Formulation and characterization of a novel pH-triggered in-situ gelling ocular system containing Gatifloxacin

*Jovita Kanoujia, Kanchan Sonker, Manisha Pandey, Koshy M. Kymonil, Shubhini A. Saraf

**Department of Pharmaceutics, Babu Banarasi Das National Institute of Technology & Management Sector I, Dr. Akhilesh Das Nagar, Faizabad Road, Lucknow, U.P. India-227105**

**ABSTRACT**

The present research work deals with the formulation and evaluation of in-situ gelling system based on sol-to-gel transition for ophthalmic delivery of an antibacterial agent gatifloxacin, to overcome the problems of poor bioavailability and therapeutic response exhibited by conventional formulations based a sol-to-gel transition in the cul-de-sac upon instillation. Carbopol 940 was used as the gelling agent in combination with HPMC and HPMC K15M which acted as a viscosity enhancing agent. The prepared formulations were evaluated for pH, clarity, drug content, gelling capacity, bioadhesive strength and in-vitro drug release. In-vitro drug release data of optimized formulation (F12) was treated according to Zero, First, Korsmeyer Peppas and Higuchi kinetics to access the mechanism of drug release. The clarity, pH, viscosity and drug content of the developed formulations were found in range 6.0-6.8, 10-570cps, 82-98% respectively. The gel provided sustained drug release over an 8 hour period. The developed formulation can be used as an in-situ gelling vehicle to enhance ocular bioavailability and the reduction in the frequency of instillation thereby resulting in better patient compliance.

**Key Words:** In-situ gelation; Gatifloxacin; Carbopol 940; HPMC K15M.

**INTRODUCTION**

Ophthalmic drug delivery is one of the most attractive and challenging field facing the pharmaceutical scientist. A significant challenge to the formulator is to circumvent the protective barriers of the eye without causing permanent tissue damage (Mitra, 2003). Most of the ocular treatments call for the topical administration of ophthalmically active drugs to the tissues around the ocular cavity (Gorle and Gatani, 2009). The most conventional ocular dosage forms for the delivery of drugs are eye drops (solution, suspension) and ophthalmic ointments. Short residence time, pulsed dosing of drug, frequent instillation, and large drainage factor are the limitation associated with conventional ocular dosage form. Newer ocular drug delivery systems are being explored to develop extended duration and controlled release strategy (Rathore and Nema, 2009). Formulation of in-situ ocular gel of gatifloxacin is a fourth generation fluoroquinolone derivative used to treat external infections of the eye, using biodegradable polymers is the approach to overcome the drawbacks of conventional eye preparations (Zhidong et al., 2006; Mishra et al., 2008; Pundir et al., 2009; Kalam et al., 2009). Carbopols are mainly used in liquid or semisolid pharmaceutical formulations as suspending or viscosity increasing agents. Formulations include creams, gels and ointments for use in ophthalmic, rectal and topical preparations. HPMC is widely used in oral and topical pharmaceutical preparations as coating agent, film formers, rate controlling polymers for sustained release, stabilizing agents, viscosifier etc. (Raymond et al., 2004; Edsman et al., 1996).

The objective of this study was to develop an optimized in-situ ophthalmic gel - a viscous liquid that shift to a gel phase upon exposure to physiological condition (Rathore and Nema, 2009; Doijad et al., 2006). To achieve the objective, independent formulation variables such as, polymer-to-polymer ratio, and different viscosity grades of HPMC (K4M and
K15M) were examined. The dependent variables included gelling capacity, percentage of gatifloxacin release at 8 hours, viscosity and bioadhesive strength was performed to identify the best formulation using 4² full factorial designs.

MATERIALS AND METHODS

Materials

Gatifloxacin was obtained as a gift sample from Syntho Pharmaceuticals Pvt. Ltd., Lucknow (India). Hydroxypropylmethyl cellulose (HPMC) and HPMC K15M were obtained from SD Fine Chemicals Limited, Mumbai, India and Carbopol 940 was obtained from Himedia Laboratories Pvt. Ltd., Mumbai, India. All other chemicals/reagents used were of analytical grade, available commercially and used as such without further processing. A UV/Vis spectrophotometer (Systronics, Double beam UV-VIS Spectrophotometer: 2201) was used for drug analysis.

Experimental Design

A 4² full factorial design was adopted to optimize the variables and 16 experiments were conducted in total. In this design, two factors were evaluated each at 4 levels (Madan et al., 2009). The polymer-to-polymer (HPMC, HPMC K15M) (X1) and the amount of bioadhesive polymer (Carbopol 940) (X2) were chosen as independent variables and Y as dependent variables (viscosity, drug content, bioadhesive strength and in-vitro drug release). The levels of independent variables are shown in Table 1 (Narendra et al., 2006).

