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Development of Thermoreversible Moxifloxacin Hydrochloride Ophthalmic Formulation

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ABSTRACT

The field of Ocular drug delivery is one of the interesting and challenging endeavors facing the pharmaceutical scientist. The most frequently used dosage forms i.e. ophthalmic solutions and suspensions are compromised in their effectiveness by several limitations, leading poor ocular bioavailability. In situ hydrogels are instilled as drops into the eye and undergoes a sol to gel transition in the cul-de-sac, improved ocular bioavailability by increasing the duration of contact with corneal tissue, thereby reducing the frequency of administration. The purpose of the present work was to develop an ophthalmic in situ gel of Moxifloxacin HCl a fluoroquinolone antibiotic. Poloxamer 407 a temperature sensitive gelling agent was employed for the formation of in situ hydrogel along with sodium alginate as a mucoadhesive polymer. *In-situ* gel was evaluated for various parameters like appearance, pH, drug content, gelling capacity, gel strength, bioadhesion, viscosity, In-vitro drug release, isotonicity, sterility, antifungal activity, ocular irritancy and stability studies. The gel strength, bioadhesion and isotonicity shown quality parameter for ophthalmic formulation. The optimized formulation containing 10% w/v poloxamer 407 and 0.1% w/v sodium alginate have shown 96.84% drug release up to 8 hrs. This is sufficient for antibacterial activity. Drug release kinetic study shown that a Korsmeyers-peppas is the best-fit model. This study found that an optimized formulation having improved viscosity and better mucoadhesive property may improve the bioavaibility of ocular administration of moxifloxacin HCl in *in-situ* gel form and can be alternative to the conventionally administered oral formulation and effectively used to prolong residence time.

Keywords: Poloxamer 407, Thermoreversible technique, Antibacterial activity

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INTRODUCTION

Ophthalmic drug delivery is one of the most interesting and challenging endeavors facing the pharmaceutical scientist. The anatomy, physiology, and biochemistry of the eye render this organ highly impervious to foreign substances. A significant challenge to the formulator is to circumvent (bypass) the protective barriers of the eye without causing permanent tissue damage. Development of newer, more sensitive diagnostic techniques and novel therapeutic agents continue to provide ocular delivery systems with high therapeutic efficacy. Conventional ophthalmic formulations like solution, suspension, and ointment have many disadvantages which result into poor bioavailability of drug in the ocular cavity.[1] A basic concept shared by most scientists in ophthalmic research and development is that the therapeutic efficacy of an ophthalmic drug can be greatly improved by prolonging its contact with the corneal surface. For achieving this purpose, viscosity-enhancing agents are added to the eye drop preparations, or the ophthalmic drug is formulated in water-insoluble ointment formulation to sustain the duration of intimate drug-eye contact. Unfortunately, these dosage forms give only more sustained drug-eye contact than eye drop solutions and do not yield a constant drug bioavailability as originally hoped. 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. The second involves maximizing corneal drug absorption and minimizing precorneal drug loss. Ideal ophthalmic drug delivery must be able to sustain the drug release and to remain in the vicinity of front of the eye for prolong period of time.[2]

MATERIALS AND METHODS

Materials

Moxifloxacin hydrochloride was obtained from the Ajanta Pharma Ltd (India), PF 127 (high molecular weight) was used as received from BASF India Ltd. (India), sodium alginate was used as received from Molycheme. All other chemicals used were of analytical grade.

Method

3^2factorial design was used for composition of different formulations (Table 1.) all different formulations were prepared as per (Table 2). Accurately weighed quantity of the Moxifloxacin HCl was dissolved in 25 mL distilled water. The sodium metabisulphite was added to above mixture with continuous stirring. The above solution was divided into 2 equal volumes, poloxamer 407 and sodium alginate were added to each of these volumes respectively then mix to each other

and was allowed to hydrate for 12 hours to produce a clear solution/ dispersion. Prepared solutions were autoclaved at 121^0 C for 20 min. The formulations were aseptically transferred to previously sterilized glass vials and sealed.

