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ORIGINAL ARTICLE

Development and Characterization of Voriconazole Loaded *In Situ* Gel Formulations for Ophthalmic Application

Oküler Uygulama için Vorikonazol Yüklü *In Situ* Jel Formülasyonlarının Geliştirilmesi ve Karakterizasyonu

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ABSTRACT

The aim of the this research was to prepare and evaluate the potential use of *in situ* gel formulations for ocular delivery of voriconazole for the treatment of fungal keratitis. An *in situ* gelling system was used to increase the residence time and thus the bioavailability of voriconazole in ocular mucosa. Temperature triggered *in situ* gel formulations were prepared by cold method using polymers like poloxamer 188, poloxamer 407 and sodium alginate. Finally, concentration of voriconazole in formulations was 0.1% (w/w). These formulations were evaluated for clarity, sol-gel transition temperature, gelling capacity, pH, viscosity and drug content. The gelation temperatures of all the formulations were within the range of 32-34˚C. All the formulations exhibited fairly uniform drug content. Furthermore *in vitro* drug release and antifungal activity of these formulations were also evaluated. Drug release study of all the formulations showed sustained release properties. In conclusion, voriconazole loaded *in situ* gels could be offered as a promising strategy for ocular drug delivery for the treatment of fungal keratitis.

Key words: Voriconazole, *In situ* gel, Ocular drug delivery, Characterization, Microbiological study

ÖZ

Bu araştırmanın amacı fungal keratit tedavisi için vorikonazolün göze hedeflendirilmiş potansiyel kullanımı olan *in situ* jel formülasyonlarını hazırlamak ve değerlendirmektir. *In situ* jelleştirici sistemi vorikonazolün oküler mukozada kalış süresini dolayısıyla biyoyararlanımını arttırmak için kullanılmıştır. Sıcaklıktan etkilenen *in situ* jel formülasyonları poloksamer 188, poloksamer 407 ve sodyum alginat gibi polimerler kullanılarak soğuk yöntemle hazırlanmıştır. Son olarak, formülasyonların içindeki vorikonazol konsantrasyonu %0.1 (a/a)dir. Bu formülasyonlar berraklık, sol-jel geçiş sıcaklığı, jelleşme kapasitesi, pH, viskozite ve ilaç içeriği açısından değerlendirilmiştir. Bütün formülasyonların jelleşme sıcaklığı 32-34˚C arasında değişmektedir. Bütün formülasyonlar oldukça uygun ilaç içeriğini göstermiştir. Ayrıca bu formülasyonların *in vitro* ilaç salımı ve antifungal aktivitesi de değerlendirilmiştir. Bütün formülasyonların ilaç salım çalışması sürekli salım özelliği göstermiştir. Sonuç olarak, vorikonazol yüklü *in situ* jeller fungal keratit tedavisinde göze ilaç hedeflendirilmesi için gelecek vadeden bir strateji olarak sunulabilir.

Anahtar kelimeler: Vorikonazol, *In situ* jel, Oküler ilaç dağılımı, Karakterizasyon, Mikrobiyolojik çalışma

INTRODUCTION

The eye is unique in terms of its anatomical and physiological nature and defence mechanisms, which make the targeting of drugs to eye tissues one of the greatest challenges in drug delivery (1,2). One of the major limitations faced in ophthalmic delivery is the attainment and retention of optimum drug concentration at the site of action within the eye (3,4,5). Poor bioavailability of drugs from conventional ocular dosage forms in mainly due to tear production, nasolacrimal drainage and transient residence time, drainage of the instilled solution, tear turnover and limited corneal area (6). 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. These ocular drug delivery systems, however, have not been used extensively because of some drawbacks such as blurred vision from ointments or lack of patient compliance are the main reasons that they have not universally accepted (6,8,9). The effective dose administered may be altered by increasing the retention time of medication into the eye by using *in situ* gel forming systems (10). *In situ* gel forming systems are liquid aqueous solutions

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before administration but turn to gel under physiological conditions. There are several possible mechanisms that lead to *in situ* gel formation, such as pH change, ionic cross-linkage, and temperature modulation (11,12).

