FORMULATION AND EVALUATION OF ION SENSITIVE OCULAR INSITU GELS FOR SUSTAINED RELEASE OF BALOFLOXACIN

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Abstract

Aim: The aim of the present investigation was to formulate and evaluate ion sensitive ocular insitu gels for sustained release of Balofloxacin, a fourth generation broad spectrum antibiotic indicated for infective ophthalmitis.

Methods: Insitu ocular gels of Balofloxacin were formulated aseptically by polymer solution method. Sodium alginate, HPMC E50 LV and HPMC K4M in different concentrations were employed. All the formulations were evaluated for pH, clarity, drug content, gelling capacity, viscosity studies, invitro drug release studies, antimicrobial activity. Formulation with good gelling capacity was selected for further studies. Drug-excipient compatibility studies in FK8 were evaluated by FTIR studies. Invitro drug diffusion studies was carried out in modified diffusion apparatus using gelatin sheet as a semipermeable membrane and comparative study of antimicrobial activity of API and formulation was assessed by cup plate technique

Results: All the developed formulations had pH in the range of 6.3-6.5 which is close to the pH of simulated tear fluid. Insitu ocular gels exhibited sustained delivery of Balofloxacin with FK8 showing 82.63% in 8 hours. The optimized gel showed higher zone of inhibition when compared to that of API.

Conclusion: The study conducted demonstrated that ocular insitu gels caused sustained delivery of Balofloxacin. The ion sensitive insitu gel is a suitable approach to enhance the bioavailability by increasing precorneal residence time.

Keywords:
Balofloxacin, sustained release, ion sensitive insitu gels, antimicrobial activity.
Introduction

Ophthalmic drug delivery is a challenging area as the unique anatomy of eye restricts the drug absorption into the deeper tissues. Existing ophthalmic drug delivery systems like inserts, ointments and suspensions had drawbacks like patient compliance, blurred vision, and heterogeneity. To overcome these drawbacks an approach to increase the viscosity of a drug formulation in the precorneal region that leading to an increased bioavailability, due to slower drainage from the cornea; several concepts for the in-situ gelling systems have been investigated. These systems can be triggered by pH, temperature, and ion activation. Insitu gels are made from polymers that exhibit phase transition due to physicochemical change in the environment. They can be conveniently used as a solution into the conjunctival sac in the eye. Upon contact with the lachrymal fluid, the polymer changes its conformation to form a gel. This delivery system has the ease of administration similar to an ophthalmic solution and has a long retention time because of the gel formation.

Ion sensitive insitu gels have the ability to cross link with the cations present in the tear fluid resulting in formation of a gel on the ocular surface. They can be formulated at optimal pH for ocular delivery using buffers, can be easily and accurately instilled at room temperature. The aim of the present study was to formulate and evaluate ion sensitive insitu gels of Balofloxacin using sodium alginate and HPMC K4M/ HPMC E50 LV in order to achieve sustained delivery of Balofloxacin by increasing the precorneal residence time.

Materials and Methods

Balofloxacin was obtained from Cirex Pharmaceuticals, Hyderabad, India as a gift sample, sodium alginate was purchased from Loba Chemie Pvt. Ltd, India HPMC E50, K4M purchased from Colorcon Asia Pvt Ltd. Other chemicals used were from SD Fine Chemicals, Mumbai. Other materials and solvents used were of analytical grade.

Preparation of Insitu gel: The polymeric solution was prepared by dispersion method. Required quantities of sodium alginate, HPMC- E 50 LV/HPMC- K4M were dispersed in water with continuous stirring until the complete dissolution. Solution of Balofloxacin was added in to the polymeric solution with continuous stirring. Preservative and isotonic agents were added. The pH of the solution was found to be 6.3-6.5.

Evaluation of insitu gels: The formulated insitu gels were evaluated for pH, clarity, viscosity, gelling capacity, drug content, invitro drug release studies and antimicrobial studies.
**FTIR studies:**

Insitu gel and pure drug were subjected to FTIR studies to observe for drug-polymer interactions.

**Syringe ability:**

A 5ml of the polymeric solution was drawn into a hypodermic syringe with a 20 gauge needle. It was checked for the ability of the formulation that can be dispensed out from the syringe.

**Gelling capacity:**

Gelling capacity of the formulated insitu gels was determined by instilling a drop of the formulation into a beaker containing freshly prepared pH 7.4 phosphate buffer and was visually observed for the time taken for gelling.

**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 formulation was diluted to 100ml with pH 7.4 phosphate buffer and the drug content was estimated spectrophotometrically at 293nm.

**Invitro drug release studies:**

The invitro drug release studies of the formulations were carried out in modified diffusion apparatus. The freshly prepared simulated tear fluid (pH7.4) was used as a diffusion medium. Gelatin sheet used as the semi-permeable membrane was soaked overnight. Two ml of formulation was accurately placed into the donor chamber which was suspended in a beaker containing 30 ml of diffusion medium such that the membrane just touches the surface of the medium. Medium was maintained at a temperature of 37 ± 2°C with a stirring rate of 50 rpm using magnetic stirrer. About 3 ml of sample was withdrawn at a predetermined time interval of 1 hour for 10 hours and replaced with an equal volume of fresh diffusion medium. The aliquots were diluted with the diffusion medium and analyzed at 293 nm spectrophotometrically.

