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RESEARCH ARTICLE

Exploration of Nanoparticulate *In Situ* Gel of Moxifloxacin Hydrochloride in Ophthalmic Delivery

Janani PRAKASH[*], Preethi SUDHEER[**°]

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SUMMARY

Moxifloxacin hydrochloride, an ophthalmic solution, treats bacterial eye infections but suffers from rapid lachrymal drainage and poor corneal penetration. This study aims to enhance corneal penetration by combining nanoparticles with an in-situ gelling system. Chitosan nanoparticles were synthesized using the ionotropic gelation method, guided by a 'Custom experimental design' approach. Key parameters, including drug entrapment efficiency, particle size, and drug release profiles, were evaluated, and the model's fit was analyzed using ANOVA. Characterization of the formulations included particle size analysis, SEM, DSC, and FTIR. Sodium alginate nanoparticle gels were analyzed for gelling capacity, viscosity, and drug diffusion and permeation studies. Sixteen formulations were created, with drug entrapment efficiency ranging from 70.9 ± 0.08% to 89.7 ± 0.09% and diffusion profiles between 67.3 ± 0.03% and 90.6 ± 0.08%. The most influential formulation had an average particle size of 497nm, and SEM revealed slightly agglomerated particles with uneven surfaces. This formulation exhibited a onefold increase in permeability coefficient and a twofold increase from the nanoparticulate in situ gel compared to marketed drops (0.5% w/v) and the pure drug in situ gel indicating its potential to penetrate deeper eye layers. The eye irritation study reports no irritation. The developed formulation also showed enhanced antimicrobial activity against E. coli and S. aureus compared to commercial samples. The Moxifloxacin hydrochloride nanoparticulate in situ gel offers a promising strategy to improve ocular penetration, prolong retention time, and potentially increase ocular bioavailability.

Key Words: *Moxifloxacin hydrochloride, nanoparticle, in situ, gel, ophthalmic.*

Oftalmik Uygulamada Moksifloksasin Hidroklorür Nanopartikül In Situ Jelinin Araştırılması

ÖZ

Oftalmik bir solüsyon olan moksifloksasin hidroklorür, bakteriyel göz enfeksiyonlarını tedavi eder, ancak hızlı lakrimal drenaj ve zayıf kornea penetrasyonundan muzdariptir. Bu çalışma, nanopartikülleri yerinde jelleştirme sistemiyle birleştirerek kornea penetrasyonunu artırmayı amaçlamaktadır. Kitosan nanopartikülleri, 'Özel deneysel tasarım' yaklaşımının rehberliğinde iyonotropik jelleşme yöntemi kullanılarak sentezlendi. İlaç tutulma verimliliği, parçacık boyutu ve ilaç salım profilleri gibi temel parametreler değerlendirildi ve modelin uyumu ANOVA kullanılarak analiz edildi. Formülasyonların karakterizasyonu partikül boyut analizi, SEM, DSC ve FTIR'ı içeriyordu. Sodyum aljinat nanopartikül jelleri, jel oluşturma kapasitesi, viskozite ve ilaç difüzyonu ve geçirgenlik çalışmaları açısından analiz edildi. İlaç tutulma etkinliği %70.9 ± 0.08 ile %89.7 ± 0.09 arasında ve difüzyon profilleri %67.3 ± 0.03 ile %90.6 ± 0.08 arasında değişen on altı formülasyon oluşturuldu. En etkili formülasyonun ortalama partikül boyutu 497 nm idi ve SEM, pürüzlü yüzeylere sahip, hafif kümelenmiş parçacıkları ortaya çıkardı. Bu formülasyon, pazarlanan damlalara (%0.5 a/h) ve saf ilaç in situ jeline kıyasla geçirgenlik katsayısında bir kat artış ve nanopartiküllü in situ jelde iki kat artış sergiledi; bu, daha derin göz katmanlarına nüfuz etme potansiyelini göstermektedir. Göz tahrişi çalışması herhangi bir tahriş rapor etmemektedir. Geliştirilen formülasyon ayrıca ticari numunelerle karşılaştırıldığında E. coli ve S. aureus'a karşı gelişmiş antimikrobiyal aktivite gösterdi. Moksifloksasin hidroklorür nanopartikül in situ jel, oküler penetrasyonu iyileştirmek, tutulma süresini uzatmak ve potansiyel olarak oküler biyoyararlanımı artırmak için umut verici bir strateji sunmaktadır.

Anahtar Kelimeler: *Moksifloksasin hidroklorür, nanopartikül, in situ, jel, oftalmik.*

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INTRODUCTION

Ocular drug delivery remains challenging, with noninvasive methods predominantly addressing anterior eye segment disorders. Targeting the anterior region, sustaining optimal drug levels, and prolonging residence time pose significant challenges in designing an effective drug delivery system to the eye. Conventional eye drops, like solutions and ointments, suffer from poor bioavailability due to rapid tear drainage and limited contact time. Overcoming these challenges is crucial for achieving successful ocular drug delivery and ensuring prolonged efficacy in treating eye disorders. Incorporating nanocarriers offers advantages such as controlled and continuous drug release, longer retention time at the target site, thus improved penetration, and enhanced bioavailability (Maharjan et al., 2019; Raj et al., 2020).

The strategies adopted in increasing drug penetration via cornea include viscosity enhancers, mucoadhesive systems, in situ gels, prodrugs, and colloidal carriers like nanoparticles and liposomes. New approaches for the eye using polymers are milestones in the delivery of drugs to the pre and intraocular tissues. In situ gels show promise as a practical approach to prolong corneal retention time and alter ocular bioavailability (Bhatia et al., 2013; Gote et al., 2019; Irimia et al., 2018).

In situ gel systems consist of polymers that exhibit sol-to-gel phase transitions with specific physicochemical responses such as pH, temperature, and ionic concentration. Consequently, the extended residence time of the system will result in a sustained drug release, enhance the ocular bioavailability, and reduce the frequent dosing regimen of the medications, resulting in improved patient compliance (Majeed & Khan, 2019; Wu et al., 2019; Yu et al., 2015).

Drug-loaded nanoparticles (DNPs) target the drug to the frontal part of the eye with enhanced bioavailability. Biocompatible-biodegradable polymers from poly(lactide-coglycolide) (PLGA), chitosan, poly lactic acid (PLA) act as permeation enhancers, thus enhancing the cellular uptake and reduced tissue clearance, and therefore offering a sustained drug delivery (Ahmed & Aljaeid, 2017; Clemens et al., 2019; Wani, et al., 2020).

Moxifloxacin hydrochloride (MOX), an 8-methoxy fluoroquinolone antibiotic, is employed in the treatment of susceptible microorganisms such as bacterial conjunctivitis. The two bacterial enzymes, topoisomerase II and topoisomerase IV involved bacterial replication translation, repair, and even recombination of deoxyribonucleic acid. The drug acts by binding to the gyrase, thus blocking the action of these enzymes (Gupta et al., 2019; Miller, 2008).

Research on MOX in situ gel systems has explored various formulations. One study incorporated MOX-loaded Eudragit RL100 nanoparticles into a gellan gum-based in situ gelling system, which exhibited prolonged ocular retention (Kesarla et al., 2016). Other investigations have examined MOX in situ gel formulations using different gelling polymers, such as sodium alginate, gellan gum, and carbopol(Shashank Nayak et al., 2012) both individually and combined with mucoadhesive polymers. Additionally, researchers have studied diverse nanosystems, including nano-emulsions in mucoadhesive gel formulations (Youssef et al., 2022). MOX niosomes have also been evaluated for their potential in controlled ophthalmic drug delivery (Kaur & Pawar, 2015).

