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Pharmaceutical Sciences **Research Article……!!!**

IN- SITU GELLING SYSTEM FOR GLAUCOMA USING β- BLOCKERS BY pH TRIGGERED AND TEMPERATURE DEPENDENT POLYMERS

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KEYWORDS:

Glaucoma; *In situ*gel; β-blocker;

Carbopol® 934; Pluronic F-127.

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ABSTRACT

The present work describes the formulation and evaluation of an ophthalmic delivery system of an anti-glaucoma agent, Brimonidine tartrate an β-blocker based on the concept of combination of pH triggered and temperature dependent polymers for *in situ* gelation, by using Carbopol^{934}(C^{934}) pH-specific polymer and Pluronic-F127(PF-127) temperature dependent polymer can be used to increase the bioavailability and therapeutic value of β-blockers(Betaxolol HCl) in glaucoma treatment by using *in situ* gelling system. Nine formulations with different concentrations of Carbopol^{934} and Pluronic F127 were tested for Clarity, pH, Gelling Capacity, Gelation Temperature, Eye irritation, Isotonicity, in-vitro diffusion, Viscosity and Sterility. Compatibility test was performed using FT-IR for all the formulations. F3 with Carbopol^{934}(0.4%w/v) and Pluronic F127 (15% w/v) passed the entire test and gave the best result. The results showed that the developed formulation were therapeutically efficacious, stable, non irritant and provided sustained release of the drug over a 10 hours period and thus, improved the bioavailability of the drug , patient compliance and its economics.

INTRODUCTION:

Glaucoma is a major cause of visual loss.In glaucomatous optic neuropathy, there is optic disc cupping and atrophy and apoptosis of retinal ganglion cells and their axons, and possibly other retinal elements, leading to irreversible visual field loss.The IOP is usually elevated with visual field loss [1, 2].

In situ gels refer to polymer solutions which can be administrated as liquid, and undergo a phase transition to semisolid gel upon exposure to physiological environments. As a result, the residence time will be increased and have enhanced ophthalmic bioavailability which cannot be achieved by conventional liquid ophthalmic formulation due to lacrimal secretion and nasolacrimal drainage.The gelation can be triggered by temperature, pH and ion change polymers[3].

Advantages of *in situ* forming gel:

- Improved local bioavailability
- Reduced dose concentration
- Less total drug
- Improved patient acceptability
- Reduced dosing frequency

MATERIALS AND METHODOLOGY:

Materials:

Betaxolol hydrochloride sample was gifted from Medigraph Pharmaceuticals (P) Ltd. (Maharashtra), Pluronic acid F-127 was purchased from Sigma Aldrich Chemicals Pvt. Ltd.,

Benzalkonium Chloride from Merk specialities private limited, Mumbai and Cellophane membrane from HiMedia Laboratories pvt.ltd were purchased. Carbopo^{934} (Rolex chemical industries),Sodium Chloride (SDFCL), Sodium Bicarbonate (Ranbaxy Laboratories limited), Sodium Hydroxide (SDFCL),Calcium Chloride (SDFCL), Fluid Thioglycollate Medium (HiMedia Laboratories pvt.ltd.), and Soyabean-Casein Medium (HiMedia Laboratories pvt.ltd.) were obtained.

Methodology:

FT-IR study [4]:

The compatibility between the drug and polymers (Pluronic F-127, Carbopol^{934}) were studied on *FT-IR* spectroscopy. Spectra of pure Betaxolol hydrochloride and physical mixture of Betaxolol hydrochloride with Pluronic F-127, Carbopol^{934} were compared at 400 to 4000cm^{-1} is shown in Fig: 2, 3 & 4.

