INTRODUCTION

In-situ gel formation is facilitated by many natural and synthetic polymers, which may find application in oral, vaginal, ophthalmic, rectal, buccal, intraperitoneal, and parenteral drug delivery. 1,2 A lot of research has been done on in-situ gels as an eye medication delivery technology to improve bioavailability and ability. 3,4 Many benefits are provided by in-situ gel forming polymeric formulations, such as prolonged and sustained activity that is comparable to that of a conventional drug delivery system. 5,6 The translated version of the Latin expression “In-situ” is “in process.” Before being dispersed into the body, drugs are distributed via in-situ gels, which undergo in-situ gelation upon administration to create a gel. It is primarily a drug delivery structure made of polymers. 7,8

The sclera is the top layer of the eye. The choroid is in the middle, the lens is in the middle, and the retina, which houses the nervous system, is at the bottom. Together, they make up the eye wall. In simple terms, the eye is round. The cornea is the clear front part of the eye that lets light in. The white part inside the eye is protected by a tough, thick, flexible sclera that covers the rest of the eye. The colored iris makes the hazel, green, grey, blue, and brown parts of the eye stand out. On the front of the eye, the choroid layer, which is in the cornea and has many blood vessels, 9–11 takes its place.

Because the eye drops come out of the eyes so quickly when you smear them, they must not be very bioavailable. 12 Because the eye is built in a way that makes it take longer for drug particles to get where they need to go, the most interesting part of a medical scientist’s job is moving drugs through the eyes. There are more than 90% eye drops on the market. For some reason, they don’t work well in the eye after topical treatment. 13–15 This is because they have a unique cleaning process. Applying medicine directly to the conjunctiva and cul-de-sac is one way to treat conditions that affect the front part of the eye. Increasing the corneal transparency and lengthening the time that drugs stay in the cul-de-sac can make them more bioavailable in the eye. There are a lot of new and different dose types on the market, like collagen, shield, and in-situ gel. Most of the time, this preparation is not appropriate because it blurs vision. This includes gels, suspensions, ointments, and polymeric implants. This problem is meant to be fixed.

ABSTRACT

The goal of this study was to create and test various in-situ gel formulas for administering posaconazole to the eye to treat fungal keratitis. They used an in-situ gelling method to help posaconazole stay in the eye mucosa longer, which made it easier for the body to use. To make in-situ gel preparations, polymers such as sodium alginate, poloxamer 407, and poloxamer 188 were used in a cold method. Finally, there was 0.2% (w/w) posaconazole in the mixtures. The pH, drug content, viscosity, gelling capacity, and temperature at which the solution turns into a gel were all checked on the recipes. It was between 32 and 34°C when each blend turned into a gel. There was about the same amount of drugs in all of them. It was also worked out how much of the antifungal and in-vitro drugs these mixtures would release. Everyone in the drug release study showed signs of long-lasting release. To sum up, in-situ gels that contain posaconazole could be a good way to use optical drug delivery to change how fungus diseases act.

Keywords: Ocular delivery, In-situ gel, Posaconazole, Corneal toxicity, Irritation testing.

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New Posaconazole-Loaded Ocular In-situ Gel Design

<table>
<thead>
<tr>
<th>Codes</th>
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<th>S2</th>
<th>S3</th>
<th>S4</th>
<th>S5</th>
<th>S6</th>
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<tr>
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<td>15</td>
<td>10</td>
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<td>10</td>
<td>15</td>
<td>20</td>
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<tr>
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<td>10</td>
<td>15</td>
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<td>10</td>
<td>15</td>
<td>20</td>
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<td>7.00</td>
<td>7.64</td>
<td>6.99</td>
</tr>
</tbody>
</table>

by the in-situ gel invention for the ocular medication release method.16–19 It is thought that making a drug layer thicker in the pre-corneal area will increase its solubility because the cornea drains slowly. However, in-situ gels must be made of fluids that don’t stick together and flow easily so that results can be repeated.20,21 This in-situ gelling arrangement has benefits such as being easy to use, causing less harm locally and generally, and helping with long-term and sustained medication relief. Some of the problems are that bigger doses can’t be directed, the automatic strength isn’t very high, and biological degradation can make the material unstable without meaning to.22–24

