

FORMULATION AND EVALUATION OF PH TRIGGERED *IN SITU* OPHTHALMIC GEL OF MOXIFLOXACIN HYDROCHLORIDE

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ABSTRACT

The aim of the current study is to design and characterize pH triggered *in situ* ophthalmic gel forming solution using a fourth generation fluoroquinolone antibiotic, Moxifloxacin hydrochloride. Polyacrylic acid (Carbopol 934) was used as gelling agent in combination with hydroxy propyl methyl cellulose(K15M) as viscosifying agent. Benzalkonium chloride in suitable concentration was used as preservative. The formulations were sterilized by autoclaving at 121^o C for 15 min at 21 psi. The prepared formulation were evaluated for visual appearance, clarity, pH measurement, gelling capacity, drug content and *in vitro* diffusion studies, sterility test, microbiological studies and stability studies. Under rheological condition both solution and gel have shown pseudo plastic behaviour. The selected formulation showed sustained release for the period of 8 hours thus showing increased residence and contact time with eye. Eye irritation test using Draize test protocol was carried out on an optimized formulation and was found to be non-irritant to rabbit eye. All studies showed favourable results thus *in situ* gelling system can be considered as alternative for conventional ophthalmic drops.

Keywords: Moxifloxacin Hcl, *In-situ* gel, *In Vitro* diffusion, Microbiological studies, Draize test

INTRODUCTION

Topical delivery of eye drops into the lower cul-de-sac is the most common method of drug treatment for ocular diseases and diagnosis¹. Eye drops that are conventional ophthalmic delivery systems often result in poor bioavailability and therapeutic response since the high tear fluid turnover and dynamics cause rapid precorneal elimination of the drug². A high frequency of eye drop instillation is associated with poor patient compliance. Inclusion of excess drug in the formulation to overcome bioavailability problems is potentially dangerous if the drug solution drained from the eye is systemically absorbed from the nasolacrimal duct^{3, 23}. Various ophthalmic vehicles such as inserts, ointments, suspensions and aqueous gels have been developed in order to lengthen the residence time of instilled dose and enhance the ophthalmic bioavailability⁴⁻⁵. These ocular drug delivery systems, however, have not been used extensively because of some drawbacks such as blurred vision from ointments or low patient compliance from inserts⁶⁻⁷.

Above mentioned problems can be overcome by the use of *in situ* gelling systems, a liquid dosage form suitable to be administered by instillation into eye, which upon exposure to physiological conditions, changes to the gel phase thus increasing the precorneal residence time of the delivery system and enhancing the ocular bioavailability. It comprises the ease of eye drop instillation and patient compliance as well as sustained release property that is described to intensify ocular bioavailability. The concept of *in situ* forming gels came into existence in early 80s⁸.

Depending on the method employed to cause sol to gel phase transition on the ocular surface, the following three types of systems have been recognized:

- > pH-triggered - The polymers used in this system are pseudo latexes - carbomer (carbopol), cellulose acetate phthalate latex (CAP-latex).
- > Temperature-dependent - Poloxamers (Pluronic, Tetronics), cellulose derivatives (MC, HPMC), Xyloglucan.
- > Ion-activated induced - Alginates, Gelrite (Gellan gum)⁹.

Moxifloxacin is a fourth-generation fluoroquinolone with a methoxy group in the C-8 position and a bulky C-7 side chain. This fourth-generation fluoroquinolone has *invitro* activity similar to that of ciprofloxacin and ofloxacin against gram-negative bacteria but enhanced activity against gram-positive bacteria including *S aureus*¹⁰.

The aim of the present study is to formulate and evaluate pH triggered *in situ* ophthalmic gel forming solution of Moxifloxacin Hcl(0.5% w/v) by using combination of hydroxy propyl methyl cellulose(HPMC) K15M as viscosifying agent and carbopol 934 as gelling agent. The dosage regimens prepared provide ease in application and provided sustained drug release with reduced frequency of administration.

