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Research Article

Studies on Formulation Development, *In-vitro* and *Ex-vivo* Characterization of Ion-Activated Ophthalmic *In-situ* Gel Containing Moxifloxacin Nanoparticles

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Abstract

Development of ion-activated ophthalmic *in-situ* gel containing moxifloxacin nanoparticles for better control of the release of the drug, minimize the dosing frequency and to improve the efficacy. Moxifloxacin nanoparticles were prepared by anti-solvent evaporation technique by using polyvinyl alcohol (PVA), Poloxomer 407 and Eudragit® RSPO. Prepared nanoparticles were loaded into the ion-activated *in-situ* gelling system (Sodium alginate) which undergoes phase transition from sol- gel in the presence of mono or divalent ions in the physiological conditions. The prepared *in-situ* gel formulations were evaluated for clarity, pH, gelling capacity, rheological properties, *in-vitro* drug release studies, *ex-vivo* corneal permeation studies, anti-microbial activity and histopathological studies. The PF3 formulation showed good gelling capacity with high % drug content. Rheological properties were acceptable for PF3 formulation when compared with the other formulations and *in-vitro, ex-vivo* release studies showed that the drug release was sustained and 65%, 62.04% was released in 4 hrs. Anti-microbial activity indicates that moxifloxacin nanoparticle loaded ophthalmic *in-situ* gel showed greater zone of inhibition when compared with the pure drug. Histopathological study revealed that there was no morphological difference between the formulations treated cornea and the healthy cornea. Hence can be concluded that formulation PF3 developed in the present study can be the successful replacement of conventional ophthalmic solutions.

Keywords: *Moxifloxacin; PVA; Eudragit® RSPO; In-situ Gel; Anti-microbial Activity; Histopathological Study*

Introduction

Eye is one of the challenging organs for drug delivery because of its anatomy and restricted absorption of drug into the deeper tissues. In case of conventional ophthalmic solutions frequent administration of drug is required due to nasolacrimal drainage and lacrimal secretion, so bioavailability is low. To overcome this excess medication is required, but side effects have been observed [1,2].

In recent years, substantial investigation have been dedicated to the development of new system of ophthalmic drug delivery to attain medication with prolonged retention time on the eye surface, reduced dosing frequency and improved trancroneal penetration, enormous attempts have been made to deliver ophthalmic drugs to the eye by using the polymeric vehicles such as sodium alginate, chitosan, gellan

gum and poloxomer [1]. *In-situ* drug delivery system contains polymers exhibit phase transition sol to gel due to changes in the environment can be instilled as a drop into the cul-de-sac of the eye and get transformed into gel (or) semisolid phase. Sodium alginate is ion-activated system which forms gel in the presence of mono (or) divalent ions.

Drug loaded nanoparticles had targeted action in the ocular tissue with enhanced ocular bioavailability. Tissue clearance can be reduced and sustained drug delivery can be achieved. moxifloxacin nanoparticles are prepared by using solvent evaporation method. Moxifloxacin is the fourth generation flouroquinolone analogue and acts by interacting with the topoisomerase II and topoisomerase IV (required for DNA replication) that has been reported for the bacterial conjunctivitis, kerato conjunctivitis [7,8].

Materials and Methods

Materials

Moxifloxacin was gifted by Hetero laboratories Ltd, Hyderabad, Poloxomer 407, eudragit® RSPO, sodium alginate, HPMC 15 CPS were obtained from Sd fine chemical Pvt. Ltd, polyvinyl alcohol was obtained from Himedia laboratories Pvt. Ltd and all other chemicals were analytical grade.

Preparation of moxifloxacin nanoparticle loaded ophthalmic i*n-situ* gel

Preparation of moxifloxacin nanoparticles: (Modification of procedure reported by Rajesh Kesarla., *et al.* [1]

Moxifloxacin nanoparticles were prepared by solvent evaporation technique. 100 mg of PVA was dissolved in 50 ml of water. It was heated on water bath until it was dissolved. Later it was allowed to room temperature. 0.5 g of drug was dissolved in aqueous solution of PVA 0.30 g of eudragit® RSPO was dissolved in acetone and this organic phase was added drop wise into the aqueous phase of PVA, Poloxomer 407 and drug. And this solution was mixed using magnetic stirrer for 3 hrs. Organic phase was evaporated using rotary evaporator at 100ºC for 30 minutes. The dried product was then passed through sieve no.120 to give free flowing particles (Table 1).

