Original Article

Formulation and evaluation of Tobramycin Sulphate

In situ gel for conjunctivitis.

Dilasha shakya, A. Geethalakshmi*, Bindu G1, Ashwini B1

The Oxford College of Pharmacy, Begur road, Bangalore, Karnataka, India.

*Corresponding Author: A. Geethalakshmi

Asst. Professor, Department of Pharmaceutics, The Oxford College of Pharmacy, Hongasandra, Bangalore, India.

E-mail address: geeshwar@gmail.com; Tel: 09901418806

Running Title: Formulation of tobramycin sulphate for conjunctivitis

Received: 16 April, 2016; Revised: 08 June, 2016 Accepted: 19 June, 2016

Available online at http://www.thescientificpub.com http://dx.doi.org/10.19046/abp.v03i03.02

Abstract

The poor bioavailability and therapeutic response exhibited by the conventional eye drops due to rapid precorneal elimination of the drug may be overcome by the use of in situ gelling systems that are instilled as drops into the eye and undergo a sol-to-gel transition in the cul-de-sac. The present study was designed to prepare and investigate the sustained release in situ ocular gels of Tobramycin sulphate using gelrite as gel forming polymer, which is used in the treatment of various bacterial infections. The formulations were evaluated with various parameters like clarity, pH, gelling capacity, rheological study, in vitro studies, sterility and isotonicity. All the formulations were transparent, having consistency and drug content was found to be in acceptable range. Among all the formulations, F3 was selected as the best formulation. The best selected F3 formulation was found to be stable at short term stability studies as per ICH guidelines. The overall results of this study supports that the Gelrite based vehicle could be used for controlled drug release that exhibits a greater potential for conjunctivitis. It also contributes to a great extent to patient compliance as the dosage is regulated as one drop of the solution to be instilled into the eye once a day.

Keywords: Conjunctivitis, Gelrite, In situ gel, sustained ophthalmic delivery, Tobramycin Sulphate.

Introduction

Conjunctivitis is an inflammation of the eyes. It describes the group of disease’s that causes swelling, itching, burning and redness of the conjunctiva, the protective membrane that lines the eyelids and covers exposed areas of the white of the eye (sclera). It is characterized by cellular infiltration and exudation. Staphylococcus aureus is the most common cause of bacterial conjunctivitis and blepharo-conjunctivitis. Many other organisms like Haemophilus influenza, Streptococcus pneumonia also cause conjunctivitis [1].

Ophthalmic drug delivery is one of the most interesting and challenging areas that are facing the pharmaceutical scientists. Ocular administration of drug is primarily associated with the need to treat ophthalmic diseases. Eye is the most easily accessible site for topical administration of a medication. This is done to prevent the risk of eye damage from high blood concentration of drug, which is not intended for the eye. The most common method of ocular drug delivery is the instillation of drops into the lower cul-de-sac. Eye drops provide pulsed entry of the drug followed by rapid decline in drug concentration [2]. Various ophthalmic vehicles such as inserts, ointments, suspensions and aqueous gels have been developed in order to lengthen the residence time of instilled dose and enhance the ophthalmic bioavailability [3, 4]. These ocular drug delivery systems, however, have not been used because of some drawbacks such as blurred vision from ointments or low patient compliance from inserts [5, 6].
Many in situ gel forming systems have been developed to prolong the pre-corneal residence time of a drug and improve ocular bioavailability. These systems consist of polymers that exhibit sol-to-gel phase transitions due to change in specific physicochemical parameters (ion, pH, temperature), in their environment; the cul-de-sac in this case. There are different methods for approaching the in situ gel form such as ion activation, pH triggering and temperature sensitive methods. By using the ion activation method we have developed the Tobramycin sulphate in situ gel system to improve the ocular bioavailability.

Tobramycin Sulphate (TOB) is an aminoglycoside antibiotic produced by Streptomyces tenebrarius. It exhibits a broad spectrum activity against aerobic gram-negative bacteria, particularly Pseudomonas aeruginosa. The bactericidal activity of TOB is accomplished by inhibiting ribosomal function leading to interruption in bacterial protein synthesis. It is used topically for treatment of eye infections, parenterally for treatment of serious bacterial infection, and also for local application in the oral cavity and stomach as part of selective decontaminant of the digestive tract.

Materials and Methods

Materials

Tobramycin sulphate was purchased from fine chemicals. Pvt. Ltd. Gellan gum was obtained from Sigma Aldrich Pvt. Ltd. and all other reagents were of analytical grade.

Methods

Preformulation studies

Solubility studies: Solubility of Tobramycin sulphate was checked with acetone, ethanol and distilled water at room temperature. 10 ml of solvent was taken and dissolved with the measured quantity of Tobramycin sulphate pure drug until the drug precipitates and noted the amount of drug dissolved in the solvent.