Several *in situ* gel forming systems have been developed to prolong the precorneal residence time of a drug and improve ocular bioavailability. Polymers are employed in such delivery systems to carry various drugs and they may demonstrate a transition from sol(liquid) to gel state once instilled in the cul-de-sac of the eye (13). *In situ* gel forming formulations current a novel idea of deliver drugs to patients as a liquid dosage form, yet achieve sustained release of drug for the desired period. Different delivery systems based on polymers have been developed, which are able to increase the residence time of the formulation at absorption site of drugs. In recent years, there has been an increasing interest in water-soluble polymers are highly advantageous compared with other polymers because, in contrast to very strong gels, they can be easily applied in liquid form to the site of drug absorption. At the site of drug absorption, they swell to form a strong gel that is capable of prolonging the residence time of drug (14).

Keratitis, a disease of the cornea, results from direct infection with viruses, bacteria, fungi, yeast, and amoebae or from immune-related complications (4). Fungal keratitis is a leading cause of serious ocular morbidity and blindness. It is worldwide in distribution, but is more common in the tropics and subtropical regions. In fungal keratitis, early diagnosis and antifungal therapy is necessary in preventing further complications such as hypopyon formation, endophthalmitis, or loss of vision (15).

Table 1. Poloxamers concentrations, gelling temperature and pH of the formulations

Codes	Poloxamer 407 (%)	Poloxamer 188 (%)	Gelling temperature (°C)	pH
F1	15	10	42-43	7.15
F2	15	15	39-40	7.31
F3	15	20	35-36	7.44
F4	15	23	34-35	7.53
F5	15	25	30-32	7.63
F6	18	15	41-42	7.51
F7	18	18	38-39	7.57
F8	18	20	33-34	7.53
F9	18	22	32-33	7.63
F10	18	25	28-29	7.85
F11	20	5	30-32	7.07
F12	20	10	33-34	7.22
F13	20	15	36-37	7.30
F14	20	20	32-33	7.34
F15	20	23	32-33	7.67

Voriconazole (VCZ), $C_{16}H_{14}F_3N_5O$, a second generation antifungal agent possesses phenomenal characteristics like broad-spectrum activity, activity against resistant fungal species, and acceptable tolerability. Almost 100% *in vitro* susceptibility was observed against various fungal isolates associated with keratitis and endophthalmitis. Moreover, studies suggested an excellent efficacy of voriconazole against several ocular mycoses following topical administration (16).

In this study, a new ocular drug delivery system based on the dispersion of voriconazole loaded *in situ* gels coating into sodium alginate was proposed. The characterization properties of the system were investigated, including clarity, gelling capasity, pH, viscosity and drug content. *In vitro* drug release and antifungal activity of these formulations were also evaluated.

EXPERIMENTAL

Materials

VCZ and sodium alginate were purchased from Sigma, Germany. Poloxamer 407 and poloxamer 188 were kind gift from BASF, Turkey. Distilled water was used throughout the study. High pressure liquid chromatography (HPLC) grade acetonitrile (Sigma, Germany) was used for HPLC studies. Roswell Park Memorial Institute (RPMI 1640) medium was puchased from Sigma-Aldrich, Germany (R65504). All the other chemicals and solvents were of analytical or HPLC grade. Dialysis membrane (Spectro/por Dialysis Mebrane, Spectra/por 4, diameter 16 mm, molecular weight of 12-14 kDa) was purchased from Spectrum.

Preparation of in situ gel formulations

Poloxamer analogs were used as the gelling agents, and the *in situ* gels were prepared by using a cold method (17). The polymeric solutions were prepared by dispersing required quantity of Poloxamer 407 and Poloxamer 188 in cold water (5 ºC) using a magnetic stirrer until the poloxamer completely dissolve (approximately 2 hours). The dispersion was kept in a refrigerator for 48 hours to get clear solution.

