

Development and Characterization of Novel *In Situ* Gel of Moxifloxacin Hydrochloride

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Abstract

Aim: Regardless of the ease of access of the anterior surface of the eye, efficient drug delivery for the treatment of various ocular disorders is always remained a challenge. Hence, development of a sustained release system is necessary which prolongs the effective therapeutic concentration moxifloxacin hydrochloride. In present work the pH and temperature triggered *in situ* gelling system for sustained ocular delivery of moxifloxacin HCl was formulated and evaluated. **Materials and Methods:** Initially preformulation studies such as description, pH, melting point, and solubility of moxifloxacin HCl and polymers (poloxamer 407, hydroxypropylmethylcellulose [HPMC] K4M, carbopol 974p, carbopol 940) were checked. Starting batches (PF1-PF4) were prepared using poloxamer 407 in combination with HPMC K4M by using the cold method. Remaining batches (CF1-CF4) were prepared using HPMC K4M in combination with carbopol 974P and carbopol 940. The prepared batches were evaluated for clarity, visual appearance, pH, drug content, and *in vitro* drug release study. **Results and Discussion:** The developed formulations were clear, stable, and isotonic. The optimized batches CF1 and PF2 sustained the drug up to 5 and 6 h, respectively. The prepared formulations were passes the tests such as antimicrobial, antibacterial, sterility, and Draize test. **Conclusion:** Batches PF1 and CF2 fulfilled the needs of pH and temperature triggered *in situ* gels of moxifloxacin hydrochloride (0.5% w/v). *In situ* ophthalmic gel will be an alternative for conventional eye drops and boon to the patients in future.

Key words: *In situ* gel, ophthalmic drug delivery, moxifloxacin HCl, sol-gel transition

INTRODUCTION

Because of rapid nasolacrimal drainage and constant lachrymal secretion conventional, liquid ophthalmic formulations show low bioavailability.^[1] The slow diffusion of water soluble drugs is an additional reason for the low bioavailability. A large number of solutions containing topically applied medicaments is instantly diluted in the tear film and over the lid margin excess fluid spills and into the nasolacrimal duct the remainder is rapidly drained.^[2] For therapeutic action, a proportion of the drug is not available as it binds to the surrounding extra orbital tissues. The cul-de-sac has 7-10 μ l of normal capacity and may be increased without blinking up to 30 μ l. From the precorneal area, the conventional eye drops are eliminated instantly and absorbed drug from topical administration is only 1-10%.^[3] Nasolacrimal drainage also may drop instillation which is related with patient noncompliance.

From the nasolacrimal duct, the drug solution drained from the eye is systemically absorbed; so an attempt to overcome bioavailability problems is addition of excess medicament in preparation is potentially dangerous. Many ophthalmic drugs are applied in high concentration because of poor ocular bioavailability which may produce ocular and systemic side effects. For the topical application to the eye, different ophthalmic dosage forms such as gels, ointments, polymeric inserts, and solutions and have been studied to prolong the ocular residence time of drugs.^[4] One of the appropriate ways to increase the contact time and bioavailability is achieved

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by use of mucoadhesive polymers. To the ophthalmic drug solutions, viscosity-enhancing polymers are usually added on the basis that a slower elimination from the precocular area will be achieved with an increased vehicle viscosity, which leads to improved precorneal residence time and hence a greater transcorneal penetration of the medicament into the anterior chamber.^[5] One of the better alternatives for conventional formulations is gel; however, for some patients, these are difficult to administer.

In situ gelling system will cover all the limitations of conventional dosage form and as a drop which is easily dropped in to the eye. This upon exposure to physiological conditions will shift to the gel phase. Various polymers such as carbomers, carbopol, or its derivatives changed from sol-to-gel as pH changes.^[6]

Thus, this type of formulations has benefit of both solutions as well gels, they may improve the retention time of drug as well the formulations, accuracy, and ease of administration.^[7-9]

In situ gelling systems consist of polymers which in the cul-de-sac exhibit sol-to-gel phase transitions due to change in specific physicochemical parameters such as temperature, pH, and ionic strength in the environment.^[10] The present work aims to formulate the sustained ocular delivery of moxifloxacin HCl in form of *in situ* gelling system and evaluation of the same by different *in vitro* studies.

MATERIALS AND METHODS

Moxifloxacin was gifted by Apotex Research Pvt. Ltd. Bangalore. Carbopol 940 and hydroxypropylmethylcellulose (HPMC) K4M were obtained from Ozone International, Mumbai. Carbopol 974P and poloxamer 407 were purchased from M.J. Biopharma Pvt. Ltd., Navi Mumbai. All other reagents were of analytical grade.

