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# DEVELOPMENT AND EVALUATION OF GELLAN GUM-BASED OCULAR GEL-FORMING SOLUTION OF OFLOXACIN USED IN THE MANAGEMENT OF OCULAR BACTERIAL INFECTIONS

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## ABSTRACT

Human eye has distinct anatomy which makes it different from other body organs. It offers several barriers to effectively deliver the drug to the desired site. Ophthalmic gel forming solutions are one such novel dosage form which remain in solution form at room temperature and at physiological conditions they undergo sol to gel transition upon contact with lacrimal fluid. The objective of the current investigation was to formulate gel forming solution of ofloxacin using Gellan gum for controlled action in the management of bacterial infections. Gellan gum was dissolved in distilled water to create a polymeric solution, which was then used to develop the formulation intended in this study. A total of six different batches containing various concentrations of gellan gum were prepared. Drug and other excipients such as mannitol, and Benzdodecinium bromide were added employing constant stirring to obtain a slightly viscous solution and pH were adjusted accordingly. Numerous characteristics such as rheological behaviour, pH, in-vitro gelling capability, drug release was assessed for the developed formulations. Based on various evaluation parameters, F5 turned out to be the best formulation amongst three formulations developed with gelation time of 10 seconds and the gel remained intact for 8 hours. The rheological behaviour of the developed formulation F5 was found to be pseudoplastic with shear thinning nature. Additionally, it was able to release 94.06% of the drug in 8 hours during the in-vitro release trial, indicating prolonged release. During histopathology study, no signs of inflammation were noted. Obtained results were satisfactory and were in specified limits. Thus, it can be concluded that the prepared formulation F5 can be further explored for effective management of bacterial infections.

**Keywords:** Ocular drug delivery system, Bacterial infections, Sustained action, Sol-to-gel, Ofloxacin, Gellan gum

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## 1. INTRODUCTION

Human eye is basically ball like spherical structure with 23 mm diameter. The anterior, posterior, and tear chambers make up the three primary chambers of the human eye. The retina, choroid, sclera, and vitreous humor make up the posterior chamber while anterior segment of eye comprises of cornea, ciliary body, iris, and aqueous humour[1]. There are a range of ocular infections mainly caused bacteria, virus, and fungi. Most commonly reported ocular infections in the world includes Conjunctivitis, Keratitis, Eye stye, Corneal ulcers, Blepharitis, Uveitis, Dacryocystitis, Endophthalmitis, Herpes Zoster ophthalmicus, Optic neuritis, Chorioretinitis, and many more.

There are oil producing glands in the eyelids, mainly functioning for lubricating the eye. When any microbe such as bacteria cause infection in this region, the oil producing gland gets obstructed and hence, appears as small bump in either upper or lower part of the eyelids. It is also referred to as hordeolum[2,3,4]. It can be caused due to unhygienic environment and lack of personal hygiene.

Ofloxacin is a second-generation fluoroquinolone antibacterial drug used commonly for the management of ocular bacterial infections. Many marketed conventional ocular dosage forms are available in the market but are unable to retain the drug for longer period of time and hence researchers have been continuously trying to develop new formulations. It exhibits bactericidal effect by binding to and inhibiting topoisomerase II (DNA gyrase) and Topoisomerase IV. This inhibition interrupts DNA replication and transcription and thus prevents cell division.

The topical route of drug administration with the help of eye drops is one of the most common and preferred routes for drug delivery to the cul-de-sac part of the eye. Diffusion, erosion, and dissolution are the three main mechanisms responsible for the availability of the drug in the tear film. Absorption of drug in the ocular surface can be majorly categorized into corneal and noncorneal[5].

Various conventional ocular dosage forms such as drops, ointments, suspensions are common ocular drug delivery regimens as they offer patient compliance due to ease of administration[6]. However, these dosage forms are unable to produce adequate therapeutic effect due to various factors attributed to the washout of the drug from the ocular surface[7].

To overcome this issue of drug washing out from the ocular surface, several strategies such as sustained release with the help of polymers so that prolonged action can be provided. Viscosity and particle size of the formulations are optimized in such a way that drug can be released over extended period of time from the formulation whereas some strategies have been developed to bypass the external barriers of the eye and deliver the therapeutic regimen directly to the anterior or posterior segment of the eye[8].

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In the field of Ocular Drug Delivery System, to overcome all the barriers and limitations in-situ gelling systems/ gel forming solution which undergo rapid phase transition from sol to gel upon stimuli activation such as change in temperature, pH, and io-activation, have been developed[9]

These novel ocular drug delivery system therapeutic regimens are transforming the landscape of ocular therapies and are more convenient and efficacious than regular conventional dosage forms

These systems solve the drawbacks of traditional dosage forms like eye drops and suspensions simply because they are able to show sol-gel transformation attributed to variety of mechanisms, including change in pH, temperature, or ionic strength, they can form gel following ocular instillation, they extend the corneal residence period and restrict the drainage of the formulation[10].

The present study endeavors to formulate ionic-triggered gel forming solution of ofloxacin employing gellan gum for the management of ocular bacterial infections. Formulation developed in this study is instilled in liquid form, and upon coming in touch with lachrymal fluid undergo phase transformation from sol-gel state due to various stimuli such as change in pH, presence of cations, or temperature. The envisioned formulation represents not only a potential alternative to already available conventional eye drops but also holds the potential to furnish sustained drug delivery.

## 2. MATERIALS AND METHODS

### 2.1.Materials

Ofloxacin was received as gift sample from Mankind Pharma, Delhi. Gellan gum was procured from Sentiss Pharma Pvt. Ltd., Gurugram, India. Mannitol (Central Drug House Pvt. Ltd.), Benzdodecinium bromide (Anmol Chemicals). All other chemicals used in the present investigation were of analytical grade.

### 2.2.Methodology

#### 2.2.1. Melting point

Melting point apparatus is used for the determination of melting point using a capillary tube. The drug is filled in the capillary tube and the temperature is elevated to such an extent that the drug filled in capillary tube starts liquifying[11] This allows precise measurement of the melting temperature of the substance and the melting temperature is noted.