Determination of sol-gel temperature (Tsol-gel)

20 g of cold sample solution were put into a beaker and placed in a temperature-controlled stirrer. A thermometer was immersed into the sample solution for constant monitoring. The solution was heated at the rate of 2 ºC/min with the continuous with stirring at 200 rpm. The temperature at which the magnetic bar stopped moving due to gelation was reported as the gelation temperature. The maximum limit for gelation was checked up to 60 ºC. Optimum poloxamer ratios were determined and selected with sol-gel temperature as 32-34 ºC which is the eye surface temperature (18). The experiments were repeated four times.

Preparation of voriconazole loaded in situ gel formulations

In situ gels were selected according to pH values and gelling temperatures of formulations. F11 (ratio of P407 and P188 were 20% and 5%, respectively) was selected as optimum formulation for preparation an ocular formulation. After detection of the optimum *in situ* gel compositions sodium alginate of different concentrations (0.1%, 0.3%, and 0.5%) and for each formulations same concentration of VCZ were added in poloxamer solutions with continuous stirring until completely dissolved. Benzalkonium chloride (0.02% w/w) was added as a preservative to the solutions. Sufficient amount of sodium chloride (0.9% w/w) was added to the mixture to maintain the isotonicity. The effect of drug and the other compositions of formulations on gel temperature were also evaluated.

Characterization of in situ gels

The prepared ocular formulations were characterized such as clarity, gelling capacity, pH, viscosity and drug content. In addition gelling temperature of formulations was determined and statistical analysis was performed using t-test. Data were considered statistically significant at $p<0.05$. The experiments were repeated four times.

Clarity of formulations

The clarity of formulations was determined by visual inspection under black and white background, and it was graded as follows: turbid, +; clear, ++; and very clear (glassy), +++.

Gelling capacity

The gelling capacity of the prepared formulation was determined by placing a drop of the formulation in a beaker at 32-34 ºC and it was visually observed for gelling time. It was graded as follows: +; gel after few minutes dissolves rapidly, ++; immediate gelation remains for few mins, +++; immediate gelation remains for nearly an hour.

Determination of pH

The pH of the gel was determined using calibrated pH meter (Mettler Toledo, Switzerland). Determinations were carried out four times and an average of these determinations was taken as the pH of the prepared gels.

Determination of viscosity

The viscosity of the developed formulations was performed with a digital viscometer (Brookfield) equipped with spindle RV2 with 50 rpm at 32±2°C.

Drug content

1 mL of the developed *in situ* gel formulation was dissolved in 100 mL pH 7.4 simulated tear fluid buffer (NaCl: 0.670g, $NaHCO_3$: 0.200g, $CaCl_2.2H_2O$: 0.008 g and distiled water q.s. to 100 g) (19) followed by HPLC estimation of the aliquot to determine drug concentration. The experiments were repeated four times.

HLPC analysis

The HPLC system consisted of a gradient pump and a UV detector supplied by Agilent 1100. C18 column (150x4.6mm,5µm) (GL Sciences, Japan) was used. The samples were analyzed at 256 nm with a 1 mL/min flow rate at 25°C. The mobile phase was a mixture of acetonitrile: ultrapure water (50:50). Retention time of drug was 4.098 min. The method was validated partially linearity, limit of detection (LOD) and limit of quantitation (LOQ), precision, accuracy and specificity, selectivity and stability.

In vitro drug release study

The *in vitro* drug release study was performed using the dialysis bag method (5). *In vitro* release study of *in situ* gel formulations was carried out in simulated tear fluid (pH=7.4) at 50 rpm. The temperature was maintained at 33±1°C to mimic eye surface temperature. 5 g formulation was separated from release media by means of dialysis membrane (Spectra/por, MW of 12-14 kDa) and capped with closures. The membrane was heated 33±1°C for 30 min in bidistilled water before use. 0.5 mL of sample was withdrawn at a predetermined time interval of 1 h to 8 h and the same volume of fresh medium was replaced. The samples were analyzed with HPLC for the drug content. The experiments were repeated three times.

