In Situ Gel for Treatment of Bacterial Conjunctivitis

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ABSTRACT

The conventional ocular drug delivery systems like solutions, suspensions and ointments possess various drawbacks, i.e., precorneal drug elimination, high variability in efficacy and blurred vision respectively. These drawbacks may be overcome by use of an in-situ gel forming system that is instilled as solution into the eye and undergo a sol-gel transition in the cul-de-sac of the eye. The aim of present work was to formulate and evaluate an ocular drug delivery system of a fluoroquinolone antibiotic, Gatifloxacin, based on the concept of an ion-activated gelation. In this study Gellan gum was used as the gelling agent in combination with Hydroxypropyl Methylcellulose (HPMC) as a release retardant. The developed formulation was isotonic and clear in the pH range 6.0-6.3. It was converted into gel in the presence of monovalent and divalent ions present in simulated lacrimal fluid and provided sustained in-vitro drug release over an 8 hr period. It can be concluded that the developed in-situ gel formulation can be viewed as a better alternative to the conventional eye drops of Gatifloxacin by virtue of its ability to enhance precorneal residence time and consequently ocular bioavailability with lesser frequency of administration. The developed product can be further explored for commercial production after successful in-vivo and scale-up studies.

Keywords: Gatifloxacin, Gellan gum, Lacrimal fluid, in-situ gel, HPMC.

INTRODUCTION

Eye is a very sensitive and important organ of the body and is considered as window hinge. There are many eye diseases i.e., conjunctivitis, uveitis, glaucoma etc that can affect even loss of vision. The bioavailability of ophthalmic drugs is, however, very poor due to efficient protective mechanisms of the eye. Blinking, baseline and reflex lachrymation, and drainage remove rapidly foreign substances, including drugs; from the surface of the eye.1 Topical administration of eye drops in the lower cul-de-sac is the most common method of drug delivery for the treatment of ocular diseases.2 There are the most commonly available ophthalmic preparations such as drops and ointments about 70% of the eye dosage formulations in market.3 However, one of the major problems encountered with the eye drops is the rapid and extensive elimination induced by tear turnover, blinking and drainage of formulation which leads to short pre-corneal residence time and poor ocular bioavailability. As a result, frequent instillation of eye drops is needed in order to achieve desired drug concentration and therapeutic effect.4 An increase in dosing frequency or use of highly concentrated solution to compensate for short ocular residence time is undesirable because of poor patient compliance and risk of toxicity due to ophthalmic absorption via the nasolacrimal duct.5

To increase ocular bioavailability and duration of drug action, various ophthalmic vehicles i.e., viscous solutions,6 ointments/gels, and polymeric inserts,8 have been used. These ocular drug delivery systems, however, have not been used extensively owing to some drawbacks, such as blurred vision from ointments, lack of patient compliance from inserts, and, sticking of eyelids from gel. As a result, an enhanced ocular bioavailability following topical drug administration remains a challenge yet to be resolved satisfactorily.

An ideal ophthalmic dosage form is one that can sustain the drug release and remain in pre-corneal contact for an extended period of time.

A significant increase in the residence time of the formulation and consequently drug bioavailability can be achieved by delivery systems based on the concept of in-situ gelation6. These delivery systems consist of polymers that exhibit sol to gel phase transition, due to change in specific physiological conditions (pH, temperature and ionic strength) in the eye9.

Depending upon the method employed to cause phase transition on ocular surface, three types of systems are recognized, i.e., pH triggered systems-Cellulose Acetate Hydrogen Phthalate latex10 and Carbopol12-14, temperature dependent systems-Pluronic15-17 and tetrionics18 and ion activated systems gellan gum19-20 and sodium alginate21.

Gatifloxacin is a fourth generation fluoroquinolone antibiotic used for the treatment of bacterial conjunctivitis. It is commercially available in the form of an eye drops and ointment.

The topical ophthalmic administration of 0.3% Gatifloxacin solution is indicated in case of severe infection.
The main objective of the present work was involved the development of an in-situ gel formulation using an ion-activated phase transition polymer to deliver the drug effectively into the eye for sustained drug release and enhanced ocular drug bioavailability.