Table 1: Composition of formulation batches as per 3^2 factorial design

Formulation code	F1	F2	F3	F4	F5	F6	F7	F8	F9
Ingredient (%)									
Moxifloxacin Hydrochloride	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Poloxamer 407	10	15	20	10	15	20	10	15	20
Sodium alginate	0.1	0.1	0.1	0.25	0.25	0.25	0.5	0.5	0.5
Sodium metabisulphite	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Distilled water	100	100	100	100	100	100	100	100	100

Table 2. Experimental Design as per 3^2 Factorial Design

Formulation Code	Coded values			
	X1	% (W/V)	X2	% (W/V)
F1	-1	10	-1	0.1
F2	0	15	-1	0.1
F3	+1	20	-1	0.1
F4	-1	10	0	0.25
F5	0	15	0	0.25
F6	+1	20	0	0.25
F7	-1	10	+1	0.5
F8	0	15	+1	0.5
F9	+1	20	+1	0.5

Evaluation of ophthalmic gel

Determination of clarity, pH and drug content[3]

The clarity was determined visually. The pH of each formulation was determined by using Digital pH meter (Digital pH meter 335). 1mL of formulation was taken in 100 mL beaker; in that beaker 100 mL distilled water was added. Aliquot 25 mL from this solution was diluted up to 50 mL with distilled water to get the final concentration of 25µg/mL. The absorbance of prepared solution was measured at 293.0 nm by using UV visible spectrophotometer.

COMPATIBILITY STUDY

Fourier Transform Infrared Spectroscopy[4, 5]

Compatibility study was carried out by using Fourier Transform Infrared Spectrophotometer (Cary 630, Agilent Technologies, USA). FTIR study was carried on pure drug and physical mixture of drug and polymers. Physical mixtures were prepared and samples were kept for 1 month at 40^0C. The infrared absorption spectrum of Moxifloxacin HCl and physical mixture of drug and polymers were recorded using a KBr disc over the wave number 4000 to 650 cm^{-1}.

Rheological study[3]

The rheological properties of gels were determined by the Brookfield viscometer; type DV-II + PRO using spindle no. SC4-18.Viscosity of the formulations were taken at two different temperatures i.e. at room temperature and at 37°C with varying shear rate.

Measurement of gelling capacity[6]

The gelling capacities of formulations were determined by placing 1 drop of the prepared formulations into a vial containing 2 mL of STF freshly prepared. Gelation was assessed visually and noting the time for gelation and the time taken for the gel formed to dissolve.

Measurement of the gel strength[7]

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. All measurements were performed in triplicate (n=3). The apparatus used for measuring gel strength at room temperature and at 37ºC is shown in Figure. 1

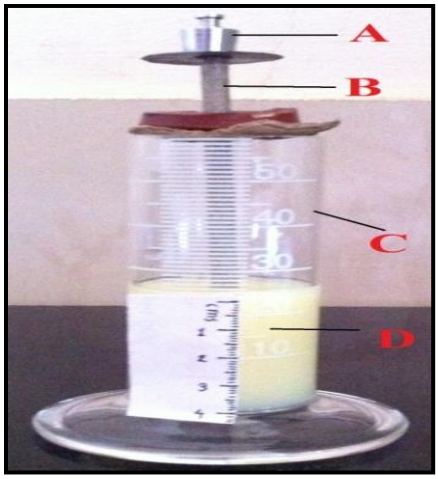


Figure. 1: Gel strength measuring device

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

Bioadhesive strength[7, 8, 9]

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

Fabrication of equipment

The equipment was fabricated by us in the laboratory as shown in Figure 2. 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. (G).

Figure. 2: - Modified bioadhesion test apparatus (Fabricated)

A-Modified physical apparatus, B-Right pan, C- Weights, D-Upper polypropylene disc, E-Lower polypropylene disc, F-Corneal membrane, G-Simulated tear fluid.

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. Immediately afterwards the membrane was placed over the surface of lower teflon cylinder (E) and secured. This assembly was placed into beaker containing simulated tear fluid 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, 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) (W1-5.25gm)) gives force required for detachment expressed in weight in grams. Procedure was repeated for two more times. Average was computed and recorded.

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 S. D. was calculated.

Force of adhesion (N)

Bioadhesive strength = (Bioadhesive strength/1000) × 9.81

Bond strength (N/m2) = Force of adhesion (N) / Surface area of disk (m^2)

The Modified Apparatus for Bioadhesive study is shown in Figure. 2

***In-vitro* drug release study[3]**

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 24 mm was used for the study. 1 mL formulation was placed in donor compartment and Freshly prepared 100 mL artificial tear fluid (sodium chloride 0.670g, sodium bicarbonate 0.200g, calcium chloride dehydrated 0.008g, potassium chloride 0.248g,distilled water q.s 100mL.) was placed in receptor compartment. Egg membrane was 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. The temperature of the medium was maintained at 37°C ± 0.5°C. 1mL of sample was withdrawn from receiver compartment after 15 min, 1, 2, 3, 4, 5, 6, 7 & 8 hrs. and same volume of fresh medium was replaced. The withdrawn samples was diluted to 10mL in a volumetric flask with distilled water and analyzed by UV spectrophotometer at 293.0nm.