Poloxamers are artificial triblock copolymers having a mass ratio of 4:24 and a core hydrophobic polyoxypropylene sequence surrounded by two hydrophilic series and two water-soluble polyoxyethylene chains.25–28 Poloxamer 188 (P188), a non-ionic direct copolymer, FLOCOR, PLURONIC F68, and RheothRx, has a mean molecular weight of 8400 Daltons. P188 has an 18-hour half-life and is safe for 72 hours. P188 should repair cell membranes,29,30

Like ravuconazole, albaconazole, voriconazole, and azaconazole, posaconazole is a member of the second generation of triazole groups. It works better than the first drugs in this group at killing germs that cause wounds.31,32 It was chosen as an active helper because it does a lot of different things. The drug has been through phase III tests and has been approved by regulators to treat and stop fungal infections that are getting in the way. Zingomycetes, Aspergillus, Candida, and Cryptococcus neoformans are some of the molds that are affected. It is made up of the same building blocks as itraconazole.33–35

Fungal keratitis is a corneal disease that can be caused by problems with the immune system or by yeast, bacteria, viruses, fungi, or amoebas getting into the eye.36 When people use steroids and broad-spectrum antibiotics, plant and dirt debris can get into corneal ulcers, people who wear contact lenses, and eye injuries are all things that can make fungal keratitis spread.37 If you want to escape complications and losing your sight, you need to treat fungal keratitis as soon as possible.38

MATERIALS AND METHODS

Materials
Posaconazole was a gift from Honour Labs Ltd. in Telangana, India, and sodium alginate was bought from Nice Chemicals in Ahmedabad, India. Loba chemistry in Mumbai, India, was where the benzalkonium chloride was bought. Poloxamers 407 and 188 were kindly given to us by BASF in Turkey. We bought a Spectra spectra/pro 4 rc dialysis membrane (12-14 kDa mw, 23.8 mm diameter, flat width-33.12 mm, 4.45 mL/cm capacity) from Himedia Laboratories Pvt. Ltd. in Mumbai, India.

Method
Preparation of in-situ gel
We use gelling agents like poloxamer Analogus in the cold way of in-situ gel formation. The in-situ gel’s polymeric solution is made up of poloxamer 407(25) and poloxamer 188 (7.5). These were mixed with cold water and a magnetic mixer for two hours or until the mixture was completely dissolved. A dispersed polymeric solution is made, and it is then put in the fridge for 48 hours to make a clear solution.

Determination of solution gel temperature-
There was water in a clear beaker that had 10 mL of polymeric solution in it. The setup was put on a magnetic mixer that turned at least 200 times per minute. The temperature of the water bath was slowly raised to a maximum of 2°C while it was being stirred all the time. A thermometer was put into the sample fluid to keep track of the evaluation as it went on. The gelation temperature is when the magnetic bar stops moving and gels. Table 1 shows pH, poloxamers concentration and gelling temperature of the formulation.

Formulation of posaconazole loaded in-situ gel
It was possible to put in-situ gels into groups based on their chemical makeup, gelling temperature, and pH levels. It was decided that S4, which had 25% P407 and 7.5% P188, was the best formulation for planning an ophthalmic formulation. Three different concentrations of sodium alginate were found to have the best in-situ gel alignments. The same amount of posaconazole was added to the liquid poloxamers for each preparation, and the mixture was mixed continuously until the posaconazole was evenly spread. It was made better to use benzalkonium chloride (0.02% w/w) as a stabilizer in the
Table 2: Formulation batches of in-situ gel