Preformulation studies

Preformulation studies were performed on the obtained sample of drug and polymers such as description, pH, melting point, solubility, ultraviolet (UV) spectroscopic study, and differential scanning calorimetry (DSC).^[11]

UV spectroscopic study

Determination of wavelength of maximum absorption

Pure moxifloxacin HCl 100 mg in 10 ml of simulated tear fluid (STF) pH 7.4 was dissolved and with the same solvent diluted to the 100 ml. Accurately measured 0.1 ml of this solution was further diluted to 10 ml with the same solvent to obtain a 10 µg/ml moxifloxacin HCl. In the

wavelength region of 200-400 nm, the solution was scanned spectrophotometrically using UV visible spectrophotometer (UV Jasco V-630).

Determination of linearity and range

The above 100 µg/ml moxifloxacin HCl solution was employed as a stock solution for linearity study. To 10 ml volumetric flask, aliquots in range of 0.2-1.4 ml from the stock solution were transferred and with the STF 7.4 pH, the volume was adjusted to 10 ml to obtain different concentrations within range of 2-14 µg/ml. Absorbance of the above solutions was taken at their working λ max of 288 nm against the blank solution prepared in the same manner without adding drug. From the calibration curve, the Beer's law was verified by plotting a graph of concentration against absorbance.^[12]

DSC

DSC (DSC 1 Star Metler Toledo) was used to study the thermal property of drug and polymers alone and in combination (1:1 physical mixture). An idea about the endothermic and exothermic parameters of drug and polymers were obtained by it.

Preparation of stimuli sensitive *In Situ* hydrogels

Following two methods were used for the preparation of different batches.

Preparation of poloxamer 407/HPMC formulation

Poloxamer 407 *in situ* gel was prepared using the cold method [Table 1]. The drug was dissolved in cold water and filtered to yield a final concentration of 0.5% w/v. The calculated amount of HPMC K4M was dispersed in the drug solution and stirred until dissolved. Solution containing benzalkonium chloride and sodium chloride was added to the polymeric solution. Appropriate amount of poloxamer 407 was added to the cold solution, refrigerated at 4°C and stirred periodically until a homogeneous solution was obtained. Distilled water was then added to make up the volume of preparation. The pH of all formulations was adjusted to 7.4 ± 0.1 by 0.1 N NaOH.

Preparation of carbopol/HPMC formulations

The HPMC K4M was added to the distilled water and stirred until dissolved. Carbopol was sprinkled over this solution and stirred with magnetic stirrer and allowed to hydrate overnight. Moxifloxacin hydrochloride was dissolved in small quantity of water and benzalkonium chloride and sodium chloride were added to this solution and filtered. The filtered solution was then added to the polymer solution and distilled water was then added to make up the volume of preparation. The prepared

Table 1: Composition of *in situ* gelling systems

Ingredients (gm/ml)	Formulation composition % w/v							
	PF1	PF2	PF3	PF4	CF1	CF2	CF3	CF4
Moxifloxacin hydrochloride	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Poloxamer 407	14	16	18	20	-	-	-	-
Carbopol 940	-	-	-	-	-	0.3	0.4	-
HPMC K4M	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36
Carbopol 974P	-	-	-	-	0.3	-	-	0.4
Disodium hydrogen phosphate	1	1	1	1	1	1	1	1
Benzalkonium chloride	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Sodium chloride	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
Distilled water	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml	100ml	100 ml

HPMC: Hydroxypropyl methylcellulose

solution was filtered through filter paper. When the drug solution and polymer solution were mixed, immediate precipitation of carbopol occurred due to decrease in pH brought about by carbopol. Hence small quantity of 0.1N NaOH solution was incorporated to the polymeric solution to get a clear solution of drug and polymer. The formulations were sterilized by membrane filtration (0.22 µm) under laminar air flow system and filled in vials under aseptic conditions (class 100).

Clarity and visual appearance

Appearance and clarity was determined by visual examination of the formulations before and after gelling under light alternatively against white and black backgrounds. The formulations was also observed for any unwanted particle or turbidity dispersed in solution.^[12]

pH

The eye formulations to be instilled into eye should be non irritating to the eye. It is essential that formulation should have same pH as that of lacrimal fluid. Formulations were taken in a beaker and 0.1M NaOH was added drop wise with continuous stirring. By using pH meter (Ecolab Digital pH meter) the pH was examined.^[13,14]

Drug content

For estimation of drug content 1 ml sample of *in situ* gel was diluted in 100 ml volumetric flask with 100 ml of STF of pH 7.4. Again 1 ml of this sample was taken and diluted with 10 ml of STF. By UV spectrometer (Jasco V-630) the absorbance was noted at 288 nm to calculate the drug percent content.^[13]