#### 2.2.2. Solubility

A simple method commonly used to assess the solubility of drug in various solvents is gravimetric method[12]. In this investigation, gravimetric analysis was used to determine the solubility of ofloxacin hydrochloride in various solvents such as (Distilled Water, methanol, ethanol, glacial acetic acid, and dichloromethane). Various small vials were taken in which 1 ml of different solvents were taken separately and then a specific amount of drug was added, then the solutions were stirred using magnetic stirrer. Temperature-controller was also used when required. Then the above stirred

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solution was filtered using filter paper and undissolved particles of ofloxacin hydrochloride remained on the filter paper. After this, the filter paper was dried and the weight of the filter paper was subtracted from the total weight of filter paper containing undissolved particles of ofloxacin hydrochloride.

### 2.2.3. UV spectrophotometric determination

First, stock solution of 100 µg/ml was prepared by dissolving 10 mg of drug in phosphate buffer pH 7.4 in a 100 ml volumetric flask and then volume was made up to the mark using phosphate buffer pH 7.4. From the stock solution prepared, various dilutions of 1-10 10 µg/ml were prepared by dissolving appropriate amount of stock solution and then diluted with phosphate buffer pH 7.4 and then, the absorbance of all dilutions were noted. UV spectrum of ofloxacin solution was obtained by scanning the sample under the range of 200-400 nm using phosphate buffer pH 7.4 as blank in double beam spectrophotometer[13].

### 2.2.4. IR spectra of ofloxacin

Ofloxacin was pressed using KBr method into pellets and scanned in the range of 4000 to 400 $cm^{-1}$ using FTIR and spectra was obtained for specific peak.

### 2.2.5. Preparation of gel-forming solution

Polymer solution was prepared by dispersing calculated amount of gellan gum in distilled water with continuous stirring using a magnetic stirrer until a clear slightly viscous solution was obtained.

Ofloxacin was dissolved in dilute acetic acid and pH was adjusted to 6.5 using 0.1 N NaOH. Benzdodecinium bromide and mannitol was added to the above solution. The resultant solution was then added to polymer solution with constant stirring. Distilled water was used to make up the volume up to 100ml. The above solution was continuously stirred using magnetic stirrer until a clear homogenous slightly viscous solution was obtained. After this, the solution was sterilized by autoclaving it at 121°C for 15 minutes. Table 1 summarizes the composition of developed ocular gel forming solutions.

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**Table 1: Formulation of gellan gum containing ofloxacin gel-forming solution**

| Ingredients | F1 | F2 | F3 | F4 | F5 | F6 |
|---|---|---|---|---|---|---|
| Ofloxacin (%w/v) | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |
| Gellan Gum (%w/v) | 0.1 | 0.2 | 0.3 | 0.4 | 0.5 | 0.6 |
| Mannitol (%w/v) | 1.8 | 1.8 | 1.8 | 1.8 | 1.8 | 1.8 |
| Benzdodecinium bromide (%) | 0.002 | 0.002 | 0.002 | 0.002 | 0.002 | 0.002 |
| Distilled Water | Q.s up to 100 ml | Q.s up to 100 ml | Q.s up to 100 ml | Q.s up to 100 ml | Q.s up to 100 ml | Q.s up to 100 ml |

#### 2.2.6. Determination of visual appearance and clarity

Prepared formulations were evaluated for its appearance, clarity, and color using sensory organs and clarity was checked using clarity apparatus.

#### 2.2.7. pH measurement

pH of the prepared formulations can be calculated using digital pH meter. A little amount of the prepared formulation was taken in a beaker and some amount of distilled water is added to it, and then subjected to determination of pH using digital pH meter.

#### 2.2.8. Rheological measurement

The viscosity of the developed formulations was determined using Brookfield viscometer. A suitable spindle was used at increasing angular velocity from 10 to 100 rpm. This level of angular velocity was reversed also. The value of viscosity was noted down from the dial present on the Brookfield viscometer[14]. The viscosity of the formulation was also calculated after sol to gel transition, this was done by placing the developed formulation with simulated tear fluid.

#### 2.2.9. Effect of sterilization on viscosity

To evaluate the impact of sterilization on viscosity, the developed formulation was sterilized using autoclaving at 121°C for 15 minutes. After sterilization, the viscosity of the developed formulations was determined using Brookfield viscometer. A suitable spindle was used at increasing angular velocity from 10 to 100 rpm. This level of angular velocity was reversed also. The value of viscosity was noted down from the dial present on the Brookfield viscometer.

#### 2.2.10. Rheological measurement after gelation

Viscosity of ocular formulations is an important parameter in assessing the efficacy of the final developed formulation. Thus, in addition to check the rheology of developed formulation in sol state, the viscosity of the developed formulation was also checked after gelation. Gelation was induced by placing the developed

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formulation in simulated tear fluid, and after gelation, the viscosity of the gel state was observed by increasing the angular velocity from 10 to 100 rpm in Brookfield viscometer.

#### 2.2.11. Gelation time

The gelation time of developed formulations can be calculated by taking the time required by the solution to convert to gel state upon coming in contact with the simulated tear fluid[15]. Gelation mainly occurs in the developed formulation due to presence of cations in the simulated tear fluid.

#### 2.2.12. *In vitro* gel formation potential

The gelling capacity of the prepared formulations can be calculated by placing a drop of formulation in a vial having 2 ml of freshly prepared simulated tear fluid. The time taken for gelling was noted. Generally, very less time is required for sol to gel conversion and the soft gel formed remain intact for long period of time.

#### 2.2.13. Time dependent *in vitro* drug release assessment

It was studied using Franz diffusion test apparatus. Semipermeable membrane was previously soaked overnight with diffusion medium (simulated tear fluid pH 7.4). 2 ml of formulation was pipetted into donor compartment. 100 ml diffusion medium (simulated tear fluid pH 7.4) was filled in the receptor compartment. The solution was homogenized at 50 rpm using magnetic stirrer present in the receptor compartment. 1 ml sample was withdrawn at every 1-hour time interval and replaced with equal volume of diffusion medium[16]. The samples withdrawn were diluted with diffusion medium and analyzed at 294 nm using UV spectrophotometer.

