Development and characterization of posaconazole loaded in situ gel formulation for ophthalmic application

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Abstract

The objective of this research was to formulate and evaluate the latent use of the *in situ* gel preparations for ocular delivery of posaconazole for the treatment of fungal keratitis. An in situ gelling system was used to rise the residence time and thus the bioavailability of posaconazole in ocular mucosa. In situ gel preparations were formulated by cold method using polymers like poloxamer 407, poloxamer 188 and sodium alginate. Finally, concentration of posaconazole in formulations was 0.2% (w/w). These formulations were evaluated for pH, solution-gel transition temperature, gelling capacity, drug content, viscosity and clarity. Gelation temperature of each and every one of the preparations was within the range of 32-34ºc. All the preparations exhibited fairly constant drug content. Also in-vitro drug release and anti-fungal action of these preparations were also estimated. Drug release study of all the preparations exhibited sustained release properties. In conclusion, posaconazole loaded in-situ gels could be presented as a promising approach for optical drug delivery for the behavior of fungal diseases.

Keywords: Ocular delivery, in situ gel, posaconazole, microbiology study

1. Introduction

Different natural and synthetic polymers undertake *in situ* gel forming and probably can be used for oral, vaginal, ocular, rectal, buccal, intraperitoneal and parenteral drug delivery [1, 2]*In situ* gels have been widely investigated as ocular drug distribution system to increase bioavailability and ability [3, 4] The *in situ* gel developing polymeric formulations offers numerous advantages like sustained and extended action in similarity to standard drug delivery system [5, 6]. "In process" is the translated form of a Latin phrase "*In situ*". *In situ* gels are drug distribution systems which are in liquid forms before dispensation in the body, when administered they undertake gelation *in situ* to form a gel. It is mainly a polymeric drug delivery structure [7, 8]

An eye is a spherical arrangement with a wall built up of three sheets: sclera the outer portion, the central part choroid layer, iris and ciliary body and the internal section nervous tissue level retina. The sclera is rough fibrous coating that shielding the inner matter of eye which stays white excluding for the transparent region at the anterior, namely cornea which permits light to pass into the eye. Choroid layer, located in the sclera, which holds many blood vessels that revised at forward-facing of the eye as pigmented iris the highlighted part of the eye (hazel, green, grey, blue, brown,)[9-11].

The eye drops need much reduced bioavailability due to their prompt washout during lachrymation in eyes [12]. Due to special structure of the eye which slows down the arrival of drug particles into the craved site, the most stimulating task for a medicinal scientist is done through the ophthalmic transfer of the drug. In the markets more than 90% of ophthalmic preparations explants to eye drops. By distinct elimination mechanism after topical direction it results in low ocular bioavailability (<5%) when they are washed off from the eye.[13-15] The therapy for the diseases of the previous segment of the eye is done when the drug in the conjunctivalon cul-de-sac is administered by topical route. The ocular bioavailability of drugs can be improved by prolonging their residence time in the cul-de-sac and by increasing their corneal permeability. There are various new dosage forms like *in situ* gel, collagen, and shield. Gels, suspension, ointment or polymeric implants produces blurred visualization on eye so that this preparation is not acceptable in most of the situations. This problem is projected by using *in situ* gel invention for ocular drug release system [16-19].

In situ gels must be free flowing; low viscous fluids to allow for reproducible believed that increasing the thickness of a drug creation in the pre-corneal area will lead to enlarged bioavailability due to gradual drainage speed from the Cornea [20, 21].

This *in situ* gelling arrangement shows advantages which deals ease of administration, reduces local and general toxicity, and help in lengthy and prolonged relief of drugs. The drawbacks are only lesser doses can be directed, it may result in early dissolution due to low automated strength, and due to biological degradation, there is a unintended of instability[22-24].

Poloxamers *in situ* forming system in optical drug delivery as of their thermo responsive developing behavior, biocompatibility and simplicity of sterilization. Poloxamer possess changeable thermo responsive forming and mucoadhesive properties. Poloxamer specifies sustained release of both minor and great drug particles. Poloxamer 407 has stayed answerable for lipidic profile change and possible renal poisonousness which compromises its expansion for parenteral use. Poloxamer 407 formulation indicates to enhanced solubilisation of weakly water soluble drugs and extended release profile in many galenic applications. Poloxamers are artificial triblock coploymers collected of a central of hydrophobic sequence of polyoxypropylle lined by two hydrophobic series of polyoxypropylene lined by two water-soluble chains of polyoxyethylene with a mass ratio of 4:24.[25-28] Poloxamer 188 (P188) is a non-ionic direct copolymer having an mean molecular weight of 8400 Daltons and is also stated to a FLOCOR, PLURONIC F68, and RheothRx, P188 takes half-life of 18 hours and remained demonstrated to be harmless when given for equal to 72 hours predictable characteristics of P188 is its skill to repair injured cell membrane[29, 30].

