

# Formulation and evaluation of an *in situ* gel-forming ophthalmic formulation of moxifloxacin hydrochloride

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## Abstract

**Background/Aim:** The aim of the present investigation is to prepare and evaluate *in situ* gel-forming ophthalmic drug delivery system of moxifloxacin hydrochloride. **Materials and Methods:** Sodium alginate, a novel ophthalmic gel-forming mucoadhesive polymer, which gets converted to gel in the presence of divalent-cations (calcium ion) present in the lachrymal fluid, was used as the gelling agent. Hydroxy propyl methyl cellulose (HPMC) is a mucoadhesive polymer used as viscosity enhancer. Suitable concentrations of buffering agents were used to adjust the pH to 6.5. All the formulations were sterilized in an autoclave at 121 °C for 15 minutes. The formulations were evaluated for clarity, pH measurement, gelling capacity, drug content estimation, rheological study, *in vitro* diffusion study, antibacterial activity, isotonicity, and eye irritation study. **Results:** The developed formulations exhibited sustained release of drug from formulation over a period of 10 hours thus increasing residence time of the drug. The optimized formulations were tested for eye irritation on albino rabbit (male). The formulations were found to be non-irritating with no ocular damage or abnormal clinical signs to the cornea, iris or conjunctiva observed. **Conclusion:** These *in situ* gelling systems containing gums may be a valuable alternative to the conventional systems..

**Key words:** Gelling capacity, *in situ* gel, *in vitro* diffusion study, moxifloxacin hydrochloride, rheological evaluation

## INTRODUCTION

Major problem in ocular therapeutics is the attainment of optimal drug concentration at the site of action, which is compromised mainly due to precorneal loss resulting in only a small fraction of the drug being ocularly absorbed. The effective dose administered may be altered by increasing the retention time of medication into the eye by using *in situ* gel-forming systems. Ophthalmic drug delivery is an extremely interesting and highly challenging endeavor.<sup>[1,2]</sup> The anatomy, physiology, and biochemistry of the eye render this organ exquisitely impervious to foreign substances. The challenge to the formulator is to circumvent the protective barriers of the eye without causing permanent tissue damage.<sup>[3]</sup> Ophthalmic ointments ensure superior drug bioavailability by increasing the contact time, minimizing the

dilution by tears, and resisting nasolacrimal drainage. Major disadvantage of ointment, providing blurred vision, due to this it could be used either night time or for treatment on the outside and edges of the eyelids. Suspension as ophthalmic delivery systems relies on the assumption that particles may persist in conjunctival sac. Precorneal drug loss can be minimal, such as retarding drainage by using diffusion-controlled, nonerodible polymeric insert. The major disadvantage of inserts is the lack of patient acceptance owing to difficult administration. The development of newer, more sensitive diagnostic techniques and therapeutic agents render urgency to the development of more successful ocular delivery systems. The primitive ophthalmic solution, suspension, and ointment dosage forms are clearly no longer sufficient to combat these diseases, and current research and development efforts to design better therapeutic systems are the primary focus of this research work. The aim of the present investigation is to formulate an *in situ* gel using novel gum system. *In situ* gel solution increases the residence time and also sustain the release mechanism of the drug.

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## MATERIALS AND METHODS

Moxifloxacin hydrochloride was obtained from Torrent Pharmaceuticals, Ahmedabad, India. Sodium alginate and HPMC-E 50LV was obtained from the nice chemicals, Ahmedabad, India. HPMC-K 4M was obtained from Signet Chemicals, Japan. Benzalconium chloride was obtained from S.D Fine chemicals, Mumbai, India. All the polymers received were of

pharmaceutical grade and were used as received. Other materials and solvents used were of analytical grade. Distilled water was prepared in laboratory using all glass distillation apparatus.

### Preparation of *in situ* gel

The polymeric solution was prepared by dispersing required quantity of sodium alginate as main polymer and HPMC- E 50 LV, HPMC- K4M as co-polymers in water using a magnetic stirrer until the polymers completely dissolve. Aqueous solution of moxifloxacin hydrochloride was added in to the polymeric solution with continuous stirring.<sup>[4]</sup> Buffering and osmolality agents were added to the resulting solution along with benzalkonium chloride. The pH of the solution was adjusted to 6.5 using 0.1 N NaOH/0.1 N HCl. The *in situ* gel formulations are depicted in Table 1.

### Physical parameters

The formulated *in situ* gel solution was tested for clarity, pH, gelling capacity, and drug content estimation. The results are as shown in Table 2.