Preparation of Formulations

Accurately weighed 0.1g of HPMC was dispersed in 50ml of purified water, HPMC K15M was added, carbopol 940 was sprinkled over this solution and allowed to hydrate overnight. The solution was stirred with an overhead stirrer and buffer salts were dissolved in the solution. Gatifloxacin was dissolved in small quantity of acidic medium (HCl in water), benzylkonium chloride (BKC) was added to this solution; the drug solution was added to the polymer solution. Purified water was then added to make up the volume to 100ml and the prepared formulations were sterilized in an autoclave at 121°C for 20 min (Mohan et al., 2009). Formulation ingredients of formulation F1 to F16 are represented in Table 2.

Evaluation Studies

Physical appearance

Physical appearance of the formulations was visually observed which included the color, homogeneity, consistency and phase separation. The prepared ophthalmic gel formulations (Figure 1) were inspected visually for physical properties (Mohan et al., 2009; Mohamed, 2004).

pH determination

0.3g gel was dissolved in 100ml distilled water and the pH was measured in triplicate (pH Meter, E I Instruments, Model 111E) (Mohamed, 2004; Quinnones and Ghaly, 2008).

Determination of viscosity

Viscosity of the instilled formulation is an important factor in determining residence time of drug in the eye. Viscosity of the samples was determined using a Brookfield digital viscometer (Model-RVT) with spindle number 3 and angular velocity run from 10-100 r/min (Abraham et al., 2009; Patel et al., 2010).

Table 1: Level of Investigated Variables.

<table>
<thead>
<tr>
<th>Independent Variable</th>
<th>Ratio of Polymer (HPMC:HPMC K15M)</th>
<th>Amount of Carbopol 940</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factors</td>
<td>1:1</td>
<td>1%</td>
</tr>
<tr>
<td>Levels</td>
<td>1:2</td>
<td>2%</td>
</tr>
<tr>
<td></td>
<td>1:3</td>
<td>3%</td>
</tr>
<tr>
<td></td>
<td>1:4</td>
<td>4%</td>
</tr>
</tbody>
</table>

Table 2: Composition of in-situ gel as per 4² Factorial Design.

<table>
<thead>
<tr>
<th>Composition</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>F6</th>
<th>F7</th>
<th>F8</th>
<th>F9</th>
<th>F10</th>
<th>F11</th>
<th>F12</th>
<th>F13</th>
<th>F14</th>
<th>F15</th>
<th>F16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gatifloxacin</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>HPMC</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>HPMC K15M</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Carbopol 940</td>
<td>0.1</td>
<td>0.2</td>
<td>0.3</td>
<td>0.4</td>
<td>0.1</td>
<td>0.2</td>
<td>0.3</td>
<td>0.4</td>
<td>0.1</td>
<td>0.2</td>
<td>0.3</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Each formulation contains 0.407g of citric acid; 1.125g of disodium hydrogen phosphate; 0.02g of benzalkonium chloride, 100ml purified water; all values are expressed in gram.
Gelling capacity
Determination of **in-vitro** gelling capacity was done by visual method. Colored solutions (1% Congo Red solution in water) of **in-situ** gel forming drug delivery system were prepared. The **in-vitro** gelling capacity of prepared formulations was measured by placing 5ml of the gelation solution (pH 7.2 buffer) in glass test tube and maintained at 37±1°C temperature. One ml of colored formulation solution was added with the help of pipette. As the solution comes in contact with gelation solution, it was immediately converted into stiff gel like structure. The gelling capacity of solution was evaluated on the basis of stiffness of formed gel and time period for which the formed gel remains as such (Figure 2).

Drug content
The drug content was determined by taking 1ml of the formulation and diluting it to 100ml with distilled water. Aliquot of 5ml was withdrawn and further diluted to 25ml with distilled water. Gatifloxacin concentration was determined at 293nm by using UV-Visible spectrophotometer (Abraham et al., 2009).

Bioadhesive strength
The bioadhesive strength was measured using a modified two arm balance as shown in figure 3. The biological membrane was fixed to the outer surface of the bottom of the 50ml beaker with cyanoacrylate adhesive and then placed in a 100ml beaker. Phosphate buffer pH 7.4 was added into the beaker up to the upper surface of the gastric mucosa such that the media remains just above the mucosa. Accurately measured 1ml gel was put between the bottom of modified stainless steel pan and beaker. A preload of 50g was placed to the pan for 5 min (preload time) to establish adhesion bonding between gel and biological membrane (Figure 3). The preload

**Figure 1**: Representative photograph of macroscopic appearance of ophthalmic gel.

**Figure 2**: Visual observation of gel formation in formulations. (+) Gel forms after few minutes, disperses rapidly (+++) Immediate gelation, remains for few hours (+++) Immediate gelation, remains for an extended period.