Incompatibility studies by FTIR

The interaction studies between drug-gelrite were done by using FT-IR spectroscopy. A spectrum of drug-gelrite was compared at 400 – 4000 cm⁻¹.

Preparation of in situ gel formulations:

Gelrite of different concentrations (0.2%, 0.4%, 0.6%, 0.8% and 1%) was added to de-ionized water and dissolved by heating to 90°C with moderate stirring. Once completely dissolved, the solution was cooled to the temperature below 30°C. Then the drug (0.03% w/v), Benzalkonium chloride (BKC) 0.01% as preservatives and glycerol (2% w/v) as viscosifying agent were slowly added to the system and then the solution was stirred instantly until a uniform solution was obtained. Final make up volume of 100ml was done by using de-ionized water [7]. (Table: 1)

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug % w/v</td>
<td>0.3%</td>
<td>0.3%</td>
<td>0.3%</td>
<td>0.3%</td>
<td>0.3%</td>
</tr>
<tr>
<td>Gellan gum % w/v</td>
<td>0.2%</td>
<td>0.4%</td>
<td>0.6%</td>
<td>0.8%</td>
<td>1%</td>
</tr>
<tr>
<td>BKC % v/v</td>
<td>0.02%</td>
<td>0.02%</td>
<td>0.02%</td>
<td>0.02%</td>
<td>0.02%</td>
</tr>
<tr>
<td>De-ionized water % w/v</td>
<td>100ml</td>
<td>100ml</td>
<td>100ml</td>
<td>100ml</td>
<td>100ml</td>
</tr>
</tbody>
</table>

Evaluation of Tobramycin sulphate in situ gel

1. Appearance, Clarity and pH: The general appearance of the formulation was observed which included color and clarity of the solution [8]. The prepared in situ gel formulations were evaluated for pH measurement by using pH meter.

2. Drug content: Estimation of drug content was done as 1ml of the formulation was diluted to 100ml of simulated tear fluid (pH7.4) (stock 1). From the above stock 5ml were taken, filtered and diluted to 25ml of simulated tear fluid. The UV absorbance was taken at 275nm in UV spectrometer [9].

   Drug content was calculated by:

   \[
   \text{Drug content (in mg)} = \frac{\text{Absorbance} \times \text{Dilution factor}}{\text{Slope} \times 1000}
   \]

   \[
   \% \text{ drug content} = \frac{\text{DC} \times 100}{\text{amount of the drug added}}
   \]

3. Test for gelling capacity: Gelling capacity of formulations was evaluated in order to identify the formulations suitable for use as in situ gelling systems. The individual ophthalmic formulations (100µl) were added into 2 ml of simulated tear fluid contained in glass vials. The phase transition of solution to viscous gel was observed. Accordingly scores were assigned [10].

4. Viscosity determination: The viscosity measurements were done by using Brookfield DV-II+ viscometer using LV-3 spindle. The developed formulations were poured into the small sample adapter of the viscometer and the angular velocity was increased gradually from 10 to 100 rpm. The angular velocity was reversed gradually by using S18 spindle. The average of the two readings was used to calculate viscosity. By adding STF (pH 7.4) the formulations were made into gel form and viscosity was determined as specified above using LV-3 spindle [10].
5. **In vitro diffusion study**: Drug release from in situ gel was determined by diffusion process. The in vitro diffusion of Tobramycin Sulphate from the formulations was studied through cellophane membrane using a Franz diffusion apparatus. The diffusion medium used was freshly prepared STF. Cellophane membrane, previously soaked overnight in the diffusion medium (STF), was placed in between the donor and receptor compartment. 1 ml volume of the formulation was accurately instilled into the donor compartment. STF was placed in the receptor compartment according to the capacity of it. The whole assembly was placed on the thermostatically controlled magnetic stirrer. The temperature of the medium was maintained at 37 ± 0.5°C. The magnetic bead was rotated in such a way that it produced a vortex and touched the cellophane membrane. Aliquots, each 1 ml volume, were withdrawn at hourly intervals and replaced by an equal volume of the receptor medium. The aliquots were diluted with STF and analyzed by UV visible spectrophotometer at 275 nm [11].

6. **Sterility test: Test for sterility**

**Method/ Procedure**: Sterility tests were performed for aerobic, anaerobic by using fluid thioglycollate medium and fungi by soyabean casein digest medium.