### Gelling capacity

The gelling capacity of the prepared formulation was determined by placing a drop of the formulation in a vial containing 2 ml of freshly prepared simulated tear fluid and visually observed. The time taken for its gelling was noted.<sup>[5,6]</sup>

### Rheological studies

The viscosity measurements were carried out using Brookfield viscometer LVDV-E model. The *in situ* gel formulations were

placed in the sampler tube. The samples were analyzed at 37°C ± 0.5°C by a circulating bath connected to the viscometer adaptor prior to each measurement.<sup>[7-10]</sup> The angular velocity of the spindle was increased 1 to 4 and the viscosity of the formulation was measured.

### Drug content estimation

The drug content estimation was carried out by diluting 1 ml of prepared formulation in 100 ml of distilled water and analyzed using UV-visible spectrophotometer (Shimadzu UV-1700 PC, Shimadzu Corporation, Japan) at 290 nm.

### *In vitro* drug release studies

The *in vitro* release of moxifloxacin hydrochloride from the prepared formulations was studied using a modified diffusion testing apparatus. The freshly prepared simulated tear fluid (pH 7.4) was used as a diffusion medium. Semi permeable membrane, previously soaked in the diffusion medium for overnight, was tied to one end of a specially designed glass cylinder (open at both ends) having inner diameter of 3.4 cm. Two milliliter of formulation was accurately pipette into the glass cylinder known as donor chamber. The cylinder was suspended in a beaker (Acceptor chamber) containing 100 ml of diffusion medium so that the membrane just touches the surface of the medium. Acceptor chamber was maintained at a temperature of 37 ± 2°C with a stirring rate of 50 rpm using magnetic stirrer. About 1 ml of sample was withdrawn at a time interval of 1 hour and replaced with an equal volume of fresh diffusion medium. The aliquots were diluted with the diffusion medium and analyzed at 290 nm using UV spectrophotometer. In a similar manner, 2 ml of pure drug solution (0.5% w/v in distilled water) and 2 ml marketed product (MILFLOX) were studied in a similar manner.

### Antimicrobial Activity

Antimicrobial activity was determined by agar diffusion test employing cup plate technique. The drug was allowed to diffuse through a solid agar medium. The standard minimum inhibitory concentration (MIC 2 µg/ml) of control and developed formulations containing moxifloxacin were prepared. A total of 60 ml of nutrient agar media was prepared and sterilized at 15 lb/sq-inch pressure for 18 minutes in an autoclave; 0.5 ml of microorganism suspension was poured into the above medium which is maintained at temperature of 52°C to 58°C. This will be done in an aseptic condition. Immediately 20 ml of the microbial agar suspension was poured into each

**Table 1: Formulation design of *in situ* gelling system**

Name of ingredient	Quantity g					
	F1	F2	F3	F4	F5	F6
Moxifloxacin hydrochloride	0.5	0.5	0.5	0.5	0.5	0.5
Sodium alginate	0.5	1.0	1.5	0.5	1.0	1.5
HPMC E 50 LV	1.5	1.5	1.5	–	–	–
HPMC K 4M	–	–	–	0.5	0.5	0.5
Benzalkonium chloride	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
Distilled water q.s to	100	100	100	100	100	100

**Table 2: Evaluation parameters of formulations**

Formulations	Visual appearance	Clarity	pH	Drug content	Gelling capacity
F1	Transparent	Clear	6.51	98.68	++
F2	Transparent	Clear	6.53	93.41	++
F3	Transparent	Clear	6.51	96.84	++
F4	Transparent	Clear	6.49	94.29	++
F5	Transparent	Clear	6.53	99.24	++
F6	Transparent	Clear	6.53	93.81	++

++: Gelation immediate remains for few hours.

petriplate. After solidification of the media, sterile solutions of moxifloxacin hydrochloride (standard solutions) and the developed formulations diluted suitably with sterile distilled water (test solutions) were poured in to the cup of sterile nutrient agar Petri plates. This was previously seeded with test organisms (*Escherichia coli* and *Staphylococcus aureus*). After allowing diffusion of the solutions for 2 hours, the agar plates were incubated at 37°C for 24 hours. The Zone of inhibition (ZOI) was measured around each cup and compared with that of control. The entire operation was carried out in a laminar airflow unit. Each formulation solution was tested in triplicate. Both positive and negative controls were maintained throughout the study.<sup>[11]</sup>

### Ocular irritancy

Ocular irritation study was performed on optimized formulation in four albino rabbits (male), each weighing about 2 to 3 kg, and 0.1 ml of the optimized sterile moxifloxacin hydrochloride formulation was instilled in to cul-de-sac twice a day for a period of 14 days. The rabbits were monitored periodically for redness, swelling, watering of the eye.<sup>[12]</sup>