Antibacterial activity[3, 10]

An agar diffusion method was used for the determination of antibacterial activity of formulations. Standard Petri dishes (9 cm diameter) containing medium to a depth of 0.5 cm were used. The sterility of the lots was controlled before use. Inoculum were prepared by suspending 1-2 colonies of *Staphylococcus aureus* from 24hr cultures in Nutrient agar medium into tubes containing 10 mL of sterile saline. The tubes were diluted with saline. The inoculum (0.5 mL) was spread over the surface of agar and the plates were dried at 35°C for 15 min prior to placing the formulation. The bores of 0.5 cm diameter were prepared and 20µL samples of formulation (1% w/v) were added in

the bores. After incubation at 35°C for 24 hrs, the zone of inhibition around the bores was measured.

Isotonicity evaluation[11, 12]

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.

Test for Sterility[3, 13]

The sterility test was carried out as per IP (2010) method. The optimized formulation was incubated for not less than 14 days at 30 -35ºC in the fluid thioglycolate medium and at 20-25ºC in soyabean casein digest medium to find out growth of bacteria in formulation.

Ocular Irritancy Test[3]

The optimized formulation was used for eye irritancy study. The protocol was approved by Institutional Animal Ethics Committee with approval no-IAEC/05/405/1. The Modified Draize Eye Irritation: The 01 Albino rabbits weighing 1.5 to 2 kg.[18] According to the draize test, the amount of formulation was applied to the eye is 100µL was placed into the lower cul-de-sac. The evaluation of ocular lesions was made at 1, 4, 24, 48, 72hrs, and 1 week after administration. 3 day washing period with saline was carried out. The sterile formulation was instilled twice a day for a period of 7 days, and a cross over study carried out. The rabbit was observed periodically for redness, swelling, watering of the eye.

Stability studies[14]

The formulations were stored at room temperature with RH 60± 5% respectively. The formulations were evaluated mainly for their physical characteristics like appearance/clarity, pH, viscosity and drug content after 6 months.

RESULTS AND DISCUSSION

Clarity, pH and Drug content

On careful visual inspection against dark and white background, all the prepared ophthalmic *in-situ* gel formulations were found to be free from any suspended particulate matter. The F1, F5, F6, F8 and F9 formulations were found to be clear. Where as F2, F3, F4 and F7 formulations were found to be opaque as it contains sodium alginate. The pH of all the formulations from F1 to F9 was found to be in the range of 6.0 to 6.6 pH values of formulations shown in Table 3. Ideally, the ophthalmic solutions should possess 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. The percentage drug content of all prepared ophthalmic formulations was found to be in the range of 98-102%. Table 3 Therefore uniformity of content was maintained in all formulation.

Table 3. Evaluation Parameters

Sr. No	Formulation code	Observed pH(±SD)	Detachment force (N)(±SD)	Drug content (%)(±SD)	Cumulative Drug Release (%)(±SD) after 8hrs
1	F1	6.53±0.005	0.0453±0.002	100.09±0.404	96.84±0.022
2	F2	6.56±0.015	0.2167±0.001	102.09±0.871	93.86±0.012
3	F3	6.41±0.01	0.3359±0.005	98.52±0.300	85.92±0.030
4	F4	6.28±0.01	0.0629±0.017	98.17±0.402	92.20±0.019
5	F5	6.00±0.011	0.2710±0.003	99.87±0.222	83.39±0.018
6	F6	6.13±0.015	0.3524± 0.026	98.88±0.715	66.34±0.039
7	F7	5.95±0.023	0.0809±0.002	101.08±0.948	85.10±0.029
8	F8	6.00±1.005	0.2752±0.004	98.34±0.266	75.75±0.030
9	F9	6.25±0.01	0.3695±0.013	102.04±4.370	52.87±0.038

Compatibility Study

Infra-red spectra of drug and polymers showed matching peaks with the drug spectra. Figure.3 The characteristic peaks of drug were also present in the spectra of all drug- polymer combinations.