S. No	Ingredients (mg)	NP
1.	Drug (mg)	0.25g
2.	Poloxomer 407	0.50g
3.	Eudragit®RSPO	0.30g
4.	PVA	0.10g
5.	Acetone	10 ml
6.	Water	50 ml

Table 1: *Composition of moxifloxacin nanoparticle preparation.*

Preparation of nanoparticle loaded ophthalmic *in-situ* gel

Different ratios of polymers sodium alginate and co polymer HPMC 15 CPS were dissolved in water by using mechanical stirrer. To this the drug loaded nanoparticles and NaCl were added with continuous stirring. Benzalkonium chloride was added at the end as preservative. pH of the solution was adjusted to 7.2 using 0.1N NaoH (Table 2).

Characterization studies of moxifloxacin nanoparticles

Percentage yield

The formulation obtained after drying was weighed. Percentage yield was calculated as follows:

$$\% \text{ Yield} = \frac{\text{Nanoparticles weight X 100}}{\text{Total solid weight}}$$

S. No.	Ingredients	PF1	PF2	PF3
1	Moxifloxacin equivalent	0.25 g	0.25 g	0.25 g
2	Sodium alginate	0.5 g	1 g	1.5 g
3	HPMC 15 cps	0.5 g	0.5 g	0.5 g
4	Sodium chloride	0.5 g	0.5 g	0.5 g
5	Benzalkonium chloride	0.01 ml	0.01 ml	0.01 ml
6	Distilled water	50 ml	50 ml	50 ml

Table 2: *Composition of drug loaded in-situ gel.*

Particle size, zeta potential

The particle size, Zeta potential of the nanoparticles was measured by Horiba scientific instrument [1,5].

Entrapment efficacy (EE) [1]

The entrapment efficiency (EE) of nanoparticles was determined by separation of nanoparticles from the aqueous medium containing free drug by centrifugation at 7,000 rpm for 1 hr. The absorbance of free drug in the supernatant was measured by UV-spectrophotometer at 288 nm and the amount of entrapped drug was calculated according to above standard curve [1,5].

Entrapment efficacy was calculated using following equation.

$$(\%\ EE) = \frac{W_{total} - W_{free}\ X\ 100}{W_{total}}$$

Evaluation of an *in-situ* gel

Clarity and pH

Clarity is the one of the most essential parameter in ophthalmic preparations. Clarity test was observed by visual inspection under a good light, viewed against a black and white background. pH was determined using calibrated digital pH meter [1,2,7,8].

Drug content

Prepared *in-situ* gel formulation (equivalent to 10 mg of pure drug) was taken and diluted to 100 ml with freshly prepared stimulated tear fluid. From this 1 ml was withdrawn and diluted to 10 ml using STF. The absorbance was measured at 288 nm against STF as blank by using UV-visible spectrophotometer [1,4,7,8].

Gelling capacity

The gelling capacity of the *in situ* gel formulation was determined by placing a drop of formulation in a test tube containing 2 ml of stimulated tear fluid and was visually observed for gelling time and capacity [1,4,7,8].

Rheological studies

Viscosity of the formulation was determined before and after gelation by using Brookfield's rheometer using spindle number 61. Formulation was taken in a small volume sample tube and viscosity was measured at 25 rpm and 50 rpm. The viscosity was measured before dilution with STF and after dilution with STF [1,4,7,8].

In-vitro drug release of *in-situ* gel system

In-vitro release studies of the formulation were studied through a franz diffusion cell apparatus consisting of 2 ml of formulation placed in donor compartment using cellophane membrane (Molecular weight:12,000 - 14,000 dalton) which was soaked for 24 hrs in

STF. The donor compartment was placed on the receptor compartment containing 50 ml of STF maintained at 37ºC ± 1ºC with constant stirring using magnetic stirrer. After every 1 hr up to 4 hrs, 1 ml was withdrawn from receiver compartment and replaced with fresh STF. All samples were withdrawn in triplicates. Samples were analyzed for amount of drug released with UV-spectrophotometer at 288 nm against STF pH 7.4 as blank. The *in-vitro* release studies were carried out with pure drug solution in order to compare its release profile with the prepared *in situ* gelling system of moxifloxacin [1,4,8].

Kinetics of drug release

To analyze the mechanism for the release and release rate kinetics of the dosage form, the data obtained was fitted into Zero order, First order, Higuchi matrix and Korsemeyer-peppas model. By comparing the R^2 values obtained, the best fit model was selected [4].