Stability of the in situ gels

In physical stability studies, VCZ loaded *in situ* gels were stored at 5±1ºC in the refrigerator and 25±2ºC and 40±2ºC for 3 months in the stability cabinets (Nüve, Turkey). After storage for 3 months visual appearance, clarity, pH, gelation time of *in situ* gels and VCZ content were investigated. The experiments were repeated three times.

Microbiological studies

Sterility studies

In situ gel formulations in the presence or absence of VCZ were prepared at Laminar air flow Cabinet (Haier HR40-IIA2).

To check the sterility of the prepared ocular formulations sterility control testing were performed. Sterility testing of the *in situ* gel formulations with or without VCZ was carried out under aseptic conditions according to the international pharmacopoeia. For anaerobic bacteria Fluid thioglycollate medium was used. For fungi and aerobic bacteria soya-bean casein digest medium was used. 1 mL of formulation solution was added to each medium and incubated at 35°C for bacteria and 25°C for fungi for 14 days.

To check the suitability of the used mediums for the sterility testing promotion test were performed. For growth promotion test of aerobes, anaerobes and fungi, fluid thioglycollate media (using separate portion of media for each microorganism) were inoculated with 100 CFU of *Staphylococcus aureus* ATCC 6538, *Clostridium sporogenes* ATCC 19404 and *Candida albicans* ATCC 10231. Media were incubated at 35°C for 48 h.

Determination of MIC of VCZ

The broth microdilution test was done in accordance with CLSI guidelines for filamentous fungi (20) and yeasts (21). VCZ was dissolved in dimethyl sulfoxide, final dilutions were made in RPMI 1640 medium buffered to pH 7.0 with 0.165 M MOPS buffer [3-(N-morpholino) propanesulfonic acid] and final concentrations were 500-0.125 µg/mL. Using the spectrophotometric method of inoculum preparation, an inoculum concentration of $1.5(\pm1.0)x10^3$ cells/mL for yeasts and $0.4\text{-}5x10^4$ spores/mL for moulds, and RPMI 1640 medium buffered with MOPS were used. *C. albicans* ATCC 10231, *C. tropicalis* RSKK 2412, *A. fumigatus* ATCC 204305 and *A. flavus* ATCC 204304 which may be possible causes of fungal keratitis were used to evaluate antifungal activity of developed formulations (22,23). A 0.1 mL inoculum was added to each well of the microdilution trays. The MICs were determined after 48 h of incubation. The plates were shaken before the comparison of growth in wells. With an aid of a reading mirror, growth in each well was compared with the VCZ free growth control well. The MIC endpoints were evaluated for the lowest drug concentration that showed a prominent reduction (50%) of the growth in the control well.

Disk diffusion testing

Disk diffusion testing was performed according to CLSI standard M44-A2 for yeasts and M51-A for filamentous fungi. Mueller-Hinton agar (Difco) supplemented with 2% glucose and 0.5 µg/mL methylene blue dye (SSI Diagnostica, Hillerød, Denmark) was used. Blank disks that were 12.1 mm in diameter were impregnated with 20 µL of formulations at final concentration of 1 µg/disk and allowed to dry at room temperature. *C. albicans* ATCC 10231 and *C. tropicalis* RSKK 2412, *A. fumigatus* ATCC 204305 and *A. flavus* ATCC 204304 which may be possible causes of fungal keratitis (22,23) were used to evaluate antifungal activity of developed formulations. The mold inocula were prepared at optical densities ranging from 80 to 82% and a suspension with a 0.5 McFarland standard was utilized for yeasts. The plates were incubated at 35˚C and inhibition zone (IZs, in millimeters) diameters were read by using a digital ruler at 24 and 48 h. Minor trailing growth in the inhibition zones was ignored.