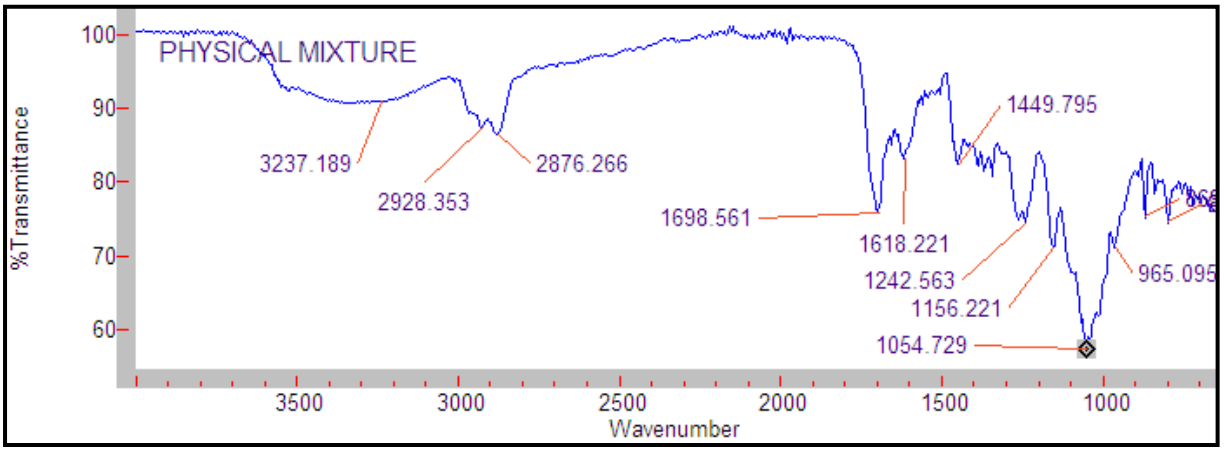


Figure. 3: Fourier Transform Infra-red of drug with polymer

Rheological study

Viscosity v/s rpm plots for all formulations shows decrease in viscosity as shear rate (rpm) was increased. Which indicate that gel has the pseudoplastic flow. As temperature was increased the increase in viscosity was observed because of temperature sensitive polymer (poloxamer 407) was used in the formulation. Concentration of poloxamer 407 was a major factor affecting viscosity of formulations. In combination with poloxamer 407 and sodium alginate has shown considerable increases in viscosity when concentration of poloxamer was 10% w/v to 20% w/v.

Table 4.Viscosity of formulations at room temperature

Rpm	Viscosity (cp) at Room Temperature								
	Formulation code								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
1.5	8	55.99	162	22	127.99	241	85.3	271.34	721.8
3	7.25	55.99	131	16	74.56	138.94	63.25	146	409.8
6	7.08	50.49	119.7	11.75	49.25	133.5	41.37	67.49	284.9
12	7	47.74	102.5	11.65	30.28	131	25.68	57.74	222.7

Table 5. Viscosity of formulations at 37°C

Rpm	Viscosity (cp) at 37°C								
	Formulation code								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
0.3	49.99	99.98	2521	120	399.98	4069	324.9	849.8	8898
0.6	29.99	84.98	1856	49.99	259.99	2514	254.9	519.9	2814
1.5	27.99	74.98	1267	34.99	141.99	1266	140	459.9	1192
3	21	73.98	1101	23	89.99	1099	70.98	110	568.9

