. Isotonicity Evaluation

The 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. The shape of blood cells were compared with standard marketed ophthalmic formulation.[12,13]

8. Test for sterility

The sterility test was carried out as per IP (2014) method. The three medium were taken for this test i.e. fluid thioglycolate medium, Artificial fluid thioglycolate and soyabean casein digest medium. The three set were prepared each set containing three tubes of each medium. The first set was negative control for this sterile media is used second set was a positive control for this sterilized media inoculated with Staphylococcus aureus was used and third set was a test. 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 formulation was incubated for not less than 14 days at 20-25° C in fluid thioglycolate medium and at 20-25° C in soyabean casein digest medium to find out growth of bacteria in formulation.[14,15]

9. 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

10. 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 (400 ± 20). 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

1. 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 4.

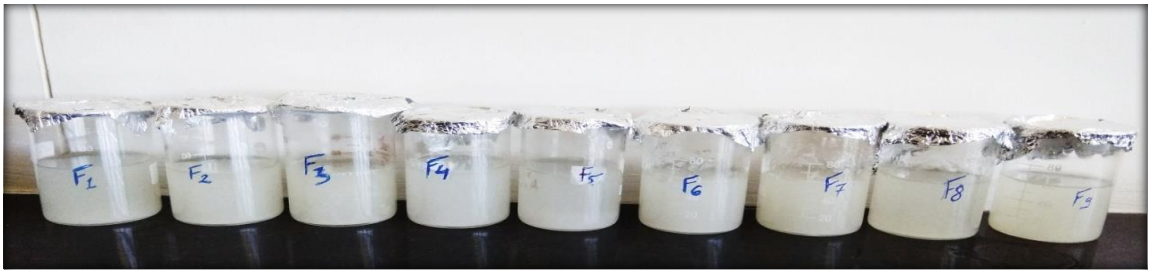


Fig.4: Prepared Formulation Batches

2. pH

The pH of all the formulations from F1 to F9 was found to be in the range of 6.67 to 6.84 pH values of formulations shown in Table 2. 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.

Table 2: pH values of formulations

Sr. No	Formulation code	Observed pH (±S.D.)
1	F1	6.83 ± 0.001
2	F2	6.84 ± 0.001
3	F3	6.79 ± 0.002
4	F4	6.68± 0.004
5	F5	6.80± 0.001
6	F6	6.85 ± 0.001
7	F7	6.81 ± 0.001
8	F8	6.78 ± 0.004
9	F9	6.67 ± 0.001

3. Rheological study

Viscosity

The Viscosity profile of formulations at pH 6.8 and pH 7.4 is shown in Fig.5 and Fig.6 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 Carbopol934 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.