Preparation of pH and temperature triggered *in situ* gelling system of betaxolol hydrochloride:

The formulations were prepared using cold method. Drug and isotonicity adjusting agent was dissolved in distilled de-ionized water and kept in refrigerator. Required quantity of PF-127 was added and kept at 4°C with periodical stirring to ensure complete dissolution. Carbopol^{934} was used as a viscosity

enhancing agent. In this case, required amount of viscosity enhancing agent was dissolved in hot water (80-90˚C) with continuous stirring for complete dissolution of Carbopol934. The drug and isotonicity adjusting agents were then added. After cooling, the required amount of PF-127 were added in Carbopol934 solutions and kept in refrigerator at 4°C for complete dissolution of PF-127. The pH of all the formulations was measured and found in the range of 6-6.5.Benzalkonium chloride (BKC) (0.02%) was added as a preservative. The formulations were then subjected to terminal sterilization by autoclaving at 121°C and 15 psi for 20 min [5].

Table:1 Formulation chart of pH and Temperature triggered *in-situ* gelling system of Betaxolol Hydrochloride:

Ingredients (gm)	Ingredient concentration(% w/v)								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
Betaxolol hydrochloride	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
PluronicF-127	15	15	15	18	18	18	20	20	20
Carbopol[934]	0.2	0.3	0.4	0.2	0.3	0.4	0.2	0.3	0.4
BKC(%V/V)	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Sodium chloride	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
Dil.water upto (ml)	100	100	100	100	100	100	100	100	100

EVALUATION OF PREPARED *IN-SITU* GELLING SYSTEM:

Clarity and pH:

The general appearance of the formulation was observed which included colour and clarity of solution. The pH of the prepared formulations was checked by using pocket pen pH meter.

Drug content:

Drug content estimation was done by pipette out 1 ml (0.25 mg) of 0.25% sample solution diluted to 100ml with simulated tear fluid [Sodium chloride 0.670 g, 0.200 g sodium bicarbonate and 0.008 g calcium chloride $2H_2O$ was dissolved in distilled water and diluted to 100.0 ml]. From the stock 1ml is pipette out diluted to 10ml with simulated tear fluid. The absorbance of the resulting sample solution was measured at 222.5 nm .The results are shown in table 2.

Gelling capacity:

The gelling capacity of the prepared formulations were determined by placing a drop of the formulation in a vial containing 2ml of freshly prepared simulated tear fluid and visually observed. The time taken for their gelling was noted [6]. The results are as shown in table 2.

Viscosity determination:

The viscosity measurements were done by using Brookfield DV-II+ viscometer using LV-2 spindle. The developed formulations were poured into the adapter of the viscometer and the angular velocity was increased gradually from 10 to 100 rpm. The angular velocity was reversed gradually. The average of the two readings was used to calculate viscosity. By adding STF the formulations were made into gel form and viscosity was determined as specified above using LV-3 spindle [6].