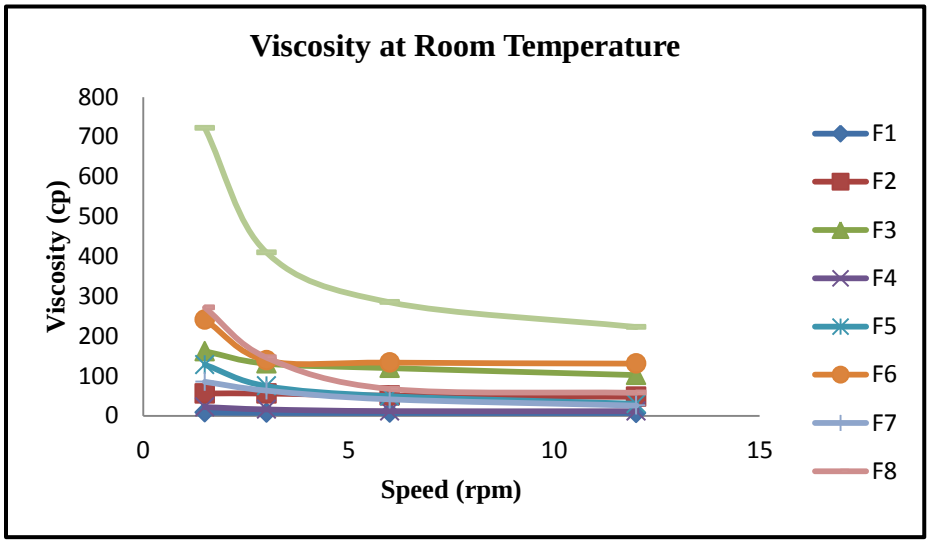


Figure. 4: Viscosity profile of formulations at room temperature

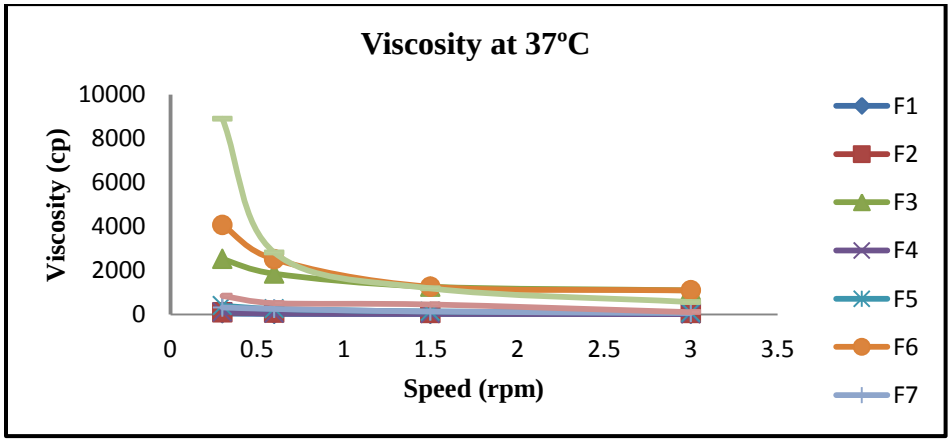


Figure. 5: Viscosity profile of formulations at 37°C

Measurement of gelling capacity

(+: Gel formed after a few minutes, dissolves rapidly, ++: Immediate gelation, remains for few hours, +++: Immediate gelation, remains for extended period.) The two main prerequisites of phase transition system are viscosity and gelling capacity (speed and extent of gelation). Moreover, the flow behavior of the formulation is an important parameter involved in utilization and in vivo performance as if it is too viscous it will lead to difficult instillation; on the contrary, if viscosity is too low it will increase drainage. From visual and manual inspection we found that all formulation coded in Table 6 underwent transition into gel phase upon contact with STF. However, it is clear that the nature of the gel formed depended upon the polymer concentration. F1, F2 and F7 formed weak gel that dissolved rapidly. The flow of F3 was liquid with very low viscosity while F6, F8 and F9 were difficult flow as gel formed during preparation. F4 and F5 had a satisfactory attributes of gelling capacity and consistency.

Table 6. Gelling capacity of ophthalmic formulation

Formulation Code	Gelling Capacity
F1	+
F2	+
F3	++
F4	++
F5	++
F6	++
F7	+
F8	+++
F9	+++

Measurement of the gel strength

The gel strength of ophthalmic formulation at room temperature and 37°C are shown in Table 7 respectively. The gel strength was found to be affected by concentrations of gelling and mucoadhesive polymers also by the temperature. Optimal mucoadhesive gel must have suitable gel strength so as to be administered easily and can be retained at ocular region without leakage after administration. Gel strength of all formulations showed comparable results as that of viscosity.

Table 7. Gel strength of formulations at room temperature and 37°C

Sr. No	Formulation code	Gel strength (sec) (±S.D.) at room temperature	Gel strength (sec) (±S.D.) at 37°C
1	F1	0.463±0.0665	0.576±0.0808
2	F2	0.666±0.1159	0.876±0.0850
3	F3	0.876±0.1234	0.916±0.0971
4	F4	0.733±0.2281	1.08±0.0953
5	F5	0.853±0.1059	1.306±0.1833
6	F6	1.18±0.0435	1.696±0.0404

7	F7	1.256±0.2706	1.86±0.1609
8	F8	1.756±1.000	2.223±0.1193
9	F9	2.03±0.3031	2.91±0.1997

Bioadhesive strength

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 force was determined for ophthalmic in situ gels. Results of this test indicate that Sodium alginate was having effect on bioadhesive strength. It shows that bioadhesive force was increased with the increasing concentration of the sodium alginate(Table 3).