#### 2.2.14. Drug content estimation

The drug content of the developed formulation can be calculated by diluting 1ml of the developed formulation with simulated tear fluid. The instant soft gel formed due to interaction of formulation and simulated tear fluid was dissolved by using glass rod until clear solution is obtained. The solution was filtered by using filter paper of pore size 0.45 mm and then absorbance of the solution was taken at 287 nm using UV-visible spectrophotometer (Shimadzu UV-1700).

#### 2.2.15. Ex vivo permeation profile of drugs through corneal tissue

Goat eye was obtained from slaughter house. The cornea was carefully excised using forceps. At least 2-4mm of surrounding sclera tissue was also removed along with cornea. The excised cornea was washed with normal saline and stored in the solution until ex-vivo corneal permeation study was started. The excised goat cornea was carefully mounted between the surface of donor and receptor compartment of the Franz diffusion cell apparatus. The placement of goat cornea between the surface of donor and receptor compartment is done in such a way that epithelial surface of cornea is faced towards donor compartment while the endothelial surface

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is faced downwards towards receptor compartment[17]. Simulated tear fluid pH 7.4 was filled in the receptor compartment and 1 ml of the developed formulation was placed in the donor compartment near the cornea. The temperature of the complete system was set at 37±2$^0$C using water bath. The diffusion medium was allowed to flow over the formulation. Samples at an interval of 15, 30, 45, 60 minutes up to 4 hours were collected and then diluted with diffusion medium and absorbance was measured at 287 nm.

**2.2.16. Corneal Hydration level test**

Corneal Hydration level can be used by gravimetric method. The weight of the goat cornea after ex-vivo corneal permeation study was taken and then the cornea was placed in a desiccator at 100$^0$ C for 6 hours, after 6 hours the weight of the dry cornea is taken[18].

The equation 2 is used to calculate corneal hydration level:

HL= (Wa-Wb) / Wb X 100

Where, HL= Hydration level

Wa= Weight of wet cornea after ex-vivo corneal permeation study

Wb= Weight of dry cornea after drying for 6 hours.

Similarly, the corneal hydration level of cornea after excision was also determined by weighing cornea after excision and then weighing after drying it for 6 hours in a desiccator.

**2.2.17. Histopathological study**

To assess the ocular irritancy of developed formulations, histopathological studies of goat cornea can be conducted. The excised goat cornea was kept in contact with the developed formulation for a period of 12 hours. After 12 hours, goat cornea was fixed with 8% w/w formalin solution, followed by drying of the issue using alcohol. After this, tissue was cut into sections and stained using eosin dye and then observed using electronic microscope at 10x magnification[19].

**2.2.18. Antimicrobial activity**

Microbes- *Staphylococcus aureus* and *Pesudomonas aeruginosa*

Agar diffusion test using cup plate technique was used to assess antimicrobial activity. Ofloxacin marketed eye drops was used as control to compare the effect of developed formulation with the control group. The minimum inhibitory concentration of control and developed formulations of ofloxacin were compared. 60 ml of nutrient agar medium was prepared and sterilized for 18 minutes in autoclave. 0.5 ml of microbe suspension was poured into the medium and maintained at 52-58$^0$ C. 20 ml of microbe agar suspension was poured in petri dishes. After solidification of this media, ofloxacin eye drops (standard solution) and developed formulation diluted with distilled water (test solutions) were poured in solidified agar plates. Diffusion of solution was allowed for 2 hours and then plates were incubated at 37$^0$ C for 24 hours. Zone of inhibition of test and control were compared. Each formulation solution was tested in triplicate.

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#### 2.2.19. Sterility test

The sterility of the formulation is assessed in order to check if any microbe grows in it after some days. For the purpose of sterility testing, the developed formulation was filtered through filter paper of pore size 0.22µm and then the developed formulation is incubated in soybean casein digest medium at 25ºC and fluid thioglycolate medium at 35ºC for 14 days, and after 14 days the presence of fungus or bacteria is observed. In case of ophthalmic preparations, there should be no growth of fungus or bacteria, as sterility is the most important parameter in ophthalmic preparations.

#### 2.2.20. Accelerated stability studies

Prepared formulations were filled in glass vials closed with rubber closures and sealed with aluminum caps. The vials were kept in stability chamber and maintained at 40±2$^0$ C and 75±5 % RH for one month. Samples were withdrawn weekly for physical evaluation. Any change in physical properties, or other evaluation parameter was noted down.

## 3. RESULTS

### 3.1. Melting point (M.P.)

The melting point (M.P.) was determined using M.P. apparatus. The M.P. of the sample (Ofloxacin) was found to be 256 º C which is in standard range of 250-257º C.

### 3.2. Solubility

During the solubility study, ofloxacin was found to be slightly soluble in distilled water, (EtOH) and (MeOH). It was slightly soluble or soluble in dichloromethane while soluble in glacial acetic acid.

### 3.3. UV spectrophotometric determination

The $\lambda_{max}$ of ofloxacin was found to be 287 nm in phosphate buffer pH 7.4 and the spectra and the calibration curve of ofloxacin in phosphate buffer pH 7.4 is shown in Fig. 1.

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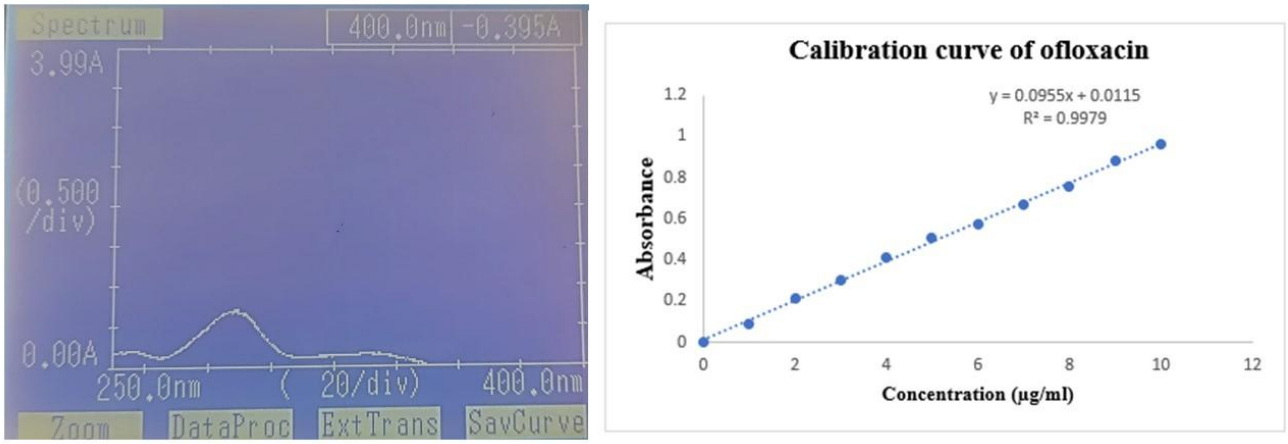