MATERIALS AND METHODS

Moxifloxacin Hcl was obtained from Micro labs pvt limited Bangalore as gift sample, HPMC K15 was provided by KAPL Bangalore. Carbopol 934 was purchased from Loba Chem, all other ingredients were of analytical grade. Albino Rabbits was provided by college (Ref:KCP/IAEC-65/2010-11) to perform Draize ocular irritation studies.

Selection of vehicle

The solubility of Moxifloxacin Hcl was checked in various solvents like Distilled water, ethanol, acetone, 2 propanol. Studies revealed that Moxifloxacin Hcl was more soluble in water than any other solvents¹¹. The solubility was confirmed by analysing the sample by quantitative determination by UV spectroscopy. Wavelength scan was done from 400-200 nm and maximum absorbance was found at 288.5 nm¹².

FTIR studies

The drug excipient compatibility study was determined by FTIR (Fourier Transformer Infrared Spectroscopy) using KBR pellets of 0.1 mm. The IR spectra of the pure drug (Moxifloxacin Hcl) is compared with IR spectrum of combination of Moxifloxacin Hcl and all the excipients to check the interaction¹⁴.

Differential scanning calorimetry (DSC) characterization

Thermal characterization of pure drug and physical mixture was performed with calorimeter. Samples were placed in sealed aluminium pans. The samples were scanned at 20^o C/min from 20^o C to 300^o C.

Preparation of *In situ* gelling system:

The detailed procedure for preparing the *in situ* gel-forming system of Moxifloxacin Hcl is outlined below table 1. Required quantity of sodium chloride was dissolved in 50 ml of distilled water, HPMC K15M was added to the above solution and stirred slowly with Magnetic stirrer, Care was taken that no lumps of

HPMC was formed during stirring. Carbopol 934 was sprinkled over this solution and allowed to hydrate overnight. The solution was again stirred with magnetic stirrer after 24 hrs. Moxifloxacin Hcl was dissolved in distilled water, benzalkonium chloride (BKC) was then added and the solution was filtered through 0.2- μ m cellulose acetate membrane filter. The drug solution was added to the carbopol -HPMC solution under constant stirring until a

uniform solution was obtained pH of the formulation was then set to 4.4 using 0.1 N NaOH. Distilled water was then added to make up the volume to 100 ml. The developed formulations were filled in 5 ml capacity amber glass vials, closed with gray butyl rubber closures and sealed with aluminium caps. The formulations in their final pack were subjected to terminal sterilization by autoclaving at 121 $^{\circ}$ C at 15 psi for 20 min¹³

Table 1: Composition of *in situ* ophthalmic gel

Ingredients %w/v	F1	F2	F3	F4	F5	F6	F7
Moxifloxacin Hcl	0.5	0.5	0.5	0.5	0.5	0.5	0.5
HPMC (K15 M)	0.1	0.2	0.3	0.3	0.3	0.3	0.3
Carbopol 934	0.1	0.2	0.3	0.4	0.5	0.6	0.7
Sodium chloride	0.9	0.9	0.9	0.9	0.9	0.9	0.9
Benzalkonium chloride	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Distilled water (ml)	100	100	100	100	100	100	100

Evaluation of the Formulation

Visual appearance, Clarity, pH, Drug content

The appearance was checked visually. The clarity of the formulations before and after gelling was determined by visual examination of the formulations under light alternatively against white and black backgrounds¹⁴. Formulation was taken in a beaker and 0.1M NaOH was added dropwise with continuous stirring. pH was checked using pH meter (Systronics digital pH meter)¹⁴.

The drug content was determined by taking 1 ml sample of *in situ* gel into 100 ml volumetric flask and diluting with 100 ml of simulated tear fluid from this further dilution was done by taking 1 ml of sample and diluting with 10 ml of simulated tear fluid. The absorbance was measured at 288.5 nm by UV-Spectrometer to calculate percentage of drug content¹⁵.