Posaconazole is a second origination triazole group associate like ravuconazole, albaconazole, voriconazole, and isavuconazole has greater effectiveness and possesses greater activity against conflict and evolving pathogens.[31, 32] Posaconazole has also stayed inspected in phase III studies and agreed by the regulatory interventions for the medication and prophylaxis of interfering fungal infections; hence , posaconazole was chosen as an active mediator considering its wide-ranging spectrum activity.[33,34] it is structurally related to itraconazole and has activity against *candida* species, *Asperigillus* species, *Cryptococcusneoformans*, the *zygomycetes* and filamentous fungi.[35]

Fungal keratitis is disease of the cornea, consequences from direct contaminations with yeast, bacteria, viruses, fungi, and amoebae or from immune linked complications [36]. Many factors responsible for expansion of fungal keratitis includes use of broad spectrum antibiotics and steroids, contamination of corneal lesions by soil and plant material, use of contact lenses, ocular trauma[37]. Initial diagnosis of fungal keratitis and its cure is important in preventing difficilties and loss of eyesight [38].

Table 1: pH, poloxamers concentration and gelling temperature of the formulation.

Codes	S1	S2	S3	S4	S5	S6	S7
Poloxamer 188	20	15	10	7.5	10	15	20
Poloxamer 407	5	10	15	25	10	15	20
pH	6.98	6.26	6.24	7.03	7.00	7.64	6.99
Gelling temperature	30-32	42-43	41-42	31-32	28-29	39-40	32-33

2. Materials and Methods-

Materials-

Posaconazole was kindly gifted from Honor Labs Ltd. Telangana, India and sodium alginate was obtained from the Nice Chemicals, Ahmedabad, India. Benzalkonium chloride was obtained from Loba chemicals, Mumbai, India. Poloxamers 407 and poloxamer188 were kind gifted from BASF, turkey. Spectrum spectra/pro 4 rc dialysis membrane (12-14 kDa mw, 23.8 mm diameter, flat width-33.12 mm, 4.45 ml/cm having approx. capacity).was purchased from Himedia Laboratories Pvt. Ltd. Mumbai, India

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Method

Preparation of *in situ* gel

Gelling agents such as poloxamer Analogus are used for the formulation of *in situ* gel with the use of cold method

In situ gel consist of polymeric solution like combination of both poloxamer 407(25) and poloxamer 188 (7.5) were dissolved in cold water with application of magnetic stirrer until it get completely dissolved for 2 hr.

Dispersed polymeric solution is formed to get a clear solution and the formed polymeric solution was kept in a refrigerator for 48 hrs.

Determination of solution gel temperature-

A transparent beaker containing 10ml of polymeric solution was kept in a water bath. The set up was placed on magnetic stirrer at minimum200 rpm.The temperature of water bath was increased slowly or moderately up to 2ºC with continuous stirring. A thermometer was dipped into the sample solution to observe continuous assessment.

Gelation temperature is known as the temperature at which movement of magnetic bar stops due to form of gelation.

Table 2: Formulation batches of *in situ* gel

Ingredients	F	F1	F2	F3	F4
Posaconazole	-	0.2	0.2	0.2	0.2
Poloxamer 188	7.5	7.5	7.5	7.5	7.5
Poloxamer 407	25	25	25	25	25

Sodium chloride	0.9	0.9	0.9	0.9	0.9
Benzalkonium chloride	0.02	0.02	0.02	0.02	0.02
Sodium alginate	-	-	0.2	0.4	0.6
Distilled water Q.S.	100	100	100	100	100

Formulation of Posaconazole loaded *in situ* gel-

In situ gels were designated according to gelling temperature and pH values and of formulations. S4 (percentage of P407 and P188 were 25% and 7.5%, correspondingly) was selected as optimal formulation for organization an ophthalmic formulation .After recognition of the finest *in situ* gel alignments of sodium alginate of various concentrations (0.2%,0.4% and 0.6%) and for every single preparations same strengths of posaconazole were supplemented in poloxamers liquids with continuous mixing until completely dispersed .Benzalkonium chloride(0.02% w/w) was enhanced as a preservative to the mixtures. Sufficient quantity of sodium chloride (0.9% w/w) was augmented to the solution to hold the isotonicity. The outcome of drug and the further compositions of preparation on gelling temperature were also estimated.