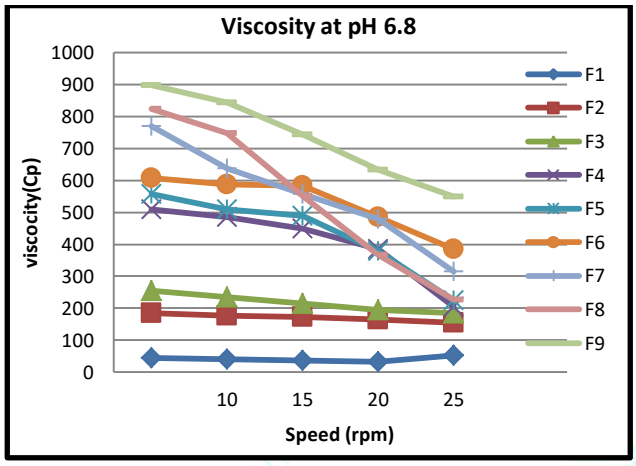


Fig. 5: Viscosity profile of formulations at pH 6.8

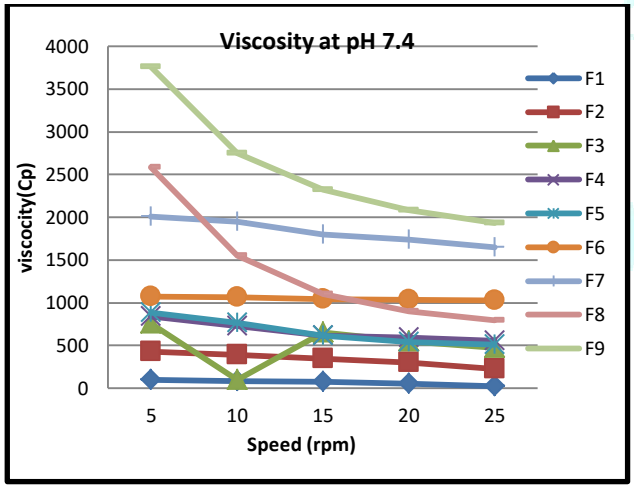


Fig. 6: Viscosity profile of formulations at pH7.4

4. 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, mucoadhesive polymers and also by the pH. Optimal mucoadhesive 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

Sr. No	Formulation code	Gel strength (sec) (±S.D.)
1	F1	0.57±0.05
2	F2	0.62 ± 0.01
3	F3	0.85 ±0.07
4	F4	1.04 ± 0.05
5	F5	1.30 ± 0.08
6	F6	1.51 ± 0.43
7	F7	1.51 ± 0.41
8	F8	2.27 ± 0.12
9	F9	2.41 ± 0.04

5. 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 934 and HPMC K100 both are having effect on bioadhesive strength. It shows that bioadhesive force was increased with the increasing concentration of the Carbopol 934 and HPMC K100.

Table 4: Bioadhesive strength of formulations

Formulation code	Detachment Force (N) (±S.D)
F1	0.1899 ± 0.035
F2	0.2018±0.027
F3	0.2525 ±0.005
F4	0.3508 ± 0.005
F5	0.4394 ± 0.005
F6	0.4870± 0.005
F7	0.5078± 0.005
F8	0.6408 ± 0.005
F9	0.6458± 0.0005

6. Drug content

The Drug content of formulations is 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 5: Percent drug content of Ophthalmic gel

Formulation Code	Drug content (%) (±S.D.)
F1	98.85± 0.12
F2	98.56± 0.17
F3	98.56±0.16
F4	98.98± 0.065
F5	99.57± 0.17
F6	97.68± 0.13
F7	98.98± 0.13
F8	98.35± 0.17
F9	97.59± 0.12