Gelling capacity

It was performed by placing 100 µl of the prepared formulation into a vial containing freshly prepared 2 ml STF

of pH 7.4. Visually gelation was assessed by observing the time for the formed gel to dissolve.^[15,16]

In Vitro drug release study

The bichambered donor-receiver compartment model (Franz diffusion cell) was used to study *in vitro* drug release. It was placed on magnetic stirrer and temperature was adjusted to 37 ± 0.5°C. On fresh goat, cornea collected from slaughter house accurately measured 1 ml of the preparation spread uniformly which was in contact with receptor medium. The receptor medium was stirred continuously at 22 rpm to simulate blinking action of eyelids. Samples were withdrawn at periodic intervals and dilution was done with 10 ml of STF of pH 7.4. Using UV spectrophotometer, the content of drug was analyzed at 288 nm against reference standard using STF as blank. With the marketed Mosi (0.5%) eye drop, the drug release study was compared.^[17]

Antimicrobial activity

It was performed with the agar diffusion medium using cup-plate technique. With the help of plastic micropipette tips, the cup was bored. Poured the 50 µl solution into the cup. Sterile solution of marketed moxifloxacin HCl eye drops was used as a standard. The developed formulations and standard solution were taken into the separate cups bored into sterile nutrient agar earlier seeded with organisms *Staphylococcus aureus*. The solutions were allowed to diffuse for 2 h and the plates were incubated at 37°C for 1 day. With the help of standard, the zone of inhibition (ZOI) was compared.^[11]

Sterility testing

Fluid thioglycolate and soybean casein digest mediums are used for aerobic, anaerobic bacteria, and fungi to perform sterility test as per Indian pharmacopoeia 1996. The study was conducted under laminar airflow and membrane filter (0.45 µm)

was used to pass the formulations using vacuum pump. The filter paper was removed after filtration from funnel and cut into two halves. One half was dropped in fluid thioglycolate media and other was dropped in soybean casein digest media. Both the media were kept for incubation for 7 days at 37°C and observed for any microbial growth. The results were compared with positive and negative control samples.

Isotonicity evaluation

To prevent eye irritation and tissue damage, isotonicity has to be maintained. Formulations CF1 and PF2 were subjected to isotonicity test. The formulations were mixed with few drops of blood and observed under microscope at ×45 magnification. With the standard marketed ophthalmic eye drop (Mosi 0.5%) containing moxifloxacin hydrochloride, shapes of blood cells were compared.

Antibacterial activity

To carryout microbiological assay, serial dilution method was employed. Test organism recommended for moxifloxacin hydrochloride antibiotic was *S. aureus*. Three samples were tested for minimum inhibitory concentration (MIC), and they are coded as A (CF1), B (pure sample), and C (PF2). The activity of compound against *S. aureus* was tested by MIC. The concentration of moxifloxacin in both standard and test taken was 5 mg/ml. 51 µl of the above solution contains 255 µg of the drug. In the rack, 14 test tubes were arranged and numbered as 1 to 14. To the 1st test tube, 2000 µl of brain–heart infusion (BHI) broth was added. To the remaining test tube, 1000 µl of BHI was added. 51 µl of BHI broth was pipette out using sterile micropipette and was discarded. Drug solution (51 µl) was added to it (2000 µl contains 256 µg of drug). The concentration in the 1st test tube was 128 µg/ml of moxifloxacin, and then, 1000 µl of the solution was transferred from tube no. 1 to tube no. 2 and mixed well. This procedure was repeated till the second last tube to obtain the concentration

of 128 µg/ml, 64 µg/ml, 32 µg/ml, 16 µg/ml, 8 µg/ml, 4 µg/ml, 2 µg/ml, 1 µg/ml, 0.5 µg/ml, and 0.25 µg/ml, respectively. The last 2 test tubes contain 1000 µl of media. One test tube was considered as media control and another test tube as drug control. 10 µl broth of the *S. aureus* was inoculated in all the test tubes except in negative control and incubated at 37°C for 24 h to observe the growth. After the incubation period, the tubes were observed for showing inhibition of growth and calculation of MIC was done and results were tabulated.

Ocular irritation study (draize test)

With the approval of Institute Animal Ethical Committee (COPH/IAEC/2012/11), the study was performed and protocol was approved as per CPCSEA guidelines. Six albino rabbit (Newzeland white rabbit) were used as test species. Right eye was designated as the test eye and left one was untreated as a control. In the lower conjunctival cul-de-sac, single drop approximately 0.1 ml was instilled and for several seconds after instillation eyelids were hold together, later normal blinking was allowed. Observations were done at 1, 24, 48, 72 h, and 1 week after exposure. Ocular changes were done by examining any alterations to the eyelids, conjunctiva, cornea, and iris.^[18]

RESULTS AND DISCUSSION

Results of preformulation studies performed on drug and polymers were given in following Table 2.

