FORMULATION OF IN-SITU GELLING OPHTHALMIC DROPS OF MOXIFLOXACIN

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ABSTRACT

The aim of the present work was to obtain an ophthalmic delivery system with improved mechanical and mucoadhesive properties that could provide prolonged retention time for the treatment of ocular diseases. The most frequently used dosage forms i.e. ophthalmic solutions and suspensions are compromised in their effectiveness by several limitations, leading poor ocular bioavailability. In-situ gels are instilled as drops into the eye and undergoes a sol to gel transition in the cul-de-sac, improved ocular bioavailability by increasing the duration of contact with corneal tissue, thereby reducing the frequency of administration. The purpose of the present work was to develop pH-triggered an ophthalmic drug delivery system using combination of gelling agents with different mechanisms for in-situ gelation of Moxifloxacin. In-situ gels were prepared by simple dispersion method using carbopol 940 along with HPMC E15 LV in factorial design and then evaluated for pH, gelling capacity, drug content, Viscosity, and in-vitro dissolution studies and Mucoadhesion study. Among formulation batches F1- F9; optimized formulation F7 imparted sustained release property to the gel formed in-situ and effective other evaluation parameters. The developed formulations were therapeutically efficacious, stable, non-irritant and provided sustained release of the drug overcoming conventional drawbacks leading to better patient acceptance.

KEYWORDS: In-situ forming systems; ophthalmic In-situ gel; Moxifloxacin; carbopol 940, HPMC E15 LV.
INTRODUCTION

Topical administration of anti-infective drug is the treatment of choice for diseases of anterior segments of the eye. When a drug solution is dropped into the eye, effective tear drainage and blinking result in a 10-fold reduction of drug concentration in 4-20 minutes. The limited permeability and rapid elimination results in low absorption and short duration of the therapeutic regimen.\textsuperscript{[1]} Ocular therapy could be significantly improved if the pre-corneal residence time of drugs could be increased. Various ophthalmic vehicles such as inserts, ointments, suspensions, and aqueous gels lengthen the residence time of instilled dose but have some drawbacks such as blurred vision from ointments or low patient compliance from inserts. This problem can be overcome by using \textit{in-situ} gel forming ophthalmic drug delivery systems prepared from polymers that exhibit reversible phase transitions and pseudoplastic behaviour to minimize interference with blinking.\textsuperscript{[2]} Depending on the method employed to cause sol to gel phase transition on the ocular surface, the following three types of systems have been recognized: pH-triggered - The polymers used in this system are Pseudolatexes - Carbomer (carbopol), Temperature-dependent-Poloxamers (Pluronic,Tetronics), Cellulose derivatives (MCHPMC), Xyloglucan. Ion-activated induced - Alginates, Gelrite® (Gellan gum). Such a system can be formulated as liquid as solution upon exposure to physiological pH condition of eye, shifts to gel phase which has a higher viscosity thus increasing the pre-corneal residence and can improve patient compliance.\textsuperscript{[2]} With the advent of new generation of fluoroquinolone,\textsuperscript{[3]} such as Moxifloxacin, the treatment of gram positive bacterial infections has been achieved. This drug shows increased potency than all other topical antibiotics making it able to eradicate methicillin-resistant \textit{Staphylococcus} \textit{61} The Pharma Innovation Journal species. Moxifloxacin penetrates at very high level into ocular tissues including the tear film, cornea, anterior chamber, and ciliary body due to its biphasic nature i.e. soluble in both lipid and aqueous solutions.\textsuperscript{[4]} Therefore, it can achieve very high concentration in the eye. Hence, it was thought of combining the benefits of the drug with pH sensitive / mucoadhesive polymers such as carbopol 940 and HPMC E15 LV viscosity enhancing agent to come out with a formulation, which might outperform the conventional eye drops of the same drug. The formulation would be useful to treat external infections of the eye such as acute and subacute conjunctivitis, bacterial keratitis, bacterial endophthalmitis, and keratoconjunctivitis.
MATERIAL AND METHOD

MATERIAL

METHOD
Determination of Absorbance of Moxifloxacin by UV Spectrophotometer
A drug solution of 8 g/ml in simulated tear fluid (pH 7.4) was prepared, scanned and UV spectrum was recorded in range of 200-400 nm.