***In-vitro* drug release study**

Out of nine formulations maximum release after 8 hrs was found for F1 formulation (Table 3). This indicates release of 96.84% drug available for antifungal activity of the drug. F1 formulation showed steady state release up to 8 hrs. Formulation F9 has shown poor release up to 8 hr. which indicate steady release because of high concentration of sodium alginate & poloxamer 407. This result also reflects the relationship of viscosity, gelling capacity, gel strength& bioadhesive strength with drug release. i.e. greater the viscosity, gelling capacity, gel strength & bioadhesive strength release was poor. *In-vitro* drug release profile of formulations shown in Figure.6

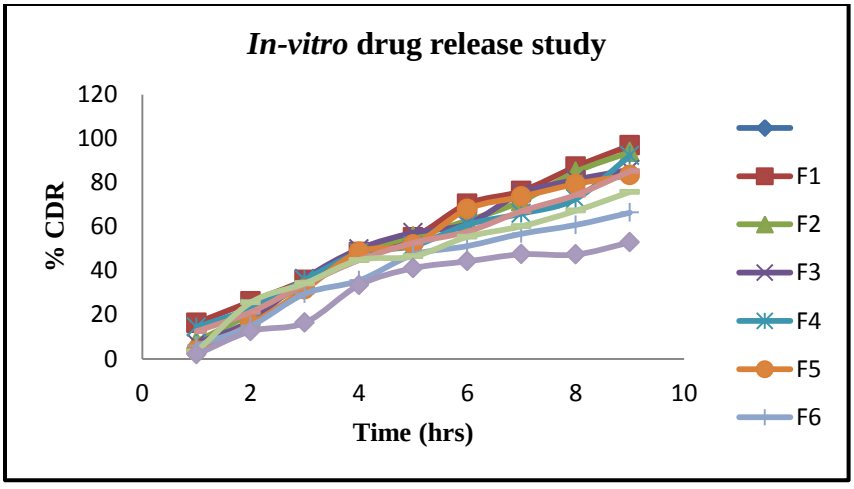


Figure. 6: *In-vitro* drug release profile of formulations

Optimization

A 3^2 full factorial design was selected and the 2 factors were evaluated at 3 levels, respectively. The percentage of poloxamer 407 (X_1) and sodium alginate (X_2) were selected as independent variables and the dependent variable was % drug release and antibacterial activity. The data obtained were treated using Design expert version 9.0.2.0 software and analyzed statistically using

analysis of variance (ANOVA). The data were also subjected to 3-D response surface methodology to study the interaction of poloxamer 407 (X_1) and sodium alginate (X_2) on dependent variable. The values of X_1 and X_2 were found to be significant at $p < 0.05$, hence confirmed the significant effect of both the variables on the selected responses. From this data optimum concentration of poloxamer 407 10%w/v and sodium alginate 0.1%w/v was found. (Figure.8 & 9)

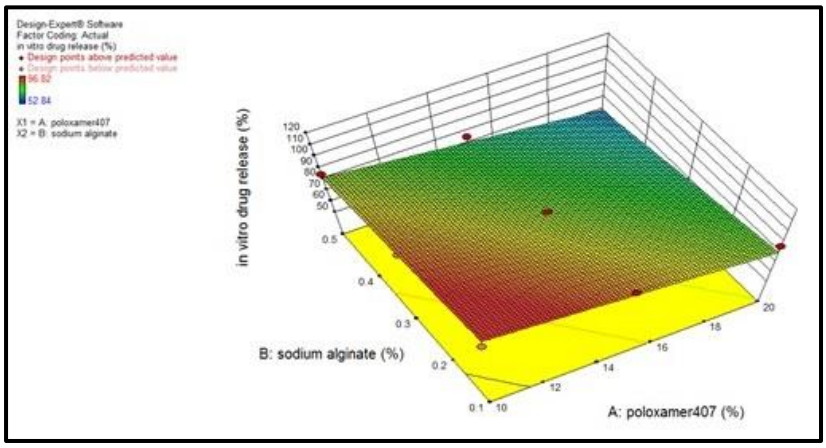


Figure. 7: Surface response plot showing effect of poloxamer 407 and sodium alginate on drug release.