UV spectroscopic study

Determination of wavelength of maximum absorption

Scanning of moxifloxacin 100 µg/ml stock solution in STF having pH 7.4 by UV spectrophotometer showed the λ max 288 nm in Figure 1.

Table 2: Preformulation study on drug and polymers

Observed parameters	Moxifloxacin	Poloxamer 407	HPMC K4M	Carbopol 974p/ Carbopol
Description	Slightly yellow to yellow crystalline powder	White, free flowing granules	White or creamy white fibrous or granular powder	White, fluffy, dry powder
pH	pH of 1% w/v aqueous solution was 4.5	pH of 2.5% w/v aqueous solution was 5.5	pH of 1% aqueous solution was 6.8	pH of 1% aqueous dispersion was 2.8
Melting point	246°C	52-57°C	70°C	Glass transition temp was 104°C
Solubility	Soluble in water, phosphate buffer (pH 7.4 and pH 4.0), 2-propanol, acetone, STF, ethanol	Freely soluble in water, propane -2-ol, ethanol	Soluble in the cold water, insoluble in ether, alcohol	Soluble in water and after neutralization soluble in glycerin, ethanol (95%)

HPMC: Hydroxypropylmethylcellulose, STF: Simulated tear fluid

Determination of linearity and range

The absorbance of moxifloxacin standard solutions containing 2-12 µg/ml of drug in STF having pH 7.4 is shown in Table 3. A representative standard calibration curve was shown in Figure 2. Linear calibration curve was obtained in the concentration range of 2-12 µg/ml at λ_{max} 288 nm and followed Beer's-Lambert's law with regression coefficient (R^2) value of 0.999.

DSC study

Using DSC, thermal properties of moxifloxacin HCl and poloxamer 407 alone and in combination (1:1 mixture) were investigated. Due to glass transition from solid to liquid phase, the curve of moxifloxacin HCl displayed an endothermic peak at 250.89°C as seen in Figure 3. The DSC curve of poloxamer 407 showed a sharp endothermic peak at 57.62°C characterized a glass transition due to melting of polymer as depicted in Figure 4. The physical mixture of poloxamer 407 and moxifloxacin HCl depicted two endothermic peaks at 58.21°C and 245°C due to glass transition temperature, respectively.

The physical mixture of moxifloxacin HCl with HPMC K4M and carbopol 974P showed characteristic peak at 250.36°C of CL formulation and 243.7°C of PL formulation [Figures 5 and 6]. The DSC revealed that moxifloxacin HCl was compatible with the polymers.

Clarity, visual appearance, pH, and drug content

The findings of these studies are presented in Table 4.

Measurement of gelling capacity

Formulations CF1, CF4, and PF2 have shown optimum gelling capacity and consistency as shown in Table 5. Figure 7 illustrates the gelling capacity due to either change in physical or chemical stimuli such as temperature before and after gelation of formulations.

In Vitro release study

It was performed using Franz diffusion cell apparatus, and fresh goat cornea was used for this study. It was found that

% controlled drug release from formulations CF1 and PF2 were up to 5 and 6 h, respectively. The drug release from the