Calibration Curve of Moxifloxacin in Simulated Tear Fluid (pH 7.4)
The stock solution was prepared by dissolving 10 mg of drug in 100 ml of STF to get 1 mg/ml concentration solution. From the above solution, remove and diluted suitably to acquire final concentration from 1 to 10 g/ml. All the solutions were scanned through UV Spectrophotometer and absorbances were taken against blank of STF at max of 288 nm.

Compatibility Study\[5],[6]\nThe formulation of a dosage form requires consideration of the physical, chemical and biological characteristics of all drug substances and excipients to be used in the formulation of the product. The drug and excipients must be compatible with one another to produce a stable, efficacious, and safe product. The interaction study of prepared in situ gel formulations was carried out using infrared spectroscopy following KBR dispersion method. The spectrum of dried mixture of drug and potassium bromide was then run followed by drug with excipients in the wavelength region between 4000 and 400 cm\(^{-1}\). The drug-polymers compatibility was confirmed by differential scanning calorimetric (DSC), which was carried out by heating drug and the physical mixture of drug with polymers separately from 25 °C to 275°C at the heating rate of 10 °C/min in a nitrogen environment. The instrument used was METTLER differential scanning calorimeter with Stare SW 8.10 software.

Formulation of In-situ gel.\[7]\nPreparation of solution A: Accurately weighed quantity (0.25 gm) of the Moxifloxacin was dissolved in 30 ml distilled water. The Mannitol and Benzalkonium Chloride were added to above mixture with continuous stirring.
**Preparation of solution B:** The Carbopol 940 and Methocel E15 LV were sprinkled over 50 ml of boiling water and was allowed to hydrate for 15 min to produce a clear solution.

**Compounding of ophthalmic solution:** The solution B was mixed slowly to solution A with continuous mechanical stirring to produce clear and transparent solution. The pH of formulation was checked and adjusted with 0.1 N HCL and volume was made up with distilled water to 100ml.

**Aseptic filling to container:** The formulation was aseptically transferred to previously sterilized glass vials and sealed with pre treated rubber closure

**Optimization by 3\(^2\) Factorial Design.**\(^{[8]}\)

A 3\(^2\) full factorial design was constructed where the amounts of carbopol 940(X1) and HPMC E15 LV (X2) were selected as the factors. The levels of the two factors were selected on the basis of the preliminary studies carried out before implementing the experimental design. All other formulation and processing variables were kept invariant throughout the study. Table 1 gives the Amount of variables in 3\(^2\) factorial design batches, Table 2 gives the Selected Concentration Ranges of Independent Variables and table 3 gives Contents of formulations.

**Table 1: Amount of variables in 3\(^2\) factorial design batches**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Variables</th>
<th>Factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Independent</td>
<td></td>
</tr>
<tr>
<td></td>
<td>X1</td>
<td>Carbopol 940</td>
</tr>
<tr>
<td></td>
<td>X2</td>
<td>Methocel E15LV</td>
</tr>
<tr>
<td>2</td>
<td>Dependent</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Y1</td>
<td>Viscosity</td>
</tr>
<tr>
<td></td>
<td>Y2</td>
<td>Release</td>
</tr>
</tbody>
</table>

**Table 2: Selected Concentration Ranges of Independent Variables**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Independent variables</th>
<th>Concentration range (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Carbopol 940</td>
<td>0.4-0.6</td>
</tr>
<tr>
<td>2</td>
<td>Methocel E15 LV</td>
<td>1.0-2.0</td>
</tr>
</tbody>
</table>

**Table 3: Contents of formulations**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Formulation Code</th>
<th>Moxifloxacin (% w/v)</th>
<th>Carbopol 940 (% w/v)</th>
<th>Methocel E15 LV (% w/v)</th>
<th>BKC (% w/v)</th>
<th>Mannitol (% w/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F1</td>
<td>0.25</td>
<td>0.4</td>
<td>1</td>
<td>0.01</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>F2</td>
<td>0.25</td>
<td>0.4</td>
<td>1.5</td>
<td>0.01</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>F3</td>
<td>0.25</td>
<td>0.4</td>
<td>2</td>
<td>0.01</td>
<td>5</td>
</tr>
</tbody>
</table>
Evaluation of Prepared In-Situ Gelling System

Visual Appearance and Clarity.\[^9\]

Clarity is one of the most important characteristic features of ophthalmic preparations. The formulations were examined for visual appearance and clarity by visual observation against a white and black background to check the presence of any particulate matter.

pH

The preparation to be instilled into eye should be non-irritating to the eye. To ensure that the preparation has same pH as that of lacrimal fluid, the pH of the prepared in-situ gelling system after addition of all the ingredients was measured using digital pH meter.