**Fig. 1 UV spectrophotometric scanning and calibration curve of ofloxacin in Phosphate buffer pH 7.4**

### 3.4. IR Spectra of ofloxacin

The IR spectra of ofloxacin is shown in Fig. 2 and found to be in standard range specified for ofloxacin.

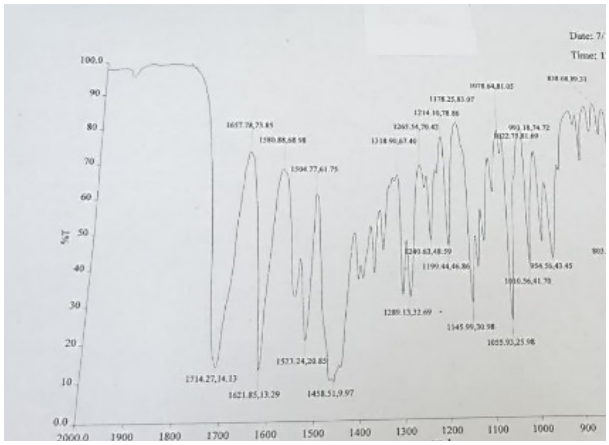

**Fig. 2 IR Spectra of ofloxacin**

### 3.5. Determination of visual appearance and clarity

All the developed gel forming solution of ofloxacin containing Gellan gum in various concentrations were found to be slightly pale yellow in colour, free flowing in nature with no presence of foreign particles or any particulate matter.

### 3.6. pH measurement

During pH determination of the developed formulations, the pH of all six developed formulations was found to be in the range of 6.4 to 6.6 which stabilizes the drug and is also favorable for ophthalmic preparations.

### 3.7. Rheological measurement

The rheological behavior of the developed formulations was found to be pseudoplastic with shear thinning nature. The viscosity of the developed formulations was found to be increasing as the angular velocity increases. Moreover, an increase in polymer concentration in the formulations was associated with a rise in viscosity, thus, F6

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possessed the highest viscosity and F1 possess least viscosity. But the viscosity range of the developed formulations F3-F5 was satisfactory for ocular administration while F1 and F2 were very or almost negligible viscous due to which sol to gel transformation may not take place and F6 was very highly viscous not suitable for ocular administration in the form of solution as it may cause blurred vision. Table 2 summarizes result obtained from Viscosity measurement of developed formulations.

### 3.8. Effect of sterilization on viscosity

It is important to assess the change is viscosity of ophthalmic preparations after sterilization as viscosity of the formulation may get altered due to several factors such as chemical degradation due to change in temperature, solvent and cross-linking effects. From the obtained results, it can be derived that viscosity of the developed formulations remain unchanged after sterilization which indicates that the developed formulations were stable during sterilization. The rheological behaviour of the developed formulations was found to be pseudoplastic with shear thinning nature. The viscosity of the developed formulations was found to be increases as the angular velocity of the spindle increases. Table 3 summarizes the results obtained from viscosity determination after sterilization.

### 3.9. Rheological measurement after gelation

For rheological measurement of developed formulations after gelation, formulations were kept in contact with simulated tear fluid to induce gelation to cation-anion interaction or ion activated mechanism. The rheological behavior of the developed formulations was found to be pseudoplastic with shear thinning nature as the angular velocity of the spindle increases from 10 to 100 rpm. Also, the viscosity of the developed formulations was found to be increasing with increase in concentration of the polymer in the formulation, thus, F6 possessed the highest viscosity and F1 possess least viscosity. The viscosity range of the developed formulations (F3-F5) was satisfactory for ocular administration while F1 and F2 were not able to show sufficient gelation required for prolonged release and F6 formed hard gel unfavorable for ocular administration. Viscosity range of developed formulations after gelation was far higher than before gelation which indicates that after gelation, the frequency of administration will be reduced. Table 4 summarizes result obtained from Viscosity measurement of developed formulations after gelation and Fig. 3 illustrates all the results related to viscosity in a graphical manner.

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**Table 2: Viscosity measurement of developed formulations F1-F3**

| Angular velocity (rpm) | Viscosity (cps) | | | | | |
|---|---|---|---|---|---|---|
| | F1 | F2 | F3 | F4 | F5 | F6 |
| 10 | 35.83±0.21 | 39.92±0.11 | 42.89±0.12 | 47.67±0.09 | 50.69±0.03 | 61.61±0.04 |
| 20 | 29.86±0.12 | 33.25±0.13 | 36.27±0.21 | 42.34±0.13 | 46.87±0.10 | 57.72±0.13 |
| 30 | 24.65±0.19 | 26.56±0.14 | 29.85±0.24 | 39.76±0.17 | 42.78±0.14 | 51.23±0.13 |
| 40 | 20.87±0.23 | 21.74±0.51 | 23.64±0.35 | 33.56±0.23 | 39.87±0.17 | 46.56±0.14 |
| 50 | 18.23±0.28 | 19.76±0.36 | 21.53±0.39 | 29.73±-.24 | 35.56±0.19 | 41.62±0.12 |
| 60 | 14.34±0.45 | 16.45±0.32 | 19.76±0.23 | 23.46±0.31 | 31.81±0.24 | 36.11±0.21 |
| 70 | 11.29±0.62 | 13.28±0.13 | 15.65±0.11 | 21.89±0.14 | 27.93±0.17 | 32.34±0.16 |
| 80 | 9.10±0.34 | 10.25±0.14 | 12.32±0.21 | 18.92±0.23 | 23.42±0.18 | 29.22±0.16 |
| 90 | 7.67±0.45 | 8.45±0.23 | 10.46±0.08 | 15.43±0.12 | 20.91±0.12 | 24.13±0.30 |
| 100 | 5.69±0.30 | 6.23±0.35 | 8.98±0.12 | 12.65±0.67 | 15.78±0.34 | 19.82±0.31 |