**Table 3**: Physicochemical parameters of **in-situ** ocular gel.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>pH</th>
<th>Clarity</th>
<th>Viscosity in cps at 100 rpm</th>
<th>Gelation Capacity</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>6.8</td>
<td>Clear</td>
<td>10</td>
<td>+</td>
</tr>
<tr>
<td>F2</td>
<td>6.4</td>
<td>Clear</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>F3</td>
<td>6.2</td>
<td>Clear</td>
<td>75</td>
<td>++</td>
</tr>
<tr>
<td>F4</td>
<td>6.1</td>
<td>Clear</td>
<td>220</td>
<td>++</td>
</tr>
<tr>
<td>F5</td>
<td>6.5</td>
<td>Clear</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>F6</td>
<td>6.3</td>
<td>Clear</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>F7</td>
<td>6.2</td>
<td>Clear</td>
<td>110</td>
<td>++</td>
</tr>
<tr>
<td>F8</td>
<td>6.2</td>
<td>Clear</td>
<td>270</td>
<td>++</td>
</tr>
<tr>
<td>F9</td>
<td>6.2</td>
<td>Clear</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>F10</td>
<td>6.3</td>
<td>Clear</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>F11</td>
<td>6.1</td>
<td>Clear</td>
<td>240</td>
<td>++</td>
</tr>
<tr>
<td>F12</td>
<td>6.0</td>
<td>Clear</td>
<td>470</td>
<td>+++</td>
</tr>
<tr>
<td>F13</td>
<td>6.3</td>
<td>Clear</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>F14</td>
<td>6.5</td>
<td>Clear</td>
<td>140</td>
<td>++</td>
</tr>
<tr>
<td>F15</td>
<td>6.4</td>
<td>Clear</td>
<td>270</td>
<td>++</td>
</tr>
<tr>
<td>F16</td>
<td>6.8</td>
<td>Clear</td>
<td>510</td>
<td>+++</td>
</tr>
</tbody>
</table>

+ gel forms after few minutes, disperses rapidly; ++ immediate gelation, remains for few hours; +++ immediate gelation, remains for extended period of time.
and preload time were kept constant for all the formulations. After completion of preload time, preload was removed from the pan and another beaker placed to the pan. The addition of water was stopped when the other pan detached from the membrane (Figure 4). The mass, in grams (weight of empty beaker and weight of beaker with water), required to detach the pan from membrane gave the measure of bioadhesive strength (Patel et al., 2010).

In-vitro release of gatifloxacin from gel
The in-vitro release of gatifloxacin from the prepared formulations was studied through cellophane membrane using a modified USP XXIII dissolution testing apparatus. The dissolution medium used was pH 7.4 buffer. Cellophane membrane previously soaked overnight in the dissolution medium was tied to one end of a specifically designed glass cylinder (open at both ends of 5 cm diameter). A 2ml volume of the formulation was accurately pipetted into this assembly. The cylinder was attached to the metallic drive shaft and suspended in 100ml of dissolution medium maintained at 37±1°C so that the membrane just touched the receptor medium surface. The shafts was rotated at 50 r/min. Aliquots each of 1ml volume, were withdrawn at hourly intervals and replaced by an equal volume of the receptor medium. The aliquots were diluted with receptor medium and absorbance was measured at 293nm using UV-Visible spectrophotometer (Mohan et al., 2009).

Statistical analysis
Statistical analysis of data was performed by one way analysis of variance (ANOVA) followed by Dunnett’s multiple comparison test to find out the effect of independent variable (concentration of carbopol 940 and HPMC K15M) on the dependent variables (in-vitro drug release, drug content, bioadhesive strength and viscosity), assuming confidence level of 95% (p<0.05) for statistical significance.

Kinetics of drug release
Three kinetic models, the zero order release equation (Eq. (1)), Higuchi equation (Eq. (2)), and first order equation (Eq. (3)), were applied to process the in-vitro data of formulation F-12 to find the equation with the best fit and to investigate the mechanism of gatifloxacin release from in-situ gel.

\[
Q = k_1 t 
\]

(1)

\[
Q = k_2 (t)^{0.5}
\]

(2)

\[
Q = 100(1 - e^{-k_3 t})
\]

(3)

where \(Q\) is the percentage release at time \(t\), \(k_1\), \(k_2\) and \(k_3\) are the rate constants of zero order, Higuchi, and first order model, respectively. Further, to confirm the mechanism of drug release, first 60% of drug release was fitted in Korsmeyer-Peppas model

\[
\frac{M_t}{M_{\infty}} = K_p t^n
\]

(4)
where $M_t/M_\alpha$ is the fraction of the drug release at time $t$, $K_p$ is the rate constant and $n$ is the release exponent. The $n$ value is used to characterize different release mechanisms and is calculated from the slope of the plot of log of fraction of drug released ($M_t / M_\alpha$) vs log of time ($t$) (Behera et al., 2008).