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>F</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Posaconazole</td>
<td>-</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Poloxamer 188</td>
<td>7.5</td>
<td>7.5</td>
<td>7.5</td>
<td>7.5</td>
<td>7.5</td>
</tr>
<tr>
<td>Poloxamer 407</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</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>
</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>
</tr>
<tr>
<td>Sodium alginate</td>
<td>-</td>
<td>-</td>
<td>0.2</td>
<td>0.4</td>
<td>0.6</td>
</tr>
<tr>
<td>Distilled water Q.S.</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

mixes. To maintain isotonicity, sodium chloride (0.9% w/w) was added. It was also determined how medicine and other mixture additives affected gelling temperature. Table 2 gives formulation batches of in-situ gel.

Characterization of in-situ gel
The developed ophthalmic formulation underwent various characterizations, including measurements of pH, gelling temperature, clarity, viscosity, drug content, and clarity.

pH
With the use of a standardized pH meter, the pH of gel was determined.

Gelling capacity
At 32 to 34°C in a beaker, a drop of ready formulation was located for determination of gelling capacity. Gelling time was visually noticed.

+ gel after few minutes the gel get quickly dissolved
++ For few minutes, instantaneous gelation retains.
+++ For approximately an hour the fast gelation rest

Drug content
To measure the medicine concentration of the posaconazole in-situ gelling combination, 100 mL of pH 7.4 simulated tear fluid and 1-mL of precisely weighed in-situ gel were mixed. Shaking for two to three minutes clarified and broke up the mixture. Posaconazole was 260 nm on the UV-visible spectrophotometer.

Viscosity
At 25 to 37°C with the help of Brookfield viscometer having spindle RV² and 100 rpm, by this method viscosity of the gel was examined.

Clarity
Below the white and black background with optical examination, the clarity of made solution was concluded. Turbid, +, clear, ++, and very clear +++.

Isotonicity
Keeping isotonicity high is crucial for ophthalmic products to avoid tissue damage and eye irritation. Salts exert osmotic pressure on water solutions. Osmometers measure osmotic pressure. The ocular product’s osmotic pressure should be 290 to 310 mOsmol/kg.

HPLC analysis
The HPLC setup had an Agilent 1100 UV detector and a gradient pump. A 4.6 x 150 mm, 5 μm C18 column from GL Sciences in Japan was used. The tests used a flow rate of 1-mL/min at 262 nm and 25°C. It was 70:30 methanol to acetonitrile in the mobile phase. The medicine was kept for two and a half minutes. The procedure’s LoQ, linearity, LoD, stability, selection, accuracy, and precision were all checked and found to be valid. Figure 1 shows HPLC chromatogram of Posaconazole.

In-vitro drug release study
For the in-vitro drug release study (Figure 2), the dialysis bag method was used. For the in-situ gel versions, they were tested for release in stirred tear fluid with a pH of 7.4 at 50 rpm. The temperature was kept between 32 and 34°C to mimic the temperature of the eye’s surface. A dialysis layer (Spectra/Pro, with a molecular weight of 12–14 kDa) was used to remove 5 g of the preparation from the discharge media. The preparation was then wrapped in ends. The sheath was cooked in bidistilled water at 33 ± 1°C for 30 minutes before it was used. At set times every 1 to 8 hours, 0.5 mL of the test were taken out and replaced with the same amount of fresh medium. Three times, the tests were seen.

Stability of the in-situ gel
Physical stability reports say that posaconazole overflows in-situ gels were kept for three months in stability cabinets at 25 ± 2°C and 40 ± C and in the fridge at 5 ± 1°C. After three months of loading, the posaconazol substance, optical presence, pH, gelation time, and in-situ gel quality were all checked. Three sets of trials were carried out.