**Table 3: Viscosity measurement of developed formulations after sterilization**

| Angular velocity (rpm) | Post-sterilization viscosity (cps) | | | | | |
|---|---|---|---|---|---|---|
| | F1 | F2 | F3 | F4 | F5 | F6 |
| 10 | 36.28±0.20 | 40.23±0.10 | 43.98±0.09 | 47.93±0.08 | 51.68±0.10 | 61.69±0.12 |
| 20 | 30.57±0.15 | 34.23±0.14 | 37.71±0.12 | 43.41±0.10 | 47.87±0.19 | 58.71±0.13 |
| 30 | 25.63±0.18 | 27.61±0.16 | 29.89±0.15 | 39.89±0.17 | 43.08±0.22 | 52.32±0.14 |
| 40 | 20.91±0.21 | 22.24±0.23 | 23.78±0.18 | 33.73±0.35 | 39.92±0.34 | 48.61±0.12 |
| 50 | 19.01±0.26 | 20.86±0.32 | 22.12±0.21 | 30.21±0.36 | 36.12±0.56 | 43.26±0.12 |
| 60 | 15.42±0.41 | 17.54±0.31 | 20.16±0.16 | 24.56±0.41 | 31.98±0.32 | 37.19±0.20 |
| 70 | 12.29±0.26 | 14.68±0.13 | 15.98±0.13 | 22.12±0.23 | 28.16±0.27 | 33.32±0.17 |
| 80 | 10.11±0.28 | 11.59±0.15 | 12.67±0.23 | 19.01±0.26 | 23.78±0.17 | 30.62±0.15 |
| 90 | 7.97±0.29 | 9.69±0.19 | 10.82±0.24 | 15.95±0.17 | 21.02±0.19 | 25.31±0.28 |
| 100 | 6.91±0.23 | 7.38±0.30 | 9.01±1.01 | 12.91±1.21 | 16.86±1.03 | 21.28±0.29 |

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**Table 4: Viscosity measurement of developed formulations after gelation**

| Angular velocity (rpm) | Post gelation viscosity (cps) | | | | | |
|---|---|---|---|---|---|---|
| | F1 | F2 | F3 | F4 | F5 | F6 |
| 10 | 272±0.21 | 287±0.12 | 310±0.21 | 420±0.19 | 515±0.22 | 523±0.21 |
| 20 | 281±0.16 | 293±0.14 | 301±0.10 | 403±0.32 | 487±0.16 | 492±0.19 |
| 30 | 253±0.18 | 265±0.20 | 278±0.22 | 382±1.13 | 421±0.32 | 435±0.28 |
| 40 | 224±0.18 | 236±0.28 | 243±0.31 | 346±1.17 | 378±0.10 | 387±0.12 |
| 50 | 184±0.28 | 201±0.38 | 210±0.42 | 305±0.26 | 346±0.21 | 352±0.16 |
| 60 | 172±0.16 | 186±0.12 | 193±0.09 | 288±0.22 | 301±1.17 | 323±1.23 |
| 70 | 165±0.21 | 171±0.27 | 178±0.33 | 261±0.16 | 277±0.26 | 289±0.45 |
| 80 | 141±0.15 | 149±0.13 | 154±0.14 | 226±0.21 | 241±0.18 | 256±0.16 |
| 90 | 133±0.13 | 138±0.11 | 142±0.15 | 201±0.34 | 220±0.23 | 232±0.28 |
| 100 | 122±0.12 | 129±0.30 | 136±0.21 | 177±0.23 | 192±0.41 | 210±0.23 |