In vitro gellation study

Gelling strength of formulations having different proportions of Carbopol-934 and HPMC K15M were evaluated by placing a drop of polymeric solution in vials containing 1 ml of freshly prepared simulated tear fluid, equilibrated at 37 $^{\circ}$ C. The gel formed and time taken for gellation was assessed visually¹⁶. The composition of artificial tear fluid used was NaCl 0.670 g, sodium bicarbonate 0.200 g, calcium chloride -2 H₂O 0.008 g, purified water q.s. 100.0 g.

Rheological studies

The rheological behaviour of the formulations were investigated as a function of pH. The relationship between contact time and the rheology was easily understood for viscosity enhanced ophthalmic solutions. Rheological studies of the prepared formulations were carried out by Brookfield viscometer (LVDVE 230 Pro) using spindle S2 at pH 4.4. and T bar spindle was used to check the pH of the formulation at pH 7.4. The viscosity of the formulations were determined at different speed conditions (5, 6, 10, 12, 20, 30, 50 rpm)¹⁷.

In Vitro drug release studies

In-vitro release studies were carried out using bichambered donor receiver compartment model (Franz diffusion cell) and this was placed on magnetic stirrer and temperature was adjusted to 37 \pm 0.5 $^{\circ}$ C. Accurately measured 1 ml of the formulation spread uniformly on a dialysis membrane, which was in contact with receptor medium. The receptor medium was stirred continuously at 20 rpm to simulate blinking action of eyelids. Samples were withdrawn at periodic intervals and dilution was done with 10 ml of STF. The drug content was analyzed using UV Spectrophotometer at 288.5 nm against reference standard using simulated tear fluid as blank¹⁸.

Sterility Studies

Direct inoculation

Preparation should be examined during usage. Sterile media was pipetted out by sterile pipette and with sterile syringe then

aseptically transferred the specified volume of sample to fluid thioglycolate medium and Soyabean casein digest medium and incubate for 7 days at 30 to 35 $^{\circ}$ C for fluid thioglycolate medium and 20 to 25 $^{\circ}$ C for Soyabean casein digest medium¹⁵ and periodic observation were carried upto seven days to check growth of microorganism.

Antimicrobial activity

Antimicrobial efficiency studies were carried out to ascertain the biological activity of sol-to-gel systems against microorganisms. This was determined in the agar diffusion medium employing Cup plate technique. Sterile solution of marketed Moxifloxacin Hcl eye drops was used as a standard. The standard solution and the developed formulations (test solution) were taken into separate cups bored into sterile SCDM Agar previously seeded with organisms (*Staphylococcus aureus* and *Pseudomonas aeruginosa*). After allowing diffusion of solutions for two hours, the plates were incubated for 24 hrs at 37 $^{\circ}$ C. The zone of inhibition (ZOI) was compared with that of the standard^{17,19,22}. Each samples were tested in triplicate.

Ocular irritation studies-Draize Test

Albino rabbit (eg: Newzeland white rabbit) are used as test species. One eye (e.g., right eye) is designated the test eye; the contralateral eye serves as a matched control and is usually left untreated. Single drop approximately 0.04 ml is instilled into the lower conjunctival cul-de-sac; normal blinking is allowed, although the eyelids can be held together for several seconds after instillation. Observations was done at 1, 24, 48, 72 hours one week after exposure. Ocular changes was graded by a scoring system that includes rating any alterations to the eyelids, conjunctiva, cornea, and iris²⁰.

Accelerated stability study

Short term accelerated stability study was carried out for the period of 45 days for the formulations. The samples were stored at different storage conditions of room temperature, elevated temperature such as 40 $^{\circ}$ C at 75% RH and refrigerator(2 to 8 $^{\circ}$ c). Samples was withdrawn on weekly interval and analysed for visual appearance, clarity, pH and drug content²¹.