RESULTS AND DISCUSSION

Preparation of in situ gel formulations

Preliminary studies were carried out using different concentrations of polymers evaluated for their gelling temperature in order to identify the compositions suitable for use in the *in situ* gelling system for ocular drug delivery. Temperature sensitive *in situ* gels were successfully prepared by cold method using poloxamer 407, poloxamer 188. Cold method is one of the preferred methods due to providing clear solution for *in situ* gel while hot process causes formation of lumps of polymer as reported and observed in literature (24).

Table 2. Compositions of the *in situ* gels (%)

Components (%)	S	S1	S2	S3	S4
Poloxamer 407	20	20	20	20	20
Poloxamer 188	5	5	5	5	5
Sodium alginate	-	-	0.1	0.3	0.5
Sodium chloride	0.9	0.9	0.9	0.9	0.9
Voriconazole	-	0.1	0.1	0.1	0.1
Benzalkonium chloride	0.02	0.02	0.02	0.02	0.02
Distiled water qs to	100	100	100	100	100

Poloxamer 407 (ethylene oxide and propylene oxide blocks) has excellent thermo-sensitive gelling properties, which is of the most interest in optimising drug formulation. Poloxamer 407 formulations led to enhanced solubilization of poorly water-soluble drugs and prolonged release profile for many applications (e.g., ophthalmic, oral, rectal, topical, nasal and injectable preparations) but did not clearly show any relevant advantages when used alone. Combination with other excipients like Poloxamer 188 or mucoadhesive polymers promotes the action of Poloxamer 407 by optimising sol–gel transition temperature or increasing bioadhesive properties (25). For this purpose poloxamer 188 and poloxamer 407 mixture were used to develop *in situ* gels.

To find out optimum gelling temperature (32±2°C), the poloxamer 407 and poloxamer 188 were mixed various concentrations. Table 1 shows poloxamer concentrations, gelling temperature and pH of the prepared formulations. F11 was selected optimum composition which contains poloxamer 407 (20%) and poloxamer 188 (5%).

After selecting F11 formulation, benzalkonium chloride (0.02%, w/w) was added as a preservative to the solution. Sufficient amount of sodium chloride (0.9% w/w) was added to the mixture to maintain the isotonicity and the formulation was coded as S. 0.1% (w/w) VCZ was loaded and different concentrations of sodium alginate of different concentrations (0.1%, 0.3%, and 0.5%) was added in poloxamer solutions with continuous stirring until completely dissolved. Finally, concentration of VCZ in formulations was 0.1% (w/w). The dispersion was kept in a refrigerator for 48 hours to get clear solution. The components of ocular *in situ* gels in absence or presence of VCZ are shown in Table 2.

Characterization of in situ gels

Characterization of the new drug delivery systems are major issues to be considered in the formulation stage, especially those intended for ocular administration. The physicochemical characterization parameters of *in situ* gels are reported in Table 3.

The clarity of all the formulations was found to be satisfactory, as shown in Table 3. Gelation temperature was changed from 32.5°C to 34.3°C with incorporation of 0.1% w/w VCZ in the poloxamer solution, while the addition of the mucoadhesive polymers played a reverse role on gelation temperature. The results showed that incorporation of sodium alginate into *in situ* gel formulation significantly decreased the gelling temperature ($p<0.05$). When the concentration of

sodium alginate was increased in the *in situ* gels, the gelling temperature was decreased (Table 3). Gelling temperature of the all formulations (S, S1-S4) were found between 32-35°C. This indicates that the formulations can be converting the gel when they installed the eye surface. At 32-34°C, the solutions are converted into gels with high viscosity. The gelling capacity data of prepared formulations presented in Table 3 represent that the formulations all formulations had immediate gelation and exist for an hour.