**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 developed formulation was transferred into a glass vial, closed with butyl rubber closures and sealed with an aluminum foil and was stored at a temperature of 45°C/75% RH for 30 days. After 30 days the formulation was checked for physical appearance, gelling capacity and drug content.

**Results and Discussion**

In the present work, the in-situ gelling systems were prepared by using different concentrations of sodium alginate and HPMC E50LV/HPMC K4M. All the formulations were evaluated for clarity, pH, gelling capacity, drug content, viscosity studies. Results have been depicted in Table1 & 2.

**Table-1: Formulation Design of Insitu Gelling System.**

<table>
<thead>
<tr>
<th>Ingredients (mg)</th>
<th>FH1</th>
<th>FH2</th>
<th>FH3</th>
<th>FH4</th>
<th>FH5</th>
<th>FK6</th>
<th>FK7</th>
<th>FK8</th>
<th>FK9</th>
<th>FK10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Balofloxacin</td>
<td>150</td>
<td>150</td>
<td>150</td>
<td>150</td>
<td>150</td>
<td>150</td>
<td>150</td>
<td>150</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>Sodium alginate</td>
<td>50</td>
<td>200</td>
<td>350</td>
<td>500</td>
<td>650</td>
<td>50</td>
<td>200</td>
<td>350</td>
<td>500</td>
<td>650</td>
</tr>
<tr>
<td>HPMC E50 LV</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HPMC K4M</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Benzalkonium Chloride(%w/v)</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>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>NaCl (%w/v)</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>
<td>0.9</td>
<td>0.9</td>
</tr>
<tr>
<td>Distilled water (volume made 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>
<td>Qs</td>
<td>Qs</td>
</tr>
</tbody>
</table>

**Table-2: Evaluation parameters of Balofloxacin ocular in-situ gels.**

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>pH</th>
<th>Clarity</th>
<th>Gelling capacity</th>
<th>Drug content</th>
<th>Visual appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>FH1</td>
<td>6.5</td>
<td>Clear</td>
<td>+</td>
<td>83.45</td>
<td>Translucent</td>
</tr>
<tr>
<td>FH2</td>
<td>6.4</td>
<td>Clear</td>
<td>+</td>
<td>86.8</td>
<td>Translucent</td>
</tr>
<tr>
<td>FH3</td>
<td>6.5</td>
<td>Clear</td>
<td>++</td>
<td>85.36</td>
<td>Translucent</td>
</tr>
<tr>
<td></td>
<td>Drug Excipient compatibility studies:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Preliminary studies:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**FH4**  
6.2 Clear ++ 90.24 Translucent

**FH5**  
6.5 Clear +++ 89.74 Translucent

**FK6**  
6.2 Clear ++ 86.44 Translucent

**FK7**  
6.4 Clear ++ 89.92 Translucent

**FK8**  
6.5 Clear +++ 96.62 Translucent

**FK9**  
6.3 Turbid +++ 90.42 Translucent

**FK10**  
6.5 Turbid +++ 89.92 Translucent

- : No gelation  
+ : Gels slowly and dissolves slowly  
++ : Gelation immediate and remains for hours  
+++ : Gelation immediate and remains for extended period of time

**Preliminary studies:**

**Drug –Excipient compatibility studies:**

- **Fourier Transform Infra Red Spectroscopy:**

FTIR spectra of pure drug and drug loaded alginate-HPMC insitu gel (FK8) were studied to understand any possible interactions. It was observed that the main functional group peaks are in the range of reported values in both the pure drug and the formulation indicating no drug-polymer interactions. 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 and gelling capacity**

All the formulations were clear, translucent with a pH range of 6.2-6.5. Based on the results of gelling capacity FH5, FK8, FK9, FK10 were selected and evaluated for drug content. From the results of drug content FK8 was selected for further studies.

**Gelling capacity:** All the formulations showed fairly good gelation properties. FK8 showed excellent gelation and hence selected for further studies.
Drug content: The results of the studies indicated that the drug content of all developed formulations was in the range of 83 to 96%. FK8 showed higher drug content of 96.62%.

Invitro drug release studies:
From the in-vitro drug release studies it was observed that cumulative percentage drug release from the developed formulations was found to be 97% for FH1 which contained about 50mg of sodium alginate keeping HPMC E50 LV as constant. FK8 showed a cumulative percentage drug release of 79% which contained 350mg of sodium alginate keeping HPMC K4M as constant. Results were shown in Figure 4. Formulation FK8-FK10 showed more sustained release compared to other formulations. This could be due to higher concentration of Sodium alginate and HPMC K4M among the developed formulations. The formulations FK9 and FK10 had a drawback of syringeability and clarity hence formulation FK8 was selected.

