DESIGN, DEVELOPMENT AND CHARACTERIZATION: IN SITU GELS OF LOMEFLOXACIN HYDROCHLORIDE FOR OCULAR DRUG DELIVERY

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ABSTRACT

The aim of the present study was to formulate and evaluate polymeric ocular in situ gel system of Lomefloxacin hydrochloride to improve its bioavailability by using in situ polymers that exhibit reversible liquid gel phase transition. In situ gels are prepared by using poloxamer 407 and hydroxyl propyl methyl cellulose as mucoadhesive polymer in different ratios. The in situ gels were evaluated for drug content, clarity, pH, gelation temperature, viscosity, in vitro drug release studies, ex-vivo studies, anti microbial activity, sterility testing, and for ocular eye irritation test. FT-IR spectroscopy was used to know drug and polymer incompatibilities. The cumulative drug release from the formulations was ranging from 91.02% to 98.31% at 10h and the drug released by diffusion mechanism. The results indicated that extent of gelation and release of drug depended on the concentration of polymers used. Optimized formulation (PH5) was found to have optimum pH and gelation temperature which was required for an in situ gel drug delivery system. The combination of poloxamer 407 and hydroxyl propyl methyl cellulose as an in situ gelling vehicle is a promising tool to enhance ocular bioavailability and to improve patient compliance for the topical treatment of ocular diseases. In vivo studies are further required to prove the efficacy of the formulations prepared.

Keywords: Gelation temperature, hydroxy propyl methyl cellulose, in situ gels, Lomefloxacin hydrochloride, poloxamer 407.
1. INTRODUCTION

Drug delivery to eye is a challenge job to the formulators because its unique anatomy restricts drug absorption into the deep tissues. The major problems with ocular route include non-productive absorption, drainage, induced lacrimation, tear turn over, impermeability of drugs to cornea [1]. Administration of drugs in the form of eye drops for treating topical eye diseases like dryness, conjunctivitis, keratitis, eye flu is the common approach and more than 90% of marketed ophthalmic formulations are still available as eye drops for water soluble drugs. Simple instillation into the eye with accuracy doses is the main criterion for using the eye drops [2, 3]. Treating eye disorders by conventional system may not be considered ideal because of rapid precorneal elimination by protective mechanisms of the eye such as blinking reflex, lacrimal fluid dilution and naso-lacrimal duct drainage [4]. Ophthalmic drug delivery systems like inserts, ointments and suspensions are developed to overcome these problems but they are not accepted by patients due to several disadvantages such as difficulty in administration of inserts, blurred vision from use of ointments and dosage heterogeneity of suspensions. Formulations with an increased viscosity may prevent some of the above drawbacks of the conventional systems. A new technology called an “in situ gel” forming formulations, which undergo phase transition from liquid to semisolid gel by an induction of environment conditions has opened up a scope in the development of ophthalmic formulations [5-9]. Thermosensitive amphiphilic block copolymers, namely poly (ethylene oxide)–poly (propylene oxide)–poly (ethylene oxide) (PEO–PPO–PEO, poloxamers), have been extensively investigated as in situ forming gels [10, 11]. These polymers form micelles in solution which can self-organize and form a viscous gel depending on polymer concentration and temperature used [12]. Though thermosensitive copolymers are widely employed, they suffer from a major drawback of having weak mechanical strength, which leads to rapid erosion [13]. Using blends of poloxamers with other biodegradable polymers like hydroxyl propyl methyl cellulose, carbopol, alginate and hydroxy propyl methyl cellulose is an alternative approach which can improve the mechanical strength of the in situ gels [14-18] by providing positively charged amine groups that could interact with the negatively charged mucous layer, conferring a mucoadhesive characteristic [19-20]. The potential of the poloxamer / hydroxyl propyl methyl cellulose gel for sustaining drug release and for overcoming cornea impermeability is needed to be evaluated. Fluoroquinolone are a class of synthetic antibacterial agents approved for ocular therapy [21] and show superior antimicrobial activity in comparison to amino glycosides and cephalothin. Hence fluoroquinolone such as Lomefloxacin hydrochloride can be beneficially prescribed for
conjunctival infections and also as prophylaxis in ocular surgery[22]. Hence, the aim of the present work was to design, formulate and evaluate the potential of Lomefloxacin hydrochloride as a model drug to form an in situ gel forming delivery system with poloxamer / hydroxyl propyl methyl cellulose as vehicles for sustaining the release and improving the bioavailability of the drug.