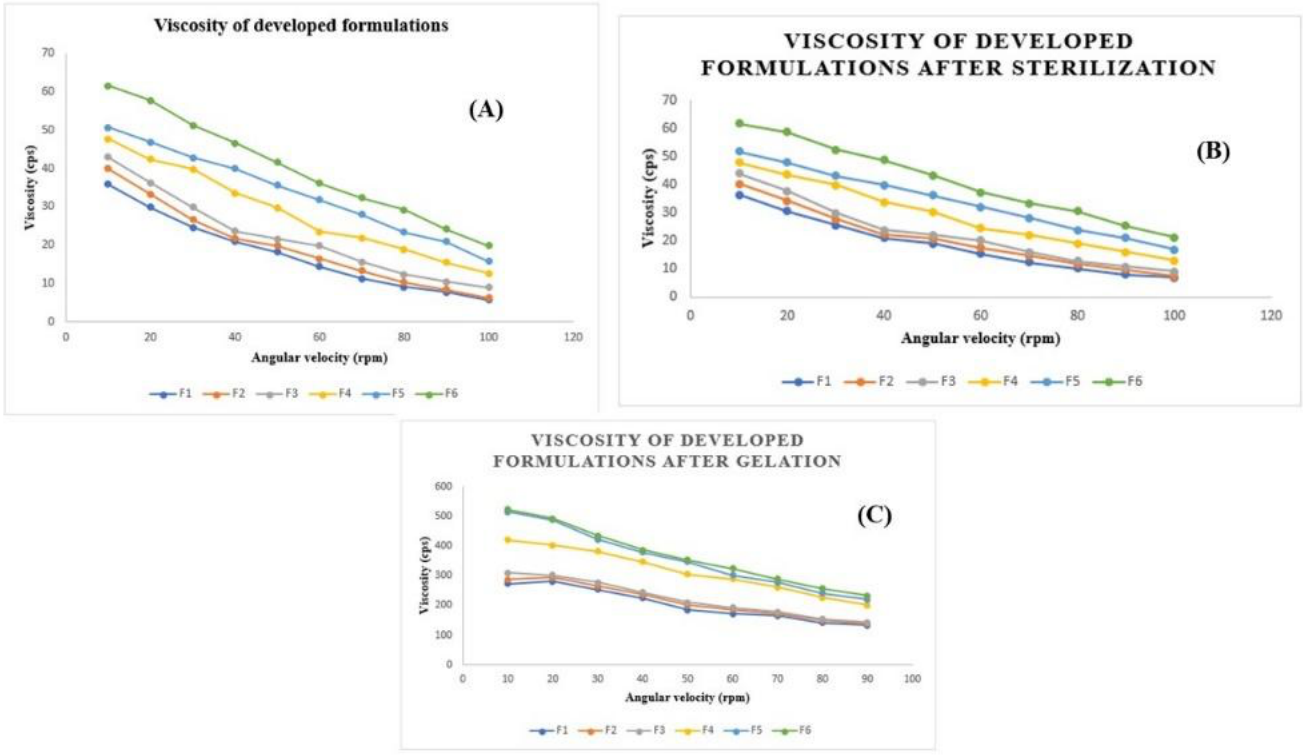


**Fig. 3: (A) Viscosity of developed formulations**
**(B) Viscosity measurement of developed formulations after sterilization**
**(C) Viscosity of developed formulations after gelation**

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### 3.10. Gelation time

All six developed formulations were assessed for gelation time. F1 and F2 were almost unable to undergo sol to gel transition due to less concentration of polymer. F3 took 15 seconds for gelation, F4 took 12 seconds for gelation F5 and F6 took the minimum time 10 seconds for gelation but F6 formed slightly hard gel as compared to F3-F5 and hence irritation can be caused upon ocular administration.

### 3.11. *In vitro* gel formation potential

For assessing the *in-vitro* gelling capacity of the developed formulations F1-F6, when developed formulation comes in contact with simulated tear fluid, sol to gel transition takes place. F1 and F2 were almost unable to undergo sol to gel transition due to less concentration of polymer. Out of all six formulations developed, F5 showed best result with gel remained intact for a period of 8 hours, while F3 and F4 formed gel which remained intact for 4-6 hours and thus, F5 turned out to be the best amongst three (F3-F5) because it prolongs the gel residence time. F6 formed hard gel not suitable for ocular administration.

### 3.12. Time dependent *in-vitro* drug release assessment

The marketed eye drops when evaluated for *in-vitro* drug release released 100% within 1 hour while the developed formulations were able to prolong the release of drug across semipermeable membrane for a period of 8 hours. Amongst all the three formulations developed providing satisfactory results (F3-F5), F5 provided the most prolonged release with a period of more than 8 hours and thus found to be most effective amongst all.

### 3.13. Quantitative estimation of drug content

The drug content of all the developed formulations was found to be in acceptable range. For F1 the drug content was found to be 94.68±2.13%, for F2 the drug content was found to be 95.98±2.30%, for F3, the drug content was found to be 96.27±3.26%, for F4 it was found to be 97.68±2.61%, for F5 the drug content was found to be 97.96±0.91%, the drug content was found to 98.89±4.12%.

### 3.14. Ex vivo permeation profile of drugs through corneal tissue

The ex-vivo corneal permeation investigation employed F5, as it exhibited most favourable *in-vitro* drug release profile. After 8 hours, the cumulative drug release percentage was discovered to be 51.28%. The slow permeation across goat cornea is also attributed to the fact that eye has complex anatomical characteristics with cornea consisting of several barriers which hinders the drugs to pass through it easily. Also, cornea is lipophilic in nature while semipermeable membrane is hydrophilic in nature, thus drug release from semipermeable membrane is comparatively faster as compared to goat cornea. Fig. 4 graphically illustrates release study of ofloxacin gel forming solution and

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corneal permeation of drug from F5 of developed ocular gel forming solution using goat cornea. **Corneal hydration test**

The value of corneal hydration levels was found to be 78.18± 1.12% and 79.1± 2.73% respectively. This result of corneal hydration level test indicates towards no corneal damage as the values for both marketed ofloxacin eye drops and developed formulation were found to be in standard range of 70-80%.

**3.4.Histopathological study**

No significant histopathological changes were observed in the cornea kept with the developed formulation and when compared to standard marketed ofloxacin eye drops, there was no different in the corneal structure. Histopathology result stated that the corneal stroma revealed dense collagen bundles arranged in parallel manner along with minimal inflammation. Fig. 5 depicts the result of histopathological study of excised goat cornea using marketed eye drops and developed formulations.

**3.4.Antimicrobial activity**

The zone of inhibition of all developed formulations was calculated using antibiotic zone reader. The zone of inhibition for F3 was found to be 30±0.29mm for *Staphylococcus aureus* while 31±0.12 mm for *Pseudomonas aeruginosa* respectively. Similarly, the zone of inhibition for F4 was found to be 36±0.18 mm for *Staphylococcus aureus* while 35±0.25 mm for *Pseudomonas aeruginosa* respectively. Out of all three formulations optimized, the maximum zone of inhibition was seen with F5. The zone of inhibition for F5 was found to be 46±0.23 mm for *Staphylococcus aureus* while 48±0.26 mm for *Pseudomonas aeruginosa* respectively. Table 5 summarizes result of antimicrobial study of ofloxacin and Gellan gum based ocular gel forming solution using *Staphylococcus aureus* and *Pseudomonas aeruginosa.*

**3.4.Sterility test**

The developed gel forming solutions successfully passed the sterility test, evidenced by the lack of turbidity. Additionally, after 14 days of incubation in soyabean casein digest media and fluid thioglycolate media, no growth of microbes or turbidity was observed.

**3.5.Accelerated stability studies**

There was no notable change in the visual appearance, pH, viscosity, isotonicity, drug content, gelation time, and *in-vitro* gelling capacity of the developed formulation during accelerated stability conditions.