\textit{In-Vitro Gelation Studies}

The gelling capacity of the prepared system containing different concentrations of carbopol 940 and HPMC E15 LV was evaluated. It was performed by placing a drop of system in vials containing 1 ml of simulated tear fluid, freshly prepared, and visually assessing the gel formation and noting the time for gelation as well as time taken for the gel formed to dissolve. The Composition of Simulated tear fluid (STF) was sodium chloride (0.67g), sodium bicarbonate (0.2 g), calcium chloride dihydrate (0.008 g) and distilled water q.s.100.0 g. Physiological pH (7.4) was adjusted by adding the required amount of 0.1 N HCl.

Drug Content Uniformity.\[^{10}\]

The vials containing the preparation were shaken for 2-3 min and 1 ml of preparation was transferred to 100 ml volumetric flask and volume was made up with simulated tear fluid pH 7.4. Aliquot of sample was withdrawn and further diluted to 10 ml with same simulated tear fluid pH 7.4. The concentration of Moxifloxacin was determined at 288 nm by using UV-Visible spectrophotometer (Pharmspec, 1700, Shimadzu, Japan).

\textit{Rheological Studies.\[^{11}\]}

The viscosity of the gel was determined using programmable viscometer (Brookfield RVDV-II) with T-bar spindle code S95 and it was operated under following conditions. The spindle

<p>| | | | | | |</p>
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</thead>
<tbody>
<tr>
<td>4</td>
<td>F4</td>
<td>0.25</td>
<td>0.5</td>
<td>1</td>
<td>0.01</td>
</tr>
<tr>
<td>5</td>
<td>F5</td>
<td>0.25</td>
<td>0.5</td>
<td>1.5</td>
<td>0.01</td>
</tr>
<tr>
<td>6</td>
<td>F6</td>
<td>0.25</td>
<td>0.6</td>
<td>1</td>
<td>0.01</td>
</tr>
<tr>
<td>7</td>
<td>F7</td>
<td>0.25</td>
<td>0.6</td>
<td>1.5</td>
<td>0.01</td>
</tr>
<tr>
<td>8</td>
<td>F8</td>
<td>0.25</td>
<td>0.6</td>
<td>2</td>
<td>0.01</td>
</tr>
<tr>
<td>9</td>
<td>F9</td>
<td>0.25</td>
<td>0.6</td>
<td>1</td>
<td>0.01</td>
</tr>
</tbody>
</table>
was attached to the lower shaft of the viscometer. The motor was turned on and spindle was rotated within the container containing 20 ml of performed gel. The helipath movement was controlled to avoid touching of the spindle to any part of the sample holder especially the bottom. A typical run involved changing the angular velocity from 0.5 to 100rpm at a controlled speed which was changed after every 10 seconds (0.5 to 100rpm). The viscosity values at each rpm were noted from the display window.

**Mucoadhesive strength measurement by Brookfield texture analyser.**[12,13]

Adhesive properties of *in-situ* gel formulations were carried out using a texture analyser with a 10gm load cell. Texture analysis is a useful tool and has been used as a valid methodology for mechanical characterization of pharmaceutical mucoadhesive dosage forms. Goat eye cornea was used as the corneal surface. Goat eye cornea was collected immediately after slaughter of the animals and was rapidly frozen (−20°C) and stored in isotonic phosphate-buffered saline pH 6.8. Before testing, goat eye cornea was defrosted at room temperature. The goat eye cornea was then placed on the base of the texture analyser with the corneal membrane facing upward. Gels which were to be tested attached to the base of an aluminium probe (using double sided adhesive tape) fixed to the mobile arm of the texture analyser as shown in fig.7.4. The area of contact on the cornea was moistened with solution. The tablet was lowered at a rate of 0.1mms⁻¹ until contact with the corneal tissue was made. A contact force of 10gm was maintained for 10 secs. After which the probe was withdrawn from the corneal membrane at a rate of 5 mms⁻¹. The peak Force of Adhesion (N) and the Mucoadhesive Force (gms) was recorded.