RESULTS AND DISCUSSION

Seven formulation of *in situ* gelling system of Moxifloxacin Hcl using combination of various concentration of HPMC and Carbopol 934 were prepared, out of which five formulation showed gellation and among them three formulation showed Sustained release pattern.

FTIR studies

FTIR spectrum of pure drug and mixture of drug and polymers are shown in figure-1, 2. From the spectral study it was observed that there was no significant change in the peaks of pure drug and drug polymer mixture. Hence, no specific interaction was observed between the drug and the polymers used in the formulations.

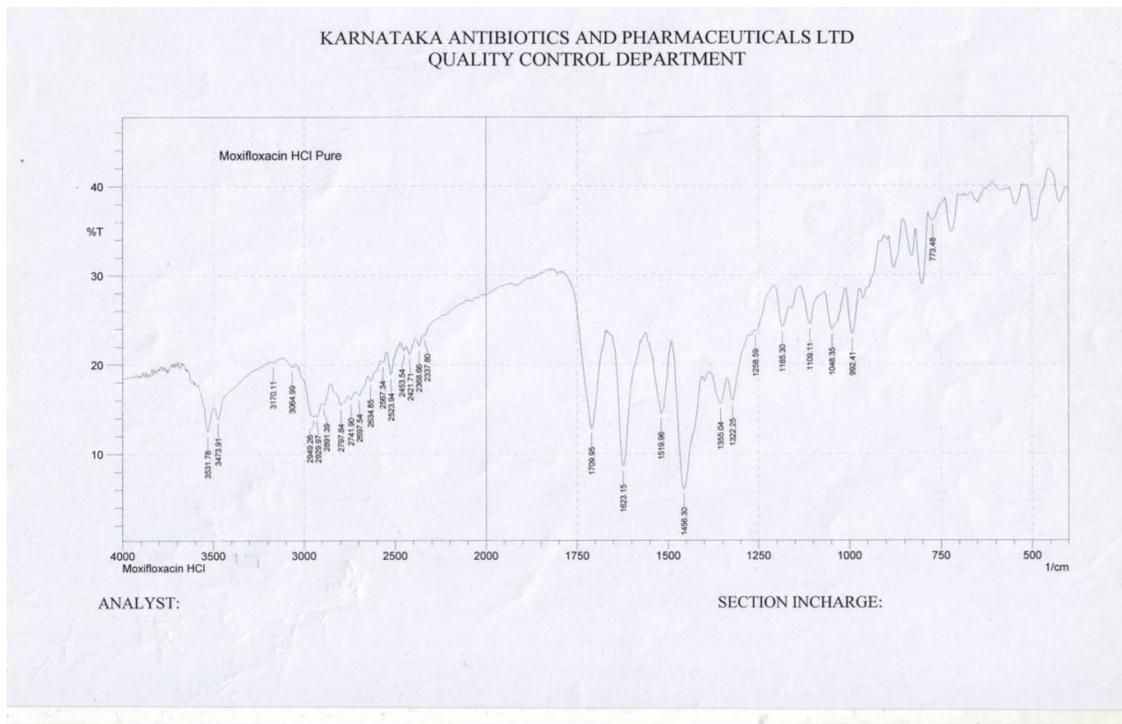


Fig. 1: FTIR of Moxifloxacin Hcl

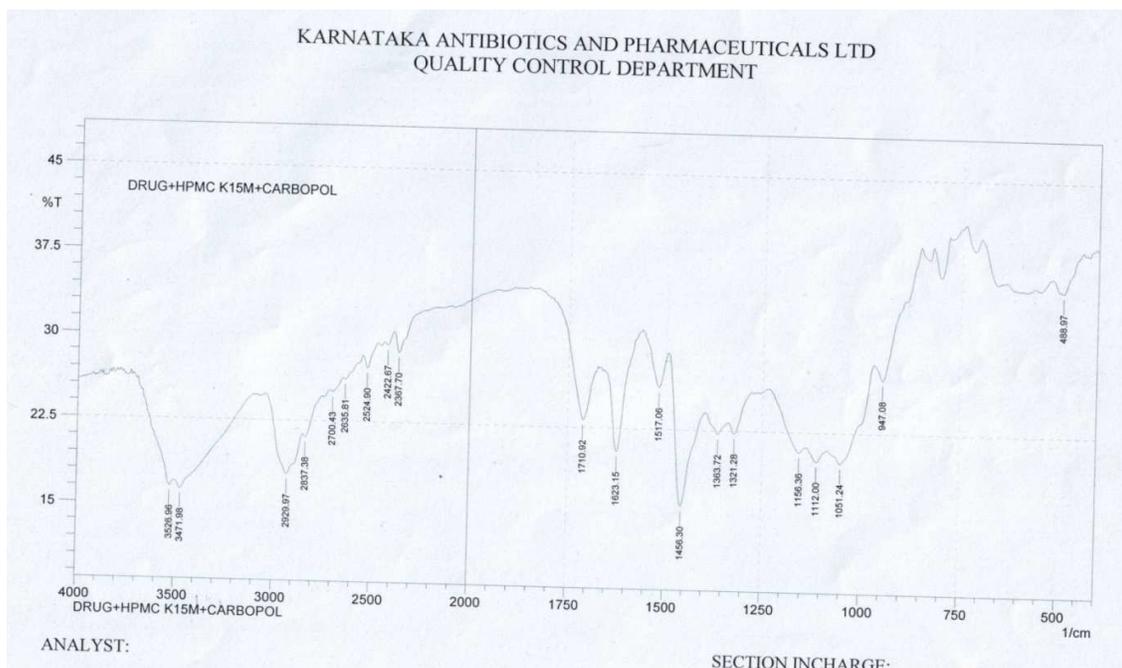


Fig. 2: FTIR of Moxifloxacin Hcl+ HPMC K15M+Carbopol 934.

Table 2: Reported and observed IR frequency of Moxifloxacin Hcl and its physical mixture.

Functional Group	Reported frequency (in cm ⁻¹)	Observed frequency in pure drug (in cm ⁻¹)	Reported frequency in physical mixture
F	1400-1000	1048.35	1051.24
C=O	1725-1680	1709.95	1710.92
N-H	3500-3310	3473.91	3471.98
O-H	3550-3450	3531.78	3526.96

Differential scanning calorimeter (DSC)

Figure 3 and 4 compares the the DSC thermogram of Moxifloxacin Hydrochloride and physical mixture of Moxifloxacin with all excipients. Moxifloxacin showed a long and characteristic endothermic peak at 250.89°C. The physical mixture of Moxifloxacin with HPMC K15M and carbopol 934 showed characteristic peak at 250.52°C. From this result, it clears that there is no interaction in between Moxifloxacin Hcl and excipients.

Visual appearance, Clarity, pH, Drug content

All the formulations were light yellow in colour and were found to be clear. The pH of the formulations was in the range of 4.40 to 4.46. Drug content was in the range of 85.6% to 98.8%. All these observations are listed in table 3

In Vitro gellation study

Out of seven formulations, five formulations showed gellation (F3 to F7) and three formulation (F5 to F7) had the best good gelling capacity. All these observation were mentioned in table 3

Rheological studies

The formulations (F5 to F7) showed Pseudo plastic behaviour that is with increase in shear rate the viscosity of the formulation were reduced (Fig- 5, 6). At pH 4.4 the formulations exhibited low viscosity and were in solution form. An increase in pH to 7.4 (pH of tear fluid) using 0.1 N NaOH transformed the solution into gel and showed increase in viscosity (figure 7, 8)