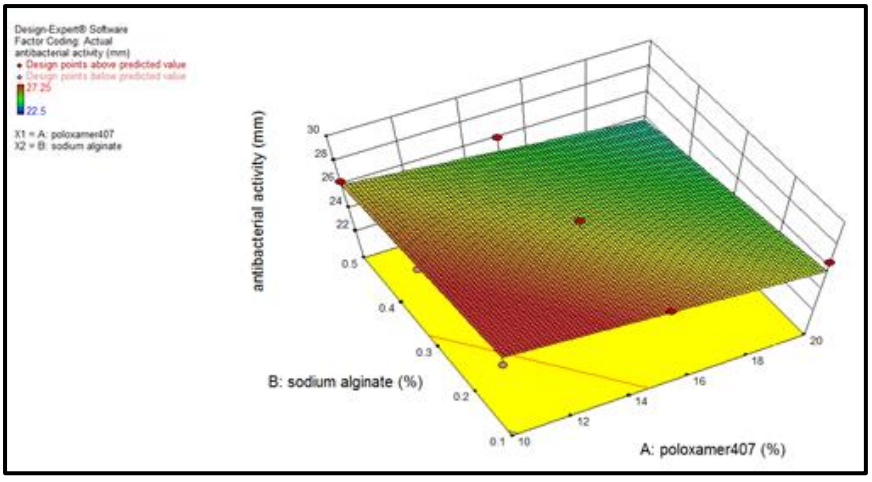


Figure. 8: Surface response plot showing effect of poloxamer 407 and sodium alginate on antibacterial activity.

Final Equation in Terms of Actual Factors

Y1 (% CDR) = 131.598 + (-2.3020)*(A)-(-52.4000) *(B)

Final Equation in Terms of Actual Factors:

Y2 (Antibacterial activity) = 30.8111+ (-0.22833)*(A) - (-4.81667)*(B)

From design expert version 9.0.2.0 one hundred and five solutions were found in which optimum batch poloxamer 407 10%w/v and sodium alginate 0.1%w/v with desirability was found to be optimum. From this data F1 batch was selected as optimum formulation.

Release kinetics[15-18]

In the present study, the drug release was analyzed by PCP Disso version v3 software to study the kinetics of drug release mechanism. The factorial design batches followed korsmeyer peppas model kinetics. The R^2value of korsmeyer peppas model was found close to one. The drug release was occurred by non fickian diffusion mechanism as reflected by its n value 0.5340 (n<0.5).

Antibacterial activity

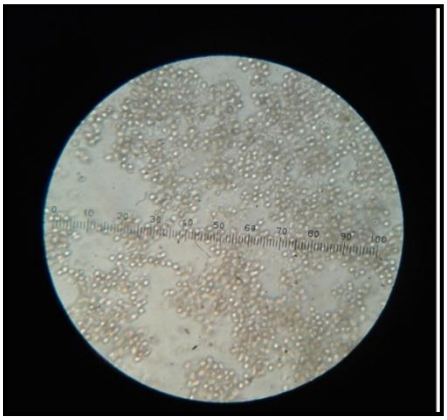
The study indicates that Moxifloxacin HCl retained its antibacterial efficacy when formulated as an ophthalmic *in-situ* gel and drug was active against selected strains of micro-organism. F1 formulation showed 27.25 mm zone of inhibition and 97.32% efficacy. Results obtained from antibacterial activity of F1 formulation resembles to release profile of drug which indicate the dependency of antibacterial activity with drug release from formulation.

Table 8. Zone of inhibition and % efficacy of formulations

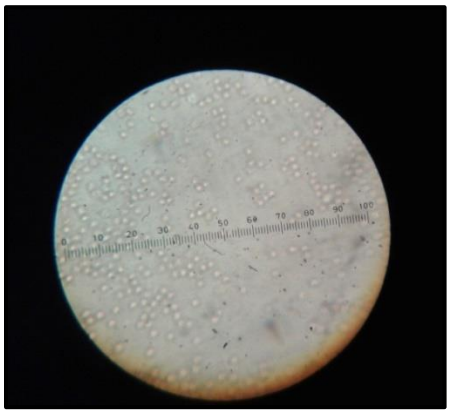
Sr.no	Formulation Code	Staphylococcus aureus	
		Zone of Inhibition (mm)	% Efficacy
1	Standard value	28	100
2	F1	27.25±0.005	97.32
3	F2	27±0.005	96.42
4	F3	26.5±0.5	94.64
5	F4	26.75±0.01	95.53
6	F5	26.5±0.5	94.64
7	F6	24.5±0.5	87.5
8	F7	26.35±0.05	94.10
9	F8	26.12±0.06	93.28
10	F9	22.5±0.5	80.35