MATERIALS AND METHODS

Gatifloxacin was used as an active pharmaceutical ingredient, Gellan gum was used as in-situ gel forming polymer and HPMC K100M was used as release retardant. All the other reagents were used in the present study were of analytical grade.

Preparation of Formulations

Boric acid and disodium edetate were dissolved in distilled water. Gellan gum and HPMC were then dissolved in the above solution. The required quantity of Gatifloxacin to give a final drug concentration of 0.3% w/v was added to the polymeric solution and stirred until dissolved and then phenyl mercuric nitrate was added to it as preservative. The formulations were filled amber colored glass vials, closed with rubber closures and sealed with aluminum caps. The formulations, in their final pack were terminally sterilized by autoclaving at 121°C temperature, 15 psi pressure for 15 min. The sterilized formulations were stored in a refrigerator until further use. Composition of formulations is mentioned in Table 1.

Evaluation of Formulations

Physicochemical Characterization

The clarity of the formulation was evaluated by visual observation against white and black back grounds. pH of the formulations was determined by pH meter and it was found to be between 6.2-6.3 (Cyberscan 510).

Drug Content Uniformity

Equivalent of 100 µl of (Gatifloxacin) the formulation was diluted to 25 ml with distilled water in sterilized volumetric flak. It was estimated spectrophotometrically using double beam UV-visible spectrophotometer and found to be between 98.7 ± 0.75 to 99.6 ± 0.84 % (Shimadzu 1700) at 286 nm as shown in Table 2.

Figure 1: (a) RBCs with Developed In-Situ Gel Formulation of Gatifloxacin, (b) RBCs with Marketed Gatifloxacin Eye Drops (Gatiquin™), (c) RBCs with Hypotonic Solution, (d) RBCs with Hypertonic Solution, (e) RBCs with Isotonic Solution
### Table 1: Composition of Prepared In-situ Gelling Formulations

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Amount (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gatifloxacin</td>
<td>F 1: 0.3</td>
</tr>
<tr>
<td>Gellan gum</td>
<td>F 2: 0.6</td>
</tr>
<tr>
<td>Boric acid</td>
<td>F 3: 1.68</td>
</tr>
<tr>
<td>Phenylmercuric nitrate</td>
<td>F 4: 0.002</td>
</tr>
<tr>
<td>Disodium edetate</td>
<td>F 5: 0.05</td>
</tr>
<tr>
<td>HPMC K100M</td>
<td></td>
</tr>
<tr>
<td>Distilled water</td>
<td>100</td>
</tr>
</tbody>
</table>

### Table 2: Drug Content Uniformity

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Drug Content (% ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F 1</td>
<td>99.3 ± 0.45</td>
</tr>
<tr>
<td>F 2</td>
<td>97.8 ± 0.11</td>
</tr>
<tr>
<td>F 3</td>
<td>98.7 ± 0.75</td>
</tr>
<tr>
<td>F 4</td>
<td>96.0 ± 0.60</td>
</tr>
<tr>
<td>F 5</td>
<td>97.1 ± 0.05</td>
</tr>
</tbody>
</table>

### Table 3: Gel Formation of Gellan Gum with Simulated Lacrimal Fluid (SLF)

<table>
<thead>
<tr>
<th>Concentration of Gellan Gum</th>
<th>Gelling Property in SLF</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1%</td>
<td>-</td>
</tr>
<tr>
<td>0.2%</td>
<td>-</td>
</tr>
<tr>
<td>0.3%</td>
<td>+</td>
</tr>
<tr>
<td>0.4%</td>
<td>+</td>
</tr>
<tr>
<td>0.5%</td>
<td>+</td>
</tr>
<tr>
<td>0.6%</td>
<td>++</td>
</tr>
<tr>
<td>0.7%</td>
<td>++</td>
</tr>
</tbody>
</table>

- = no gelation + = immediate gelation for short period of time; ++ = immediate gelation and remains for extended period of time