**Microbiological study.**[14,15]

2.5 gm of dextrose agar was weighed and transferred in a 250 ml of conical flask containing 100 ml of purified water and was heated to dissolve it completely. Further, it was sterilized at 121°C and 15 lb pressure in autoclave for about 20 min. Then, it was cooled to room temperature and the bacterial strain (*Staphylococcus aureaus*) was dispersed in the medium (1 ml). The medium (0.1 ml) was poured in the petridish and allowed to cool until it solidifies at room temperature. The cups were bored in petridish with the help of sterile steel bore of 6mm. The test formulations were added to these wells and plates were incubated for 48 h at 37°C in incubators. The zone of inhibition was observed and the radius of the zone of inhibition was calculated.
In-Vitro Release Studies\textsuperscript{[16,17]}

The in-vitro release of formulation was studied through dialysis membrane using a modified USP XXIII dissolution testing apparatus. The dissolution medium used was PBS 7.4 freshly prepared. Dialysis membrane, previously soaked overnight in the dissolution medium, was tied to one end of a specifically designed glass cylinder (open at both ends and of 1.75 cm diameter). 1 ml volume of the formulation was accurately added into this assembly. The cylinder was attached to the metallic driveshaft and suspended in 50 ml of dissolution medium so that the membrane just touched the receptor medium surface. The shaft was rotated at 50 rpm. Aliquots, each of 1 ml volume, were withdrawn at one hour intervals and replaced by an equal volume of the receptor medium. The aliquots were diluted with receptor medium and analyzed by UV spectrophotometry at respected maximum wavelength.

RESULT AND DISCUSSION

Estimation of Moxifloxacin by UV Spectrophotometer

The drug solution was scanned for UV absorption between 200-400 nm. The spectrum was recorded, which showed the absorbance maxima (max) at 288 nm.

Construction of Calibration Curve of Moxifloxacin

Calibration curve of the drug in simulated tear fluid (pH 7.4) was plotted by recording the absorbance of solutions of different concentrations (1-10 μg/ml). The Beers and Lamberts range was found to be in the range of 1-10 μg/ml and the coefficient of correlation was 0.998 and slope 0.089 as shown in fig.1.

![Calibration curve of moxifloxacin in STF](image)

Fig. 1: Calibration curve of Moxifloxacin in simulated tear fluid IR Spectroscopy of Moxifloxacin
The moxifloxacin were subjected to FT-IR studies for the purpose of characterization. The infrared spectra of the drug were recorded by potassium bromide dispersion technique using FTIR with diffuse reflectance attachment (FTIR-8400S). Drug was mixed with potassium bromide and spectra were obtained in range of 400-4000 cm\(^{-1}\). The baseline correction was carried out using dried potassium bromide.

**Fig. 2: IR spectrum of Moxifloxacin**

**Evaluation of Prepared In Situ Gelling System**

**Visual appearance, Clarity, and pH**

The clarity of all formulations was found to be satisfactory. The formulations were light yellow in colour. Terminal sterilization with autoclaving had no effect on the physicochemical properties of the formulations. PH of the formulations did not vary considerably.

**Drug Content Uniformity**

The drug content was found to be in the acceptable range for all the formulations. Percent drug content for all nine formulations was in the range of 98.53-100.0.5\% indicating uniform distribution of the drug in Table 4.

**Table 4: Drug content and gelling capacity of formulations**

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Formulation Code</th>
<th>% Drug content</th>
<th>Gelling ability</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F1</td>
<td>99.17±0.91</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>F2</td>
<td>99.17±1.90</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>F3</td>
<td>98.79±1.90</td>
<td>++</td>
</tr>
<tr>
<td>4</td>
<td>F4</td>
<td>100.05±1.48</td>
<td>++</td>
</tr>
<tr>
<td>5</td>
<td>F5</td>
<td>99.54±1.15</td>
<td>++</td>
</tr>
</tbody>
</table>
In-vitro Gelation Studies

Table 4 indicatets F1, F2 and F3 exhibited very weak gelation. F4 and F5 showed more suitable gelling capacity, which completed the gelation immediately and remained for few hours, compared with the F6, F7, F8, and F9, which gelled instantaneously but remained for extended period of time. These can also be reflected in the viscosity F9 had greater viscosity, which would cause the gel difficult to spread out on cornea and would make vision blurring.