MOX, being a hydrophilic substance, is anticipated to have restricted passage through the corneal membrane. Chitosan, a naturally occurring polymer, exhibits antibacterial qualities, forms gels, and possesses mucoadhesive characteristics. Furthermore, scientific literature has documented its ability to enhance penetration across biological membranes.

Considering the characteristics of both the drug and polymer, a decision was made to develop a nanoparticulate system of MOX using chitosan. This approach offers several advantages. The unique adhesive properties of chitosan allow for proximity to the corneal membrane, enabling penetration into deeper corneal layers in cases of severe bacterial infections. Additionally, the gradual release of the drug

from nanoparticles may result in prolonged therapeutic effects. Combining nanoparticles with an in-situ gel-forming polymer can enhance penetration by maintaining close contact between the nanoparticles and the ocular surface. Furthermore, utilizing an ion-sensitive in situ polymer can address precorneal clearance issues and extend drug action through the controlled release of nanoparticles from the gel matrix.

Thus, to achieve an optimal drug concentration and therapeutic potential, we combined a nanoparticulate chitosan system with in situ gels to deliver moxifloxacin hydrochloride. This approach enhances drug permeation through the chitosan-based nanosystem and increases the corneal retention time through the sol-gel system, thereby ensuring higher ocular bioavailability.

MATERIAL AND METHODS

Materials

MOX was obtained from Yarrow Chemicals Pvt Ltd, Mumbai, India; chitosan (>85% deacetylated, (molecular weight of 161.16 kDa) was obtained from Indian Fine Chemicals India; Sodium tripolyphosphate and sodium alginate were obtained from Loba Chemie Pvt Ltd, Mumbai, India. The animal study was approved by protocol (Institutional Animal Ethics Committee (IAEC) (Ref No: KCP/IAEC/PCOL/PCEU/62/2020).

Preparation of nanoparticles by ionotropic gelation method

Firstly, the polymer chitosan was dissolved in 1% v/v acetic acid, then the drug was added under magnetic stirring and continued stirring for one h. The required crosslinking agent STPP solution was added to the chitosan solution, homogenized (Polytron Homogenizer) for 30 min to get nano-sized particles, and continued stirring. The product was transferred into a centrifuge tube and centrifuged at 3000 rpm for 30 minutes. From the supernatant, the particles were filtered and washed with three 10 ml portions of water. The particles were then dried to obtain nanoparticles (Mohammadpour Dounighi et al., 2012; da Silva Furtado et al., 2020)

Experimental design

Custom design using Design Expert software 13 was used to optimize the trials. The concentration of chitosan (%) and STPP (%) were continuous factors. Stirring speed (rpm) and stirring time (h) were used as categorical factors, which were chosen as independent variables on the responses as given in Table 1(Elmizadeh et al., 2013), and the experimental layout is shown in Table 2.

Table 1. Experimental design with factors and responses

Factors	**Values**	
	Low level	**High level**
Chitosan (%)	0.25	1
STPP (%)	0.5	1
Stirring speed (rpm)	7500	15000
Stirring time (h)	6	24
Responses	**Goal**	
Particle size (nm)	100	500
Drug entrapment efficiency (%)	80	90
Drug release (%) (24h)	80	90

Table 2. Custom Design Experimental Runs

Formulation code	Chitosan (%)	STPP (%)	Stirring speed (rpm)	Stirring time (h)
T1	0.69625	0.64	7500	6
T2	1	1	7500	24
T3	0.25	0.5	7500	6
T4	0.2575	0.64	15000	6
T5	0.8575	0.5454	15000	6
T6	1	0.5	7500	6
T7	0.55	0.9955	15000	24
T8	0.7	0.5	7500	24
T9	1	1	15000	6
T10	0.25	1	7500	6
T11	0.6962	0.5	15000	6
T12	0.992	0.46	15000	24
T13	0.3856	0.554	15000	24
T14	0.2575	0.6355	7500	24
T15	0.69625	0.64	7500	6
T16	0.25	0.5	15000	24

Evaluation of nanoparticles

Drug content

The amount of moxifloxacin in nanoparticles was assayed by solubilizing the nanoparticles (10 mg) in 1% acetic acid, diluted suitably, and drug concentration was determined by UV spectrophotometrically (Shimadzu) analytical India at 293 nm (Desai, 2016).

Drug entrapment efficiency

The free drug concentration of a 10 mg formulation was determined after dissolving the free drug by centrifugation at 2500 rpm. The spectrophotometric determination of the supernatant was carried out at 293 nm after suitable dilution using water as blank. The following formula calculates entrapment efficiency (Shelake et al., 2018; Yurtdaş-Kirimlioğlu et al., 2018).

$$EE = \frac{Total\ drug - free\ drug}{Total\ drug} x100$$

Particle size analysis

The Horiba SZ-100 nanoparticle dynamic light scattering (DLS) system determined the mean particle size and size distribution. The particle size was analyzed after a suitable dilution in double distilled water at a scattering method at 90° at 25.2 °C (Mahor et al., 2016; Mahmood et al., 2017).

Drug diffusion studies from nanoparticles

A specially designed glass cylinder, open at both ends, had one end fitted with a pre-soaked dialysis membrane (pore size of 70). This setup was immersed in 50 ml of receiver fluid maintained at 37.0°C ± 0.2°C and stirred magnetically at 100 rpm. The volume of the diffusion medium withdrawn hourly over 24-hours period was replaced with an equal volume under sink conditions. After appropriate dilution, the drug concentration was analyzed spectrophotometrically at a λmax of 293 nm (Gadad et al., 2016).

Preparation of *in situ* gel

Polymeric solutions were prepared by dispersing the necessary amount of sodium alginate until it dissolved. The polymeric solution was examined to assess the impact of concentration on gelling behavior in the presence of simulated tear fluid (Nanjawade et al., 2007).

Physical evaluation of gels

Two parameters, transparency and clarity, were assessed. Clarity was evaluated by visually inspecting the samples under appropriate lighting against a dark background. After gently shaking, the samples were examined for particle presence. The pH of the

formulations was measured using a calibrated digital pH meter to ensure compatibility with the ocular environment (Nanjawade et al., 2007).

Viscosity of gel

The viscosity measurements describe the drop's retention time in the eye. A Brookfield viscometer is used at different angular velocities, 10-100 rpm 37 °C, to record the viscosity (Gadad et al., 2016)

Gelling capacity

Considering that the volume of fluid retained in the non-blinking eye is about 30 µl, 3 ml of simulated tear fluid was used to study the gelling capacity. A 0.5 ml freshly prepared gel was mixed with a 3 ml volume of simulated tear fluid at 37°C. Time for sol-gel conversion is noted (Mandal et al., 2012).

Preparation of *nanoparticulate* gel system

Weighed quantity of drug-loaded optimized nanoparticles formulation equivalent to the prescribed dose of (MOX Equivalent to 0.5% w/v) in commercial ophthalmic drops) was taken and dispersed into an in-situ gel (1% w/v sodium alginate solution). The formulation was further scaled up to obtain the same concentration to form a nanoparticle-loaded in-situ gel (Ahmed & Aljaeid, 2017; Anish Wani et al.,2020).

Compatibility study by FT-IR Spectra

An ATR-FTIR was employed to study the compatibility of MOX, chitosan, tripolyphosphate, and sodium alginate. Using an IR spectrophotometer (Alpha-II (Bruker)), samples were examined in the 4000-400cm^{-1} range. The same study was extended to formulation in the later stage (Gadad et al., 2016).

Zeta potential

Zeta potential was carried out for optimum formulation, approximating their surface charge. Zeta potential was determined using Horiba SZ-100, which utilizes an electrophoretic light scattering method, where specific electrodes contain cuvettes at 20 µg/ml concentrations. The measurements were carried out after diluting with distilled water at 25°C at a 90° angle (Mahmood et al., 2017).