2. EXPERIMENTAL

2-1 Materials
Lomefloxacin Hydrochloride was kindly gifted by M/S Nakoda Pharmaceuticals (Hyderabad, India). Poloxamer 407 and hydroxyl propyl methyl cellulose (HPMC) were purchased from Sigma Aldrich (Gattefosse, India). All other chemicals, reagents and solvents used were of analytical grade.

2-2 Preparation of in situ gels
Lomefloxacin hydrochloride in situ gels were prepared using cold method. Briefly, weighed amount of poloxamer (14–18% w/w) was dissolved in cold ultrapure water and stored in refrigerator at 6°C for 12 h. Weighed quantities of HPMC, lomefloxacin hydrochloride (0.3% w/v) benzalkonium chloride (0.9% w/v) and sodium chloride (0.01% w/v) (Table 1.) were added to soaked polymers solution and stirred for a period of 15 minutes on a magnetic stirrer at 300 rpm. The pH of the formulations was adjusted to 7.4 using 0.1N sodium chloride solution. The final volume was adjusted to 2ml with distilled water. All the formulations after preparation were stored in refrigerator till evaluation.

2-3 Fourier transform infrared (FT-IR)
FT-IR spectra were taken using an optical bench (Shimadzu FT-IR 8400S, Japan) to determine interactions between the drug and polymers. The drug, individual polymer (poloxamer 407 and HPMC), 1:1 physical mixture (5 mg) were taken and mixed properly with 100 mg of KBr. About 50 mg of this mixture was compressed to form a pellet using a hydraulic press at 15 tonnes pressure. The prepared pellets were scanned from 4,000 to 400 cm⁻¹ using FT-IR spectrophotometer.

2-4 Determination of absorption maxima (λ max) for Lomefloxacin HCl
Accurately 10mg drug weighed was dissolved in 10ml volumetric flask with STF to get concentration of 1mg/ml. From the stock solution suitable dilutions were made to obtained concentrations ranging from 2 to10µg/ml. After scanning the absorbance of solutions was
determined at λmax 281nm against STF as blank. The experiment was repeated three times and a calibration curve was plotted from the mean value.

2-5 Drug content
The drug content was determined by accurately placing 100µl of formulations in a test tube and suitably diluted with STF to obtain a concentration of 10µg/ml. By using UV-Visible spectrophotometer at 281nm the drug concentration was determined. All experiments were performed in triplicates.

2-6 Test for appearance/ clarity
The prepared in situ gel formulations were observed for general appearance i.e. color, odour and for the presence of suspended particulate matter. The clarity of the preparation was checked using against black and white background.

2-7 Determination of pH
The pH of all formulations was recorded using a calibrated digital pH meter immediately after preparation. All experiments were performed in triplicates.

2-8 Gelation temperature
Gelation temperature was performed by placing 1ml of prepared formulations in a test tube. The test tubes were sealed with a parafilm then immersed in a water bath at 4°C. The temperature of the water bath was increased in increments of 1°C and left to equilibrate for 15 min at each new temperature setting. The samples were examined for gelation (occurs when meniscus of the test tube would no longer move when tilted more than 90° C) and the temperature was recorded. All experiments were performed in triplicates.

2-9 Rheological studies
The prepared formulations were allowed to gel in the simulated tear fluid at 25°C, 35°C and the viscosity was determined using cone and plate Brookfield viscometer (R/S PLUS) with cone C50-1 with angular velocity run from 10 to 100 rpm. The experiment was performed in triplicate.

