FORMULATION AND EVALUATION OF THERMOSENSITIVE OCULAR INSITU GELS FOR SUSTAINED RELEASE OF BALOFLOXACIN

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ABSTRACT

The aim of the present study was to formulate and evaluate ophthalmic drug delivery system of Balofloxacin based on the concept of thermo-sensitive gelation. Balofloxacin is a fourth generation broad spectrum fluoroquinolone antibiotic indicated for infective ophthalmitis. Insitu gel was formulated by cold method using different concentrations of Poloxamer and Chitosan as thermoreversible polymers. Prepared formulations were evaluated for gelling capacity, drug content, rheological studies, gelation time, gelation temperature, in-vitro diffusion studies and antimicrobial activity. The pH of all the formulations was found to be 7.4. Formulation (G7) was optimized based on drug content and in vitro drug release studies. All the formulations were found to be clear and devoid of particulate matter. With the increase in concentration viscosity of the solution increases which renders sustained release of the drug from the formulation over a period of 10h. Gelling capacity studies revealed that G7 showed good gelation characteristics. The results of in-vitro drug release studies indicated that with the increase in the polymer concentration the more was the sustained delivery of drug. The zone of inhibition for both Staphylococcus aureus and E.coli was found to be significantly greater for tested formulation than the pure API. Stability studies concluded that the developed systems were stable for 3 months. Hence thermosensitive insitu gels were concluded to be a suitable approach by improving the bioavailability and thereby increases the precorneal residence time.

KEYWORDS: Insitu gelling system, thermosensitive, Balofloxacin, antimicrobial studies.
INTRODUCTION\textsuperscript{[1,2]}

The field of ocular drug delivery is one of the interesting and challenging endeavors facing the pharmaceutical scientist. Various ophthalmic drug delivery systems such as inserts, ointments, suspensions, and aqueous gels lengthen the residence time of instilled dose but have some drawbacks such as blurred vision and therefore low patient compliance. A suitable approach to these problems was to formulate insitu gels. Insitu gels are instilled as drops into the eye and undergo a sol to gel transition in the cul-de-sac, thus cause improved ocular bioavailability by increasing the duration of contact with corneal tissue, thereby reducing the frequency of administration. There are several possible mechanisms that lead to insitu gel formation: solvent exchange, UV-irradiation, ionic cross-linkage, pH change, and temperature modulation. Those approaches, which do not require organic solvents, copolymerization agents or an externally applied trigger for gelation, have gained increasing attention, such as a thermosensitive approach for insitu gel formation. Rajalakshmi et al (2013)\textsuperscript{[4]}, successfully developed insitu gels of Gemifloxacin mesylate which improved the mucoadhesive and mechanical properties which increased the retention time. Bhushan et al (2011)\textsuperscript{[10]} developed thermosensitive insitu gels of Ciprofloxacin resulting in sustained delivery of drug.

Thermoreversible polymers: They include pluronics and tetronics.

Poloxamer/Pluronics, a class of block copolymer of polyoxyethylene and polyoxypropylene, tetronics, ethyl (hydroxyl ethyl) cellulose, methyl cellulose exhibit thermoreversible gelation.

Poloxamer is a nonionic surface active agent, and block copolymers consisting of polyethylene oxide and polypropylene oxide units. Their relatively low toxicity and capacity to form clear gels make them particularly suitable for dermatological or ophthalmic formulations as well as in the area of controlled drug delivery systems and it is known for exhibiting the phenomenon of reverse thermal gelation under a certain concentration and temperature and temperature. Though thermosensitive copolymers are employed widely they suffer from a major drawback of having weak mechanical strength which leads to rapid erosion of polymer. This problem can be solved by using blends of poloxamer with chitosan.\textsuperscript{[3]} Chitosan a natural polysaccharide derived from naturally abundant chitin, having excellent ocular compatibility. Chitosan is a polycation on interacting with the poly anionic surface of mucosal surface of cornea it enhances the mucoadhesive properties.\textsuperscript{[4]} Gupta et al,
developed timolol maleate isotonic solution using chitosan/poloxamer that converted into gel at temperatures 37°C.

Balofloxacin a fourth generation broad spectrum fluoroquinolone antibiotic indicated for infective ophthalmitis, belongs to BCS class II category.

The objective of the present investigation was the development of a sustained release ophthalmic drug delivery system of Balofloxacin a fourth generation fluoroquinolone antibiotic, with more residence time in the eye which may result in increased bioavailability, reduction in the dosing frequency and improved patient compliance.