### Table 4: Diameter Zone of Inhibition

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Mean ± S.D (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gatifloxacin In-situ Gel formulation (F 3)</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>6.8 ± 0.28</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>4.5 ± 0.28</td>
</tr>
</tbody>
</table>

### Table 5: Observations for HET-CAM Test

<table>
<thead>
<tr>
<th>Test substance</th>
<th>Score</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.9% NaCl</td>
<td>0</td>
<td>Non-irritant</td>
</tr>
<tr>
<td>Formulation (AN/GFXGFS/07)</td>
<td>0</td>
<td>Non-irritant</td>
</tr>
<tr>
<td>1 % NaOH</td>
<td>14.27</td>
<td>Severe irritant</td>
</tr>
</tbody>
</table>
Rheological Studies

Viscosity of the formulation was determined by Brookfield viscometer (LVT model). To assess the gelation of formulation on instillation and mixing with lacrimal fluid, the viscosity measurements were also taken after diluting the formulation with the simulated lacrimal fluid (SLF). SLF comprised of 0.670 g sodium chloride, 0.200 g sodium bicarbonate and 0.008 g calcium chloride dihydrate and distilled water q.s to 100 g\(^{15,15,22}\) which simulated the cation content of lacrimal fluid.

Gelation Studies

The gelation studies were carried out in cylindrical tubes which were filled with 5 ml of simulated lacrimal fluid (SLF). 50 µl of formulation containing ponceau red dye was added with a conventional dropper to a tube containing SLF and was then examined visually for gel formation as shown in Table 3.

Isotonicity Evaluation

Isotonicity, which is an important characteristic of the ophthalmic formulations, had to be maintained to prevent tissue damage and irritation to the eye.\(^{23}\) Smear of re suspended RBCs with Gatifloxacin in-situ gel formulation was prepared and observed under the polarizing microscope (Leica) at 45x magnification. Same procedure was followed for the marketed Gatifloxacin eye drops (Gatiquin\(^{TM}\)), isotonic solution (negative control) as well as hypertonic and hypotonic solution (positive controls). Size and shape of the RBCs with developed Gatifloxacin in-situ gel formulation was compared to that with marketed Gatifloxacin eye drops (Gatiquin\(^{TM}\)) as well as with the positive and negative controls.

Gel Consistency Test

Gel consistency was studied using Texture Analyzer TA-XT Plus (Stable Microsystems). Gelation of the formulation was induced by adding SLF to it. Test was carried out in a standard back extrusion rig using 50 mm (diameter) container filled approximately up to 75% with the formulation. An extrusion disc of 45 mm diameter was positioned centrally over the sample container.

In-vitro Drug Release Study

The in-vitro drug release of the Gatifloxacin in-situ gel formulation was estimated using modified USP dissolution apparatus-1 (Electrolab). Whatman\(^{®}\) filter paper No 41 was placed inside the USP basket. It was then wetted by dipping in a solution of simulated lacrimal fluid for one minute to ensure the intimate contact of release medium with the formulation. Then 100 µl of the formulation was applied to it.

Fifty ml of simulated lacrimal fluid was filled in a beaker and basket was rotated over its surface. A 3-3 ml aliquots of samples were withdrawn at regular time intervals and replaced with an equal volume of fresh simulated lacrimal fluid. The samples were analyzed spectrophotometrically for Gatifloxacin content using double beam UV-visible spectrophotometer (Shimadzu 1700) at 286 nm.