Characterization of *In Situ* Gel-

Various characterization were performed for the prepared ophthalmic formulation like pH, gelling capacity, clarity, viscosity, drug content, gelling temperature was determined.

pH-

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

Gellingcapacity-

At 32 - 34ºC in a beaker a drop of ready formulation was located for determination of gelling capacity. Gelling time was visually noticed. It was graded as follows:

+ Gel after few minutes the gel get quickly dissolved

++ For few minutes instantaneous gelation retains.

+++ For approximately an hour the fast gelation rests

Drug Content-

In 100 ml simulated tear fluid having pH 7.4 1 ml of accurately weighed quantity of developed in situ gel was dissolved to determine the drug content of posaconazole in situ gelling formulation. To dissolve it completely the formulation was shaken for 2 to 3 min to get a clear solution. By referring the UV visible spectrophotometer the concentration of posaconazole was determined at 260nm.

Viscosity-

At 25 to 37ºC with the help of Brookfield viscometer having spindle RV^2 and 100 rpm, by this method viscosity of gel was examined.

Clarity-

Below white and black background with optical examination the clarity of made solution was concluded. It was graded as: turbid, +; clear, ++; and very clear +++.

Isotonicity-

Isotonicity is main characteristics of ophthalmic preparation which has to be preserved to avoid irritation to the eye or any tissue damage. It denotes to the osmotic pressure applied by salts in aqueous solutions. Tonicity is measured by using osmometer. Ophthalmic preparation must have osmotic pressure within the range of 290-310 mOsmol/kg.

HPLC analysis-

The HPLC arrangement consisted of a UV detector supplied by Agilent 1100 and gradient pump. C18 column (5µm, 4.6x150mm) (GL sciences, Japan) was used. The tests were analyzed at flow rate of 1 ml/min at 25ºC and at 262 nm. The mobile phase with a mixture of methanol: acetonitrile (70:30). Retention time of drug was 2.6 min. The method was validated for limit of quantitation

(LOQ), linearity and limit of detection (LOD) stability and selectivity, specificity, accuracy and precision.

In Vitro Drug Release Study

In vitro drug release analysis was done by using the method of dialysis bag. *In vitro* release reading of *in situ* gel preparations was carried out in stirred tear fluid at 50rpm(pH-7.4). The temperature was retained at 32 to 34 °C to represent eye external temperature. 5gm of preparation was divided from discharge media by processes of dialysis layer (Spectra/Pro, molecular weight of 12-14 kDa) and covered with endings. The sheath was heated for 30 minutes at 33±1ºC in bidistilled water earlier use. 0.5ml of test was withdrawn at a prearranged time pause of 1 hour to 8 hour and the similar volume of new medium was exchanged. For three times the experiments were frequented.

Stability of the *in-situ* gel-

In physical stability reports, posaconazole overloaded *in situ* gels at 5±1ºC were kept in the refrigerator and in the stability cabinets 25±2°C and 40±°C for 3 months were stored After loading for 3 months optical presence, pH, gelation time, clarity of *in situ* gels and posaconazol substance were inspected. The experiments were repeated for 3 times.

Microbiological Studies:

Sterility Studies

In situ gel creation in the existence or absence of posaconazole stayed equipped at laminar air movement cabinet(Haier HR40-IIA2).To the check infertility of the prepared visual formulations sterility regulator testing were implemented. Sterility analysis of the *in situ* gel with or without posaconazole was approved out under sterile conditions allowing to the universal pharmacopoeia. For aerobic and fungi microorganisms soya bean casein abstract medium was used. For anaerobic bacteria liquid thioglycollate vehicle was used. 1 ml of formulation mixtures was put on to every single medium and incubated at 25ºC for fungi and 35ºC for bacteria for 14 days.

To detect the solubility of the habituated mediums for the sterility studying advancement test were completed. For growth elevation test of aerobes, anaerobes and yeasts, fluid thioglycollate vehicles (using separate quantity of media for each microbe) were injected with 100 CFU of

Clostridium sporogenes ATCC 19404, Staphylococcus aureus ATCC 6538, and Candida albicans ATCC 10231.methods were protected for 48 hr at 35ºC.