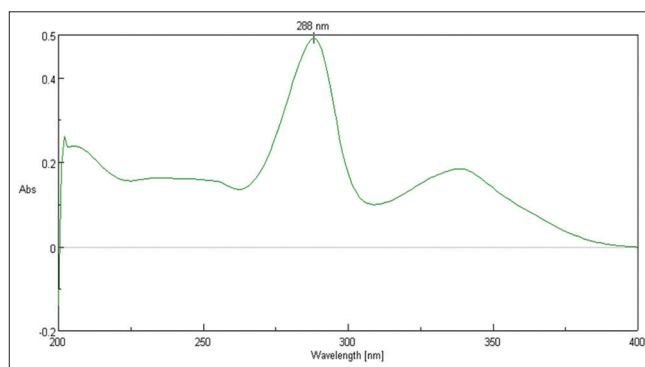


Figure 1: Ultraviolet spectra of moxifloxacin HCl

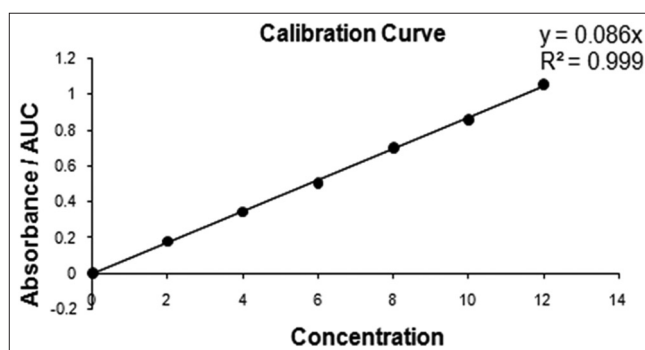


Figure 2: Calibration curve of moxifloxacin HCl

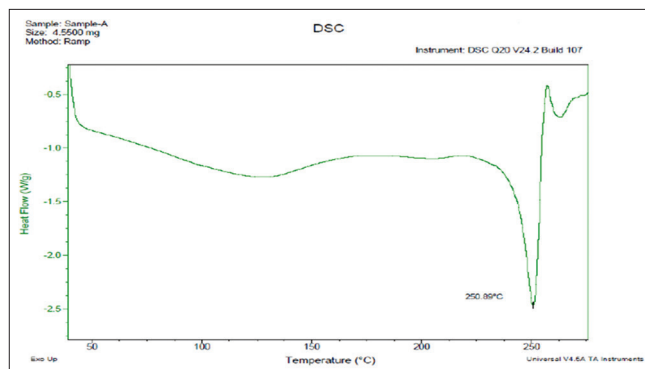


Figure 3: Thermal analysis of moxifloxacin HCl

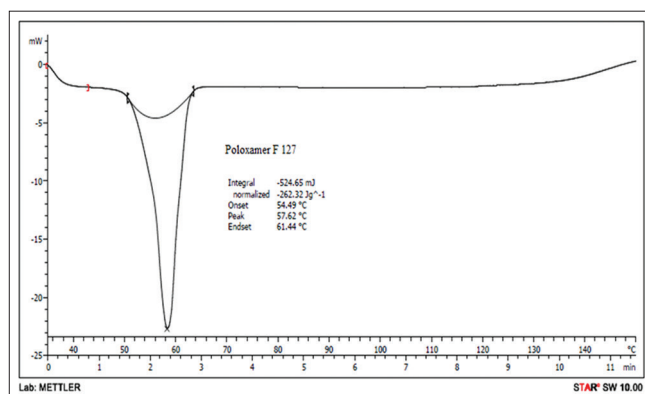


Figure 4: Thermal analysis of poloxamer 407

Table 3: Absorbance of moxifloxacin HCl

Concentration in µg/ml	Absorbance at 288 nm
2	0.178
4	0.342
6	0.502
8	0.698
10	0.859
12	1.055

Table 4: Determination of visual appearance, pH, and drug content

Formulations	Appearance	Clarity	pH	% drug content \pm SD*
CF1	Light yellow	Clear	6.8	99.54 \pm 0.7292
CF2	Light yellow	Clear	6.6	99.32 \pm 0.3245
CF3	Dark yellow	Clear	6.7	98.45 \pm 0.7328
CF4	Dark yellow	Cloudy	6.4	99.43 \pm 0.3055
PF1	Light yellow	Cloudy	6.7	99.41 \pm 0.4725
PF2	Light yellow	Clear	6.9	100 \pm 0.2598
PF3	Dark yellow	Clear	6.7	99.28 \pm 0.2950
PF4	Dark yellow	Cloudy	6.6	99.72 \pm 0.5783

SD: Standard deviation

Table 5: Gelation capacity of formulations

Formulations	Gelling capacity* n=4
CF1	+++
CF2	++
CF3	++
CF4	+++
PF1	+
PF2	+++
PF3	++
PF4	++

+: No gel formation, ++: Gelation immediate at room temperature only, +++: Gelation immediate and remains for an extended period

optimized batches is good as compared to marketed sample. The graphs are shown in Figures 8 and 9.