Figure 2: In vitro Drug Release Study

In-vitro Transcorneal Permeation Study

Freshly excised goat corneas (collected from the slaughter house) were used to study the permeation of Gatifloxacin across the corneal membrane. Whole eyeballs of goat were procured from slaughter house and corneas were carefully removed along with 5-6 mm of surrounding sclera and washed with saline solution.\(^{24}\)

Cornea was tied to one end of the hollow cylindrical glass tube and the corneal surface was wetted by applying 14 µl of simulated lacrimal fluid to simulate the physiological condition of eyes. 100 µl of developed Gatifloxacin in-situ gel formulation was spread over the corneal surface. The cylinder was then dipped into 50 ml of SLF maintained at 37 ± 0.5°C so as to keep corneal surface in contact with SLF stirred by rotation at 50 rpm. A 3-3 ml aliquots of fluid were withdrawn at fixed time intervals over a period of 7 hr and was replaced with an equal volume of fresh SLF maintained at same temperature. Aliquots of withdrawn samples were analyzed for drug content using a double beam UV-visible spectrophotometer (Shimadzu 1700) at 286 nm.

In-vitro Antimicrobial Efficacy Study

Figure 3: Zone of Inhibition Exhibited by the Developed Gatifloxacin In-situ gel Formulation and Marketed Eye Drop (Gatiquin\(^{TM}\))
In-vitro antimicrobial efficacy was performed by agar diffusion test using a cup-plate technique\(^\text{19}\) developed Gatifloxacin in-situ gel formulation, marketed eye drops of Gatifloxacin (Gatiquin\(^\text{TM}\)) and placebo were poured in cups bored into sterile nutrient agar previously seeded with *P. aeruginosa* (ATCC 27853) and *S. aureus* (ATCC 25923). After allowing diffusion of solution for 2 h, the agar plates were incubated at 37 ± 0.5°C for 24 h. The diameter of zone of inhibition (ZOI) measured around each cup was compared with marketed eye drops as well as positive and negative controls. The entire operation except the incubation was carried out under laminar air flow unit.

**Ocular Irritation Study (HET-CAM Test)**

For the present study, HET-CAM test (Hen’s Egg Test-Chorio Allantoic Membrane) was carried out.\(^\text{25}\) This test is used for the detection of ocular corrosives and irritants. The potential ocular irritancy of a test substance was measured by its ability to induce toxicity in the Chorio Allantoic Membrane of a chick embryo. Fertilized hen’s eggs weighing between 50-60 g were procured from poultry farm. The eggs were then candled to discard the defective ones and were then incubated in a humidified incubator at 37°C temperature and 75 ± 5% RH. The trays containing eggs were rotated manually in a gentle manner every hour. After ninth days, a window (2x2 cm) was cut on pointed end of eggs through which 0.2 ml of Gatifloxacin in-situ gel formulation was instilled. A 0.9% NaCl solution was used as a negative control because it is reported to be practically non irritant being isotonic and physiologically compatible and 1% NaOH as positive control in present study. After instillation of the formulations, the scores were recorded.

**RESULTS**

The compositions of various formulations of eye drops are shown in Table 1. These formulations were evaluated for drug content uniformity as shown in Table 2. Different concentrations of gellan gum, i.e., 0.1-0.7% were studied for the gelling property in physiological conditions. Only 0.6% gellan gum solution exhibited desired flow characteristics and resulted in instantaneous gelation in simulated lacrimal fluid which was retained for an extended period of time as shown in Table 3. HPMC K100M was incorporated as a release retardant in the formulation. All the formulations were clear, having pH 6.0-6.3 and resulted in gel formation in SLF, clearly indicating phase transition behavior in physiological conditions of eye. Gatifloxacin in-situ gel formulation was found to be isotonic, as it exhibited no change in the size and shape of RBCs as shown in Fig. 1 and in-vitro drug release as shown in Fig. 2. Combination of 0.6% gellan gum and 0.4% HPMC was selected, as it had satisfactory attributes of in-situ gelling property, flow characteristics and prolonged in-vitro release over the duration of 8 hr with 90.6% release in 8 h. Viscosity of the formulation was found to be 52 cps in solution form and 325 cps in gel form. Gel was further evaluated for its consistency.