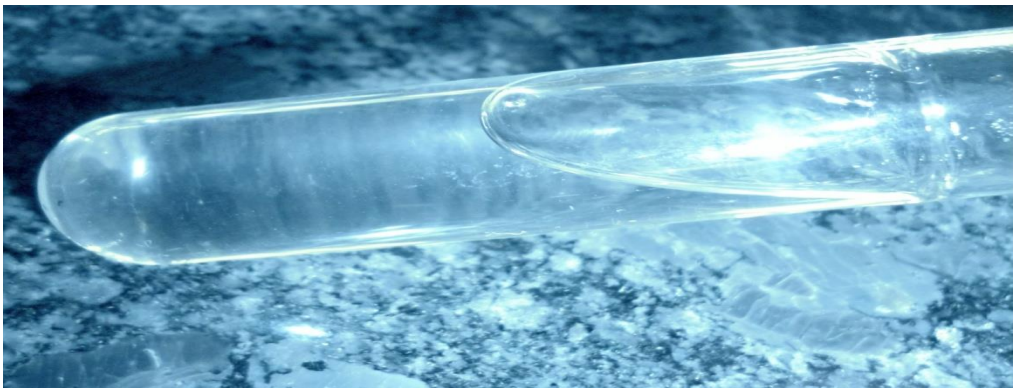

Fig1: Formation of gel on addition of STF

***In vitro* drug diffusion studies:**

The *in vitro* diffusion of Betaxolol Hydrochloride from the formulations was studied through cellophane membrane using a Franz diffusion apparatus. The diffusion medium used was freshly prepared STF. Cellophane membrane, previously soaked overnight in the diffusion medium (STF), was placed in between the donor and receptor compartment. 1 ml volume of the formulation was accurately instilled into the donor compartment. STF was placed in the receptor compartment according to the capacity of it. The whole assembly was placed on the thermostatically controlled magnetic stirrer. The temperature of the medium was maintained at 37 ± 0.5°C. The magnetic bead was rotated such that it produced a vortex and touched the cellophane membrane. Aliquots, each 1 ml volume, were withdrawn at hourly intervals and replaced by an equal volume of the receptor medium. The aliquots were diluted with STF and analyzed by UV visible spectrophotometer at 222.5 nm [7].

Test for sterility:

Method/ Procedure: Tests for sterility were performed for aerobic and fungi by using fluid thioglycollate medium and soyabean casein digest medium.

Preparation of fluid thioglycollate medium: 29.3 g of fluid thioglycollate medium was dissolved in 1000 ml distilled water by boiling. Sterilized by autoclaving at 15 lbs pressure at 121°C for 20 min.

Preparation of soyabean-casein digest medium: 30 g of soyabean-casein digest medium was dissolved in 1000 ml distilled water. The medium was boiled to dissolve completely. Sterilized by autoclaving at

15 lbs pressure at 121°C for 20 minutes. The media used should comply with the following tests carried out before or in parallel with the test on the preparation being examined.

i) Sterility (negative control) test: Fluid thioglycollate media was incubated at 30-35°C and soyabean casein digest medium at 20-25°C for not less than 7 days. No growth of organisms was observed.

ii) Growth promotion (positive control) test: Here, the sterile media was inoculated with about 100 viable micro-organisms and incubated according to the conditions specified. The test media were satisfactory, if clear evidence of growth appears in all media within 7 days.

Ophthalmic preparations should be sterile and must be checked for the presence of any bacteria or fungi before it is used. In each test, three sterile test tubes were used in the study and are labelled as 'negative control', 'test' and 'positive control'.

Test for aerobic bacteria:

20 ml each of sterile fluid thioglycollate was transferred to 3 tubes aseptically. The tube labeled as positive control was inoculated with viable aerobic microorganism *Bacillus subtilis* aseptically. 2.5 ml of the ophthalmic preparation was added to the tube labeled as test. Then all three test tubes were incubated at 30-35°C for not less than 7 days.

Test for fungi:

20 ml each of sterile soyabean-casein digest medium was transferred to 3 tubes aseptically. The tube labelled as positive control was inoculated with *Candida albicans* aseptically. 2.5 ml of the ophthalmic preparation was added to the tube labelled as test. Then all the three test tubes were incubated at 20-25°C for not less than 7 days. The sterility testing of ophthalmic drug delivery system were performed for aerobic bacteria and fungi by using fluid thioglycollate medium and soyabean casein digest medium as per the IP Procedure[8].

Isotonicity evaluation:

Isotonicity is important characteristic of the ophthalmic. Isotonicity has to be maintained to prevent tissue damage or irritation of eye. F3 was subjected to isotonicity testing, since they exhibited good release characteristics and gelling capacity and the required viscosity. Formulations were mixed with few drops of blood and observed under microscope at 45X magnification and compared with standard marketed ophthalmic formulation. The shape of blood cell was compared with standard marketed ophthalmic formulation [9].

Eye irritation studies:

Two albino rabbits of both sexes weighing 2.0 to 2.5 kgs were used for the study. 0.1 ml of the selected formulation- F3 was instilled in the conjunctival sac of right eye of each rabbit and readings were observed at 1, 24 and 48 h. Eye was evaluated for injuries to the cornea, conjunctiva and the iris were

scored separately. In the above studies, the left eye was served as control (without drug-placebo) and the right eye was served as test (sterile formulation). The scoring was given according to Draize irritancy scale [10].