Ex-vivo corneal permeation

Goat corneas were used to study the permeation across the corneal membrane. Whole eye ball of goat were procured from a slaughter house and transported to laboratory in cold condition. They were maintained in normal saline at 4ºC. The cornea was then carefully removed along with a 5 - 6 mm of surrounding scleral tissue and washed with cold saline. The study was carried out using franz diffusion cell in such a way that corneal side remains continuously in contact with the formulation in the donor compartment, 2 ml of the optimized formulation was taken and mounted on goat cornea. The receptor compartment was filled with STF pH 7.4 at 37ºC ± 1ºC. The receptor medium was stirred on the magnetic stirrer. 5 ml of samples was withdrawn at every 1 hr and absorbance was taken by UV spectrophotometer. Receptor compartment was replenished with an equal volume of STF (pH 7.4) at each time interval. The percent drug release was plotted against time to get dissolution curves [4].

Anti-microbial efficacy studies

Antimicrobial efficacy was determined by the agar diffusion test by employing cup plate technique. The microbiological studies ascertained the biological activity of the optimized formulations and pure drug against different microorganisms like *Staphylococcus aureus*, *E. coli, Bacilli,* and *Pseudomonas*. Cups were bored into sterile nutrient agar previously seeded with *Staphylococcus aureus*, *E. coli, Bacilli* and *Pseudomonas* organisms with the help of sterile borer with 4 mm diameter. After allowing diffusion of solution for 2 hrs, the plates were incubated for 24 hrs at 37ºC. The zone of inhibition (ZOI) of prepared formulation was compared with the pure drug. The ZOI was for the prepared gel [4,11].

Histological study

To study the effect of *in-situ* formulation on corneal structure and study the irritation potential, cornea are removed from the eyes of the freshly sacrificed goat and incubated at 37ºC for 5 hrs in formulation. Sodium -dodecylsulfate (SDS) solution in phosphate buffer saline (PBS) 0.1% (w/w) as the positive control. After incubation, corneas were washed with PBS and immediately fixed in formalin (8%, w/w). Tissues are dehydrated in an alcohol gradient, placed in melted paraffin and solidified in block form. Cross sections are cut, stained with haematoxylin and eosin (H&E). Cross sections are observed microscopically for any modification [1,11].

Results and Discussion

Percentage yield

The prepared nanoparticles were collected and accurately weighed. The % yield of preparation was calculated.

The % yield of prepared moxifloxacin nanoparticles was found to be 74.24% which is acceptable however there is a loss of about 25%.

Particle size

The particle size was determined was found to be 155 nm. Rajesh Kesarla., *et al.* [1] prepared moxifloxacin nanoparticles by solvent evaporation method and the particle size was reported to be 152 to 172 nm. Nagarwal., *et al.* [5] prepared modified nano *in-situ* gel with 5-FU by solvent evaporation method and the particle size was reported to be 128 to 194 nm (Table 3).

S. No	S.P. area ratio	Mean	S.D	Mode
1	1.00	155.5 nm	12.8 nm	154.4 nm
2	-	- nm	- nm	- nm
3	-	- nm	- nm	- nm
Total	1.00	155.5 nm	12.8 nm	154.4n

Table 3: *Particle size of Moxifloxacin nanoparticles.*

Zeta potential

Zeta potential of the moxifloxacin nanoparticles was determined as -21 mv. Rajesh Kesarla., *et al.* prepared moxifloxacin nanoparticles by solvent evaporation method and zeta potential was reported in the range of 5.3mv to 8.89 mv [1] (Table 4).

Peak No.	Zeta potential	Electrophoretic Mobility
1	-13.7 mV	-0.000106 cm^2/Vs
2	-29.2 mV	-0.000226 cm^2/Vs
3	-mV	- cm^2/Vs

Table 4: *Zeta potential of moxifloxacin nanoparticle*

Entrapment efficiency

The % entrapment efficiency of the moxifloxacin nanoparticle prepared was determined as 64.05%. Rajesh Kesarla., *et al.* 2016 the entrapment efficacy of prepared moxifloxacin nanoparticles was 71% [1].

Evaluation of an *in-situ* gel

Clarity and pH

Clarity of various formulations was determined by visual inspection under a good light, pH was determined using digital pH meter and reported as follows [8,10] (Table 5).

S.no	Formulation	Clarity	pH
1	PF1	Clear	7.2 ± 0.01
2	PF2	Clear	7.2 ± 0.02
3	PF3	Clear	7.2 ± 0.01

Table 5: *Data of clarity and pH of prepared formulations.*

Drug content

The drug content of all the formulation from PF1, PF2 and PF3 was calculated and the yield was in the range of 61.73 - 65%. PF3 was found to have maximum % drug content (65%) [1]. The % drug content of the prepared nanoparticles of moxifloxacin using anti-solvent evaporation method within the range of 77.83% (Table 6 and Figure 1).