**Preparation of fluid thioglycollate medium**: 29.3 g of fluid thioglycollate medium was dissolved in 1000 ml distilled water by boiling and sterilized by autoclaving at 15 lbs pressure at 121°C for 20 min.

i) **Sterility (negative control) test**: Fluid thioglycollate media was incubated at 30-35°C for not less than 7 days.

ii) **Growth promotion (positive control) test**: The sterile media was inoculated with about 100 viable micro-organisms and incubated according to the conditions specified. The test media were satisfactory, if clear evidence of growth appears in all media within 7 days. Ophthalmic preparations should be sterile and must be checked for the presence of any bacteria before it is used. In each test, three sterile test tubes were used in the study and labeled as ‘negative control’, ‘test’ and ‘positive control’.

**Test for aerobic bacteria**

20 ml each of sterile fluid thioglycollate was transferred to 3 tubes aseptically. The tube that was labeled as positive control was inoculated with viable aerobic microorganism *Bacillus subtilis* aseptically. 2.5 ml of the ophthalmic preparation was added to the tube labeled as test and incubated at 30-35°C for not less than 7 days [12].

**Test for anaerobic bacteria**

20 ml each of sterile alternative thioglycollate was transferred to 3 tubes aseptically. The tube labeled as positive control was inoculated with viable anaerobic microorganism *bacteroides vulgatus* aseptically. 2.5 ml of the ophthalmic preparation was added to the tube labeled as test and incubated at 30-35°C for 7 days.

**Test for fungi**

20 ml each of sterile soyabean casein digest medium was transferred to 3 tubes aseptically. The tube labeled as positive control was inoculated with *candida albicans* (ATCC No.10231) aseptically. 2.5 ml of the ophthalmic preparation was added to the tube labeled as test and were incubated at 20-25°C for not less than 7 days.

7. **Isotonicity**: Isotonicity is an important characteristic of the ophthalmic formulations. Isotonicity has to be maintained to prevent tissue damage or irritation of eyes. The selected in situ gel formulation F3 was subjected to isotonicity testing, since it exhibited good gelling capacity, the required viscosity and release characteristics. Formulation was mixed with few drops of blood and observed under microscope at 45X magnification and compared with standard marketed ophthalmic formulation. The shape of blood cells was compared with standard marketed ophthalmic formulation [13].

8. **Stability studies**: Stability studies were carried out on most satisfactory formulation F3 as per ICH guidelines Q1C. Sterile gel forming ophthalmic solution (Formulation F3) was filled in autoclavable transparent plastic bottles, closed with autoclavable rubber closures and sealed with aluminum foils. The formulation was kept in stability chamber at 40±2°C & 75±5% RH for 6 months. Samples were evaluated for pH, Clarity, gelling capacity, drug content, viscosity studies, in vitro diffusion, isotonicity and sterility [14].

**Results and Discussion**

The efforts in the present study were directed to develop and optimize Tobramycin sulphate in situ gel for conjunctivitis treatment using polymers such as Gelrite by ion activation method in situ gelling phenomenon. The optimization data of in situ gel formulations containing different polymer concentrations are shown in **Tables**.
Drug polymer compatibility studies were carried out using FT-IR spectroscopy to establish the possible interaction of polymer and excipients with the drug in the formulation. The FT-IR spectrum of drug alone as well as combination of drug with polymer and excipients were obtained and analyzed for the compatibility. FT-IR study revealed that there was no interaction between drug and polymer as it showed the characteristic peak of the drug and excipients; hence the drug and polymer are compatible with each other as shown in Figure: 2.

**Table 2: Solubility of Tobramycin Sulphate (pure drug)**

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>Solvent</th>
<th>Solubility observed</th>
<th>Solubility (g/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Water</td>
<td>Freely soluble</td>
<td>198mg/10ml</td>
</tr>
<tr>
<td>2</td>
<td>Ethanol (95%)</td>
<td>Slightly soluble</td>
<td>78mg/10ml</td>
</tr>
<tr>
<td>3</td>
<td>Acetone</td>
<td>Sparingly soluble</td>
<td>89mg/10ml</td>
</tr>
</tbody>
</table>

**Visual appearance and clarity** was studied and the appearance of all the formulations was found to be transparent and clear. Terminal sterilization by autoclaving had no effect on the formulations. The pH of all the formulations was found to be within the range of 5.8-6.3, which is desirable for the ophthalmic formulations. The results are presented in (Table 3).

Drug content in all the formulations were found to be within the range as per IP limits as shown in (Table 3).

**In situ** gelling systems were evaluated for the in vitro gelation capacity and all the formulations were found to be satisfactory (Table 3).