RESULTS AND DISCUSSIONS

Physical appearance and pH
The formulations were light yellowish in color and clear. The pH value of all the prepared formulations ranged from 6.0 to 6.8, which is considered acceptable to avoid the risk of irritation upon application to the eye. Physicochemical data presented in table 3 shows pH, clarity, viscosity and gelation capacity of the prepared gels.

Viscosity & gelling capacity
The two main fundamentals of gelling system are viscosity and gelling capacity. The viscosity of the different formulations was compared as shown in Table 4. The viscosity was directly dependent on the polymeric content of the formulations. The data indicated that the viscosity increased with increase in concentration of HPMC K15M and carbopol 940 (1 to 4%). F16 showed the maximum viscosity of 510cps at 100rpm (HPMC:HPMC K15M:Carbopol 940 was 1:4:4) whereas the minimum viscosity at 100 rpm was shown by F1(HPMC:HPMC K15M:Carbopol 940 was 1:1:1). Except for the formulations F1, F2, F5, F6, F9, F10 and F13, all the formulations gelled instantaneously on addition to the simulated tear fluid and extended for few hours. The in-situ formed gel should preserve its integrity without dissolving or eroding for prolonged period to facilitate sustained release of drugs locally.

Drug content, bioadhesive strength and in-vitro release studies
On the basis of physicochemical properties (viscosity and gelation capacity) nine formulations (F3, F4, F7, F8, F11, F12, F14, F15 and F16) were selected and evaluated for drug content, bioadhesive strength and in-vitro dissolution. The drug content of all the formulations was in range (82-98%). The bioadhe-
sive strength was found to be satisfactory, maximum bioadhesive strength was 40gm for formulation F16. The evaluation results are shown in figure 5 and 6.

Figure 7 shows the cumulative amount of gatifloxacin released versus time profiles for different drug-containing solutions. In the case of formulation F12, approximately 74% of gatifloxacin was released from the solution (1% HPMC, 3% HPMC K15M, 4% Carbopol 940 1:3:4) after 90 min. This indicates that formulation F12 has a better ability to retain drugs than the individual polymer solution. These results also suggest that the HPMC, HPMC K15M, Carbopol 940 aqueous system can be used as an *in-situ* gel-forming system for ophthalmic drug delivery systems. The release of drug from these gels was characterized by an initial phase of high release (burst effect). However, as gelation proceeds, the remaining drug was released at a slower rate followed by a second phase of moderate release.

**Statistical analysis**

The results obtained from the experiment were statistically analyzed for response variables by using Graph Pad Prism Demo 5 (version 5.03, Graph Pad Software Inc.). Statistical analysis (One way ANOVA) however revealed that batches were significantly different (calculated F value is greater than tabulated F value). Dunnett’s multiple comparison tests predicted that there was a significant effect of independent variable (concentration of carbopol 940 & HPMC K15M) on the dependent variables (*in-vitro* drug release, drug content, bioadhesive strength and viscosity) as shown in table 4.

**Kinetics of release**

The *in-vitro* release profiles were fitted to various kinetic models in order to find out the mechanism of drug release. The rate constants were calculated from the slope of the respective plots. High correlation ($R^2=0.9031$) was observed in the Higuchi plot rather than first-order ($R^2=0.3273$) and zero-order ($R^2=0.6485$) models. The drug release was proportional to square root of time, indicating that the drug release from *in-situ* gel was diffusion controlled. The data obtained was also fit in Korsmeyer-Peppas model in order to find out n value, which describes the drug release mechanism. The n value (0.8029) obtained from Korsmeyer-Peppas was more than 0.5, which indicated that the mechanism of the
drug release was Anomalous and Non Fickian diffusion controlled.

**CONCLUSION**

HPMC, HPMC K15M, Carbopol 940 ocular in-situ gel of Gatifloxacin showed appreciable gel forming properties on application in eye. The gels were found to be uniform, clear, viscous and bioadhesive. On the basis of in-vitro drug release, drug content and gelation capacity studies, it could be concluded that Gatifloxacin could be successfully administered through gel forming controlled release ocular formulation for treatment of bacterial keratitis and conjunctivitis and also important is the ease of administration afforded and decreased frequency of administration resulting in better patient acceptance. The statistical analysis revealed that the factor, concentration of carbopol 940 and HPMC K15M did significantly affect the studied dependent variables.

**REFERENCES**