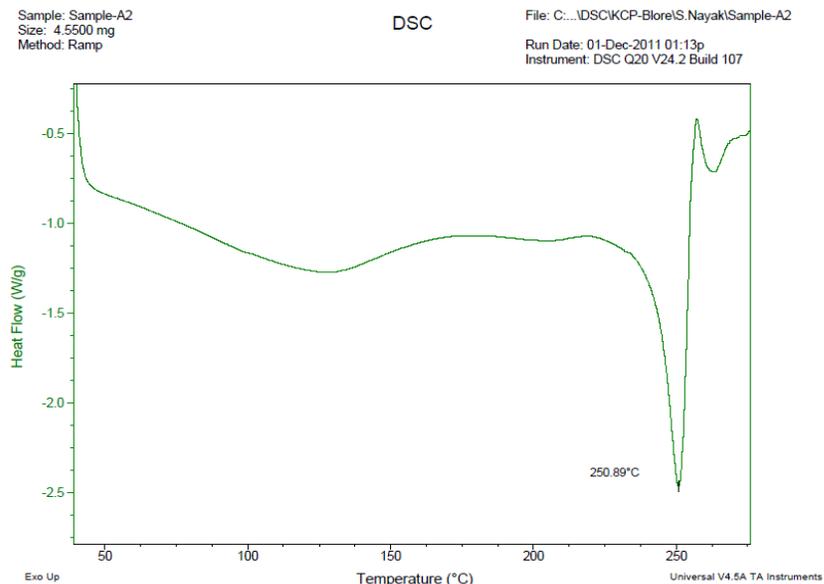


Fig. 3: Thermal analysis of Moxifloxacin Hydrochloride

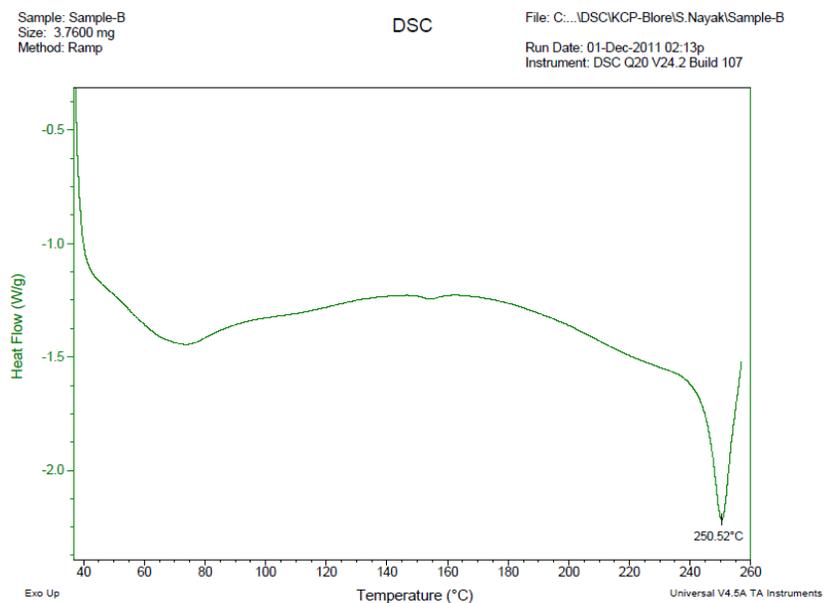


Fig. 4: Thermal analysis of Moxifloxacin Hydrochloride+Excipients.