Scanning electron microscopy (SEM)

The surface topography of optimum formulation was studied using SEM Jeol Japan functioned 15KV acceleration voltage. After gold sputtering the surface photographs were captured (Mahor et al., 2016).

Differential scanning calorimetry (DSC)

The heat transition behavior of pure drug, excipients, and the optimum formulation was deliberated by weighing 5mg of the MOX, excipients, and drug equivalent formulations into a non-hermetically sealed aluminium pan of the calorimeter (Perkin Elmer 4000) and crimped. It was melting transitions and changes in heat capacity of the drug and polymers performed under nitrogen at a 50 mL/min flow rate at 50-300 °C with an increased rate of 10 °C (Gadad et al., 2016).

***Ex- vivo* permeation studies**

An isolated goat cornea (from a previously stored eyeball at four °C in saline) sample (n=3) was used to assess the nanoparticle gel's permeation characteristics. The cornea and a thin layer of sclera tissue were isolated, and the nanoparticulate gel formulation was instilled on the cornea that was mounted on a diffusion cell assembly (diffusional area of 3.39cm^{-2}). A one ml volume of simulated tear fluid wets the donor compartment. In comparison, phosphate buffer pH 7.4 (100 ml) was used as the receiver medium, and its temperature was regulated at 350C. The assembly was magnetically stirred at 50 rpm. The sample concentration in the receiver fluid was determined at regular time intervals using spectroscopic analysis. A similar procedure was performed using commercially available MOX drops and a pure drug-loaded gel. Both the permeability coefficient and flux were calculated from the permeation profiles (ElMeshad & Mohsen, 2016). The results of the *ex vivo* permeation studies were subjected to statistical analysis using one-way ANOVA to understand the results to understand the level of significance.

Ocular irritancy

All animal experiments complied with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines and were carried out under the Prevention of Cruelty to Animals (PCA) Act, 1960. This study used male New Zealand white rabbits ((Ref. No. KCP/IAEC/PCOL/PCEU/62/2020), weighing 1.5 and 2 kg from the institutional animal facility. The experimental animals were familiarized for four days before beginning the study. One eye of each rabbit was used to administer the drops, whereas the other eye served as a control to evaluate the extent of irritation. After administering the nanoparticulate in situ drops into the eye cul-de-sac, the eyes were monitored at 1, 24, 48, and 72 h and continued to be observed for up to 7 days. Parameters such as eye-watering, redness, mucosal discharge, and swelling were evaluated at these time intervals and throughout the week (Gadad et al., 2016).

Stability studies

International conference on harmonization. (ICHQ1 A R_2) guidelines, assisted stability conditions at 25°C±2° //65%± 5% RH (40°C ±2°C 75%± 5% RH) for six months. The formulations were stored in glass vials and evaluated after six months for physical nature, viscosity, and gelling capacity (Gupta et al., 2019).

Antimicrobial studies

The antimicrobial study utilized Mueller Hinton agar medium using the agar cup method, where S. aureus and *E. coli* were incubated in broth media to gain their colony. The medium, after sterilization, was transferred to Petri plates. The medium was allowed to solidify under aseptic conditions; after solidifying the medium, the lawn was made with 0.1ml microorganisms, both strains, in separate Petri plates. After preparing cups using a sterile borer, nanoparticulate gel, and marketed moxifloxacin drops (0.1 ml) were added and incubated for 48 h at 37°C. The zone of inhibition was measured for both formulations (Swain et al., 2019).

RESULTS AND DISCUSSION

The custom design assisted in optimization trials utilized concentration of chitosan (%), STPP (%) was chosen as continuous factors, stirring speed (rpm), and stirring time (h) as categorical factors, for the responses such as drug entrapment efficiency (%), particle size (nm), drug release (%). The design generated 16 experimental trials.

The drug entrapment ranged between 70.9±0.08 to 89.7±0.09 %, as shown in Table 3. The NPs comprised chitosan and STPP; the coexistence of these two provides high loading efficiency. The pH of the STPP solution of about 9 provides OH- and phosphoric ions, which may react with cationic NH3+ groups of chitosan, resulting in cross-linking at acidic conditions. The OH- groups responsible for CS deprotonation compete with TPP. A higher concentration of TPP disclosed a significant effect on drug entrapment efficiency (> 85%.) Similarly, a lower concentration of TPP reduced the drug entrapment within the nanoparticles by < 70%. However, chitosan concentration had a variable effect on drug entrapment efficiency values. We could not conclude the effect of chitosan on drug entrapment efficiency, as it was invariably different according to the conditions performed. The drug release ranged between 67.3±0.03 to 90.6±0.08% (Figure 1.) and was affected by the structure of nanoparticles, concentration of chitosan, and STPP. As seen in T2 and T9, where the concentration of chitosan and crosslinker is higher, the dug release was maximum. Formulation with higher cross-linking capacity showed significant swelling; therefore, the drug release greatly depended on the extent of cross-linking. The lowest drug release was observed for T11, for which all factors except stirring speed were at the highest level. Perhaps we can say that the responses are contributed by permutation and combination effects. The particle size of the formulations ranged from 350-647 nm. In all the formulations, except T1, T2, T7, and T13, the particle size was > 500nm. The properties and concentrations of polymer, cross-linking agents, and processing

parameters greatly affected the particle size. A lower concentration yielded a low viscosity and thus might have promoted smaller particles' formation. Hence, a cross-linking agent, especially STPP, offers the additional effect of avoiding aggregation of the fine particles.

Table 3. Results of responses

Formulation code	Average Particle size (nm)	Drug release at 24 h (%)	Drug entrapment efficiency (%)
T1	430.5±2.24	84.6±0.6	80.5±0.4
T2	350.7±1.89	90.6±0.08	89.4±0.05
T3	597.1±1.4	78±0.01	75.7±0.1
T4	553.3±2.09	77.7±0.8	85.7±0.02
T5	589.3±3.12	77.3±0.06	83.3±0.06
T6	506.8±1.45	84.3±0.01	70.9±0.8
T7	420.5±3.11	81.6±0.05	89.7±0.09
T8	647.3±2.12	85.8±0.9	72±0.02
T9	542.8±2.12	86±0.06	88±0.03
T10	592.8±1.89	80.5±0.07	88.3±0.07
T11	570.2±1.45	67.3±0.3	76.9±0.0
T12	600.2±1.34	74.4±0.06	79±0.05
T13	433.2±1.90	78.1±0.04	85±0.02
T14	580.3±1.11	83.4±0.09	86±0.08
T15	530.2±1.89	84.8±0.01	88±0.4
T16	430.5±1.09	70.6±0.05	78±0.01

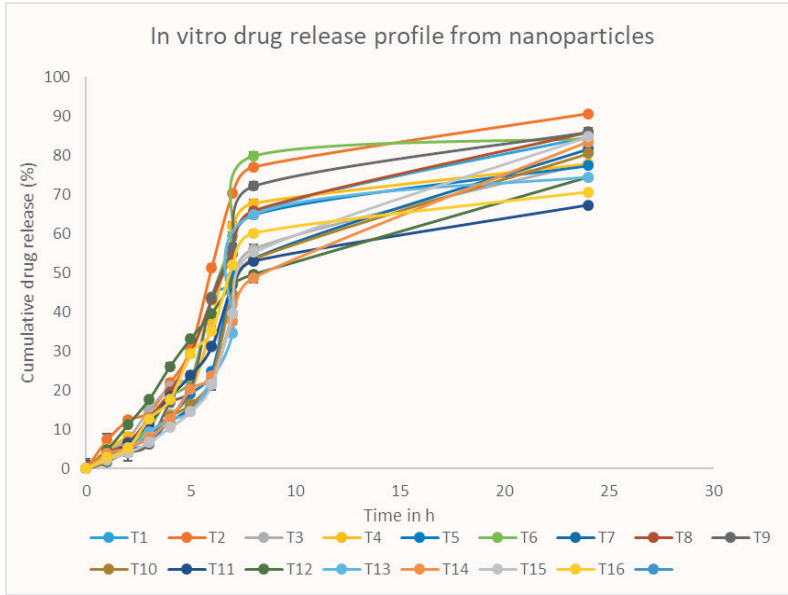

Figure 1. *In vitro* drug release profile from nanoparticles (T1-T16)

A custom design is an experimental approach that addresses various challenges within a structured framework. Fit statistics suggest (Table 4) 2FI for particle size and drug release and a linear model for drug entrapment efficiency. A summary of the responses is shown in Table 5.