MATERIALS AND METHODS
Balofloxacin was kindly gifted from Cirex Pharmaceuticals, Hyderabad, India. Poloxamer, Chitosan and other chemicals used were from SD Fine Chemicals, Mumbai, India. Other materials and solvents used were of analytical grade.

Preparation of thermosensitive insitu gel
Thermosensitive insitu gels were prepared by cold method. The chitosan solutions of various concentrations were prepared by dispersing the required amount of polymer in 2% w/v acetic acid solution with continuous stirring until completely dissolved, similarly poloxamer solutions were prepared by dispersing required amount of polymer in distilled water with continuous stirring for 1 hour at room temperature. The partially dissolved solutions were stored in refrigerator for 24 hours until the entire polymer was completely dissolved. Then the poloxamer solutions were dispersed in the chitosan solution with continuous stirring for 1 hour and again kept for refrigeration for about 24 hours. Balofloxacin (0.1%) was added to the polymeric solutions with continuous stirring until completely mixed. Benzalkonium chloride (0.002% w/v) was added as a preservative in all the solutions. The pH of all the solutions was adjusted to 7.4±0.1 using 0.1 N NaOH.

Table 1: Formulation Design of Thermosensitive In situ Gels

<table>
<thead>
<tr>
<th>Ingredients (w/v)</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>G4</th>
<th>G5</th>
<th>G6</th>
<th>G7</th>
<th>G8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Balofloxacin (mg)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Poloxamer</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Chitosan</td>
<td>0.05</td>
<td>0.1</td>
<td>0.15</td>
<td>0.25</td>
<td>0.05</td>
<td>0.1</td>
<td>0.15</td>
<td>0.25</td>
</tr>
<tr>
<td>Benzalkonium chloride</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>0.9</td>
<td>0.9</td>
<td>0.9</td>
<td>0.9</td>
<td>0.9</td>
<td>0.9</td>
<td>0.9</td>
<td>0.9</td>
</tr>
<tr>
<td>Distilled water (upto 100ml)</td>
<td>qs</td>
<td>qs</td>
<td>qs</td>
<td>qs</td>
<td>qs</td>
<td>qs</td>
<td>qs</td>
<td>qs</td>
</tr>
</tbody>
</table>

qs = quantities sufficient
EVALUATION OF BALOFLOXACIN INSITU GELS\textsuperscript{[5-8]}

FTIR studies: The drug polymer containing insitu gel and the pure drug were subjected to the Fourier transform infrared spectroscopy in order to check the possible drug-polymer interactions.

Visual appearance and clarity: The formulated insitu gels were evaluated for pH, clarity, viscosity, gelling capacity, gelation temperature, drug content. Results were depicted in Table 2.

pH: The pH of all the formulations was measured using digital pH meter.

Rheological studies: The viscosity was measured using Brookfield viscometer. 25ml of the insitu gel formulation was placed in a sampler tube and analyzed at 37˚C by a circulating bath connected to the viscometer adaptor. The angular velocity of the spindle was increased from 1 to 30 and the viscosity of the formulation was measured.

Estimation of drug content
0.1ml of the optimized formulation was taken into a clean conical flask and the volume was made upto 100ml with pH 7.4 buffer and analyzed spectrophotometrically at 293nm.

Gelling capacity: Gelling capacity was determined by placing a drop of polymeric solution in vials containing 1 ml of simulated tear fluid and time taken for gelation as well as time taken for the gel formed to dissolve was noted.

Gelation temperature: 10 ml of formulation was taken into a 25ml beaker and placed in a low temperature water bath. A thermometer was immersed into the sample solution for constant monitoring. The solution was heated with stirring at 100 rpm using a magnetic bar. The temperature at which the magnetic bar stopped moving due to gelation was noted as the gelation temperature (\(T_{gel}\)). Each sample was measured in triplicate.

In-vitro drug diffusion studies: The in-vitro release of Balofloxacin from the prepared formulations was carried out in modified diffusion apparatus. The diffusion medium used was pH 7.4 buffer. Gelatin sheet with a pore size of 250μ was previously soaked overnight in the dissolution medium and tied to one end of a specifically designed open end glass cylinder. A 1ml volume of the formulation was accurately transferred into this assembly. The cylinder was placed in a beaker containing 30ml of the diffusion medium which is called as the acceptor chamber, so that the membrane just touches the receptor medium surface. Acceptor
chamber was maintained at a temperature of $37 \pm 2^\circ C$ with a stirring rate of 50 rpm using magnetic stirrer. Aliquots (each of 3ml) were withdrawn at hourly intervals and replaced by an equal volume of the receptor medium. The aliquots were diluted using pH 7.4 buffer and the samples were analyzed at 293nm using UV Visible spectrophotometer.