Microbiological Studies

Sterility studies
The gel formation continued in a Haier HR40-IIA2 cabinet with laminar airflow, with or without posaconazole. The visual recipes were examined for sterility regulators to prevent reproduction. A clean environment sterility analysis of the in-situ gel with or without posaconazole authorized the universal pharmacopeia. Soybean casein abstract media was employed for aerobic bacteria and fungi. A liquid thioglycollate container held anaerobic bacteria. Each medium received 1-mL of formulation mixtures daily for 14 days. Fungi were kept at 25°C and bacteria at 35. The advancement test determined how effectively standard testing fluids dissolved in the sterility research. For the aerobe, anaerobe, and yeast growth elevation test, fluid thioglycollate carriers were filled with 100 CFU of Clostridium sporogenes ATCC 19404, Staphylococcus aureus ATCC 6538, and Candida albicans ATCC 10231. Microbes received different media quantities. For 48 hours, the procedures were maintained at 35°C.

Determination of MIC of posaconazole
The broth micro reduction assay followed CLSI standards for fungi and filamentous yeasts. Posaconazole was combined with dimethyl sulfoxide at a final concentration of 500 to
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Table 3: pH, gelling capacity, drug content, viscosity and clarity of *in-situ* gel

<table>
<thead>
<tr>
<th>Formulations</th>
<th>F</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ph</td>
<td>6.26 ± 0.02</td>
<td>6.99 ± 0.01</td>
<td>7.00 ± 0.01</td>
<td>7.03 ± 0.02</td>
<td>7.64 ± 0.01</td>
</tr>
<tr>
<td>Gelling capacity</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Drug content</td>
<td>-</td>
<td>84.24 ± 0.26</td>
<td>83.18 ± 0.34</td>
<td>81.21 ± 1.05</td>
<td>93.47 ± 0.49</td>
</tr>
<tr>
<td>Viscosity</td>
<td>338 ± 2.1</td>
<td>354 ± 3.6</td>
<td>378 ± 9.27</td>
<td>414 ± 11.35</td>
<td>457 ± 8.56</td>
</tr>
<tr>
<td>Clarity</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

0.125 µg/mL 0.0165M MOPS buffer (3-(N-morpholino)propane sulfonic acid) raised the RPMI 1640 intermediate buffer pH to 7.0. Spectrophotometric inoculum production involved 0.4 to 5×10⁴ spores/mL for molds and 1.5 (± 1.0) × 10³ cells/mL for yeasts. MOPS-buffering RPMI 1640 was employed. The proposed mixture was examined for its antifungal effectiveness against fungal keratitis-causing *A. flavus* (ATCC 204304 and 204305), *C. tropicalis* (RSKK 2421), and *C. albicans* (ATCC 1031). Each microdilution plate received 0.1 mL inoculum to strengthen it. Developable MICs were repaired after 48 hours. The same-rate well expansion rattled the plates. Each well’s development was compared to the posaconazole-free control well using an interpretive mirror. The MIC endpoints were calculated using the final medication dose that inhibited control well growth by 50%.

RESULT AND DISCUSSION

Several concentrations of poloxomers, which are made by polymers, were used to keep an eye on preliminary tests. The *in-situ* gelling organization is made up of different pH components, such as poloxamer 407, poloxamer 188, and sodium alginate. Poloxamer 407 and poloxamer 188 are very heat-sensitive, which helps make heat-sensitive *in-situ* gels by using the cold method. Ethylene oxide and propylene oxide gel too easily when heated too much, but poloxamer 407 can make drugs that don’t dissolve well in water dissolve better.

Determining appropriate gelling temperature both poloxamer 407 and poloxamer 188, sodium alginate, sodium chloride, and posaconazole are to be combined together at various concentrations. Table 3 displays various terms, including pH, concentration and gelling temperature of poloxamer.

**Description of *In-situ* Gel**

When describing thermosensitive *in-situ* gel formation, things like clarity, pH, viscosity, drug amount, gelling temperature, and gelling capacity are all taken into account.

**pH**

This pH is compatible with eyes and would not induce irritation inside the eye. The pH of the prepared polymeric solution is displayed in Table 3. The pH of the prepared polymeric solution ranges between 6.2 to 7.6.