Table 4. Fit Summary of the Responses

Parameter	Source	Sequential P-value	Lack of Fit P-value	Adjusted R^2	Predicted R^2	Model suggested
Particle Size	2FI	0.0073	0.9970	0.8496	0.8109	Suggested
Drug Entrapment	Linear	< 0.0001	0.9878	0.8632	0.8073	Suggested
Drug Release	2FI	0.0022	0.0971	0.9745	0.8130	Suggested

Table 5. Statistical Evaluation of the responses

Source	p-value							
	Particle Size		**Drug release (%)**			**Drug Entrapment Efficiency (%)**		
Model	0.0114	Significant	Model	0.0002	Significant	Model	< 0.0001	Significant
A-Chitosan conc	0.6303	NS	A	0.0034	S	A	0.0548	NS
B-TPP	0.0053	S	B	< 0.0001	S	B	< 0.0001	S
C-Stirring speed	0.8743	NS	C	< 0.0001	S	C	0.2117	NS
D-Stirring time	0.3789	NS	D	0.0541	S	D	0.9329	NS
AB	0.0207	S	AB	0.0868	NS			
AC	0.0030	S	AC	0.0029	S			
AD	0.0693	NS	AD	0.1564	NS			
BC	0.1091	NS	BC	0.0003	S			
BD	0.0431	S	BD	0.0397	S			
CD	0.0238	S	CD	0.0337	S			
Residual			Residual					
Lack of Fit	0.9970	Not significant	Lack of Fit	0.0971	Not significant	Lack of Fit	0.9878	Not significant
S=Significant					NS: Non-significant			

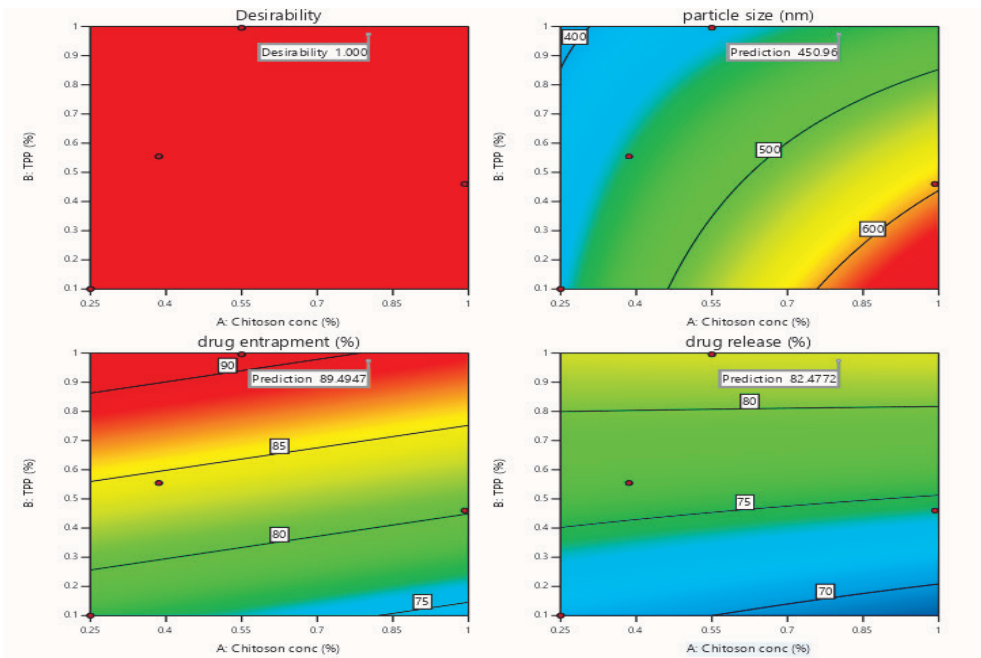

Figure 2. Predication profiler for the experimental runs

As per the surface response graphs (Figure 2), the chitosan concentration positively affected the particle size. Meanwhile, with a higher concentration of TPP, particle size was low and gradually increased as the concentration increased, with an additional curvature effect from high-order interactions. However, drug entrapment efficiency was a linear factor of TPP concentration, and chitosan concentration had a reverse effect. A similar response was observed in the drug release profile; an increase in the concentration of chitosan resulted in a decrease of this parameter; however, the interactive effect of the cofactor, chitosan concentration, had a noteworthy impact on drug entrapment efficiency.

The desirability approach specifies how to deal with multiple response processes and how the ranges are close to the optimum, which has values of zero to one. At a maximum desirability value of 1, the optimized formulation exhibited a particle size, zeta potential, and in vitro drug release profile of 503.1nm, -41.6mV, and 90.7%± 1.01%, respectively.

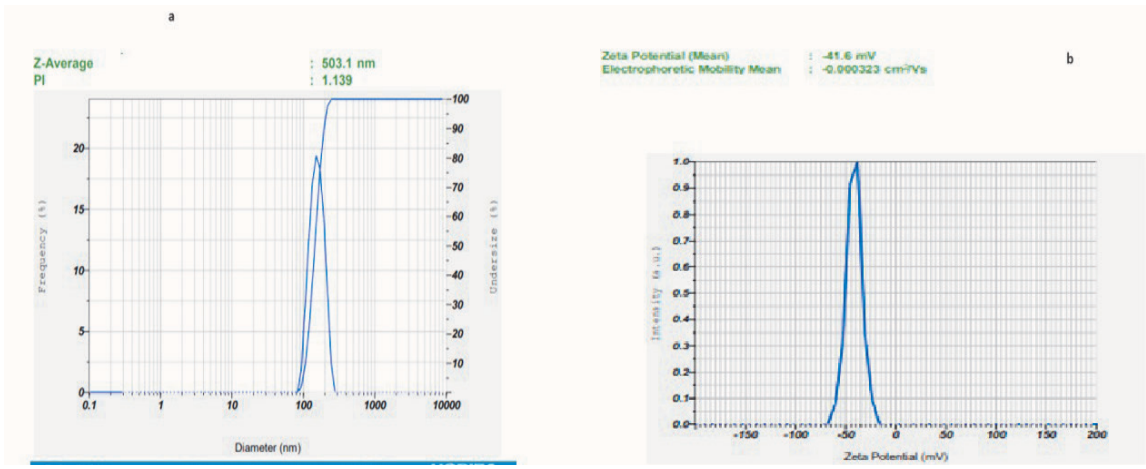

Figure 3. a) Particle size distribution of optimum formula b) Zeta potential

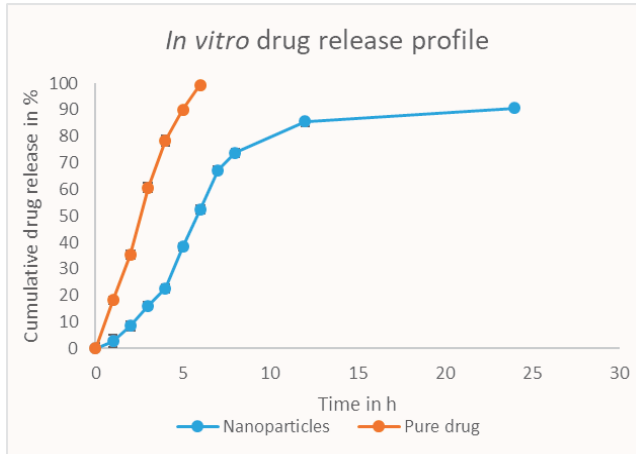

Figure 4. Comparative *in vitro* drug release profile of pure drug vs Nanoparticles

A comparative drug release profile of optimum nanoparticle formulation (Figure 4) against marketed drops (Moxicip) showed a rapid drug release from marketed drops and 99.34±1.8% drug release in 6 h compared to a sustained drug release from optimised nanoparticle formulation. It took 24 hours for 90.7±1.6% of the drug to get released. The delay in drug release suggests a crosslinked form of chitosan, which may require time to deprotonate and release the drug from the networks.