Determination of MIC of posaconazole

The broth micro reduction assay was done in understanding with CLSI guidelines for filamentous yeasts and fungi. Posaconazole was liquefied in dimethyl sulfoxide, ending dilution were completed RPMI 1640 intermediate buffered to pH 7.0 with 0.165M MOPS buffer [3-(N-morpholino) propane sulfonic acid] and last concentrations were 500-0.125µg/ml. By means of the spectrophotometric process of inoculum planning, an inoculum attention of 0.4-5x10^4 spores/ml for moulds and 1.5 (±1.0)x10^3 cells/ml for yeasts and RPMI 1640 average buffered with MOPS were expended. A. flavus. ATCC 204304, A. flavus. ATCC 204305, C.tropicals RSKK 2421. C.albicans ATCC 1031 which might be possible reasons of fungal keratitis remained used to estimate antifungal action of developed preparation. A 0.1 ml inoculum was enhanced to each fine of the micro dilution plates. The MICs were fixed after 48 hr. of maturation. The plates were trembled before the similarity of growth in wells. With a help of an interpretation mirror, development in each well was releated with the posaconazole free growth control well. The MIC endpoints were estimated for the final drug concentration that displayed a protruding reduction (50%) of the development in the control well.

3. Result and Discussion

Preparatory studies were monitored by using several concentrations of poloxomers which is resulting by polymers. *In situ* gelling organization contain different compositions pH components like poloxamer 407, poloxamer 188, sodium alginate, Poloxamer 407 and poloxamer 188 are extremely thermo-sensitive in nature that help in preparation of heat sensitive *in situ* gels by cold method. Ethylene oxide and propylene oxide having excessive heat sensitizing gelling feature while poloxamer 407 has ability to intensify solvability of poorly water soluble drug.

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

Characterization of *in situ* gel

Characterization of thermo-sensitive *in situ* gel formation present clarity, pH, viscosity, drug content, gelling capacity, gelling temperature.

pH

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

Gelation temperature and gelling capacity

The prepared thermo-sensitive *in situ* gel formulation having appropriate viscosity which helpful for easy instillation in the eye as liquid drop and would also allow the formulations to goes into rapid solution to gel formed *in situ*. If concentration of gelling agent increases then gelling capacity will goes on increasing.

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

Drug content

The drug content of the prepared formulation was ensured dose uniformity and within acceptable limits. Drug content of the prepared formulation was found to be 81.21 to 93.47%

Viscosity

Viscosity of prepared polymeric solution was displayed in Table 3.The viscosity of prepared polymeric solution of *in situ* gel having specific viscosity primarily *in situ* gel is in solution form but when *in situ* gel instilled into conjuctival de-sac it goes to forming gel due to lower bioavailability of eye.

Clarity

Simultaneously before and after gelling of *in situ* gels undergoes visual inspection against white or black background to get clan solution without disturbance and unwanted particles or opalescence. Ideal in-situ gel carry transparent property at temperature ranges 4-25^{0}C forms clear solution.

Isotonicity-

Tonicity is measured by using osmometer. Tonicity was found to be 0.298 Osmol/kg i.e. 298 mOsmol/kg which is within the acceptable limit.

HPLC

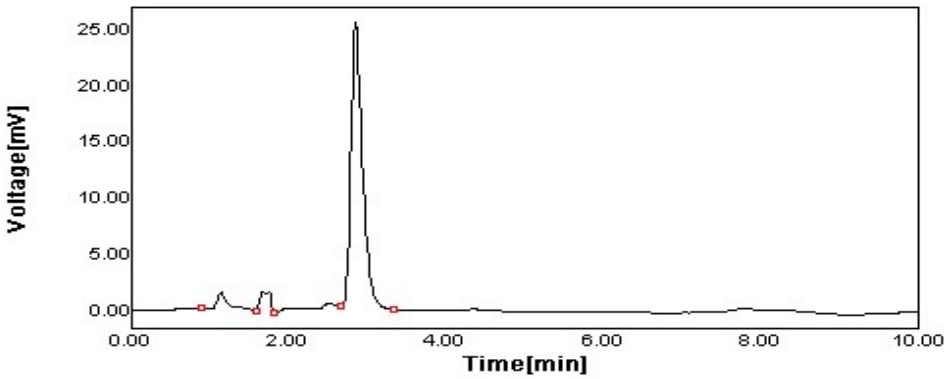

Figure 1 HPLC Chromatograph of Posaconazole

***In vitro* drug release study**

The *in situ* gelling preparation of posaconazole, F1-F4, were expose to in vitro release studies, which were passed out using simulated tear fluid of pH7.4 as release vehicle. Posaconazole formulations showed sustained drug release for a time of 8 hrs. At the end of 8 hr., in vitro posaconazole release from F1,F2 and F3 formulations was found as 68%, 62% and 64% correspondingly ($p>0.05$). F4 formulation was exhibited slower release than the other

preparations. This could be aim of higher concentration of sodium alginate between the developed formulations.