Antimicrobial efficacy study

Measurement of ZOI by cup plate method

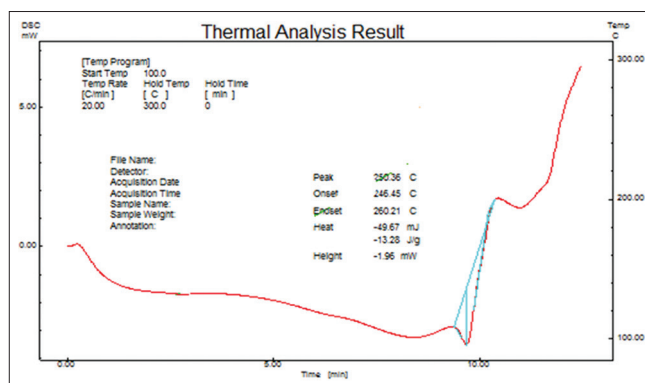
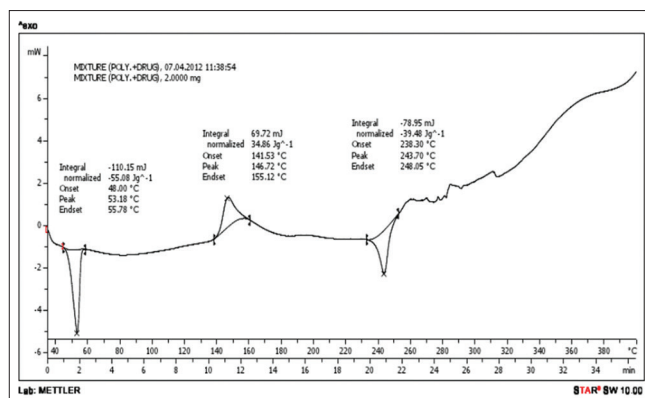
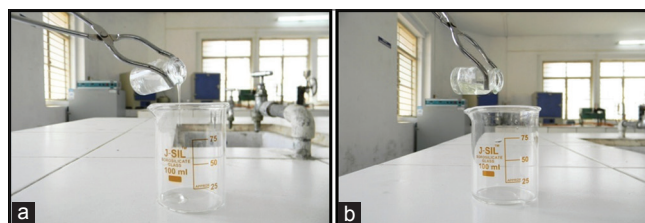
The size of the zone found lesser as a drug was more effective to the organism. In first few concentrations, the diameter of the zone was more due to high sensitivity of the drug. Readings of this study are tabulated in Table 6. Images of ZOIs of marketed, CF1 and PF2 formulations are depicted in Figure 10.

Sterility test

No turbidity was observed, and hence, no evidence of microbial growth when the formulations were incubated for prescribed time and temperature. The preparations being examined therefore passed the test for sterility as shown in Figure 11.

Isotonicity testing

Isotonicity test revealed no change in the shape of blood cells, i.e., bulging or shrinking in CF1 and PF2 formulations


Figure 5: Thermal analysis of moxifloxacin + carbopol + hydroxypropyl methylcellulose K4M physical mixture

Figure 6: Thermal analysis of moxifloxacin + poloxamer + hydroxypropyl methylcellulose K4M physical mixture

Figure 7: Gelation capacity before and after gelation. (a) Before gelation. (b) After gelation

[Figure 12]. Hence, formulations were found to be fully isotonic with the blood.

Table 6: Diameter of zone of inhibition against *Staphylococcus aureus* on marketed (Mosi 0.5%), CF1, PF2 formulations

Conc (µg/ml)	Marketed (mm)	Sensitivity	CF1 (mm)	Sensitivity	PF2 (mm)	Sensitivity
1:10	50	Sensitive	31	Sensitive	33	Sensitive
1:20	39	Sensitive	27	Sensitive	27	Sensitive
1:30	30	Sensitive	25	Sensitive	22	Sensitive
1:40	23	Sensitive	20	Sensitive	12	Sensitive
1:50	17	Intermediate	17	Intermediate	0	Resistant
1:60	13	Sensitive	14	Sensitive	0	Resistant
1:70	0	Resistant	0	Resistant	0	Resistant
1:80	0	Resistant	0	Resistant	0	Resistant
1:90	0	Resistant	0	Resistant	0	Resistant
1:100	0	Resistant	0	Resistant	0	Resistant