S.no	Formulation code	% Drug content*
1	PF1	61.73% ± 0.18
2	PF2	63.90% ± 0.27
3	PF3	65% ± 0.40

Table 6: *% drug content.*

**n = 3.*

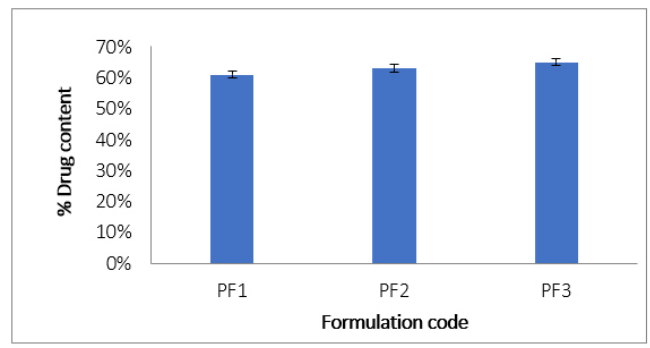

Figure 1: *% Drug content.*

Gelling time and gelling capacity

The gelling time, gelling capacity of all ion- sensitive *in-situ* gel formulations is as shown in table 10. Both PF2 and PF3 formulation showed the immediate gelation and remained for extended period of time. The formulations should have good gelling capacity, and then only it would preserve its integrity without eroding for a prolonged period of time. Gelling capacity is coded as described in table 7. (+) gelation after few minutes and remains for few seconds, (++) immediate gelation and remains for few hours, (+++) immediate gelation and remains for extended period [1,8]. From the above data in table 10 indicates that PF3 formulation had good gelling capacity when compared with PF1, PF2 (Table 7).

S. No	Formulation code	Gelling time	Gelling capacity
1	PF1	30 mints	+
2	PF2	30 sec	++
3	PF3	20 sec	+++

Table 7: *Gelling time and gelling capacity.*

Rheological studies

An ophthalmic formulation should have an optimum viscosity that will allow easy installation into the eye as liquid drops and which undergo rapid sol- gel transition upon instillation into the eye. Viscosity of all the formulations is shown in the table 8 and 9, figure 2 and 3. All the formulation showed the shear thinning behavior. The high viscosity at lower shear rate helps to increase the contact time of *in-situ* gel on the corneal surface [1,4,8]. From the table 8 and 9 all the formulations (PF1, PF2 and PF3) showed the pseudo plastic behavior that is with increase in the angular velocity there is decrease in the viscosity. This pseudo plastic behavior of gel is necessary as shear rate that is experienced during blinking of eyes. Among all the formulation PF3 formulation had high and acceptable viscosity [4]. The increase in the viscosity after gelling is prerequisite for any *in-situ* gel formulations which had to maintain its integrity for prolonged period of time. Rajesh Kesarla., *et al.* [1] reported the viscosity of the moxifloxacin loaded gellan gum ophthalmic *in-situ* gel as 128 cps before gelation, 856 cps after gelation. Sonjoy Mandal., *et al.* [8] prepared moxifloxacin *in-situ* gel and the rheological property was reported to be in the range of 170 cps-1462 cps. However, for the optimized formulation the reported value was 1462 cps.

S. No	Formulation code	Rheological studies (before gelation) cps	
		25 rpm	50 rpm
1	PF1	78	44.24
2	PF2	324	198
3	PF3	359	264

Table 8: *Rheological studies of formulation before gelation.*

S. No	Formulation code	Rheological studies (after gelation) cps	
		25 rpm	50 rpm
1	PF1	211	131
2	PF2	716	347
3	PF3	890	516

Table 9: *Rheological studies of formulation after gelation.*

Time (Min)	% Drug released			
	PF1	PF2	PF3	Pure
0	0	0	0	0
15	9.5± 0.1	9.8 ± 0.2	7.55 ± 0.4	28.87± 0.1
30	27.04 ± 0.5	23.36 ±0.4	16.02 ± 0.1	40.91± 0.4
60	38.87 ± 0.2	38.67 ± 0.1	27.14 ± 0.3	79.5 ±0.3
120	52.24 ± 0.4	50.306 ± 0.4	36.73 ± 0.2	92 ± 0.3
180	77.85± 0.1	62.75± 0.3	51.73 ± 0.1	
240	85.30 ± 0.4	74.89± 0.5	65.40 ± 0.1	

Table 10: *Comparative % in-vitro diffusion profile of prepared formulations and pure drug.*