Rheological Studies

The viscosity of formulations was measured as the change of shear rate under physiological (pH 7.4) conditions to investigate the rheology of these formulations. At pH 5.0 the formulations were in a liquid state and exhibited low viscosity. An increase in the pH to 7.4 caused the solutions to transform into gels with high viscosity. The formulations exhibited pseudo plastic rheology. Fig. 3

![Fig. 3: Rheological studies for F1-F9](image)

In-vitro drug release study of formulation

These F1-F3 formulations exhibited these release at 8 hr they exhibited sustained release effects and this could be due to increase in HPMC concentration. F4, F5, and F6 showed 86.27%, 87.91%, and 88.54% drug release. 93.91% and 90.54% of the drug was released from F7 and F8. This more sustained release was seendue to higher concentration of both carbopol 940 (0.5% w/v) and HPMC E15 LV. F9 showed least drug release (84.85%). The developed formulations obviously outperformed the marketed eye drop by releasing drug over a long period of time and lead to prolonged therapeutic activity.
Table 5: Mucoadhesive force of optimized batch F7 ophthalmic formulation

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Mucoadhesive strength (dynes)</th>
<th>Force of adhesion (dynes/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F7</td>
<td>7.0</td>
<td>87.47</td>
</tr>
</tbody>
</table>

The force of adhesion for formulations F7 was found to be 87.47. When HPMC E15LV was added to Carbopol, mucoadhesion was increased significantly. This can be attributed to interaction of hydroxyl group of cellulose derivatives with hydroxyl/ carboxyl group of biological membrane leading to formation of hydrogen bond between gel formulation and corneal membrane. This might result into increase in mucoadhesion. Thus, indicating the chances of prolonged retention of formulation on ocular surface.

Fig. 5 – Mucoadhesion strength measurement of F7 ophthalmic gel

Microbiological Study

In these studies, the diameter of the zone of inhibition of optimized formulations was compared with that of standard drug solution. It is evident that the zone of inhibition value of standard drug solution is comparative to that of optimized batch of same concentration. The
study indicated that Moxifloxacin retained its antimicrobial efficacy when formulated as an
in- situ gelling system.

![Image of zone inhibition](image.png)

**Fig. 6. Zone for drug soln and optimized batch of moxifloxacin**

**Table 6: Measurement of zone of inhibition**

<table>
<thead>
<tr>
<th>Code</th>
<th>Zone of inhibition area (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug solution</td>
<td>1.43</td>
</tr>
<tr>
<td>F7</td>
<td>0.61</td>
</tr>
</tbody>
</table>

**Statistical Analysis**

**3² Full Factorial Design Batches**

A 3² full factorial design was used in the present study. Two factors were evaluated, each at 3 levels, and experimental trials were performed at all 9 possible combinations. Carbopol 940 with HPMC E15LV. An optimized combination of these two able to achieve desired drug release. Hence amount Carbopol 940 and amount of HPMC E15LV were assumed as independent variables in a 3² full factorial design. The amount of carbopol 940 was taken as 0.4%, 0.5%, 0.6% and while that of HPMCE 15 LV was taken as 1%, 1.5%, 2% which responded as -1, 0 or 1 levels respectively. The factorial design batches were evaluated for Viscosity and % drug release study. The thickness, % cumulative drug release at 8 hrs and drug diffusion were taken as dependent variables.

**Viscosity (Y₁)**

\[
\text{Viscosity (Y₁)} = +93444.44 + (-3833.33 \times X₁) + (+5916.67 \times X₂) + (-3750.00 X₁ X₂) + (-20666.7X^2) + (-20666.67X^2) \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldOTS
Response Surface and Contour Plot
The quadratic surface model obtained from the regression analyses was used to build up 3D surface and 2D contour plots in which the responses were represented by curvature surface as a function of independent variables. The relationship between the response and independent variables can be directly visualized from the response surface plots.

Fig. 7- Different plots showing effect of independent variables on Viscosity of In-situ gelling eye drop.

Fig. 8- Different plots showing effect of independent variables on % drug release of In-situ gelling eye drop

CONCLUSION
The optimized formulation (F7) contained 0.6 % w/w carbopol 940 P and 1 %w/w HPMC E15 LV wherein carbopol caused initial fast release of drug due to its hydrophilic nature later on hydroxypropyl methylcellulose imparted sustained release property to the gel formed in situ. The in situ gelling system will get good patient acceptance because it is easy to instill
and gradually erodes by dissolution of the gel, avoiding the need for removal. Hence, it can be concluded that in-situ gels are a viable alternative to conventional eye drops by providing sustained release of medicament resulting in decreased frequency of administration leading to better patient acceptance.

ACKNOWLEDGEMENTS
Authors gratefully acknowledge to SAVA Pharmaceutical pvt. ltd. for providing gift sample of Moxifloxacin.

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