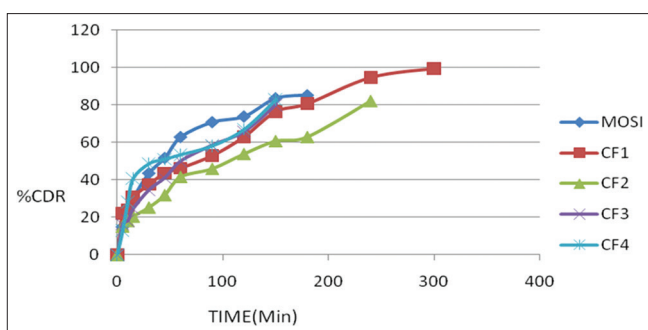


Figure 8: % drug release of carbopol formulations with Mosi eyedrop

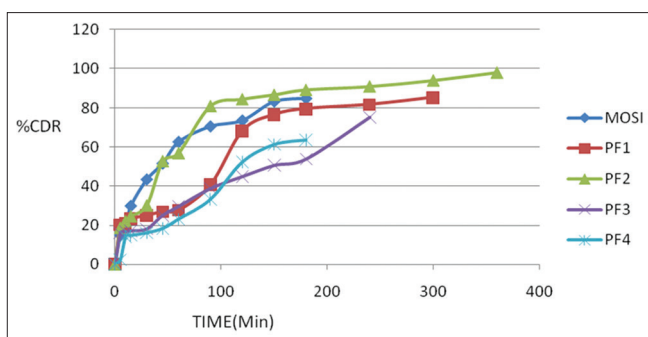


Figure 9: % drug release of poloxamer formulations with Mosi eyedrop

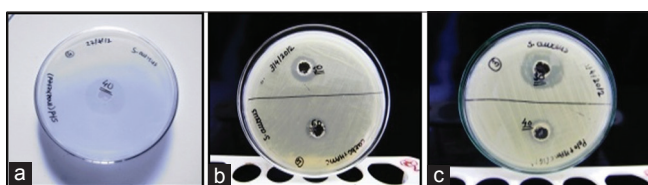


Figure 10: Zone of inhibitions of marketed, CF1 and PF2 formulations. (a) Marketed. (b) CF1. (c) PF2

Antibacterial sensitivity test

In A and B samples, the MIC concentration was found to be 1 mcg/ml and 16 mcg/ml as shown in Table 7. Hence, the

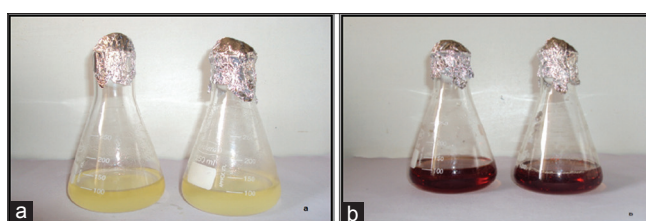


Figure 11: Sterility testing of moxifloxacin in soybean casein media and thioglycolate media. (a) Soybean casein media. (b) Thioglycolate media

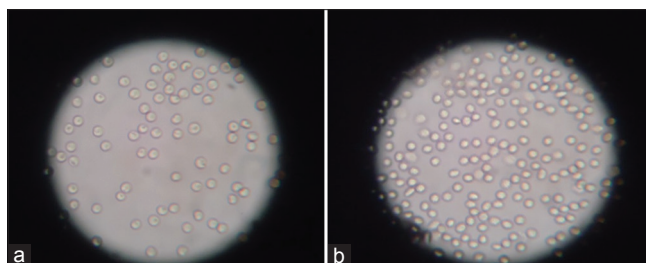


Figure 12: Isotonicity CF1 and of PF2 formulations. (a) Isotonicity of CF1. (b) Isotonicity of PF2

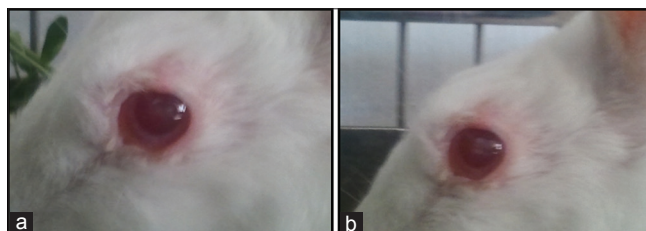


Figure 13: Observations on rabbit eye before and after instillation of formulations. (a) Before instillation. (b) After instillation

turbidity in the formulations below this concentration indicates the growth of microorganism. No signs of turbidity and growth were observed in Poloxamer formulations. Hence, it was not sensitive to *S. aureus* strain of bacteria but sensitive to *Pseudomonas aeruginosa* strain. In marketed and CF1 formulations, clear solution was seen in concentration from 128 to 16 mcg/ml.

Table 7: MIC determination of CF1, pure drug, and PF2

Concentration mcg/ml	Turbidity in CF1	Turbidity in pure drug (standard)	Turbidity in PF2
128	-	-	-
64	-	-	-
32	-	-	-
16	-	-	-
8	-	+	-
4	-	+	-
2	-	+	-
1	-	+	-
0.5	+	+	-
0.25	+	+	-
0.125	+	+	-
NC	+	-	-
MC	-	-	-
DC	-	-	-
PC	+	+	+

NC: Negative control, MC: Media control, DC: Drug control, PC: Positive control, +: Presence of turbidity (no inhibition), -: Presence of clear solution (inhibition), MIC: Minimum inhibitory concentration

Ocular irritation study

Animal study revealed that there was no irritation into both eyes, no watering from eyes, or no physiological change or any severe damage to the eyes. The iris was normal; neither congestion nor hemorrhage was observed. Details of iris were clearly visible and cornea was found with no opacity as seen in Figure 13.