Drug release kinetics: On application of different release kinetic model the formulation showed higher linearity (R² = 0.928-0.992). It was observed that formulation showed the zero order kinetics.

Rheological evaluation:
All the formulations exhibited Newtonian flow before gelling and exhibited pseudo-plastic flow after gelling in the eye. There was an increase in the viscosity after gelling. Increase in the shear rate decreased the viscosity of the formulation. Results are depicted in Table 3 and Figures 5 and 6.

Table-3: Viscosity (in cps) profile of Balofloxacin insitu gels.

<table>
<thead>
<tr>
<th>Angular Velocity (rpm)</th>
<th>FH1</th>
<th>FH2</th>
<th>FH3</th>
<th>FH4</th>
<th>FH5</th>
<th>FK6</th>
<th>FK7</th>
<th>FK8</th>
<th>FK9</th>
<th>FK10</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>195</td>
<td>215</td>
<td>235</td>
<td>257</td>
<td>298</td>
<td>314</td>
<td>345</td>
<td>369</td>
<td>394</td>
<td>401</td>
</tr>
<tr>
<td>2</td>
<td>156</td>
<td>201</td>
<td>211</td>
<td>232</td>
<td>276</td>
<td>285</td>
<td>323</td>
<td>355</td>
<td>386</td>
<td>399</td>
</tr>
<tr>
<td>5</td>
<td>132</td>
<td>189</td>
<td>197</td>
<td>219</td>
<td>259</td>
<td>267</td>
<td>319</td>
<td>343</td>
<td>365</td>
<td>372</td>
</tr>
<tr>
<td>10</td>
<td>102</td>
<td>175</td>
<td>186</td>
<td>193</td>
<td>209</td>
<td>234</td>
<td>259</td>
<td>298</td>
<td>342</td>
<td>356</td>
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<tr>
<td>20</td>
<td>98</td>
<td>154</td>
<td>173</td>
<td>187</td>
<td>196</td>
<td>221</td>
<td>246</td>
<td>274</td>
<td>312</td>
<td>324</td>
</tr>
<tr>
<td>30</td>
<td>75</td>
<td>130</td>
<td>165</td>
<td>178</td>
<td>165</td>
<td>201</td>
<td>212</td>
<td>245</td>
<td>289</td>
<td>318</td>
</tr>
</tbody>
</table>
Fig 1: IR spectrum of Balofloxacin pure drug.

Fig 2: IR spectrum of Balofloxacin insitu gel (optimized formulation FK8).

Fig 3: Comparison of drug content of formulations.

Figure-4: Combined graph of in-vitro drug release profiles of Balofloxacin in-situ gels.
Fig 5: Viscosity (cps) profile of Balofloxacin insitu gels (before gelation)

Fig.6 Viscosity profile of FK8 before and after gelation.

Fig .7: Comparative antimicrobial activity of optimized insitu gel FK8 and pure Balofloxacin solution
(a) Staphylococcus aureus (b) Escherichia coli

Antimicrobial activity:

Antimicrobial efficacy study was performed on FK8 formulation using Gram positive Staphylococcus aureus and gram negative E. coli organism. The zone of inhibition of FK8 ophthalmic formulation was found to be 31mm & 44mm and 29mm & 42mm, respectively, for Gram positive S. aureus and Gram negative E. coli organism. The results of
antimicrobial activity are as shown in the Table 4 & 5. The study indicated Balofloxacin which showed more activity when formulated as a gel forming ophthalmic system against both selected S. aureus and E. coli.

Table-4: Microbial studies of various formulations of Balofloxacin in situ gels.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Concentration of standard and test (µg/ml)</th>
<th>Zone of inhibition of standard (mm)</th>
<th>Zone of inhibition of test (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.aureus</td>
<td>1</td>
<td>22</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>27</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>31</td>
<td>44</td>
</tr>
<tr>
<td>E.coli</td>
<td>1</td>
<td>24</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>25</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>29</td>
<td>42</td>
</tr>
</tbody>
</table>

Table-5: Antimicrobial activity of optimized formulation for both Staphylococcus aureus and E.coli.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Zone of inhibition of Balofloxacin in situ gel against S.aureus</th>
<th>Zone of inhibition of Balofloxacin in situ gel against E.coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>FK8</td>
<td>44</td>
<td>42</td>
</tr>
</tbody>
</table>

Accelerated stability studies: Accelerated stability studies were carried out at 40 ± 2°C at 75 ± 5 % RH for 1 month. The samples were analyzed periodically every week, and found that there are no changes in visual appearance, clarity, pH, and gelation. Assay values after 1 month of storage are found almost same (deviating not more than one percent).

Conclusion

The main objective of the present study was to improve the bioavailability of Balofloxacin thereby improving precorneal residence time, and sustain the drug release by utilizing the approach of in situ gelling systems using polymers, sodium alginate and Hydroxy propyl methyl cellulose to enhance bioavailability by increasing precorneal residence time and ability to sustain drug release. The polymers used are inexpensive and easily available. The formulation also helps to reduce the frequency of drug administration, thus improving patient compliance.

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References


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