**Antimicrobial activity**

Antimicrobial activity of Balofloxacin was determined by Cup plate technique. Standard sample of pure drug and test solution of the formulation was prepared in the concentration of 1, 1.5, 2µg/ml and pH 7.4 was used as a control. Plates were inoculated with 0.3ml of cultures of Staphylococcus aureus and E.coli. After solidification of the media, wells were punched using sterile borer, standard and test solutions were added into the wells and allowed to diffuse for some time and incubated for 24hrs. The zone of inhibition was measured around each well and compared with that of standard. The entire procedure was carried out in a laminar air flow chamber. Each measurement was taken in triplicate.

**Accelerated stability studies**

The accelerated stability studies were carried out according to the ICH guidelines. Optimized formulation G7 was transferred into a glass vial which is capped and covered by aluminum foil and this packed formulation was stored in stability chamber which is maintained at $40^\circ C \pm 2^\circ C$ and 75% RH $\pm 5 \%$ for 1 month. The formulations were evaluated before and after periodic interval for change in appearance, the drug content, and in-vitro drug release.

**RESULTS AND DISCUSSION**

**Preliminary studies**

**FTIR studies**

![Fig.1: IR Spectrum of Pure Balofloxacin](image-url)
From the FTIR studies it was depicted that the characteristic principal peaks of Balofloxacin were observed are N-H stretching (3037 cm$^{-1}$), C=O stretching (1581 cm$^{-1}$), C-F stretching (1535 cm$^{-1}$), C-N stretching (1271 cm$^{-1}$), C-H stretching (879 cm$^{-1}$). Similar peaks were observed in spectra of different combinations of excipients and in optimized formulation (Balofloxacin insitu gel), along with absence of interfering peaks indicating there is no unwanted reaction between Balofloxacin and other excipients used in the study.

**Appearance, clarity, pH, drug content**

The appearances of all formulations were yellow in color and were clear without any particulate matter. The pH of all the formulations was found to be 7.4. The pH, clarity, appearance, drug content of all the formulations was depicted in table 2.

**Table 2: Evaluation Parameters of Balofloxacin Thermosensitive Insitu Gels**

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Appearance</th>
<th>Clarity</th>
<th>pH</th>
<th>Drug content</th>
<th>Gelling capacity</th>
<th>Gelation temperature (˚C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>Yellow</td>
<td>Clear</td>
<td>7.4</td>
<td>76.8</td>
<td>+</td>
<td>36.4</td>
</tr>
<tr>
<td>G2</td>
<td>Yellow</td>
<td>Clear</td>
<td>7.4</td>
<td>78.2</td>
<td>++</td>
<td>36.0</td>
</tr>
<tr>
<td>G3</td>
<td>Yellow</td>
<td>Clear</td>
<td>7.4</td>
<td>80.4</td>
<td>++</td>
<td>37.1</td>
</tr>
<tr>
<td>G4</td>
<td>Yellow</td>
<td>Clear</td>
<td>7.4</td>
<td>83.80</td>
<td>+++</td>
<td>36.8</td>
</tr>
<tr>
<td>G5</td>
<td>Yellow</td>
<td>Clear</td>
<td>7.4</td>
<td>75.2</td>
<td>+</td>
<td>37.0</td>
</tr>
<tr>
<td>G6</td>
<td>Yellow</td>
<td>Clear</td>
<td>7.4</td>
<td>82.3</td>
<td>++</td>
<td>37.0</td>
</tr>
<tr>
<td>G7</td>
<td>Yellow</td>
<td>Clear</td>
<td>7.4</td>
<td>89.82</td>
<td>+++</td>
<td>37.2</td>
</tr>
<tr>
<td>G8</td>
<td>Yellow</td>
<td>Clear</td>
<td>7.4</td>
<td>81.34</td>
<td>++++</td>
<td>37.0</td>
</tr>
</tbody>
</table>

- : No gelation  
+: Gels slowly and dissolves slowly  
++: Gelation immediate and remains for hours  
+++: Gelation immediate and remains for extended period of time
Results shown in Table 3 revealed that the drug content of all developed formulations was in the range of 76 to 89%. Formulation G7 showed higher drug content. All the formulations exhibited fairly uniform drug content.