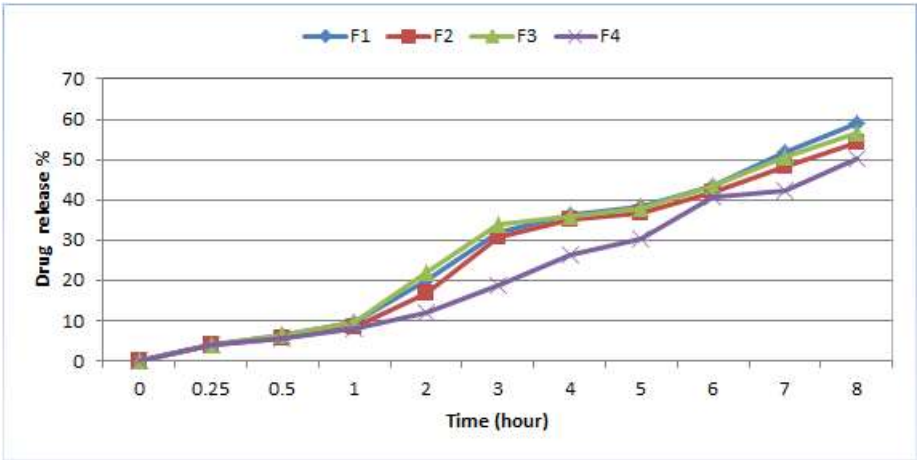

Figure 2 Posaconazole release study from in situ gel

Stability

The stability studies were carried out at 5 to 25ºC for 3 months using stability cabinets. The samples were analyzed periodically on every month and found that there are no changes in visual appearance, clarity and gelation time.

Microbiological Studies

The improved in situ gels approved the test for purity as there was no development of turbidity and hence no indication of microbial progress when incubated at 35ºC for 14 days in case of liquid thioglycollate medium and at 20-25ºC in the case of soya bean casein digest medium. Moreover to control the exhausted mediums for appropriateness of sterility test development promotion test were achieved and it was to create that both microorganisms showed visible in all media.

MIC of Posaconazole

The MIC endpoints were calculated for the final drug concentration that displayed a prominent decrease (90% and 50%) of the progress in the control well. MIC_{90} values of posaconazole against C. tropicals, C. albicans, A. flavus and A. fumigatus were 1, 2, 0.5 and 0.5 µg/ml, correspondingly and MIC_{50} standards were 0.25µg/ml for all of the microbes. In unity with our study also calculated the MIC_{90} results in Asperigillus sp. as 1 mg/L determined the MIC values for both environmental

strains and clinical of A. flavus and A. fumigatus and the MIC ranges were 0.25-2µg/ml for both of the microbes and MIC ranges of A. flavus and A. fumigatus detaches were 0.125-1 and 0.25-1µg/ml, correspondingly. For Candida species conferring to CLSI M27-A3 MIC values were≤1µg/ml are recognized as liable; 2µg/ml is dose in need of susceptible and ≥4µg/ml is known as tough.

Table 3: pH, gelling capacity, drug content, viscosity and clarity of *in situ* gel

Formulations	**F**	**F1**	**F2**	**F3**	**F4**
pH	6.26±0.02	6.99±0.01	7.00±0.01	7.03±0.02	7.64±0.01
Gelling capacity	+++	+++	+++	+++	+++
Drug content	-	84.24±0.26	83.18±0.34	81.21±1.05	93.47±0.49
Viscosity	338±2.1	354±3.6	378±9.27	414±11.35	457±8.56
Clarity	++	+++	++	+++	+++

4. Conclusion:

In a development of *in situ* gel poloxamer 407 is an essential component showing ideal property such as thermo-sensitive copolymer so that we preferred to use poloxamer 407. However, since the combination poloxamer 407 and poloxamer 188 are used in a formulation polymeric solution. The *in situ* gel is successfully prepared by cold method. Various parameters like pH, viscosity, clarity, gelation, temperature, drug content, stability and in vitro studies were performed. In this study shows the prepared *in situ* gel formulation could be used for ocular delivery of posaconazole.

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