2-10 Autoclaving sterilization
To study the effect of autoclaving sterilization on physicochemical properties of in situ gels, the selected formulations were subjected to the autoclaving sterilization conditions following
recommendation by the United States Pharmacopeia (USP). In Brief, screw cap test tubes containing 10g of drug loaded in situ gel were placed in an autoclave and subjected to autoclaving at 121˚C, under a pressure of about 15 psi, for 20 min. The formulations were evaluated for physicochemical properties like flow ability, percentage labeled amount, pH, sol–gel transition temperature and in vitro drug release and compared with the formulations before subjecting to autoclaving.

2-11 In vitro drug release studies

In vitro drug release studies for formulations were carried by using modified USP apparatus II paddle method with STF (pH 7.4) as dissolution medium by using 2 cm diameter cylinder test tubes open at both ends for placing test sample under study. Dialysis membrane (cut off 12000 MW) previously soaked for 12h in STF (pH 7.4) was tied to one end of the glass cylinder and 0.5ml volume of the formulation was accurately weighed and placed. The glass cylinder was attached to the shaft of USP apparatus II, in place of paddle. The cylinder were then suspended in 50 ml of dissolution medium maintained at 34 ± 0.5°C and at 25 rpm such that the dialysis membrane just touched the dissolution medium. Samples were withdrawn at regular intervals of 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 h and replaced by equal volumes of medium analyzed by UV Spectrophotometer at 281nm for its drug content at respective time after suitable dilution with STF. The percent release of the drug was computed. Experiment was performed in triplicate.

2-12 Ex vivo corneal permeation

Ex vivo corneal permeation studies were performed for the optimized formulations and for the plain drug solution. Corneas from goat eyes were used as the permeation membrane to mimic in vivo conditions. Ex vivo drug release studies for formulations were carried out by using modified USP apparatus II paddle method with STF (pH 7.4) as dissolution medium. Isolated cornea previously soaked in STF (pH 7.4) was taken and tied on to one end of the glass cylinder and placed 0.5ml of the formulation. Permeation studies were performed as discussed in in vitro drug release method. Experiment was performed in triplicate.

2-13 Antimicrobial efficacy testing

For optimized formulation antimicrobial efficacy test was determined using agar diffusion method. The sterile nutrient agar media previously seeded with test organisms (Pseudomonas aeruginosa and Staphylococcus aureus) was placed in Petri plates and 0.5 ml of sterile standard solution and optimized formulation were placed into the bored cups of the media.
The plates were incubated at 37°C for 24 h and the zone of inhibition (ZOI) was measured and compared with the control plate. Experiment was performed in triplicate.

2-14 Sterility testing
Sterility testing was performed by aseptically transferring 2ml of optimized formulation into 20ml thioglycolate medium and soya bean - casein digest medium in two separate test tubes. The inoculated medias were incubated at 30 to 35°C (thioglycolate medium) and 20 to 25°C (soya bean - casein digest medium) for 14 days and observed for turbidity and microbial growth.

2-15 Eye irritation test
Acute eye irritation was performed according to OECD guidelines on three New Zealand white rabbits weighing between 1.5 to 2.5 kg. All the experimental protocols were approved by IAEC bearing CPSCEA number (1548/PO/1/11 CPCSEA dated 15.02.2012). The animals were divided into two groups. The animals in group I are subject to Initial test (One rabbit) and animals in group II will act as confirmatory test (Two rabbits). The animals were positioned in cages and the eyes were marked as test and control respectively. The test eye received 0.1ml formulation of Lomefloxacin and the other eye which was untreated act as a control. After instillation of the formulation the eyes were examined for ocular irritancy by ophthalmoscope and observed for opacity, conjunctivitis, inflammation, lacrimation and damage to iris for 1h, 24h, and 72h and on 7th day after administration.

2-16 Accelerated stability studies
Stability testing was performed as per ICH guidelines for optimized formulation (PH5) at 40 ± 2°C and 75 ± 5 % RH, at 4°C and at room temperature for a period one month by placing them in previously sterilized glass eye drops bottles. For every 7th day samples were withdrawn and estimated for drug content, pH, visual appearance, gelation temperature and in vitro drug release.