7. In-vitro drug release study

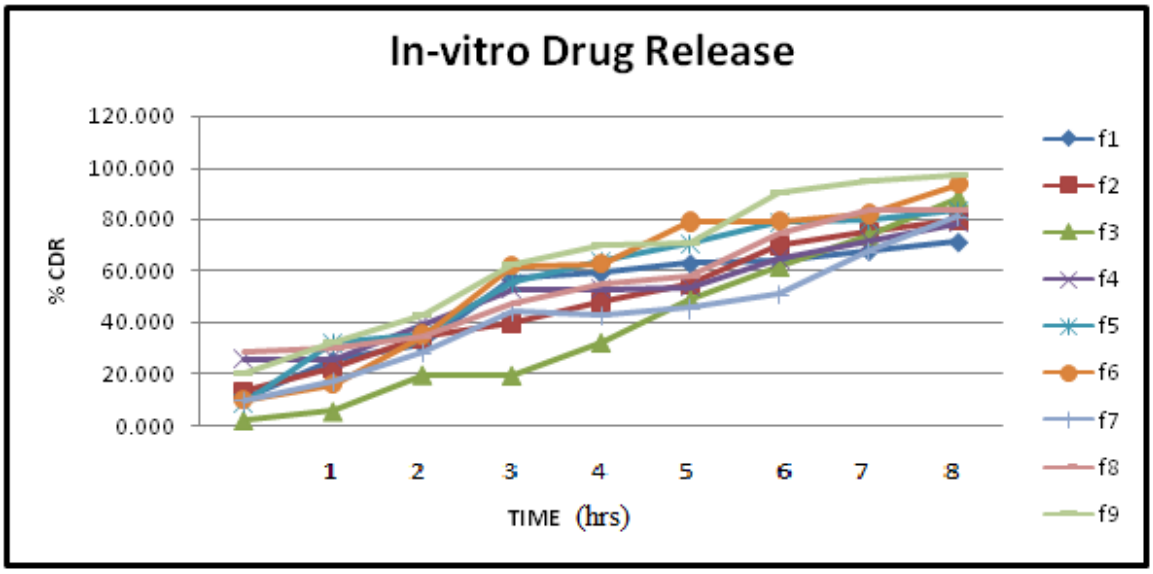
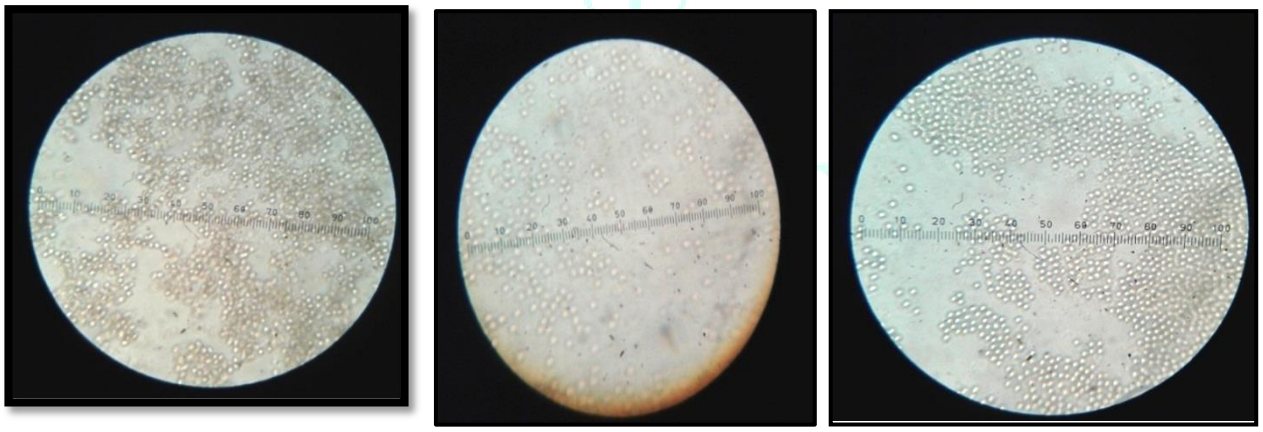


Fig.7: In-vitro drug release profile of formulations

Out of nine formulations maximum release after 8 hour was found for F5 formulation. This indicates release of 99.1 % drug availability. In-vitro drug release profile of formulations shown in Figure 6.

8. Isotonicity Evaluation

The shape of blood cells, blood cells with Bepotastine Besilate F5 and blood cells with Bepreve as marketed formulation are shown in figure 7. 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.**