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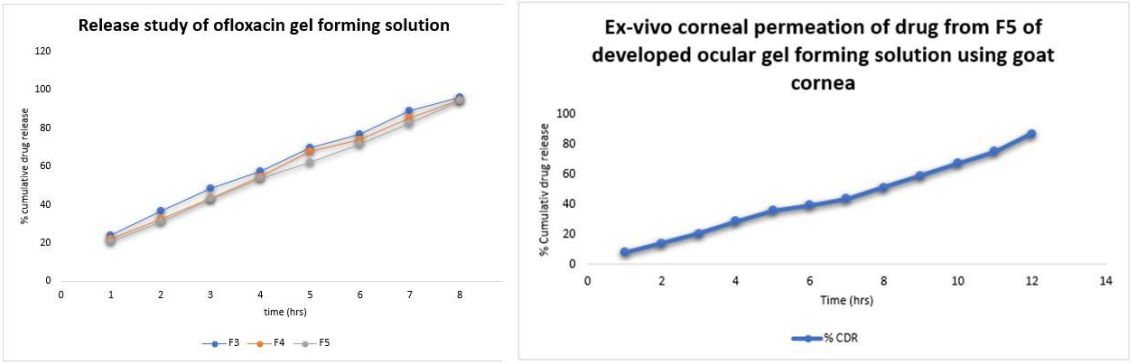


(A) (B)

**Fig. 4 Graphical illustration of (A) Release study of ofloxacin gel forming solution (B) corneal permeation of drug from F3 of developed ocular gel forming solution using goat cornea**

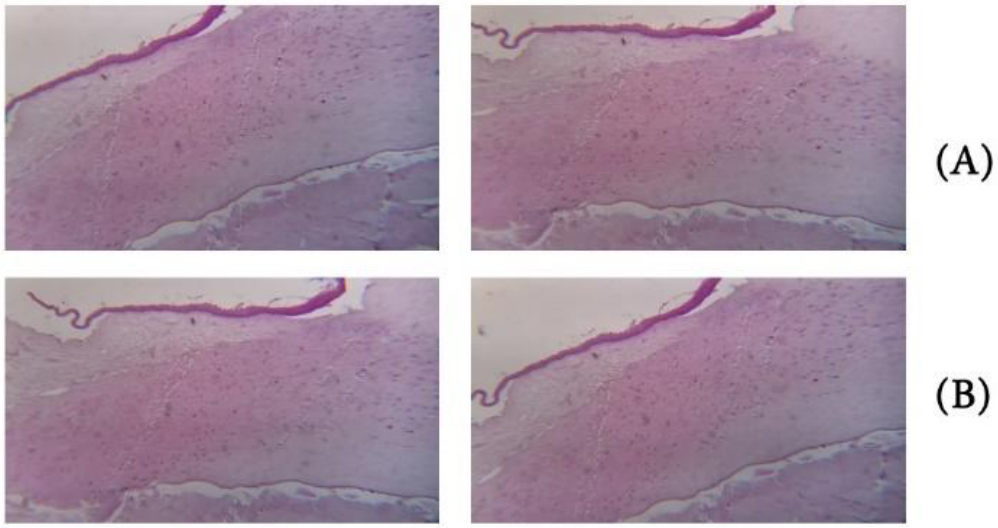


**Fig. 5 Histopathological study of excised goat cornea**
(A) **Developed gel forming solution**
(B) **Marketed ofloxacin eye drops**

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**Table 5: Result of Antimicrobial study of ofloxacin and Gellan gum based ocular gel forming solution using *Staphylococcus aureus* and *Pseudomonas aeruginosa***

| Formulation | Inhibition zone diameter (mm) | | Inhibition zone diameter (mm) | |
|---|---|---|---|---|
| | Standard (ofloxacin eye drops) | Test (developed formulation) | Standard (ofloxacin eye drops) | Test (developed formulation) |
| | *Staphylococcus aureus* | | *Pseudomonas aeruginosa* | |
| F3 | 28±0.42 | 30±0.29 | 29±0.24 | 31±0.12 |
| F4 | 34±0.21 | 36±0.18 | 33±0.28 | 35±0.25 |
| F5 | 44±0.16 | 46±0.23 | 46±0.19 | 48±0.26 |

## 4. DISCUSSION

During preformulation study, melting point of Ofloxacin was determined to be 256 ° C, falling within reference range of 250-257° C and thus ensures purity of drug used in investigation. It was noted that the $\lambda_{max}$ of ofloxacin was found to be 287 nm.

It was observed that the color of all developed formulations was somewhat pale yellow and free flowing in nature with no presence of foreign particles or any particulate matter. pH values of formulations were found to be within 6.4 to 6.6 range, indicating a favourable range for the stability of the drug.

The viscosity of F3 at angular velocity of 10 rpm was found to be 42.89±0.12 while at 100 rpm it was found to be 8.98±0.12, similarly for F4, the viscosity at angular velocity of 10 rpm was found to be 47.67±0.09 while at 100 rpm it was found to be 12.65±0.67. F5 had the viscosity range of 50.69±0.03 to 15.78±0.34. F3 showed the best viscosity range favorable for ocular formulations.

All six developed formulations were assessed for gelation time. F1 and F2 were almost unable to undergo sol to gel transition due to less concentration of polymer. F3 took 15 seconds for gelation, F4 took 12 seconds for gelation F5 and F6 took the minimum time 10 seconds for gelation but F6 formed slightly hard gel as compared to F3-F5 and hence irritation can be caused upon ocular administration.

Amongst all the three formulations developed providing satisfactory results (F3-F5), F5 provided the most prolonged release with a period of more than 8 hours and thus found to be most effective amongst all. The ex-vivo corneal permeation investigation employed F5, as it exhibited most favourable *in-vitro* release profile of 51.28% after 8 hours which shows that it was able to prolong the drug release. The value of corneal hydration levels was found to be 78.18± 1.12% and 79.1± 2.73% respectively. This result of corneal hydration level test indicates towards no corneal damage.

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Histopathology result stated that the corneal stroma revealed dense collagen bundles arranged in parallel manner along with minimal inflammation suggesting no histopathological changes. The zone of inhibition for F3 was found to be 30±0.29mm for *Staphylococcus aureus* while 31±0.12 mm for *Pseudomonas aeruginosa* respectively. Similarly, the zone of inhibition for F4 was found to be 36±0.18 mm for *Staphylococcus aureus* while 35±0.25 mm for *Pseudomonas aeruginosa* respectively. Out of all three formulations optimized, the maximum zone of inhibition was seen with F5. The zone of inhibition for F5 was found to be 46±0.23 mm for *Staphylococcus aureus* while 48±0.26 mm for *Pseudomonas aeruginosa* respectively.