**Table 3: Evaluation of ophthalmic in situ gel**

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Drug content (%)</th>
<th>Visual appearance</th>
<th>Clarity</th>
<th>Gelling capacity</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>97.6±0.57</td>
<td>Transparent</td>
<td>Clear</td>
<td>+</td>
<td>5.9</td>
</tr>
<tr>
<td>F2</td>
<td>98.3±0.47</td>
<td>Transparent</td>
<td>Clear</td>
<td>++</td>
<td>5.8</td>
</tr>
<tr>
<td>F3</td>
<td>98.4±1.66</td>
<td>Transparent</td>
<td>Clear</td>
<td>+++</td>
<td>6.0</td>
</tr>
<tr>
<td>F4</td>
<td>98.56±0.85</td>
<td>Transparent</td>
<td>Clear</td>
<td>+++</td>
<td>6.3</td>
</tr>
<tr>
<td>F5</td>
<td>99.24±0.47</td>
<td>Transparent</td>
<td>Clear</td>
<td>+++</td>
<td>5.8</td>
</tr>
</tbody>
</table>

(+): gels formed after few minutes and disappeared rapidly. (++): gels formed and remained for few minutes. (+++): gelation immediate remains for an extended periods.

**Furthermore** the results of rheological studies revealed that, the viscosities of all formulations at physiological (pH-7.4) conditions were decreased, as cut off rate increased. Hence, the formulations may possess the characteristics of pseudoplastic fluid as shown in (Figure 3 and 4).
In-vitro drug release studies of selected Tobramycin sulphate in-situ gel formulations showed that, formulations released their drug contents in acceptable range over a period of 7 h. The prolonged period of drug release may be due to slow diffusion of drug from combined effect of polymers. The prolonged release may be probably due to the formation of hydrogen bonds between drug and polymers, which have helped in rate control release of drug. The results are shown in (Figure 3).

Figure 4: Viscosity of formulation in gel form

Figure 5: In vitro drug releases of the formulations

Table 4: Release Kinetics studies of Formulations F1-F5

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Zero order</th>
<th>First order</th>
<th>Higuchi</th>
<th>Kosermeyer-Peppas</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>0.978</td>
<td>0.59</td>
<td>0.959</td>
<td>0.052</td>
</tr>
<tr>
<td>F2</td>
<td>0.981</td>
<td>0.54</td>
<td>0.946</td>
<td>0.052</td>
</tr>
<tr>
<td>F3</td>
<td>0.973</td>
<td>0.45</td>
<td>0.951</td>
<td>0.051</td>
</tr>
<tr>
<td>F4</td>
<td>0.965</td>
<td>0.45</td>
<td>0.936</td>
<td>0.196</td>
</tr>
<tr>
<td>F5</td>
<td>0.969</td>
<td>0.39</td>
<td>0.922</td>
<td>0.235</td>
</tr>
</tbody>
</table>

Figure 6: In vitro release of F1, F2, F3, F4, F5, in situ gelling formulations of % CDR vs. time

Figure 7: In vitro release of F1, F2, F3, F4 F5 in situ gelling Log% drug remaining vs. time.

Figure 8: In vitro release of F1, F2, F3, F4, F5, in situ gelling % CDR vs. √T.
The higher regression co-efficient values in the table 3 for each formulation suggested that all the formulations followed Zero order release rate kinetics type of drug release. The 'n' value obtained from Peppa’s equation were more than 0.5, which indicated that formulations F1 – F5 showed drug release by Non-Fickian diffusion mechanism. From the in vitro diffusion studies F3 formulation was selected for further studies like sterility, isotonicity.

In vitro drug diffusion studies of marketed eye drops

The gelrite based system (F3) was compared with marketed product which showed that the marketed product released the drug within 2 hours whereas F3 formulation showed 7 hours release than Tobrasaf (marketed product). So the selected formulation (F3) in in situ gel form was having prolonged release characteristics than the marketed product. In addition there was no microbial growth in the selected formulation F3 after 7 days of incubation period and that the method used for sterilization was reliable. Further stability studies of the formulations showed no change in appearance, clarity and pH. Additionally, was observed that the gelling capacity of the formulations was least affected.
As the isotonicity has to be maintained to prevent tissue damage or irritant of eye. We finally subjected the F3 formulation to isotonicity testing and found that there were no signs of cremation or swelling of blood cells when compared with the marketed formulation.

**Conclusion**

Tobramycin sulphate was successfully formulated as ion activated in situ gel forming eye drops. The method used for preparations of in situ gel solution was simple and cost effective. This is a new approach used in the treatment of conjunctivitis of the eye. The formulations showed decreased frequency of administration and better patient acceptance. The formulation is a viable alternative to conventional eye drops by virtue of its ability to enhance bioavailability through its longer precorneal residence time and ability to sustain the drug release and also the ease of its administration afforded and decreased frequency of administration resulting in better patient acceptance, since only one drop is needed twice a day.

**Acknowledgements**

The authors are thankful to the management of The Oxford College of Pharmacy, Hongasandra, Bangalore for providing the necessary facilities to carry out this research project.

**Conflict of Interest**

The authors declare that there is no conflict of interest.

**References**