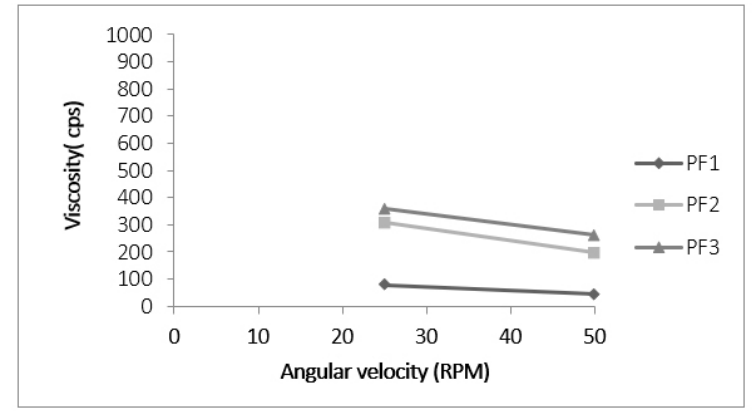

Figure 2: *Viscosity before gelation.*

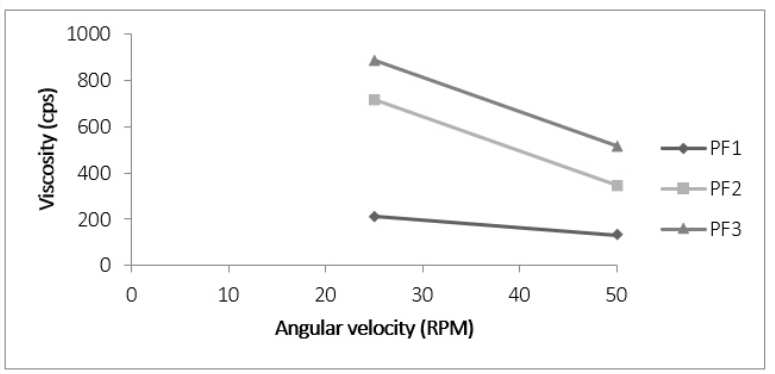

Figure 3: *Viscosity after gelation.*

In-vitro drug release of *in-situ* gel system

The *in-vitro* drug diffusion of formulations (PF1, PF2 and PF3) was studied. The samples were withdrawn at definite time intervals and were analyzed for the drug concentration by UV- spectrophotometer at 288 nm. The results showed that the formed gels had the ability to retain the drug for the period of 4 hrs (duration of study). It was found that drug release of the formulation were in the range of 85% - 65%. The release rate showed that with increase in the concentration of the sodium alginate there was decrease in the release rate of the drug. The following results indicated that the structure of gel functioned as a barrier to drug release [1,4,8]. Based on the data obtained from gelling capacity, Viscosity, *in-vitro* drug release studies of formulations PF1, PF2 and PF3 the gelling capacity and viscosity is high for PF3 formulation. And also % cumulative drug release is low when compared with the PF1, PF2 and pure. This indicates that PF3 formulation is optimized formulation. Figure 4 shows % *in-vitro* drug release of the formulation PF1, PF2 and PF3 was in the range of 85% - 65%. The release rate showed that with increase in the concentration of the sodium alginate there was decrease in the release rate of the drug. The results indicated that the structure of gel functioned as a barrier to drug release. Among the formulation prepared PF3 formulation showed more sustained release of the drug when compared to other. The % *in-vitro* drug release of PF3 was compared with pure drug solution. The % cumulative drug release at the end of 2 hrs was found to be 36.73% and 92.95% for PF3 formulation and pure drug respectively. The PF3 formulation clearly showed a better sustenance of the drug release [8].

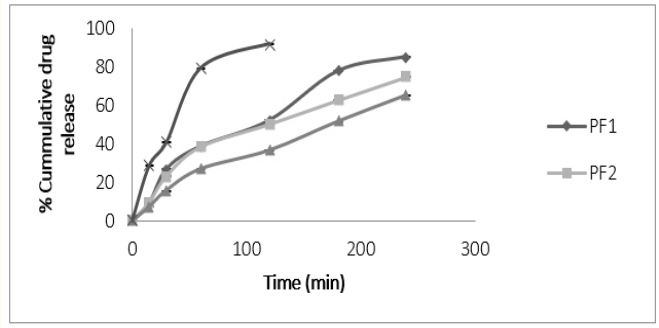

Figure 4: *Comparative in-vitro diffusion profile of prepared formulations and pure drug.*

In view of the results obtained it can be concluded that the developed formulation is capable of releasing the moxifloxacin nanoparticle in sustainable manner. Sonjoy Mandal., *et al.* [8] prepared moxifloxacin *in-situ* gel and the *in-vitro* drug release was reported as 78.71% for 10 hours [8].