Table 3: Evaluation of the formulation

Formulations	Appearance	Clarity	pH	Gelling capacity	Drug content
F1	Light yellow	Clear	4.41	-	94.2 %
F2	Light yellow	Clear	4.41	-	95.1 %
F3	Light yellow	Clear	4.44	+	91.6%
F4	Light yellow	Clear	4.40	+	95.0%
F5	Light yellow	Clear	4.40	++	98.8 %
F6	Light yellow	Clear	4.41	+++	98.8 %
F7	Light yellow	Clear	4.43	+++	85.6%

- : No gellation
 + : Gels slowly and dissolves
 ++ : Gellation immediate and remains for hours
 +++: Gellation immediate and remains for extended period of time

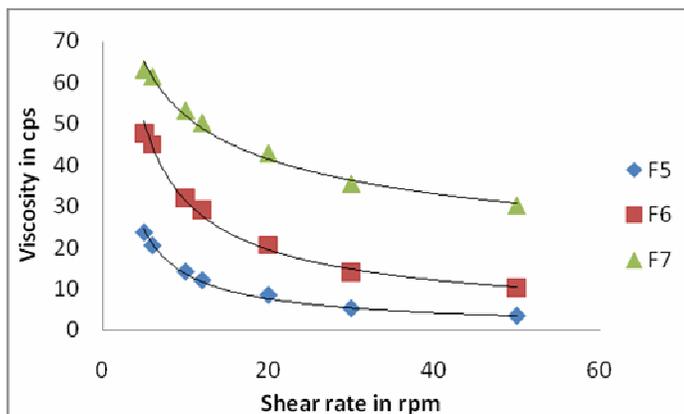


Fig. 5: Viscosity of the formulation at pH 4.4.

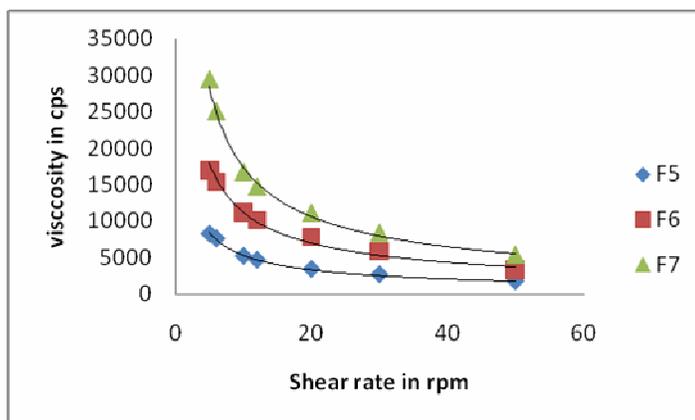


Fig. 6: Viscosity of the formulation at pH 7.4.



Fig. 7: Before gellation at Non Physiological condition at pH 4.4



Fig. 8: After gellation at Physiological condition at pH 7.4

In Vitro drug release studies

In vitro drug release of the formulation F5 to F7 is showed in figure 9. The drug release data were subjected to various Pharmacokinetic parameters like Zero order, First order, Higuchi square root and Krosmeier Peppas model to know the Pattern of drug release table 4. The formulation F5 showed good sustained release for the period of 8 hours and finally comparative study with marketed formulation was done.

Sterility studies

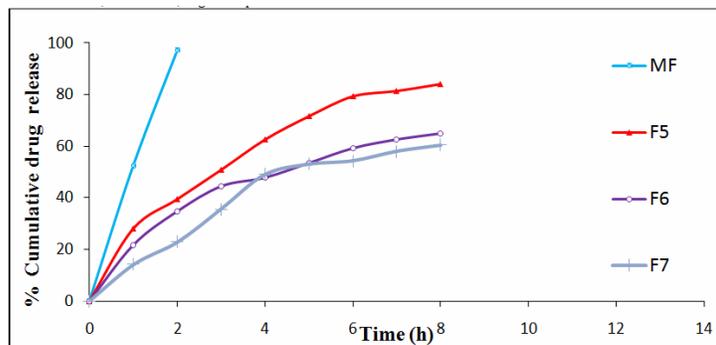
All the formulations were found to be sterile when subjected to sterility study by direct inoculation and no growth of any forms of microorganisms were observed in the formulations

Antimicrobial activity

The Zone of Inhibition was better with *Staphylococcus aureus* (gram positive micro organism) when compared to *Pseudomonas aeruginosa* for the formulations and Marketed product Fig-10, 11. The zone of inhibition of marketed and prepared formulations was found to be almost similar. The results of the antimicrobial efficacy tests are represented in table 5. The present study results indicate that Moxifloxacin hydrochloride retained its antimicrobial efficacy when formulated as an *in situ* gelling system.