Gatifloxacin in-situ gel of viscosity 325 cps exhibited consistency of 216.592 g/sec, firmness 28.344 g, index viscosity-37.465 g/sec, and cohesiveness-17.896 g. The in-vitro trans-conorneal permeation study across excised goat’s cornea exhibited 74.8% drug permeability in 7 hr with apparent permeability coefficient of 8.25 x 10\(^{-5}\) cm/sec. Diameter of zone of inhibition observed with the developed formulation was higher than that of marketed preparation Gatiquin\(^\text{TM}\) as shown in Table 4 and graphically represented in Fig.4. Gatifloxacin in-situ gel formulation was found to be non irritant to eyes exhibiting mean score of zero in HET-CAM test for ocular irritancy for 5 min as shown in Table 5.

**DISCUSSION**

Gellan gum is an anionic heteropolysaccharide which forms clear gel in the presence of monovalent and divalent ions present in cul-de-sac of the eye. Different concentrations of gellan gum, i.e., 0.1-1% were evaluated for the gelling property in physiological conditions out of which 0.6% resulted in instant gelation, and retained for an extended period of time. HPMC K100M was employed as a release retardant gave desirable results in concentrations range of 0.3-0.5%. As, isotonicity is a desirable attribute of an ophthalmic formulation, sodium chloride and boric acid were studied as an isotonicity adjusting agents. Sodium chloride imparted gelation of the formulation in-vitro, hence, boric acid was selected as an isotonicity adjusting agent. Phenyl mercuric nitrate was used as a preservative in the formulation. 0.05% of disodium edetate was also added to enhance the solubility of Gatifloxacin in water and prevent its crystallization in freeze thaw conditions. Formulations were sterilized by autoclaving at 121°C temperature, 15 psi pressure for 15 min.

Combination of 0.6% gellan gum and 0.4% HPMC K100M was selected as an optimum composition, as it exhibited desirable flow characteristics, physico-chemical properties and in-vitro drug release. By the in-vitro drug release study, it was found that gel has ability to retain Gatifloxacin for the entire duration of study (8 hr).

There was 6 folds increase in the viscosity of gel as compared to the sol form. It exhibited pseudoplastic behavior as there was shear thinning with the increase of angular velocity.

Gatifloxacin in-situ gel formulation (F 3) was evaluated for the isotonicity and was also compared with the marketed eye drops, isotonic solution (negative control), hypertonic and hypotonic solution (positive controls). Hypertonic solution resulted in shrinkage of the cells and hypotonic solution caused bursting of the cells. Hence, it was confirmed that formulation is isotonic to eye. It was also compared with that of marketed Gatifloxacin eye drops (Gatiquin\(^\text{TM}\)).

Transcorneal drug permeation study for Gatifloxacin in-situ gel formulation was performed in the SLF (pH 7.4 and 37 ± 0.5°C) which exhibited the delayed release of drug
from the polymeric matrix. Developed formulation was also found to be non-irritant to eyes. It was tested for irritation on the Chorio Allantoic Membrane of the chick embryo, which is a complete tissue including veins, arteries and capillaries and responds to injury with a complete inflammatory process, a process similar to that induced in the conjunctival tissue of rabbit eyes.

Overall values of the diameter of zone of inhibition against *P. aeruginosa* (for F3 it was 6.6 cm) were higher than that of *S. aureus* (for F3 it was 4.3 cm).

The higher values obtained for the developed formulation in comparison to the marketed eye drops which was 5.3 cm for *P. aeruginosa* and 4 cm for *S. aureus* could be attributed to slow and prolonged diffusion of the drug from the polymeric solutions.

**CONCLUSION**

It can be concluded on the bases of results and observations that the developed Gatifloxacin *in-situ* gel formulation can overcome the drawbacks of the conventional ocular dosage forms.

The developed formulation provided efficient therapy through prolonged drug release of the drug over an 8-hr period *in-vitro*. It exhibited better antimicrobial efficacy when compared with the marketed eye drops. Formulation was isotonic and devoid of any irritant effect to the eyes.

The ease of administration along with its ability to provide sustained release could result in decrease in frequency of administration thus enhancing the patient compliance.

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**REFERENCES**


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*# Erratum in page 51:* Corresponding author affiliation details are included based on corresponding author request, which were not included in initial publication. Article with errata is re-published on September 27, 2020.