CONCLUSION

The present work was carried out to develop pH and temperature triggered *in situ* gel of moxifloxacin hydrochloride (0.5% w/v). Attempts were made to design the formulations with low concentration of HPMC K4M (0.36% w/v). The formulations were in solution form at pH 4.4 which underwent sol-to-gel transformation when instilled in eyes at pH 7.4, showed increased in precorneal residence time, ocular bioavailability, and patient compliance with decreased in dosing frequency. It was 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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REFERENCES

- Gupta H, Jain S, Mathur R, Mishra P, Mishra AK, Velpandian T. Sustained ocular drug delivery from a temperature and pH triggered novel *in situ* gel system. *Drug Deliv* 2007;14:507-15.
- Palani S, Nisha MJ, Goda CC, Anish Z, Zelalem A. Ocular drug delivery: A review. *Int J Pharm Sci Res* 2010;1:1-13.
- Laddha UD, Mahajan HS. An insight to ocular *in situ* gelling systems. *Int J Adv Pharm* 2017;6:31-40.
- Gratieri T, Gelfuso GM, de Freitas O, Rocha EM, Lopez RF. Enhancing and sustaining the topical ocular delivery of fluconazole using chitosan solution and poloxamer/chitosan *in situ* forming gel. *Eur J Pharm Biopharm* 2011;79:320-7.
- Patel PB, Shastri DH, Shelat PK, Shukla AK. Ophthalmic drug delivery system: Challenges and approaches. *Syst Rev Pharm* 2010;1:113-20.
- Kumar KK, Swathi M, Srinivas L, Basha N. Formulation and evaluation of floating *in situ* gelling system of losartan potassium. *Pharm Lett* 2015;7:98-112.
- Mandal S, Thimmasetty MK, Prabhushankar G, Geetha M. Formulation and evaluation of an *in situ* gel-forming ophthalmic formulation of moxifloxacin hydrochloride. *Int J Pharm Investig* 2012;2:78-2.
- Vijaya C, Goud KS. Ion-activated *in situ* gelling ophthalmic delivery systems of azithromycin. *Indian J Pharm Sci* 2011;73:615-20.
- Majithiya RJ, Ghosh PK, Umrethia ML, Murthy RS. Thermoreversible-mucoadhesive gel for nasal delivery of sumatriptan. *AAPS PharmSciTech* 2006;7:67.
- Liu Z, Li J, Nie S, Liu H, Ding P, Pan W. Study of an

- alginate/HPMC-based *in situ* gelling ophthalmic delivery system for gatifloxacin. *Int J Pharm* 2006;315:12-7.
11. Jyothirmai KS, Manasa M, Sravani D, Devi GT, Mounika M, Santhi PN, *et al.* Formulation and evaluation of ocular *in situ* hydrogels of acyclovir. *Int J Res Pharm Chem* 2017;7:162-70.
 12. Hemalata D, Ashok H, Vikram S, Sanket G, Omprakash G. Formulation and evaluation of pH triggered *in situ* ophthalmic gel of moxifloxacin hydrochloride. *Indo Am J Pharm Res* 2016;6:6790-9.
 13. Mandal S, Thimmasetty MK, Prabhushankar G, Geetha M. Formulation and evaluation of an *in situ* gel-forming ophthalmic formulation of moxifloxacin hydrochloride. *Int J Pharm Investig* 2012;2:78-2.
 14. Patil S, Kadam A, Bandgar S, Patil S. Formulation and evaluation of an *in situ* gel for ocular drug delivery of anticonjunctival drug. *Cellulose Chem* 2015;49:35-40.
 15. Makwana SB, Patel VA, Parmar SJ. Development and characterization of *in situ* gel for ophthalmic formulation containing ciprofloxacin hydrochloride. *Results Pharma Sci* 2015;6:1-6.
 16. Wagh VD, Deshmukh KH, Wagh KV. Formulation and evaluation of *in situ* gel drug delivery system of *Sesbania grandiflora* flower extract for the treatment of bacterial conjunctivitis. *J Pharm Sci Res* 2012;4:1880-4.
 17. Harpinder KG, Shweta B. A novel *in situ* gel for sustained ophthalmic delivery of ciprofloxacin hydrochloride and diclofenac sodium: Design and characterization. *World J Pharm Pharm Sci* 2015;4:1347-56.
 18. Akshata IS, Bolmal UB, Gadad AP. Formulation and evaluation of pH triggered *in situ* ophthalmic gel containing natamycin cyclodextrin inclusion complex. *Indian J Pharm Sci Res* 2016;6:94-103.

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