2-17 Release mechanisms
To examine the mechanism of drug release from formulated in situ gels, in vitro and ex vivo permeability data were fitted to zero order, first order, Higuchi release model, and Korsemeyer and Peppa’s model and the model with higher correlation coefficient was considered to be the best model to know its release mechanism.
3 RESULTS AND DISCUSSION

3-1 Preparation of in situ gels

The in situ gels of Lomefloxacin hydrochloride were formulated by cold technique using combination of polaxomer 407 and HPMC. The prepared in situ gels were found to be transparent confirming that the used ingredients were completely dissolved in the vehicle at refrigerated and room temperature. All formulations were found to be clear, translucent and free from any suspended particulate material.

3-2 Interaction studies

Interaction study between the drug and the excipients used was performed with FT-IR spectrophotometer from 4,000 to 400 cm\(^{-1}\). The characteristic peaks of the pure drug at 3055.2 cm\(^{-1}\) indicated the OH group in acid functional group, 1020 cm\(^{-1}\) indicated CN group, 1007 cm\(^{-1}\) CF with alkyl halide, 1725 cm\(^{-1}\) indicated C=O in acid, 1524 cm\(^{-1}\) indicated C=O aromatic ketone (Figure 1). No major differences in the respective peaks were observed in the IR spectra of the pure drug and the physical mixture indicating good compatibility of drug with the used excipients. (Fig:1)

3-3 Drug content, pH and Gelation temperature

Drug content was determined for the prepared formulations by UV-Visible spectrophotometer at 281 nm using simulated tear fluid as a blank. The drug content of was in the range 83%-92%. The pH of all the formulations was ranging from 7.41±0.02 to 7.37±0.04 and gelation temperature of was in the range of 27.0±0.13°C to 38.5°±0.12°C. The data of the same is given in Table 2.

3-4 Rheological studies

The prepared formulations were studied for rheological behaviors as a function of temperature (25°C and 35°C) by allowing in situ gel to gel in simulated tear fluid at an rpm of 10 to 100. As the shear rate increased from 10 to 100 the viscosity changed from 1800 Cps to 350 Cps at 25°C, from 3900 Cps to 1000 Cps at 35°C and 2993 Cps to 600 Cps after dilution with STF at 35°C. The formulations were shear thinning as the shear rate was increased. The high viscosities of the gel at low shear rates will aid in maintaining a good contact between the corneal surface and the in situ gel delivery system and the shear thinning property of the gel is responsible for uniform distribution of the gel over the surface of the eye (Figure: 2).
3-5 Autoclaving sterilization

The optimized formulation was autoclaved at 121°C and 15 lb pressure for a period of 20 min and the effect of autoclaving on the formulation was examined for drug content and pH of the formulation before and after autoclaving. From the results the drug content and pH did not vary significantly (Table. 3) indicating the formulation was stable in terms of pH and drug content after autoclaving.

3-6 In vitro drug release method

The cumulative percent released versus time profiles of lomefloxacin in situ gel containing poloxamer 407 and hydroxyl propyl methyl cellulose in different ratios are shown in Fig. 3. The in vitro drug release was ranging from 91.03 to 98.31% in 10 h. (Figure:3) From in vitro drug release studies and gelation temperature for all formulations PH5 showed sustained release up to 10 h and have gelation temperature closer to the ocular surface temperature i.e. 35°C hence the formulation was selected for further study. Plain drug was released in 1 h from the reference suspension almost instantaneously after the start of release experiment, indicating that sink conditions were appropriately maintained in the apparatus.

The in vitro drug release data was fitted into different release kinetics to study the release mechanism. K and r² values were the parameters considered to evaluate the model that best suits the release kinetics of the drug. Peppa’s model with r² value 0.99 was found to be the best model. The drug release from in situ gel may occur partly via diffusion from the gels and partly due to simultaneous dissolution of the gels into the surrounding dissolution media (Table 4). Drug release conditions may be very different from in vitro to the in vivo condition of the eye. However, the in vitro results clearly indicate that the gels have the ability to retain the drug for prolonged period. In the cul-de-sac, the gel may undergo faster dissolution because of the shearing action of the eyelids and eyeball movements.