The pH of an ophthalmic formulation is important for patient compliance. The pH of the prepared formulations ranged between 7.07 and 7.13. The pH of the formulations was appropriate for ocular delivery since they were iso-hydric. This indicated the nonirritancy of the formulation in ocular mucosa. When a formulation is administered to the eye, it stimulates the flow of tears. Tear fluid is capable of quickly diluting and buffering small volumes of added substances, thus eye can tolerate a fairly wide pH range. Ophthalmic solutions may range from 6.5 to 8.5 (26,27).

The formulation should have an optimum viscosity, which will allow its instillation into the eye as a liquid, which will then undergo rapid sol-gel transition due to temperature change. When the *in situ* solutions were installed the 32-34°C surface, the solutions were converted to gel form after few seconds.

All the formulations reflected fairly uniform drug content ensuring adequacy in the method of preparation of the *in situ* gel. Drug content was found to be within the range of 82.68-92.58%. The drug content of the prepared formulations was within acceptable limits and ensured dose uniformity.

In vitro drug release study

The *in situ* gelling formulations of VCZ, S1-S4, were subjected to *in vitro* release studies, which were carried out using simulated tear fluid of pH 7.4 as release medium. Formulations showed sustained drug release for a period of 8 hours (Figure 1). At the end of the 8 h, *in vitro* VCZ release from S1, S2 and S3 formulations was found as 63%, 60% and 60%, respectively ($p>0.05$). S4 formulation was showed slower release than the other formulations. This could be the reason of higher concentration of sodium alginate among the developed formulations. Mandal et al. prepared moxifloxacin hydrochloride loaded *in situ* gel using sodium alginate and hydroxy propyl methyl cellulose as polymers. They were found that when sodium alginate and hydroxy propyl methyl cellulose concentration was increased, release rate was decreased (28).

Stability

The stability studies were carried out at 5±1°C, 25±2°C and 40±2°C for 3 month 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. After 3 month pH values of S1, S2 S3 and S4 formulations were found as 7.12±0.002, 7.18±0.001, 7.20±0.001 and 7.21±0.002, respectively. In addition 92-98% of initial drug content of formulations was kept its stability for in 3 month.

Microbiological studies

The optimized *in situ* gels passed the test for sterility as there was no appearance of turbidity and hence no evidence of microbial growth when incubated for 14 days at 35°C in case of fluid thioglycolate medium and at 20-25°C in the case of soya-bean casein digest medium. Furthermore to control the used

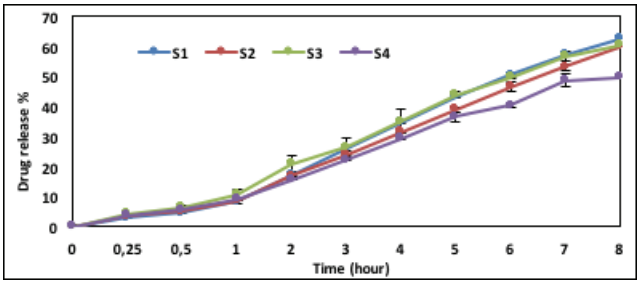

Figure 1. *In vitro* release of voriconazole from *in situ* gels at pH 7.4 simulated tear fluid buffer

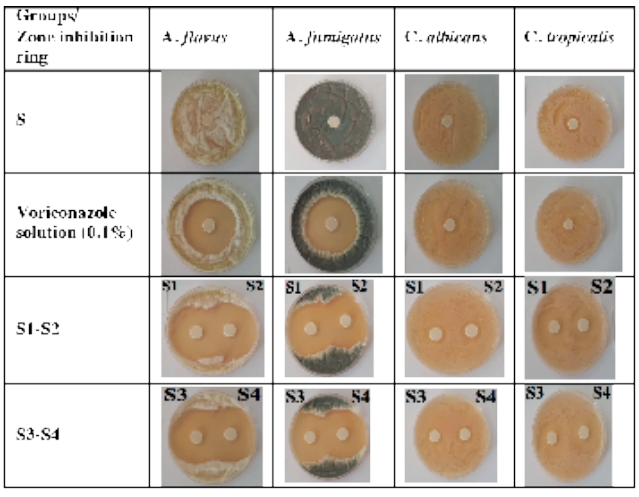

Figure 2. Zone inhibition diameters of formulations against *A. flavus, A. fumigatus, C. albicans and C. tropicalis*