The developed gel forming solutions successfully passed the sterility test, evidenced by the lack of turbidity. Furthermore, there was no turbidity or microbial development after incubation for a period of 14 days in culture media.

## 5. CONCLUSION

In the present investigation, ocular gel forming solution was developed. The major gelling polymer utilized was Gellan gum. Gellan gum acts through ion-activated mechanism which stimulates via contact in cations. Before developing formulations, Preformulation investigations were carried out, demonstrating the compatibility of the medication and polymer employed. UV spectra confirmed the purity of the drug as results obtained were comparable to reference values of drug mentioned in pharmacopoeia. The developed formulations were assessed based on a number of factors, including pH, drug content, *in-vitro* gelling ability, gelation time, drug release, ex-vivo permeation, histopathology, antimicrobial and sterility testing. All formulations produced utilizing different amounts of Gellan gum were liquid with slight viscosity easily flowable in nature before instillation. The formulations could undergo phase transition within seconds and gel remained intact for long period of time as obtained from result of *in-vitro* gelling capacity. The findings of additional investigations revealed that above parameters were determined to be within the range limit. Prolonged release of drug is mainly due to the presence of in situ gelling polymer which holds drug in its matrix. testing revealed that all three formulations developed passed sterility test as no turbidity was seen after 14 days. Amongst the six formulations developed, F5 containing 0.5% of Gellan gum proved to be the most effective due to extended release and its minimum or nonexistent inflammatory response, as determined by a histopathology analysis. The present investigation was a successful attempt at developing ocular gel forming solution of ofloxacin for management of bacterial infections. Establishing *in-vitro* and in-vivo correlation is essential to ensure bioavailability from developed formulations.

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**Disclosure statement**

The authors report there are no competing interests to declare.

**REFERENCES**

1. Tiwari M, Pradhan M, Badwaik H, Verma A, Garg A, Singh MK. Exploring the Benefits of Preservative-Free Ophthalmic Solutions in the Management of Glaucoma: A Comprehensive Review. International Journal of Pharmaceutical Sciences and Nanotechnology (IJPSN). 2024 Jun 30;17(3):7415-37.
2. Jawad MA, Mohammed TK. Stye, Bacterial Eye Infection among the Students of the Technical Medical Institute, AL-Mansour. Prof.(Dr) RK Sharma. 2020 Oct;20(4):4764.
3. Byrd LB, Gurnani B, Martin N. Corneal ulcer. InStatPearls [Internet] 2024 Feb 12. StatPearls Publishing.
4. Davies NM. Biopharmaceutical considerations in topical ocular drug delivery. Clinical and experimental pharmacology and physiology. 2000 Jul 17;27(7):558-62.
5. Tangri P, Khurana S. Basics of ocular drug delivery systems. International Journal of Research in Pharmaceutical and Biomedical Sciences. 2011 Oct;2(4):1541-52.
6. Gaudana R, Ananthula HK, Parenky A, Mitra AK. Ocular drug delivery. The AAPS journal. 2010 Sep;12:348-60.
7. Patel PB, Shastri D, Shelat P, Shukla A. Ophthalmic drug delivery system: challenges and approaches. Systematic Reviews in Pharmacy. 2010 Jul 1;1(2):113.
8. Shivhare R, Pathak A, Shrivastava N, Singh C, Tiwari G, Goyal R. An update review on novel advanced ocular drug delivery system. World Journal of Pharmacy and Pharmaceutical Sciences. 2012 Jul 15;1(2):545-68.
9. Dhyani A, Kumar G. A new vision to eye: Novel ocular drug delivery system. Pharmacophore. 2019;10(1-2019):13-20.
10. Meshram S, Thorat S. Ocular in Situ gels: Development, evaluation and advancements. Sch. Acad. J. Pharm. 2015;4:340-6.
11. Giordano F, Rossi A, Pasquali I, Bettini R, Frigo E, Gazzaniga A, Sangalli ME, Mileo V, Catinella S. Thermal degradation and melting point determination of diclofenac. Journal of thermal analysis and calorimetry. 2003 Aug;73:509-18.
12. Qureshi A, Vyas J, Upadhyay UM. Determination of solubility by gravimetric method: A brief review. Nat. J. Pharm.. 2022;2:1-3.

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13. El-Yazbi FA. Spectrophotometric and spectrofluorimetric determination of ofloxacin. Spectroscopy letters. 1992 Mar 1;25(2):279-91.
14. Kumar S, Haglund BO, Himmelstein KJ. In situ-forming gels for ophthalmic drug delivery. Journal of ocular pharmacology and therapeutics. 1994;10(1):47-56.
15. Gözcü S, Polat HK, Gültekin Y, Ünal S, Karakuyu NF, Şafak EK, Doğan O, Pezik E, Haydar MK, Aytekin E, Kurt N. Formulation of hesperidin-loaded in situ gel for ocular drug delivery: a comprehensive study. Journal of the Science of Food and Agriculture. 2024 Aug 15;104(10):5846-59.
16. Abraham S, Furtado S, Bharath S, Basavaraj BV, Deveswaran R, Madhavan V. Sustained ophthalmic delivery of ofloxacin from an ion-activated in situ gelling system. Pakistan journal of pharmaceutical sciences. 2009 Apr 1;22(2).
17. Abdelbary GA, Amin MM, Zakaria MY. Ocular ketoconazole-loaded proniosomal gels: formulation, ex vivo corneal permeation and in vivo studies. Drug delivery. 2017 Jan 1;24(1):309-19.
18. Jain P, Jaiswal CP, Mirza MA, Anwer MK, Iqbal Z. Preparation of levofloxacin loaded in situ gel for sustained ocular delivery: in vitro and ex vivo evaluations. Drug development and industrial pharmacy. 2020 Jan 2;46(1):50-6.
19. Khan N, Aqil M, Ameeduzzafar, Imam SS, Ali A. Development and evaluation of a novel in situ gel of sparfloxacin for sustained ocular drug delivery: in vitro and ex vivo characterization. Pharmaceutical development and technology. 2015 Aug 18;20(6):662-9.