### Accelerated stability studies

Optimized sterile formulation was subjected to stability testing. Sterile optimized ophthalmic formulation was filled in glass vials, closed with gray butyl rubber closures and sealed with an aluminium caps. The vials contain optimized formulation were kept in stability chamber, maintained at  $40 \pm 2^\circ\text{C}$  and  $75 \pm 5\%$  RH for one month. Samples were withdrawn weekly

and estimated for drug content, pH, visual appearance, gelling capacity and *in vitro* drug release.<sup>[13]</sup>

## RESULTS AND DISCUSSION

In the present investigation, efforts were made to prepare the sustained release moxifloxacin hydrochloride *in situ* gel forming ophthalmic solution using polymers such as sodium alginate and HPMC. Sodium alginate a novel ophthalmic gel-forming mucoadhesive polymer, which gets converted to gel in the presence of divalent-cations (calcium ion) present in the lachrymal fluid (pH 7.4), was used as the gelling agent.

The prepared *in situ* gel formulations were evaluated for clarity, pH measurement, gelling capacity, drug content estimation, rheological study, *in vitro* diffusion study. The pH of *in situ* gel solution was found to be around 6.5 for all the formulations. The formulation should have an optimum viscosity that will allow for easy instillation into the eye, which would undergo a rapid sol to gel transition (triggered by ion exchange) as shown in Table 2.

Rheological evaluation of all the formulation exhibited Newtonian flow before gelling and exhibited pseudoplastic flow after gelling in the eye. There was increase in the viscosity after gelling. Additionally, the gel formed *in situ* should maintain its integrity without dissolving or eroding for a prolonged period. Results are as shown in Table 3 and Figures 1 and 2.

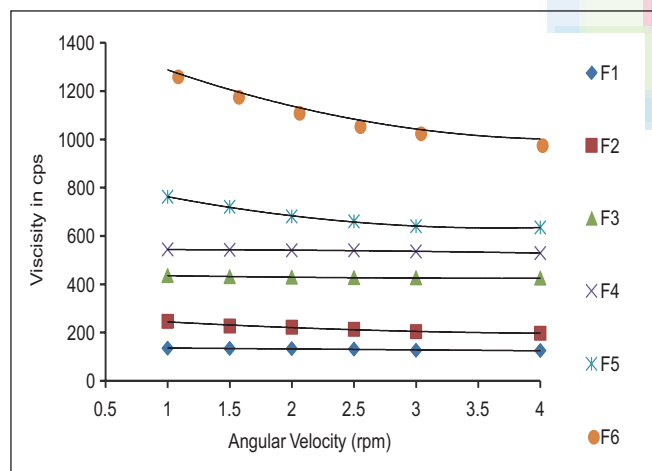


Figure 1: Formulations F1 to F6 (Before gelling)

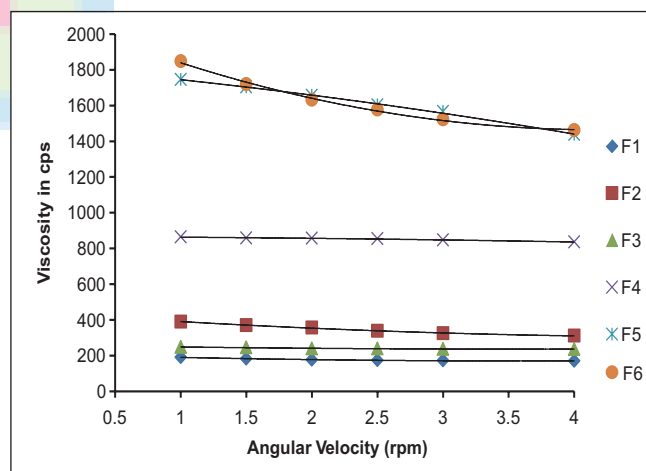


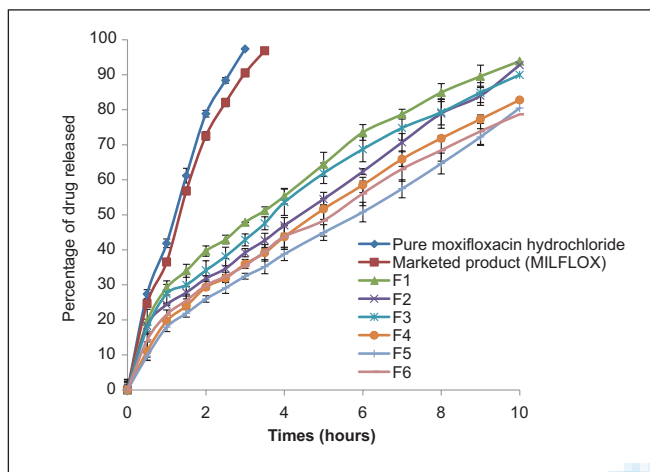
Figure 2: Formulations F1 to F6 (After gelling)