Stability studies:

Stability studies were carried out on most satisfactory formulations (F2, F3, and F5) as per ICH Guidelines. Sterile gel forming ophthalmic solution were filled in autoclavable transparent plastic bottles, closed with autoclavable rubber closures and sealed with aluminium foils. The formulations were kept in stability chamber of at 40 ± 2°C & 75 ± 5% RH for 2 months. Samples were evaluated for drug content, pH and Clarity, gelling capacity, isotonicity and *in vitro* diffusion shown in table:3.

RESULTS:

Drug and excipients compatibility study by *FT-IR*:

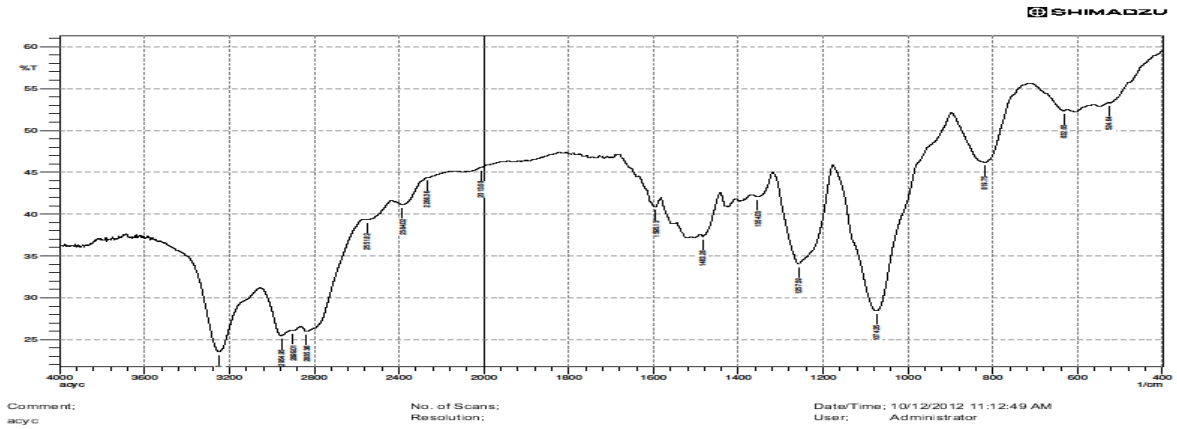

Fig 2:*FT-IR*spectra of pure Betaxololhydrochloride

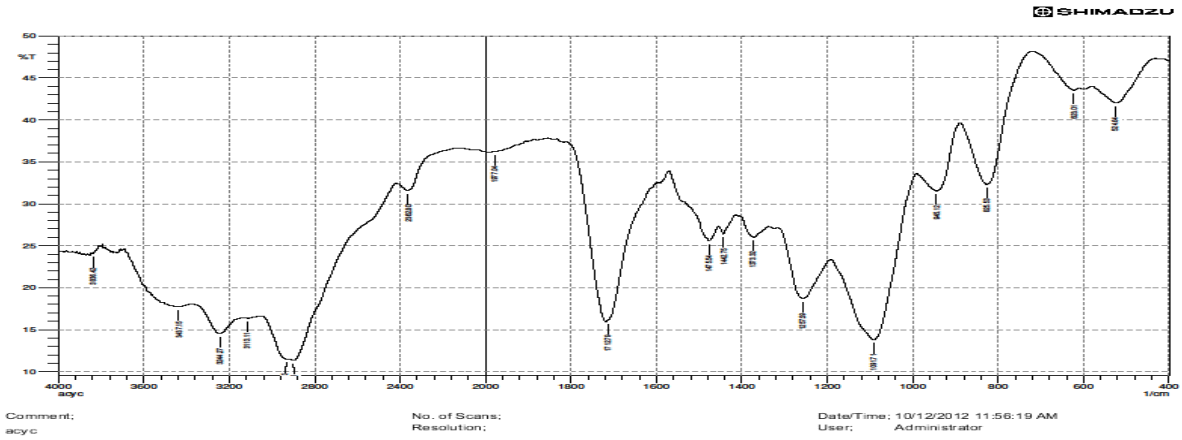

Fig3: *FT- IR* spectra of pure Betaxolol Hydrochloride, Pluronic F-127 & Carbopol[934]