Kinetics of drug release for optimized formulation PF3

The following table 11 shows the r^2 values for zero order, first order, higuchi, Korsmeyer-Peppas and Hixson Crowell models and Hixson-Crowell models. The corresponding plots and r^2 values are shown in table 11. When the release data was analyzed as per zero and first order models, the r^2 values were relatively higher in first order model so the formulation follows the first order kinetics. It shows the diffusion dependent release. Values of Korsmeyer peppas are superior to Higuchi model. The release kinetics indicates that the drug release was swelling erosion which always associated with diffusion mechanism. It can be analogous transportation i.e. non fickian kinetics (pure diffusion controlled release).

Formulation code	Zero order	First order	Higuchi	Korsmeyer peppas model	Hixson Crowell
	r^2	r^2	r^2	r^2	r^2
PF3	0.9753	0.9876	0.9831	0.9566	0.9101
				n = 0.735	

Table 11: *r^2 values for zero order, first order, higuchi, Korsmeyer-Peppas and Hixson Crowell model.*

Ex-vivo corneal permeation studies

Ex-vivo corneal permeation for all the formulations developed along with the pure drug was carried out. The drug permeation through the cornea of formulation PF1, PF2 and PF3 was comparison with pure which shown in the following table and graph [4,11]. Figure 5 shows % cumulative *ex-vivo* corneal permeation of the formulation PF1, PF2 and PF3 was in the range of 86.02% to 62.04%. The % *ex-vivo* corneal permeation of the PF3 formulation was compared with the pure solution. The % cumulative drug permeation at the end of 2 hrs was found to be 39.59% and 92.95% for PF3 formulation and pure. Anand Panchakshri Gadad., *et al*. [4] conducted corneal permeation of the thermo-sensitive *in-situ* gel of moxifloxacin and reported the 79.28% after 7 hrs [4]. Further there are no reports in literature about *ex-vivo* permeation studies of moxifloxacin nanoparticle loaded *in-situ* gel (Table 12).

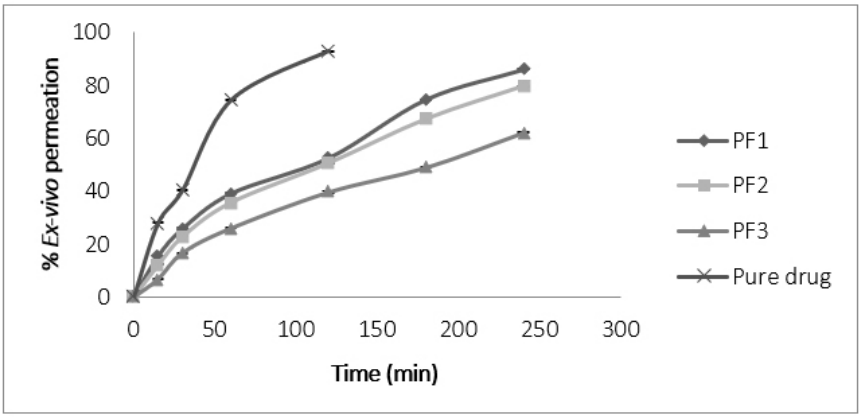

Figure 5: *Comparative ex-vivo corneal drug permeation profile of PF1, PF2 and PF3 with pure drug.*

Time (min)	% Drug permeation			
	PF1	PF2	PF3	Pure drug
0	0	0	0	0
15	15.51 ± 0.1	12.14 ± 0.3	6.83 ± 0.1	27.94 ± 0.3
30	25.81 ± 0.5	22.85 ± 0.2	16.73 ± 0.1	40.30 ± 0.4
60	39.18 ± 0.3	35.81 ± 0.2	26.02 ± 0.3	74.38 ± 0.4
120	52.55 ± 0.1	50.71 ± 0.1	39.59 ± 0.5	92.95 ± 0.1
180	74.69 ± 0.1	67.44 ± 0.5	48.87 ± 0.2	
240	86.02 ± 0.4	79.8 ± 0.1	62.04 ± 0.3	

Table 12: *Ex-vivo corneal permeation profile of formulations and pure drug.*

Comparison of *in-vitro* drug release profile with *ex-vivo* corneal permeation

The *in-vitro* drug release profile of the optimized PF3 formulation was 65% after 4 hrs whereas *ex-vivo* corneal permeation of the optimized PF3 formulation was found to be 62.04%. The drug diffuse through the corneal membrane was less when compared with the diffusion membrane. This may be because of cornea is made up of lipophilic and hydrophobic barriers [1,4]. It was found that there is a good correlation between *in-vitro* and *ex-vivo* studies (Figure 6).