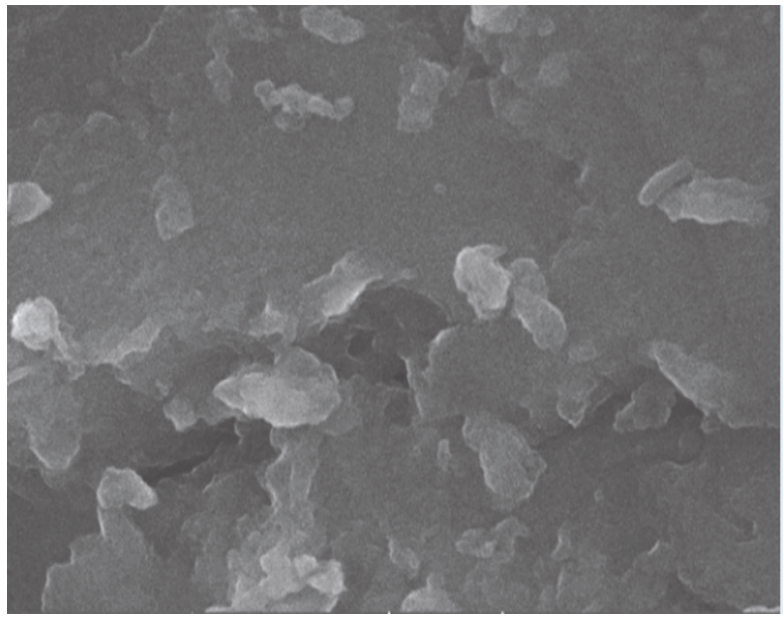

Figure 5. Surface photograph of optimized formulation

The surface photograph of the nanoparticles in Figure 5 reveals slightly distorted particles with uneven surfaces. The high stirring speed may have disoriented the structure of the chitosan, contributing to this unevenness.

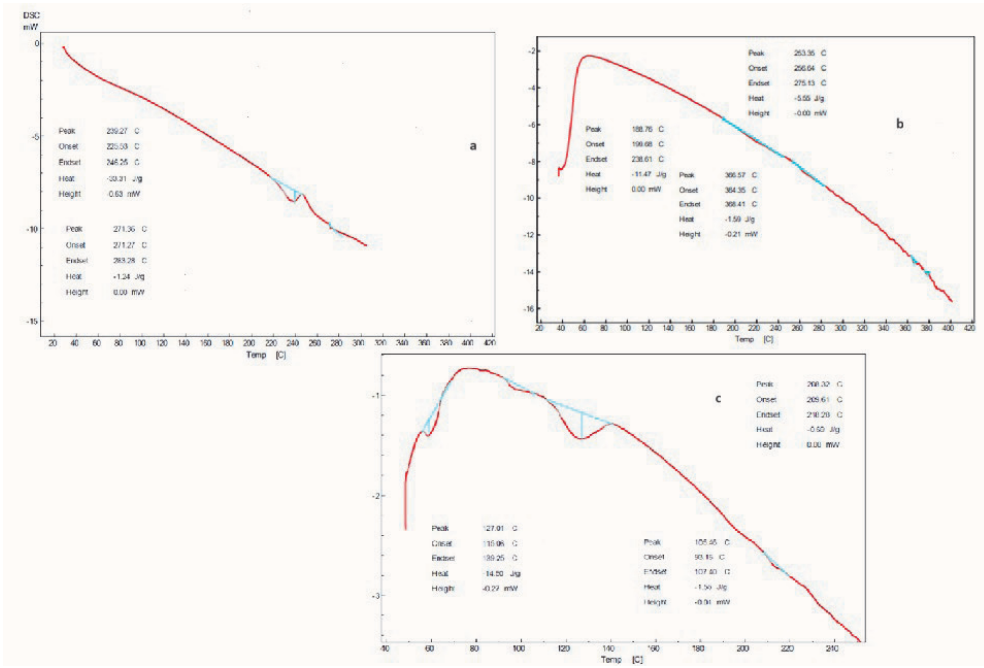

Figure 6. DSC thermogram of a) Pure drug, b) Physical Mixture c) Optimized NP formulation

The thermal behavior of Moxifloxacin hydrochloride (MH) and its nanoparticles (NP) is directed to the heat impact on both the polymer and MH. The DSC thermogram of MH displays an endothermic peak at 239˚C, while in the physical mixture, the peak shifts slightly to 259˚C. An endothermic peak appears at 208˚C for the optimized formulation, as optimized Figure 6. These results

suggest that in nanoparticle form, the drug's solubility decreases due to chitosan, a solubilizing agent, contributing to MH's melting point. Consequently, this promotes the retention of the drug within the polymer matrix. Upon contact with an aqueous medium (pH 7.4), the drug release increases due to the reversal of these effects.

Polymer solutions (0.5%, 1%, and 1.5% w/v) were studied for their gelling, physical, and viscosity. The 0.5% w/v concentration remained liquid under physiological conditions, whereas the 1% w/v concentration exhibited good gelling action. The 1.5% w/v concentration showed high viscosity under the studied conditions, as detailed in Table 6. Sodium alginate is an ion-responsive polymer. It contains monomeric sugar units such as mannuronic acid (M moiety) and guluronic acid (G moiety), with gelation depending on the ionic interaction between the cation and the carboxyl functional group of the G moiety. In the presence of divalent calcium ions, the sodium alginate solution undergoes a sol-to-gel conversion, which is ion and concentration-dependent. The gelling properties were evaluated using scores: (+: slow), (++: immediate and short-time effect), (+++: immediate and extended period).

Table 6. Evaluation of *In Situ* Gel

Sl.no	Concentration %	Clarity	pH	Viscosity (Cp)	Gelling capacity
1.	0.5	Clear	7.4±0.04	15.2	+
2.	1	Clear	7.4±0.07	34.8	+++
3.	1.5	Turbid	7.4±0.02	59.3	++

Note: + Gelled slowly and lost its consistency immediately, +++, Spontaneous gelling retained its consistency up to 8.5 h, ++ Spontaneous gelling, retained its consistency up to 5 h.Optimized nanoparticle-loaded in situ gel formulation was studied for its clarity, pH, drug content, and viscosity to know the extent of drug retention on the eye. The nanoparticulate gel (1w/w alginate gel base) was clear without any gritty particles by visual observation, had a pH of 7.4±0.04, and drug content of 87.5±0.2%. The viscosities of the gel formulation before and after the addition of STP at various angular velocities are given in Figure 7. As seen, in the absence of STF, the viscosity was increased by increasing the rpm, whereas after combing with STF, there was a decrease in viscosity with an increase in rpm.