3-7 Ex vivo corneal permeation

Ex vivo studies were conducted for the optimized formulations (PH5). The amount of the drug that permeated through the 10 mm diameter of the cornea was calculated by maintaining the simulated eye conditions. Cumulative percent released during corneal permeation was 58.94% in 10 h for the optimized formulation and in case of pure drug it was released about 85.74 % in 2 h. The ex vivo permeation data was fitted into different release kinetics to study the release mechanism. Peppa’s model with r² value 0.99 was found to be the best model (Fig
no.4 and Table 4). Based on the n value, diffusion was considered to be the major mechanism of the release.

3-8 Antimicrobial efficacy testing

The selected formulation was tested for antimicrobial activity. The percentage efficiency was 99.1% for gram positive bacteria and 94.7% for gram negative bacteria. The antimicrobial effect of the optimized in situ gelling formulation was probably due to constant release of drug from the polymer drug reservoir complex. (Fig:5)

3-9 Sterility testing

Sterility testing is an important requirement of ophthalmic preparations to confirm the absence of viable microorganism which may harm the eye when formulation instilled into the eye. There was no sign of microbial growth observed in both fluid thioglycolate medium and soya bean casein digest medium confirming that sterilization by autoclaving as a suitable method for achieving sterility (Fig no: 6).

3-10 Eye irritation test

The likelihood of eye irritation due to the instillation of in situ gel was evaluated in rabbits. The eyes of the rabbits were examined for the ocular irritancy by ophthalmoscope for opacity, conjunctivitis, inflammation, lacrimation and damage to iris if any after administration. The results indicated that there were no sign of irritation to the eye or lacrimation, inflammation and damage to iris when compared with the control group. Thus the designed in situ gel as an ocular drug delivery system is evidently proved to be free from ocular irritancy and conjunctiva defects. Hence, the in situ gels can be regarded as a safe delivery system.

3-11 Accelerated stability studies

Accelerated stability studies were performed at 4°C, 25°C, 40°C and 75% RH in triplicates. The parameters like pH, drug content, gelation temperature and in vitro drug release were performed. The pH and gelation temperature was not affected during the stability study but the drug content and in vitro release was affected but not significant difference in initial sample and samples stored at 28 days was observed hence the values were not included.
Table 1 Formulae of poloxamer 407 and hydroxy propyl methyl cellulose in situ gel formulations

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Poloxamer 407 (% w/v)</th>
<th>Hydroxy propyl methyl cellulose (% w/v)</th>
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<tbody>
<tr>
<td>PH1</td>
<td>14</td>
<td>0.5</td>
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<tr>
<td>PH2</td>
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<td>1</td>
</tr>
<tr>
<td>PH3</td>
<td>14</td>
<td>1.5</td>
</tr>
<tr>
<td>PH4</td>
<td>16</td>
<td>0.5</td>
</tr>
<tr>
<td>PH5</td>
<td>16</td>
<td>1</td>
</tr>
<tr>
<td>PH6</td>
<td>16</td>
<td>1.5</td>
</tr>
<tr>
<td>PH7</td>
<td>18</td>
<td>0.5</td>
</tr>
<tr>
<td>PH8</td>
<td>18</td>
<td>1</td>
</tr>
<tr>
<td>PH9</td>
<td>18</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Table 2 Evaluation of poloxamer 407 and hydroxy propyl methyl cellulose in situ gels (n=3)

<table>
<thead>
<tr>
<th>Code</th>
<th>Drug content (%)</th>
<th>pH</th>
<th>Gelation temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>PH1</td>
<td>85.3±1.5</td>
<td>7.37±0.02</td>
<td>38.5⁰C±0.71</td>
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<tr>
<td>PH2</td>
<td>83.0±1.24</td>
<td>7.4±0.07</td>
<td>38.0⁰C±0.14</td>
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<tr>
<td>PH3</td>
<td>87.6±1.5</td>
<td>7.32±0.01</td>
<td>37.5⁰C±0.15</td>
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<tr>
<td>PH4</td>
<td>92.0±1.86</td>
<td>7.40±0.02</td>
<td>35.5⁰C±0.75</td>
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<tr>
<td>PH5</td>
<td>87.3±1.32</td>
<td>7.41±0.01</td>
<td>34.5⁰C±0.14</td>
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<tr>
<td>PH6</td>
<td>87.0±1.78</td>
<td>7.38±0.05</td>
<td>35.0⁰C±0.17</td>
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<tr>
<td>PH7</td>
<td>86.6±2.04</td>
<td>7.42±0.05</td>
<td>27.5⁰C±0.72</td>
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<tr>
<td>PH8</td>
<td>92.0±2.56</td>
<td>7.40±0.12</td>
<td>28.0⁰C±0.03</td>
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<td>PH9</td>
<td>91.3±1.85</td>
<td>7.41±0.03</td>
<td>27.0⁰C±0.76</td>
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</table>