Rheological studies

Table 3: Viscosity Measurements of Balofloxacin Thermosensitive Insitu gel formulations

<table>
<thead>
<tr>
<th>Angular Velocity (rpm)</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>G4</th>
<th>G5</th>
<th>G6</th>
<th>G7</th>
<th>G8</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>392</td>
<td>435</td>
<td>478</td>
<td>493</td>
<td>515</td>
<td>527</td>
<td>553</td>
<td>586</td>
</tr>
<tr>
<td>2</td>
<td>365</td>
<td>412</td>
<td>455</td>
<td>468</td>
<td>501</td>
<td>519</td>
<td>532</td>
<td>565</td>
</tr>
<tr>
<td>5</td>
<td>321</td>
<td>392</td>
<td>423</td>
<td>434</td>
<td>489</td>
<td>497</td>
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<tr>
<td>10</td>
<td>298</td>
<td>365</td>
<td>412</td>
<td>420</td>
<td>465</td>
<td>475</td>
<td>496</td>
<td>502</td>
</tr>
<tr>
<td>20</td>
<td>256</td>
<td>323</td>
<td>399</td>
<td>407</td>
<td>422</td>
<td>443</td>
<td>468</td>
<td>487</td>
</tr>
<tr>
<td>30</td>
<td>229</td>
<td>313</td>
<td>386</td>
<td>398</td>
<td>403</td>
<td>421</td>
<td>432</td>
<td>465</td>
</tr>
</tbody>
</table>

Fig. 3: Drug content profile of Balofloxacin insitu gels

Fig. 3: Viscosity Profile of Insitu gel formulations before gelation
Rheological evaluation of all the formulations exhibited Newtonian flow before gelling and exhibited pseudo-plastic flow after gelling in the eye. Increase in the shear rate decreases the viscosity of the formulation. Additionally, the gel formed in situ should maintain its integrity without dissolving or eroding for a prolonged period. Results are as shown in Table 3 and Fig (3).

**Invitro Drug Release Studies**

The invitro drug release studies revealed that the cumulative percentage drug release of all the formulations was found to be in between 84 -95%. Slow release of the drug from the polymeric solution containing in situ gel was due to increase in the concentration of the polymers thereby increase in the viscosity which renders sustained drug release.
**Antimicrobial activity**

The results of the antimicrobial efficacy test were shown in table 4. Balofloxacin insitu gel formulation and Balofloxacin API showed measurable difference in area of zone of inhibition (Fig.7). The higher zone of inhibition values obtained for the formulations in comparison to the standard could be attributed to the slow and prolonged diffusion of the drug from the polymeric solution due to its higher viscosity. The study indicated that Balofloxacin insitu gel showed significantly high zone of inhibition than the standard API.

| Table 4: Microbial Studies of Various Formulations of Balofloxacin Insitu gels |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| **Organism**                  | **Concentration of standard and test (µg/ml)** | **Zone of inhibition of standard (mm)** | **Zone of inhibition of test (mm)** |
| S.aureus                      | 1 | 20 | 28 |
|                               | 1.5 | 21 | 33 |
|                               | 2 | 24 | 42 |
| E.coli                        | 1 | 17 | 25 |
|                               | 1.5 | 21 | 29 |
|                               | 2 | 23 | 32 |

**Fig.7: Comparative antimicrobial activity of Optimized Insitu Gel (G7) and pure drug solution**

**Accelerated stability studies**

From stability study it reveals that formulation G7 showed no change in physical appearance, but both formulation showed very less decrease in drug content and in vitro release. From this study it has been revealed that tested formulation was satisfactorily stable.

**CONCLUSION**

Thermoreversible insitu gels of Balofloxacin were successfully formulated using poloxamer 407 and chitosan. The prepared formulations were evaluated for pH, clarity, gelling capacity, gelation temperature, drug content, in-vitro drug release studies, antimicrobial studies and
drug-compatibility studies. The in-vitro drug release studies demonstrated that the developed formulation released for more than 10 hours thus improving the precorneal residence time. The antimicrobial studies revealed that Balofloxacin insitu gel showed higher zone of inhibition than pure API. Thus the designed insitu gel was a suitable approach to improve bioavailability by increasing the pre-corneal residence time.

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