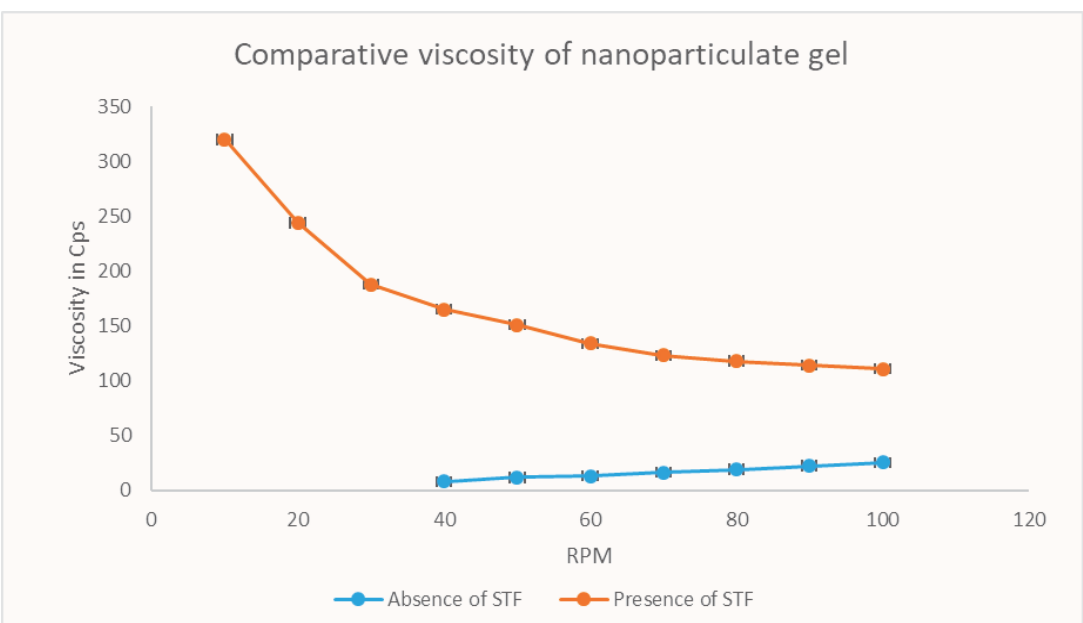

Figure 7. Comparative viscosities of nano particulate gel in the presence and the absence of SNF

The drug permeation rate from the NP in situ gel was 91.5 ± 0.02%, compared to 70.4 ± 0.05% for the pure drug-loaded gel and 69.12 ± 0.7% for the marketed Moxicip drops (0.5%) over a 24-hour release study, as illustrated in Figure 8. The NP gel formulation showed sustained activity for up to 24 hours, with a peak at six hours, indicating prolonged action of the in-situ gel. While both the marketed formulation and the pure drug gel achieved a maximum drug permeation of approximately 70%, the developed NP in situ gel demonstrated a faster onset of action, comparable to the marketed drops. The *ex-vivo* permeation results showed a higher flux of 3.02 ± 0.3 cm/h x 10^3, a permeability coefficient of $0.73cmh^{-1}10^{3}$. and rapid permeation for the NP in situ gel. In comparison, the pure drug gel had a flux of 0.64 ± 0.04, a permeability coefficient of 0.30, and the marketed Moxicip drops had a flux of 1.64 ± 0.08 μg/cm²/h and a permeability coefficient of 0.60. ANOVA results shows that there was statistical difference between the permeation profiles of the three formulations studied p>0.05). The significant mucoadhesive properties of chitosan, due to electrostatic interactions and hydrogen bonding with anionic mucin, prolong corneal residence time. The pH-responsive nature of the polymer can also help control drug delivery and extend drug release. Additionally, chitosan's permeation-enhancing properties and the colloidal size contribute to its effectiveness.

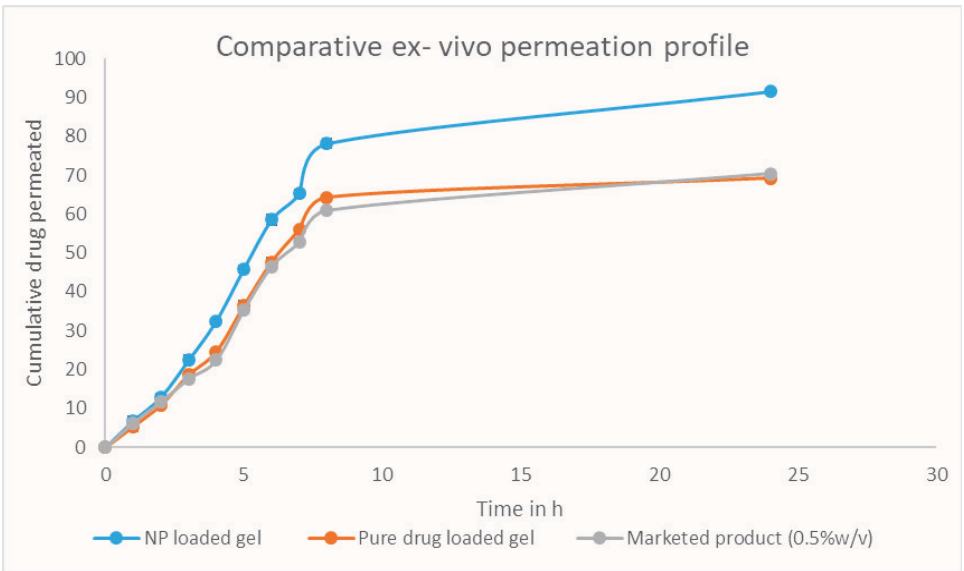

Figure 8. *Ex-vivo* permeation profile of NP-loaded gel, pure drug-loaded gel and marketed product

The optimum MOX-loaded gel formulation (OF) was subjected to short-term stability studies for 3 m at 25°C ± 2°CRH and 40°C ± 2°CRH. The result of physical appearance shows that there was no change at different storage conditions. No notable changes were observed in viscosity and gelling capacity at both storage conditions, as shown in Table 7.

Table 7. Stability Studies

SLNo	Days	Physical appearance			Viscosity (cps)			Gelling capacity (min±SD)		
		OF	25±2°C	40±2°C	OF	25±2°C	40°±2°C	OF	25±2°C	40°±2°C
1	0	Free-flowing and clear			111±1.1			0.5±0.03		
2	180	Free-flowing and clear			111±1.1	145±1.9	200±2.2	0.5±0.03	0.5±0.07	0.6±0.02

The FTIR of the drug, drug/excipients, and the optimum formula are given in Figure 9. Compatibility studies showed that there is no interaction between drugs and excipients used. Peaks for drug and excipients were observed within the stretching range 1500-1700 for carboxylic Acid (C=O), 1550-1500 for nitro compound N-O, 840-790 for aromatic Substitution C, 1400-1000 for fluoro compound C. The observed ranges for optimum formulation were within the stretching range 3550-3200 for Alcohol O-H, 2000-1650 for Aromatic compound C-H, 1550-1500 for Nitro compound N-O, and 1400-1000 for Fluoro compound C-F.