Isotonicity evaluation

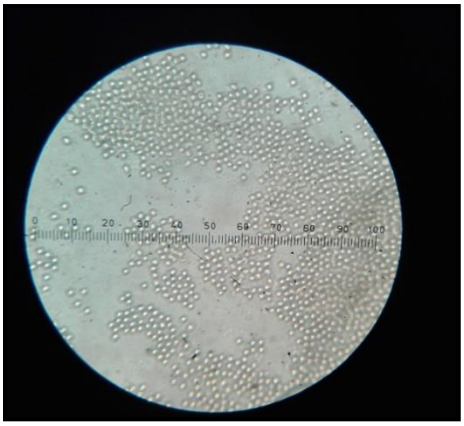
Isotonicity testing of formulations (F1) exhibited no change in the shape of blood cells. The blood cell size was found in 7-8µm range which reveals the isotonic nature of the formulations as compared with standard marketed ophthalmic formulation. This indicates the maintenance of tonicity in prepared formulations. Isotonicity was maintained to prevent tissue damage of eye.



a. Blood cells



b. Blood cells with Moxifloxacin HCl Formulation (F1)



c. Blood cells with Moxicip as marketed formulation

Figure. 9: Shape of Blood cells

Test for sterility

There was no appearance of turbidity and hence no evidence of bacterial growth when optimized formulation was incubated for not less than 14 days at 30ºC to 35ºC in case of fluid thioglycolate medium and at 20ºC to 25ºC in case of soyabean-casein digest medium. The preparations examined, therefore, passed the sterility test.

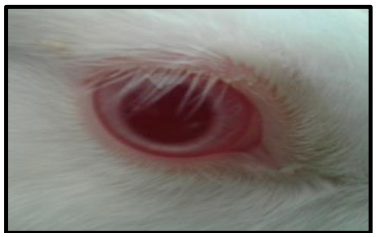
Ocular irritancy test

The results of the ocular studies indicate that the formulation F1 was non-irritant and no ocular damage or abnormal clinical signs were visible. The ocular irritancy study on rabbit eye is shown in Figure.10.

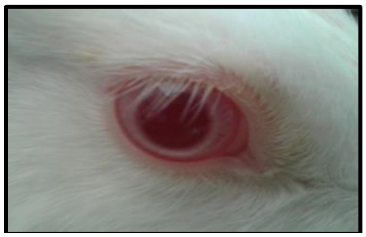
Table 9. Observations of ocular irritancy study

Time	Redness		Swelling		Watering	
	1 Left Eye	1 Right Eye	1 Left Eye	1 Right Eye	1 Left Eye	1 Right Eye
At the time of installation (0hr)	1	1	0	0	1	1
30min	0	0	0	0	0	0
1hr	0	0	0	0	0	0

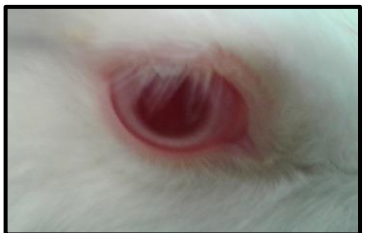
4hr	0	0	0	0	0	0
24hr	0	0	0	0	0	0
48hr	0	0	0	0	0	0
72hr	0	0	0	0	0	0
1 week	0	0	0	0	0	0



Normal right eye



Right eye after 15 min



Right eye after 1week

Figure.10: Ocular irritancy study

Stability study

Stability study of optimized F1 formulation at room temperature shown in Table 10. Formulations at room temperature were found to be stable up to 6 months. There is no change in drug content, clarity, pH and viscosity.

Table 10. Stability study of optimized F1 formulation at room temperature (25ºC ± 2ºC)

Sr. No	Observations	Before Stability Testing				After Stability Testing 3 months				After Stability Testing 6 months			
1	Clarity	Clear				Clear				Clear			
2	visual appearance	Transparent				Transparent				Transparent			
3	pH	6.28				6.25				6.23			
4	viscosity at room temp and at 37^0C	**rpm**	**RT**	**rpm**	**37ºC**	**rpm**	**RT**	**rpm**	**37ºC**	**rpm**	**RT**	**rpm**	**37ºC**
		1.5	8	0.3	49.9	1.5	8.0	0.3	50.1	1.5	8.9	0.3	50.2
		3	7.2	0.6	29.9	3	7.1	0.6	31.3	3	7.1	0.6	32.1
		6	7.0	1.5	27.9	6	7.8	1.5	28.0	6	7.8	1.5	28.8
		12	7	3	21	12	7.1	3	23.1	12	7.1	3	23.8
5	drug content	100.09				99.87				99.71			

CONCLUSION

This ophthalmic *in-situ* gel formulation fulfills all necessary parameters required for ophthalmic use. This optimized formulation having improved viscosity and better mucoadhesive property may improve the bioavailability of ocular administration of moxifloxacin HCl in *in-situ* gel form and can be alternative to the conventionally administered oral formulation.

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