**Gelation Temperature and Gelling Capacity**

The created thermo-sensitive *in-situ* gel formulation has the right viscosity, making it easy to instill as a liquid drop in the eye and enabling the formulations to dissolve quickly into the *in-situ* gel. If the concentration of gelling agent increases then gelling capacity will go on increasing.

Gelling capacity based on which type of polymers were added with their different concentration gelation temperature of prepared polymeric solution ranging between 31 to 34ºC. Sodium alginate is accountable (responsible) for reducing gelation temperature.

**Drug Content**

It was ensured that the medication content in the produced formulation was within permissible limits and that the dose was uniform. The produced formulation’s drug content ranged from 81.21 to 93.47%.

**Viscosity**

Table 3 shows the viscosity of the produced polymeric solution. When *in-situ* gel is injected into a conjunctival decubitus, its viscosity changes to a gel-forming state because the eye’s bioavailability is reduced. The produced polymeric solution of *in-situ* gel has a specified viscosity.

**Clarity**

*In-situ* gels are simultaneously subjected to visual inspection before and after gelling against a white or black backdrop in...
order to obtain a clan solution free of undesirable particles or opalescence. At temperature ranges of 4 to 25°C, ideal in-situ gels with transparent properties create clear solutions.

**Isotonicity**

The osmometer is used to measure tonicity. The measured tonicity of 0.298 or 298 mOsmol/kg, is within permissible bounds.

**HPLC**

*In-vitro drug release study*

Posaconazole gelling products F1 through F4 were put through in-vitro release tests. The release vehicle was simulated tear fluid with a pH of 7.4. Posaconazole formulations showed constant drug release for 8 hours. The amount of posaconazole released in-vitro from formulations F1, F2, and F3 was found to be 68, 62, and 64%, respectively, after 8 hours (p > 0.05). In contrast to the other formulations, the F4 one showed a delayed release. This could be an attempt to get more sodium alginate into the formulas that are being made.

**Stability**

Stability cabinets were used for the three-month stability investigations, which were conducted at 5 to 25°C. The samples’ visual appearance, clarity, and gelation time were all confirmed to be unchanged by the monthly periodic analysis.

**Studies on microbiology**

The superior in-situ gels accepted the purity test since the liquid thioglycollate medium at 35°C for 14 days and the soya bean casein digest medium at 20 to 25°C showed no turbidity growth or bacterium growth. To ensure the growth promotion test and sterility test media worked, both species of bacteria had to be present in all mediums.

**MIC of posaconazole**

The MIC values for the final medication concentration that slowed control well progress by 90 and 50% were discovered. Posaconazole has MIC90 values of 1, 2, 0.5, and 0.5 µg/mL against C. tropicalis, C. albicans, A. flavus, and A. fumigatus, in order. The MIC50 values for all species were 0.25 µg/mL. The MIC values for A. flavus and A. fumigatus from outdoor strains and clinical isolates matched our investigation. Aspergillus sp. has a 1-mg/L MIC90. Both bacteria had MICs between 0.2 and 5 µg/mL. For A. flavus detachments, the range was 0.125 to 1-µg/mL and 0.25 to 1-µg/mL. Candida species with CLSI M27-A3 MIC values ≤1-µg/mL are considered liable, 2 µg/mL requires susceptibility, and 4 µg/mL or higher is challenging.

**CONCLUSION**

We chose to use poloxamer 407 because it is an important part of making in-situ gel and has great qualities, such as being a thermo-sensitive copolymer. Poloxamer 188 and poloxamer 407, on the other hand, are used together in a polymeric solution mixture. The in-situ glue is successfully made using the cold method. Many measurements were taken, such as pH, viscosity, clarity, gelation, temperature, medication content, stability, and study that was done in a lab. Based on this study, the developed in-situ gel product might be able to deliver posaconazole to the eyes.

**REFERENCES**


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