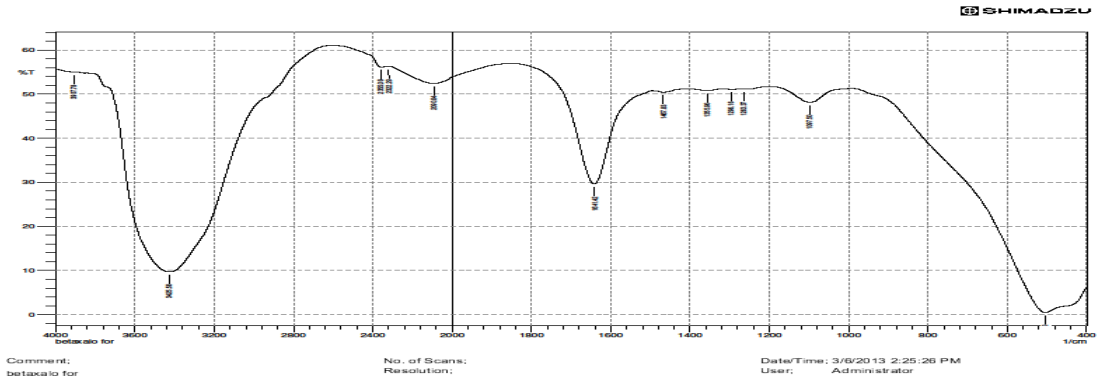

Fig 4: *FT-IR* of F3 after stability study of F3

Table 2: Evaluation of ophthalmic pH triggered & temperature dependent *in situ* gelling systems of Betaxolol hydrochloride:

Formulation Code	Drug Content (%)	Visual appearance	Clarity	Gelling capacity	Gelation temp.(˚C)	pH
F1	96.71±0.339	Transparent	Clear	+	32.15±0.07	6.1
F2	95.16±0.523	Transparent	Clear	++	30.66±0.91	6.0
F3	93.98±0.127	Transparent	Clear	+++	29.07±0.10	6.0
F4	92.45±0.381	Transparent	Clear	++	37.85±0.07	6.3
F5	96.98±0.374	Transparent	Clear	+++	36.3±0.14	6.0
F6	96.28±0.155	Transparent	Clear	+++	36.55±0.21	6.5
F7	94.97±0.297	Transparent	Clear	+	36.50±0.14	6.2
F8	93.08±0.098	Transparent	Clear	+++	35.60±0.14	6.1
F9	96.51±0.198	Transparent	Clear	+++	34.75±0.21	6.4

+: Gels after few min, remains for upto 2-3 h. **++:** Gelation immediate remains for upto 4-6 h.

+++: Gelation immediate remains for upto 7-9 h.

Table 3: Stability study readings after 3 months at 40 ± 2°C& 75 ± 5% RH (best formulation)

Formulation code	Drug content (%)	Clarity	pH of the solution	Gelling capacity	Isotonicity
F2	95.53±1.45	partial turbid	5.8	++	Isotonic
F3	94.0±2.78	clear	6.0	+++	Isotonic
F5	97.17±1.45	partial turbid	6.2	+++	Isotonic