Fig.8: Shape of Blood Cells

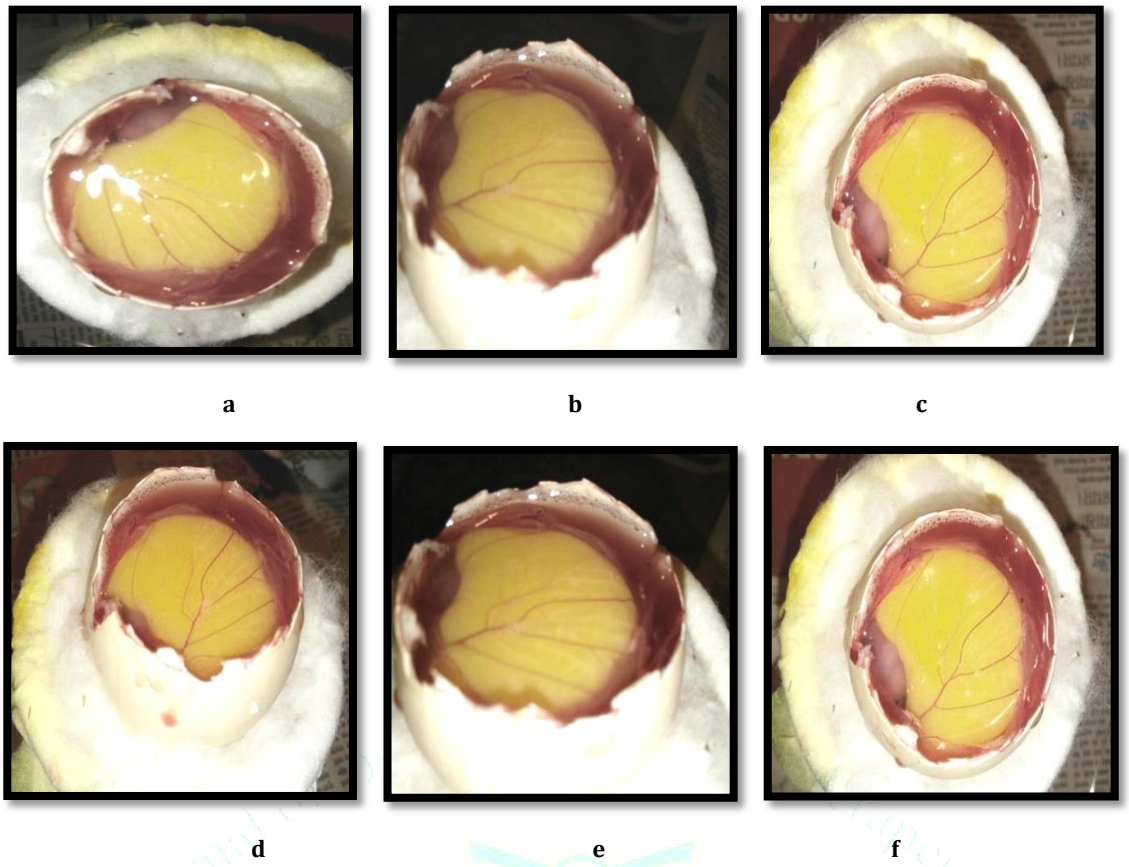
a, b and c are the image of blood cells, blood cells with bepotastine besilate in situ ophthalmic gel blood cells with marketed formulation.

9. 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 c in case of soyabean-casein digest medium. The preparations examined therefore passed the sterility test.

10. HET-CAM Test

The result 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 Fig. 8



a b c

d e f

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 and 5 min respectively.

11. Stability study

Stability study of optimized F5 formulation at room temperature shown in Table 6. Formulations at room temperature were found to be stable upto 3 months. There is no change in drug content, pH, clarity.

Table 6: Stability study data for F5 batch

Sr. No.	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%

CONCLUSION

1. Preformulation evaluation study has shown the identity and purity of Bepotastine Besilate.

2. Infrared spectroscopy studies of Bepotastine Besilate alone and their physical mixture with Carbopol 934 and HPMC K100 revealed that Bepotastine Besilate is compatible with all polymers used.

3. The clarity of the prepared formulations was found satisfactory.

4. pH of all the formulations was found to be in between the ophthalmic pH range (6.5-8.5) which is in tolerable range in contact with ocular tissues.

5. The viscosities of the all formulations were greatly affected by concentration of Carbopol 934 and HPMC K100.

6. Gel strength and bioadhesive strength of formulations resembles to the viscosity results.

7. Drug content of all formulations was found to be in between 97-99.56% which was in acceptable range.

8. The release kinetics results obtained indicate that formulation containing 0.2%w/v Carbopol 934 and 0.8% w/v HPMC K100 showed highest release i.e. 99.1 after 8hours which indicates that the formulation have shown prolong release. This optimized formula was also confirmed by design expert 11 optimization software.

9. The optimized formulation has shown the maintenance of tonicity.

10. The optimized formulation has passed sterility test.

11. The optimized formulation showed no ocular irritancy.

12. The optimized formulation F5 showed good stability and no change in any physical characteristics over a 3 months period.

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