Table 3 Zone of inhibition and percentage efficiency comparision for standard and test compound

<table>
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<tr>
<th>Type of microorganism</th>
<th>Species</th>
<th>ZOI (Standard)</th>
<th>ZOI (Test)</th>
<th>Percentage efficiency</th>
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<td>Gram positive</td>
<td>Staphylococcus aureus</td>
<td>48mm</td>
<td>47.6±1.52 mm</td>
<td>99.1%</td>
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<tr>
<td>Gram negative</td>
<td>Pseudomonas aeruginosa</td>
<td>70mm</td>
<td>66.3±1.55 mm</td>
<td>94.7</td>
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Table 4 *In vitro* release kinetics of *in situ* gels

<table>
<thead>
<tr>
<th>Formulation</th>
<th>ZERO ORDER</th>
<th>FIRST ORDER</th>
<th>HIGUCHI</th>
<th>PEPPA’S</th>
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<tbody>
<tr>
<td></td>
<td>r²</td>
<td>K</td>
<td>r²</td>
<td>K</td>
</tr>
<tr>
<td>PH1</td>
<td>0.951</td>
<td>21.30</td>
<td>0.939</td>
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<tr>
<td>PH2</td>
<td>0.981</td>
<td>16.37</td>
<td>0.908</td>
<td>2.150</td>
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<td>PH3</td>
<td>0.961</td>
<td>18.21</td>
<td>0.960</td>
<td>2.089</td>
</tr>
<tr>
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<td>0.941</td>
<td>18.02</td>
<td>0.954</td>
<td>2.112</td>
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<tr>
<td>PH5</td>
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<td>14.25</td>
<td>0.875</td>
<td>2.232</td>
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<td>PH6</td>
<td>0.959</td>
<td>11.46</td>
<td>0.979</td>
<td>2.090</td>
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<td>PH7</td>
<td>0.977</td>
<td>9.136</td>
<td>0.971</td>
<td>2.095</td>
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<tr>
<td>PH8</td>
<td>0.989</td>
<td>5.563</td>
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<tr>
<td>PH9</td>
<td>0.982</td>
<td>5.488</td>
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<td>PH5(ex vivo)</td>
<td>0.978</td>
<td>6.488</td>
<td>0.973</td>
<td>1.990</td>
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Table 5 Effect of autoclave on *in situ* gels (PH5)

<table>
<thead>
<tr>
<th></th>
<th>Before autoclaving (n=3)</th>
<th>After autoclaving (n=3)</th>
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<tr>
<td>Assay (%)</td>
<td>pH</td>
<td>Assay (%)</td>
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<tr>
<td>87.3±1.32</td>
<td>7.41±0.01</td>
<td>85.6±1.5</td>
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</table>

Fig 1 FT-IR spectra of drug, excipients and physical mixture.
Fig 2 Rheological study of *in situ* gel

Fig 3 *In vitro* release of Lomefloxacin HCl *in situ* gels

Fig 4 *Ex vivo* release of Lomefloxacin HCl *in situ* gels
**4 CONCLUSION**

Thermoreversible *in situ* gel formulation of Lomefloxacin Hydrochloride were formulated with poloxamer 407 and hydroxy propyl methyl cellulose. The prepared formulations were evaluated and compatibility studies were performed. The *in vitro* and *ex vivo* studies demonstrated that the developed formulation release for more than 10h thus maintaining the concentrations for a longer duration. The designed *in situ* gel is a realistic alternative to conventional eye drops by virtue of its ability to improve bioavailability and in improving the patient compliance.
REFERENCES