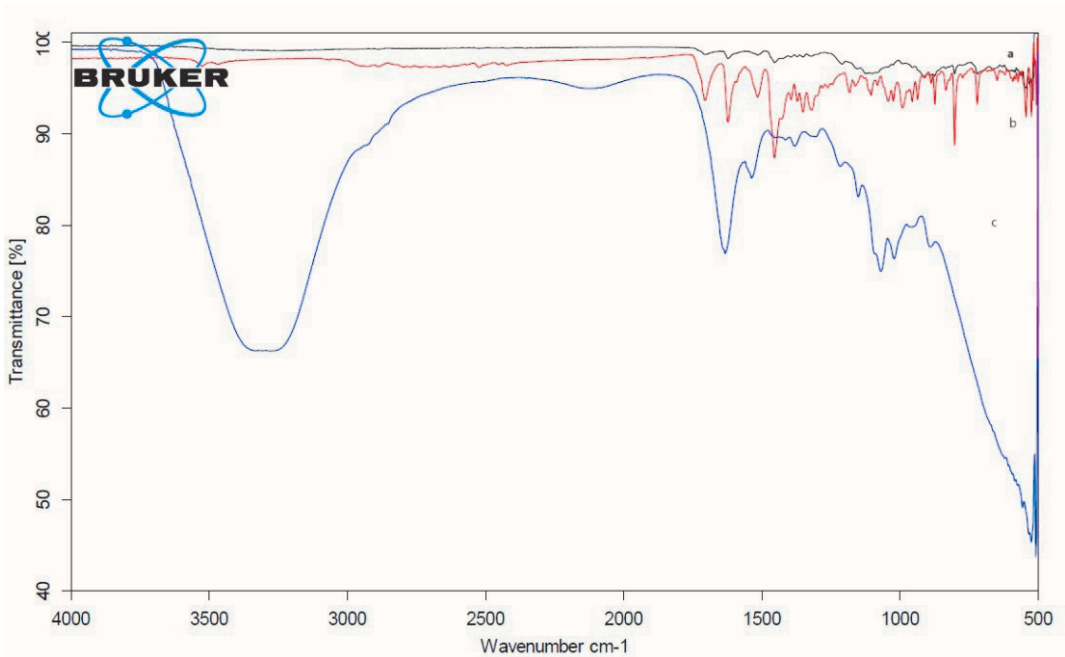

Figure 9. FTIR spectrum of NP formulation

The optimum MOX-loaded was studied for its irritation on rabbit eye against as a control—seven days to see the eye's irritation and redness (Figure 10). There were no signs of watering or inflammation.

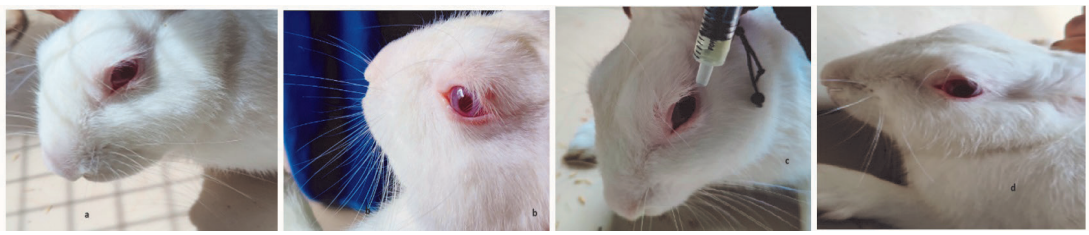

Figure 10. *In vivo* eye irritation study (a) Control, (b) Normal rabbit eye, before instillation of the drops, (c) Instillation of optimum formulation, (d) After instillation, observation for 7 days.

Table 8. Antimicrobial Study

Organism	Zone of Inhibition*(mm)	
	Marketed Formulation	**Developed formulation**
S aureus	34.6±0.4	38.6±0.4
E coli	38±0.8	45.6±0. 9

The antimicrobial study indicates that MOX retained its antimicrobial property when formulated into nanoparticle-loaded in situ gel, as shown in Table 8. Also, the study showed that the developed formulation reflected more antimicrobial activity compared to the marketed formulation, as shown in (Figure 11) this may be due to the combined effect of polymer, gel, and the drug and because of its more penetration power

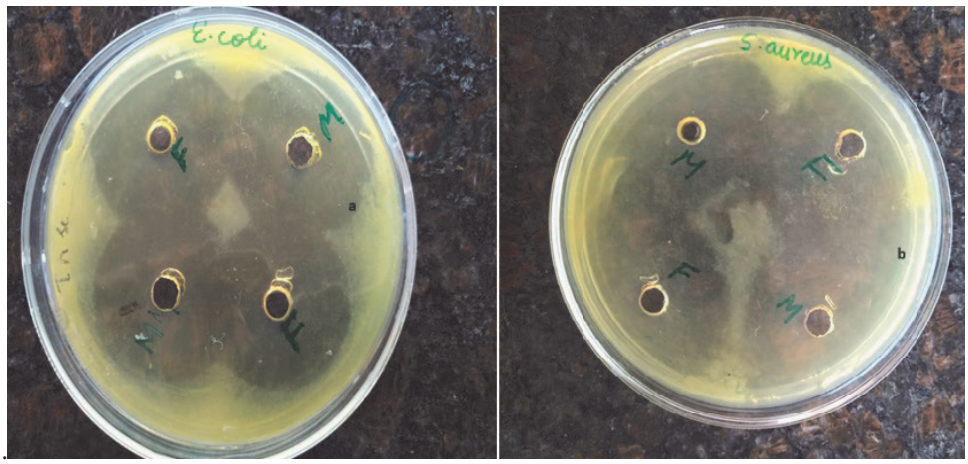

Figure 11. Zone of inhibition of (a) organism E. Coli (b) Organism S. aureus, (F): Developed formulation (M): Marketed formulation.

CONCLUSION

The study aimed to develop a colloidal carrier-based in situ gel for ophthalmic delivery of moxifloxacin hydrochloride to treat deep corneal infections. Chitosan-MX nanoparticles, prepared through experimental design, were incorporated into a sodium alginate in situ gel. The *ex vivo* permeation profile demonstrated that the optimized formulation provided higher permeation and extended drug action compared to the marketed eye drop (Moxicip - 0.5% w/v). The formulation exhibited superior antimicrobial activity against both gram-positive and gram-negative bacteria, comparable to that of marketed formulations, and was non-irritating to the eye. The combination of chitosan nanoparticles-loaded in situ gel showed enhanced ocular bioavailability, increased corneal retention time, and reduced frequency of drug administration, thereby improving patient compliance. Additionally, this approach offers clinicians a new, cost-effective, safe, and efficient option for ocular drug delivery.

AUTHOR CONTRIBUTION STATEMENT

PS: Concept, Design, Supervision and Manuscript Writing

JP**:** Literature Search, Data Collection, and Processing

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

Ahmed, T. A., & Aljaeid, B. M. (2017). A potential in situ gel formulation loaded with novel fabricated poly(Lactide-co-glycolide) nanoparticles for enhancing and sustaining the ophthalmic delivery of ketoconazole. *International Journal of Nanomedicine*, *12*, 1863–1875. https://doi.org/10.2147/IJN.S131850

Bala, H.B., Ajay, S., Anil, B. Studies on Thermoreversive Mucoadhesive Ophthalmic in Situ Gel of Azithromycin. (2013). 3(5),106–109.