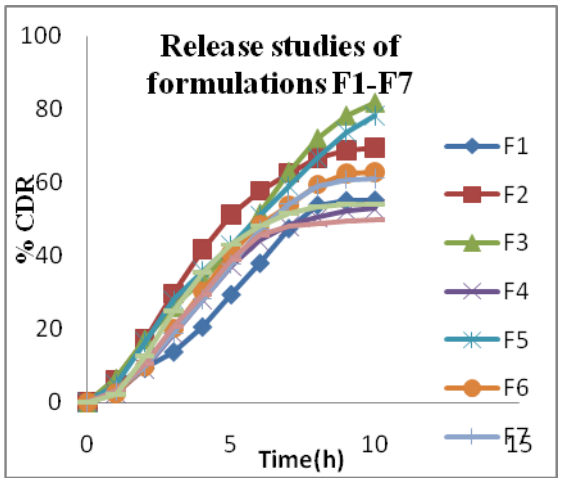

Fig 5: Release studies of Formulations F1-F9

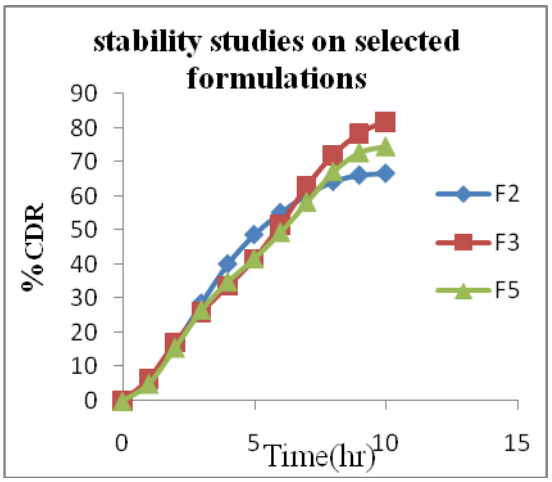

Fig 6: Stability studies on selected formulations

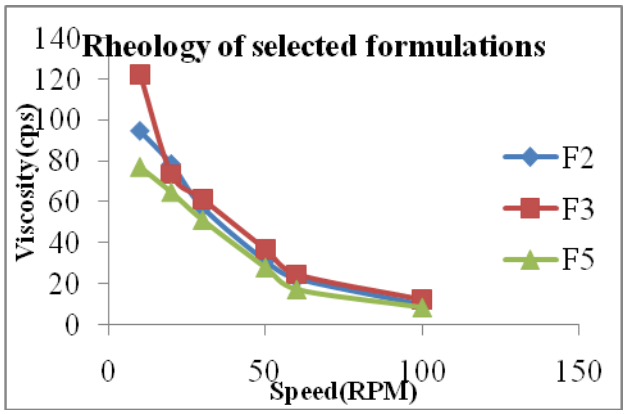

Fig 7: Viscosity of the selected formulation in gel

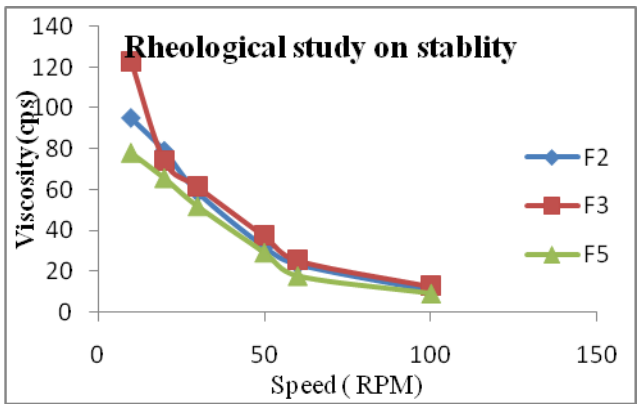

Fig 8:Viscosity study after 3 months stability

Table 5: Observations of sterility testing

Sterility Tests	Results Obtained																			
	Negative control							Test							Positive control							
	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7
Test for aerobic bacteria	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+
Test for Fungi	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+

- (-) sign suggests negative results (No growth of microorganisms)
- (+) sign suggests positive results (Formation of colonies of microorganisms)

Table: 6- Ocular irritation scores obtained

Section	Tissues	Total Scores	Total Maximum Scores
Section-I	Cornea	**05**	**80**
Section-II	Iris	**05**	**10**
Section-III	Conjunctivae	**06**	**20**

DISCUSSION

Betaxolol is a selective β1-blocking agent. It lowers the intraocular tension by reducing aqueous formation. This probably results from down regulation of adenylylcyclase due to β receptor blockade in the ciliary epithelium & a secondary effect due to reduction in ocular blood flow. This agent has a high specificity for glaucoma treatment.