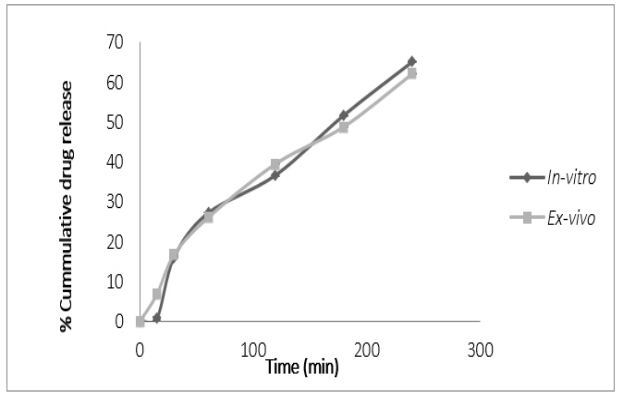

Figure 6: *Comparison between in-vitro and ex-vivo drug release profile drug.*

Anti-microbial efficacy studies

Antimicrobial test was conducted using *Staphylococcus aureus, E. coli, Bacillus subtilis* and *Pseudomonas aeruginosa* bacterial strains for the formulations PF1, PF2 and PF3. The microbial activity of standard moxifloxacin solution was also measured (in diameter) and a graph was drawn for gram +ve and gram-ve organism for comparison. Antimicrobial activity was compared and shown in following table and graph. From the table the antimicrobial efficacy test indicates that moxifloxacin nanoparticle loaded ophthalmic *in-situ* gel showed greater zone of inhibition when compared with the pure drug. The zone of inhibition of the optimized PF3 formulation showed 6.3 cm, where pure showed 4.8 cm against *Staphylococcus aureus*. This indicated that the prepared moxifloxacin nanoparticles loaded in sodium

alginate *in-situ* gel was more efficacious when compared with pure drug. As it is a broad spectrum antibiotic it is also effective against *E. coli, Bacillus subtilis, Pseudomonas aeruginosa*. Rajesh Kesarla., *et al.* [1] the zone of inhibition of the prepared moxifloxacin nanoparticle loaded gellan gum ophthalmic *in-situ* gel was 1.5 cm for standard and 1.4 cm for test. Sonjoy Mandal., *et al*. [8] reported the zone of inhibition of the prepared moxifloxacin *in-situ* gel was reported as 28 mm for test and 30 mm for standard against *Staphylococcus aureus.* In the view of above results obtained and reported literature values it can be concluded that the formulation developed in the present study is superior with respective anti-microbial activity (Figure 7 and 8, Table 13).

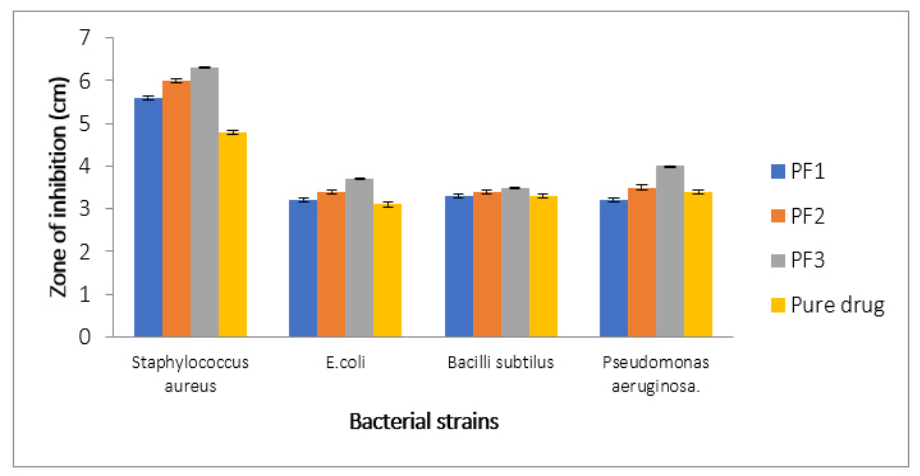

Figure 7: *Comparison of antimicrobial activity of prepared formulations and pure drug.*