Bhatia, HB., Sachan, A., Bhandari, A. (2013) studies on thermoreversive mucoadhesive ophthalmic in situ gel of azthromycin. Journal of Drug Delivery and Therapeutics, 3(5), 106-109.

da Silva Furtado, G. T. F., Fideles, T. B., de Cassia Alves Leal Cruz, R., de Lima Souza, J. W., Barbero, M. A. R., & Fook, M. V. L. (2020). Chitosan/NaF Particles Prepared Via Ionotropic Gelation: Evaluation of Particles Size and Morphology. *Materials Research*, *21*(4). https://doi.org/10.1590/1980-5373-MR-2018-0101

Desai, K. G. (2016). Chitosan nanoparticles prepared by ionotropic gelation: An overview of recent advances. *Critical Reviews in Therapeutic Drug Carrier Systems*, *33*(2), 107–158. https://doi.org/10.1615/CritRevTherDrugCarrierSyst.2016014850

ElMeshad, A. N., & Mohsen, A. M. (2016). Enhanced corneal permeation and antimycotic activity of itraconazole against Candida albicans via a novel nanosystem vesicle. *Drug Delivery*, *23*(7), 2115–2123. https://doi.org/10.3109/10717544.2014.942811

Elmizadeh, H., Khanmohammadi, M., Ghasemi, K., Hassanzadeh, G., Nassiri-Asl, M., & Garmarudi, A. B. (2013). Preparation and optimization of chitosan nanoparticles and magnetic chitosan nanoparticles as delivery systems using Box-Behnken statistical design. *Journal of Pharmaceutical and Biomedical Analysis*, *80*, 141–146. https://doi.org/10.1016/j.jpba.2013.02.038

Gadad, A. P., Wadklar, P. D., Dandghi, P., & Patil, A. (2016). Thermosensitive in situ gel for ocular delivery of lomefloxacin. *Indian Journal of Pharmaceutical Education and Research*, *50*(2), S96–S105. https://doi.org/10.5530/ijper.50.2.24

Gote, V., Sikder, S., Sicotte, J., & Pal, D. (2019). Ocular drug delivery: Present innovations and future challenges. *Journal of Pharmacology and Experimental Therapeutics*, *370*(3), 602–624. https://doi.org/10.1124/jpet.119.256933

Gupta, C., Juyal, V., & Nagaich, U. (2019). Formulation, optimization, and evaluation of in-situ gel of moxifloxacin hydrochloride for ophthalmic drug delivery. *International Journal of Applied Pharmaceutics*, *11*(4), 147–158. https://doi.org/10.22159/ijap.2019v11i4.30388

Irimia, T., Ghica, M. V., Popa, L., Anuţa, V., Arsene, A. L., & Dinu-Pîrvu, C. E. (2018). Strategies for improving ocular drug bioavailability and cornealwound healing with chitosan-based delivery systems. *Polymers*, *10*(11). https://doi.org/10.3390/polym10111221

Kaur, V., & Pawar, P. (2015). Formulation and Evaluation of Moxifloxacin Hydrochloride Niosomes for Controlled Ophthalmic Drug Delivery. *Journal of Pharmaceutical Technology, Research and Management*, *3*(1), 11–28. https://doi.org/10.15415/jptrm.2015.31002

Kesarla, R., Tank, T., Vora, P. A., Shah, T., Parmar, S., & Omri, A. (2016). Preparation and evaluation of nanoparticles loaded ophthalmic in situ gel. *Drug Delivery*, *23*(7), 2363–2370. https://doi.org/10.3109/10717544.2014.987333

Maharjan, P., Cho, K. H., Maharjan, A., Shin, M. C., Moon, C., & Min, K. A. (2019). Pharmaceutical challenges and perspectives in developing ophthalmic drug formulations. *Journal of Pharmaceutical Investigation*, *49*(2), 215–228. https://doi.org/10.1007/s40005-018-0404-6

Mahmood, S., Mandal, U. K., Chatterjee, B., & Taher, M. (2017). Advanced characterizations of nanoparticles for drug delivery: Investigating their properties through the techniques used in their evaluations. *Nanotechnology Reviews*, *6*(4), 355–372. https://doi.org/10.1515/ntrev-2016-0050

Mahor, A., Prajapati, S. K., Verma, A., Gupta, R., Iyer, A. K., & Kesharwani, P. (2016). Moxifloxacin loaded gelatin nanoparticles for ocular delivery: Formulation and in-vitro, in-vivo evaluation. *Journal of Colloid and Interface Science*, *483*, 132–138. https://doi.org/10.1016/j.jcis.2016.08.018

Majeed, A., & Khan, N. A. (2019). Ocular in situ gel: An overview. *Journal of Drug Delivery and Therapeutics*, *9*(1), 337–347. https://doi.org/10.22270/jddt.v9i1.2231

Mandal, S., Prabhushankar, G., Thimmasetty, M., & Geetha, M. (2012). Formulation and evaluation of an in situ gel-forming ophthalmic formulation of moxifloxacin hydrochloride. *International Journal of Pharmaceutical Investigation*, *2*(2), 78. https://doi.org/10.4103/2230-973x.100042

Miller, D. (2008). Review of moxifloxacin hydrochloride ophthalmic solution in the treatment of bacterial eye infections. *Clinical Ophthalmology*, *2*(1), 77. https://doi.org/10.2147/opth.s1666

Mohammadpour Dounighi, N., Damavandi, M., Zolfagharian, H., & Moradi, S. (2012). Preparing and characterizing chitosan nanoparticles containing hemiscorpius lepturus scorpion venom as an antigen delivery system. *Archives of Razi Institute*, *67*(2), 145–153.

Nanjawade, B. K., Manvi, F. V., & Manjappa, A. S. (2007). In situ-forming hydrogels for sustained ophthalmic drug delivery. *Journal of Controlled Release*, *122*(2), 119–134. https://doi.org/10.1016/j.jconrel.2007.07.009

Raj, V. K., Mazumder, R., & Madhra, M. (2020). Ocular drug delivery system: Challenges and approaches. *International Journal of Applied Pharmaceutics*, *12*(5), 49–57. https://doi.org/10.22159/ijap.2020v12i5.38762

Shashank Nayak, N., Sogali, B. S., & Thakur, R. S. (2012). Formulation and evaluation of pH triggered in situ ophthalmic gel of Moxifloxacin hydrochloride. *International Journal of Pharmacy and Pharmaceutical Sciences*, *4*(2), 452–459.

Shelake, S. S., Patil, S. V., Patil, S. S., & Sangave, P. (2018). Formulation and evaluation of fenofibrate-loaded nanoparticles by precipitation method. *Indian Journal of Pharmaceutical Sciences*, *80*(3), 420–427. https://doi.org/10.4172/pharmaceutical-sciences.1000374

Swain, G. P., Patel, S., Gandhi, J., & Shah, P. (2019). Development of Moxifloxacin Hydrochloride loaded in-situ gel for the treatment of periodontitis: In-vitro drug release study and antibacterial activity. *Journal of Oral Biology and Craniofacial Research*, *9*(3), 190–200. https://doi.org/10.1016/j.jobcr.2019.04.001

Wani, M., Jagdale, S., Khanna, P., Gholap, R., Baheti, A. (2020). Formulation, and evaluation of ophthalmic In-situ gel using moxifloxacin coated silver nanoparticles. Research J. Pharm. and Tech., 13(8), :3623-3630. 10.5958/0974-360X.2020.00641.1%0A

Wu, Y., Liu, Y., Li, X., Kebebe, D., Zhang, B., Ren, J., Lu, J., Li, J., Du, S., & Liu, Z. (2019). Research progress of in-situ gelling ophthalmic drug delivery system. *Asian Journal of Pharmaceutical Sciences*, *14*(1), 1–15. https://doi.org/10.1016/j.ajps.2018.04.008

Youssef, A.A.A., Thakkar, R., Senapati, S., Joshi, P.H., Dudhipala, N., Majumdar, S. (2022). Design of Topical Moxifloxacin Mucoadhesive Nanoemulsion for the Management of Ocular Bacterial Infections. Pharmaceutics, 14(6), 1246. https://doi.org/10.3390/pharmaceutics14061246

Yu, S., Wang, Q. M., Wang, X., Liu, D., Zhang, W., Ye, T., Yang, X., & Pan, W. (2015). Liposome incorporated ion sensitive in situ gels for opthalmic delivery of timolol maleate. *International Journal of Pharmaceutics*, *480*(1–2), 128–136. https://doi.org/10.1016/j.ijpharm.2015.01.032

Yurtdaş-Kirimlioğlu, G., Özer, S., Büyükköroğlu, G., & Yazan, Y. (2018). Formulation and in vitro evaluation of moxifloxacin hydrochloride-loaded polymeric nanoparticles for ocular application. *Latin American Journal of Pharmacy*, *37*(9), 1850–1862.