In the present research work, Betaxolol Hydrochloride *in situ* gel systems were prepared by Temperature dependent and pH triggered methods and were evaluated for several parameters like viscosity, drug-polymer interaction, clarity, pH measurement, drug content(%), sterility, *in vitro* drug release, and ocular safety or eye irritation.

PREFORMULATION STUDY:

Drug-Polymer Interaction Studies:

FT-IR study showed that there is no interaction between drug and polymer hence the drug and polymer are compatible.

Standard Calibration Curve:

Betaxolol Hydrochloride showed maximum absorption at wavelength 222.5 nm in simulated tear fluid. Standard calibration curve in simulated tear fluid obeyed Beer's law in the concentration range of 2-20 µg/ml. The correlation co-efficient for the standard curve was closer to 1 at the range 2-20 µg/ml, the regression equation generated was slope=0.034 and R2=0.998.

Clarity, pH and Gelling time:

The formulations from F1 to F9 were transparent. The pH of all the formulations was within the acceptable range and hence would not cause any irritation upon administration. The drug content of all the formulations were in the range. Except for the formulations F1 and F7 all the formulations gelled instantaneously with a translucent matrix on addition to STF, and extended for few hours. The evaluation results are mentioned in table 3.

Drug content studies:

The drug content of all the formulations lies in the range of 92.82% to 97.17%, indicating the greater uniformity of the dosage in the formulations. The evaluation results are mentioned in table 3.

***In vitro* drug diffusion studies:**

The *in vitro* release studies indicated that amongst all the formulations F3 showed sustained drug release for 10 h, which may be due to optimum concentration of Pluronic F-127 and Carbopol^{934}. The evaluation results are shown in Fig: 5

Viscosity studies:

The viscosity of the formulation F3 ranged from 5-130 cps. All the formulations exhibited pseudo-plastic rheology, as shown by shear thinning and a decrease in the viscosity with increase in angular velocity. The evaluation results are shown in Fig: 7

Stability studies:

Stability studies of the formulations were carried out as per the ICH guidelines. Gelling capacity, Drug content, pH and clarity of F3 formulation did not show any significant change as compared to the F2 and F5 formulations which developed haziness and turbidity when stored for 30 days at 40 ± 2°C & 75 ± 5% RH. So formulations F2 and F5 were discarded. The results showed that there were no significant changes in the *in vitro* drug diffusion studies of the formulation F3. (fig:6).

Sterility test:

The formulation passed the sterility test as there was no appearance of turbidity and hence no evidence of microbial growth when incubated for not less than 7 days at 30-35°C in case of fluid thioglycollate medium and at 20-25°C in case of soyabean casein digest medium. The overall results of the sterility test showed that, the prepared ophthalmic formulation passed the sterility test. The evaluation results are mentioned in table 5.

Irritation study:

The results of ocular irritation studies indicated that the formulations were non-irritant. Excellent ocular tolerance was noted. No ocular damage or abnormal clinical signs to the cornea, iris or conjunctivae were observed. The evaluation results are mentioned in Table 6.

CONCLUSION:

A novel drug delivery system by the using Betaxolol hydrochloride entrapped in an *in situ* gel forming systems was formulated in a solution form such that the Betaxolol hydrochloride drops when instilled into the eye undergo a solution-gel transition in cul-de-sac.The study adapted the following stages: - Designing a master formula of the liquid dosage form by pH triggered and temperature dependent polymer methods using polymers like combination of Pluronic F-127 and Carbopol^{934}. Different

evaluation parameters were checked such as pH, gelation temperature, drug content, clarity, gelling capacity, viscosity, sterility study, isotonicity and *in vitro* drug diffusion.

Screening of stability studies was also taken up. The best formulations selected by keeping in focus, the key parameters such as gel formation, gel retention and *in vitro* drug diffusion studies, out of which the most suitable batch falling under all the basic criteria's involved *in situ* gel drug delivery system batch F3 was taken up for the final study. The last part of the study after identification of good formulation, stability study as per ICH guidelines and eye irritation studies on animals (Rabbits) were carried out to select the most ideal formulation.

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