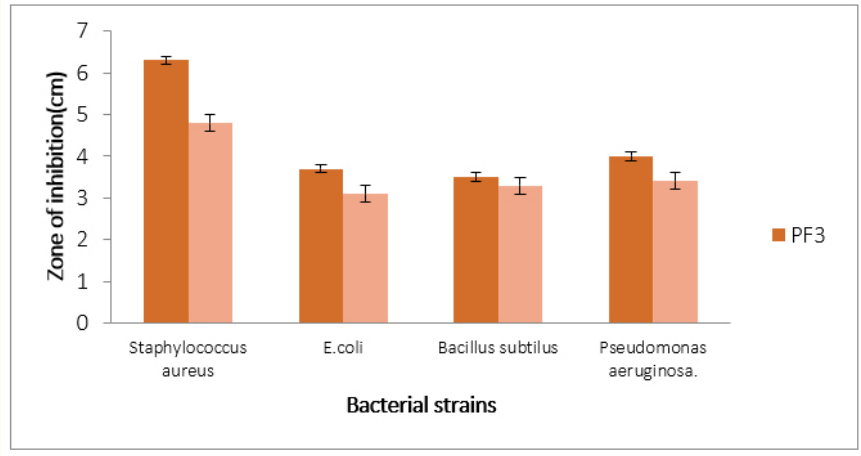

Figure 8: *Comparison of antimicrobial activity of optimized (PF3) with pure drug.*

Formulation code	Zone of inhibition			
	Staphylococcus aureus	*E. coli*	*Bacillus subtilis*	*Pseudomonas aeruginosa*
PF1	5.6 ± 0.2	3.2 ± 0.2	3.3 ± 0.1	3.2 ± 0.1
PF2	6 ± 0.1	3.4 ± 0.1	3.4 ± 0.2	3.5 ± 0.2
PF3	6.3 ± 0.1	3.7 ± 0.1	3.5 ± 0.2	4 ± 0.2
Standard	4.8 ± 0.2	3.1 ± 0.3	3.5 ± 0.1	3.4 ± 0.2

Table 13: *Comparison of zone of inhibition by different bacterial strains at standard concentration 5 µg/ml.*

Ocular irritation test

Histopathological study was conducted by exposing goat cornea to the formulation there was no morphological changes was observed in the goat cornea when compared with positive control. By this we can conclude that the formulation is not causing any irritation to the eye. Rajesh Kesarla., *et al.* [1] reported the similar results (Figure 9 and 10).

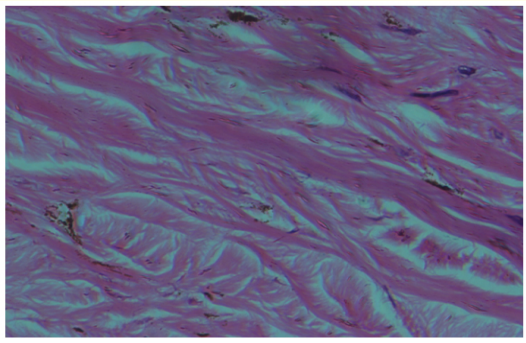

Figure 9: *Histopathological result of healthy eye.*

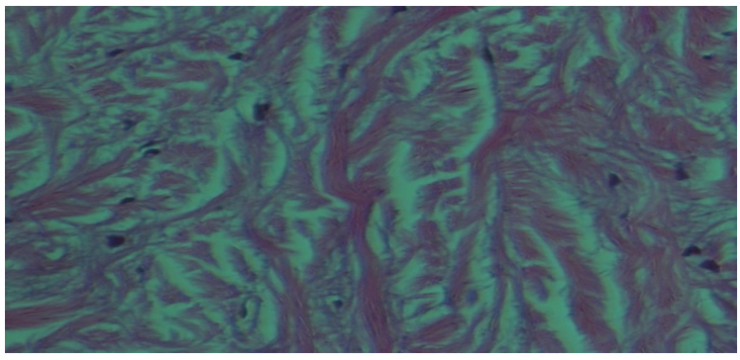

Figure 10: *PF3 formulation treated goat cornea.e.*

Conclusion

The developed formulation of moxifloxacin nanoparticles loaded ion-activated *in-situ* gel system was found to be liquid at formulated pH and undergone phase transition in the presence of mono or divalent ions at physiological conditions. From the rheological studies it was demonstrated that all the formulations exhibited pseudo plastic behavior i.e. decrease in viscosity with increasing in angular velocity. All the evaluation tests like % drug content, gelling capacity, rheological properties are good for PF3 formulation. *In-vitro* drug release studies indicated that for PF3 formulation showed 65% within 4 hours where pure showed 92% drug release. So, this indicated that PF3 formulation showed the sustained drug release. The optimized formulation followed the first order release kinetics and diffusion controlled release.

The optimized PF3 formulation showed higher antibacterial activity against both gram +ve and gram-ve bacteria when compared with pure one. Histopathological study revealed that there was no morphological difference between the formulations treated cornea and the healthy cornea. Hence the formulation PF3 developed in the present study is successful in meeting the objective.

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Conflict of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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