Ocular irritation studies-Draize Test

Formulation F5 was used for this test. The formulation was found to be non irritating with no ocular damage or abnormal clinical signs to the cornea, iris or conjunctivae observed fig 12, 13. Hence the formulation was suitable for the eye instillation. The scores are mentioned in table 6.



MF-Marketed formulation

Fig. 9: In vitro drug release of the formulations and marketed product (Moxicip 0.5% W/V)

Table 4: Pharmacokinetic release of formulations

Formulation	Zero order	First order	Higuchi	Krosmeier Peppas	n value	Best fit model
F5	0.9171	0.9914	0.9913	0.9906	0.5595	1 st order
F6	0.8887	0.9647	0.9937	0.9837	0.5155	1 st order
F7	0.9119	0.9630	0.9630	0.9611	0.7294	1 st order



Fig. 10: ZOI of Formulation F5 and marketed product (Moxicip 0.5% w/v) seeded with *Staphylococcus aureus*.

S-Formulation F5

C-Moxicip eye drops 0.5% w/v



Fig. 11: ZOI of Formulation F5 and marketed product (Moxicip 0.5% w/v) seeded with *Pseudomonas aeruginosa*.

S-Formulation F5

C-Moxicip eye drops 0.5% w/v

Table 5: ZOI with *Staphylococcus aureus* and *Pseudomonas aeruginosa*

Microbial strain	Zone of inhibition (mm) Standard(marketed product)	Zone of inhibition(mm) F5
<i>Staphylococcus aureus</i>	23.40±0.35	22.41±0.41
<i>Pseudomonas aeruginosa</i>	17.23±0.12	16.54±0.29

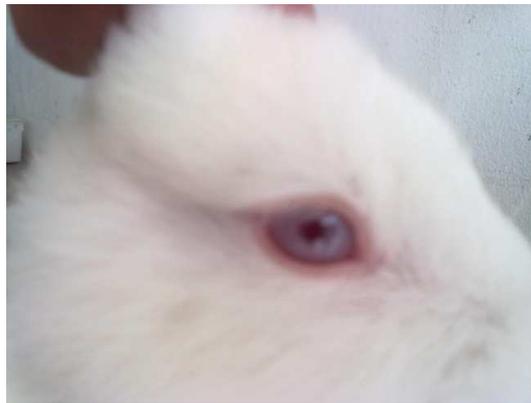


Fig.12: Before instillation.



Fig. 13: One week after instillation.

Table 6: Ocular irritation study as per the Draize test protocol

Eye part	Cornea	Iris	Conjunctiva	Total
Score	0	0	0	0

Accelerated stability study

The stability data at the end of the 45 days revealed that formulations was found to be stable and efficacious. No change in pH, drug content, gelling capacity were observed.

CONCLUSION

The present work was carried out to develop pH triggered *in situ* gel of Moxifloxacin Hydrochloride (0.5% w/v) using combination of HPMC K15M and carbopol 934. Attempts were made to design the formulation with low concentration of HPMC K15M (0.3%w/v). The formulations were in solution form at pH 4.4, which underwent sol-gel transformation when instilled into eye (pH 7.4) indicating increase in precorneal residence time of drug thus increasing ocular bioavailability, reducing the dosing frequency and improved patient compliance. The three formulations showed sustained release for a period of 8 hours. Because of higher viscosity of F6 and F7 the formulation F5 was optimized. Finally it can be concluded that *in situ* ophthalmic gel is an alternative for conventional eye drops and it will be boon to the patients in the future.

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