Table 3: Rheological studies of formulations

RPM	Viscosity in cps. (Before Gelling) (spindle no 61)						Viscosity in cps. (After Gelling) (spindle no 61)					
	F1	F2	F3	F4	F5	F6	F1	F2	F3	F4	F5	F6
1	135	246	245	543.8	762	1290	191	390	431	864	1746	1848.3
1.5	134.1	227	240	542.1	720	1204	182	370	429	858	1704	1720
2	132.8	222	239	540.8	681	1137	176.4	357	427	857	1656	1632
2.5	131	213	237	540	660	1080	174	338	426.1	854	1603	1577.7
3	126	204	236	536	640	1050	172.4	325	424.8	847	1566	1522.1
4	125	197	245	529	635	1000	170	311	431	836	1438	1462

**Table 4: Zone of inhibition values of formulation F1 to F6 and pure drug at the concentration of 2 µg/ml against *Staphylococcus aureus* and *Escherichia coli***

Microorganism	Concentration (mcg/ml)	Zone of Inhibition (mm)						
		Standard (Pure Drug)	F1	F2	F3	F4	F5	F6
Gram +ve <i>Staphylococcus aureus</i>	2 µg/ml	29.66	28	28.66	29	28.33	28	28.66
Gram -ve <i>Escherichia coli</i>	2 µg/ml	31.0	28.7	29.4	30.33	30.88	30.77	30.99

**Figure 3:** Comparative *in vitro* diffusion profile of pure drug, marketed product and F1 to F6 formulations

From the *in vitro* results it was observed that percentage release of the drug from the developed formulations F1 (93.86%), F2 (92.78%), F3 (89.97%), F4 (82.80%), F5 (80.49%), and F6 (78.71%) as shown in Figure 3. Formulation F6 showed more sustained release compared to other formulations. This could be the reason of higher concentration of Sodium alginate and HPMC K4M among the developed formulations. By observing the drug release profile it can be conclude that release is not stagnant even end of 10 hours. Formulation F6 showed highest zone of inhibition values against *S. aureus* (28.66 mm) and *E. coli* (30.99 mm), respectively, compared to other developed formulations. Hence, F6 formulation was taken for further study.

Antimicrobial efficacy study was performed on F6 formulation using Gram +ve *S. aureus* and Gram -ve *E. coli* organism. The zone of inhibition of F6 ophthalmic formulation found to be 28.66 and 30.99 mm, respectively, for Gram +ve *S. aureus* and Gram -ve *E. coli* organism. The results of antimicrobial activity are as shown in the Table 4. The study indicated moxifloxacin hydrochloride retained its antimicrobial activity when formulated as gel forming ophthalmic system against both selected *S. aureus* and *E. coli*.

Ocular irritation study was performed using healthy albino rabbits after getting prior permission from the institutional animal ethics committee. The eyes of each rabbits were examined at particular time interval after instillation of the optimized formulation (F6). There was no redness, continuous blinking, swelling or watering of eyes. No ocular damage or abnormal clinical signs to the cornea, iris or conjunctiva were

visible. The result of ocular irritation studies indicates that formulations containing all ingredients are non-irritant to rabbit eye.

Accelerated stability studies were carried out at  $40 \pm 2^\circ\text{C}$  at  $75 \pm 5\%$  RH for 1 month using stability chamber. The samples were analyzed periodically on every week, and found that there are no changes in visual appearance, clarity, pH, and gelation. Assay values after 1 month of storage are found almost same (deviating not more than one percent). Release profiles were similar to that of zero days.

## CONCLUSION

Moxifloxacin hydrochloride, a broad spectrum antibacterial agent used in the treatment of ocular infections, was successfully formulated as *in situ* gel-forming eye drops using Sodium alginate as a gelling agent in combination with HPMC as a viscosity enhancing agent. Thus, the developed formulation is a viable alternative to conventional eye drops by virtue of its ability to enhance bioavailability through its longer precorneal residence time and ability to sustain drug release. Also important is the ease of administration afforded and decreased frequency of administration resulting in better patient acceptance.

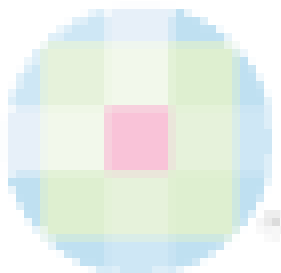
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