Table 3. Clarity, gelling temperature, pH, drug content, *in vitro* gelation capacity, viscosity, and drug content of *in situ* gels

Formulations	S	S1	S2	S3	S4
Clarity	+++	+++	+++	++	++
Gelling temperature (oC)	32.5±0.03	34.3±0.01	33.1±0.023	32.6±0.012	32.4±0.001
Gelling capacity	+++	+++	+++	+++	+++
pH	7.07±0.002	7.08±0.001	7.1±0.002	7.11±0.001	7.13±0.001
Viscosity (cP)	358±3.1	362±6.21	400±11.32	424±23.15	440±9.17
Drug content (%)	-	83.14±0.36	82.68±1.06	84.11±0.7	92.58±0.35

mediums for suitability of sterility test growth promotion test were performed and it was found that both microorganisms showed visible growth in all media.

The MIC endpoints were evaluated for the lowest drug concentration that showed a prominent reduction (90% and 50%) of the growth in the control well. MIC_{90} values of VCZ against *C. albicans*, *C. tropicalis*, *A. fumigatus* and *A. flavus* were 2, 1, 0.5 and 0.5 µg/mL, respectively and MIC_{50} values were 0.25 µg/mL for all of the microorganisms. In accordance with our study (29) also evaluated the MIC_{90} results in *Aspergillus sp.* as 1 mg/L. (30) determined the MIC values for both clinical and environmental strains of *A. fumigatus* and *A. flavus* and the MIC ranges were 0.25-2 µg/mL for both of the microorganisms and (31) MIC ranges of *A. fumigatus* and *A. flavus* isolates were 0.25-1 and 0.125-1 µg/mL, respectively. For *Candida* species according to CLSI M27-A3 MIC values ≤ 1 µg/mL are accepted as susceptible; 2 µg/mL are dose dependent susceptible and ≥4 µg/mL are accepted as resistant.

The efficacy of groups was investigated using the inhibition zone diameters in disc diffusion test. Disk diffusion testing was performed according to CLSI standard M44-A2 for yeasts and M51-A for filamentous fungi. *C. albicans*, *C. tropicalis*, *A. fumigatus* and *A. flavus* were used as they are the common organisms causing ocular fungal infections (22,23). Figure 2 shows inhibition zone (IZ) diameters of formulations. The developed VCZ loaded *in situ* gels were found to be more effective on more effective on moulds in comparison with yeasts (*A. flavus* than *C. albicans*) (Table 4). In addition to this, sodium alginate is not effective on the *C. albicans*, *C. tropicalis*, *A. fumigatus* and *A. flavus*, because addition of sodium alginate into the *in situ* gel (S1) did not changed the IZ diameter. While absence of voriconazole (S) in the *in situ* gel (blank *in situ* gel) is not effective on *C. albicans*, *A. fumigatus* and *A. flavus*; a minor inhibition zone was seen in *C. tropicalis* which may be caused by benzalkonium chloride (0.02%, w/w) or other components of the gel. Since the interpretive breakpoints of VCZ for susceptible species is ≥17 mm and for resistant is ≤13 mm, we can say that all of organisms are susceptible for all of the formulations (32).

CONCLUSION

In the present study, the potential of VCZ loaded *in situ* gels as drug carriers for ocular delivery was investigated. The present study showed that *in situ* gels of VCZ can successfully be prepared with cold method. The clarity, pH, gellation time and drug content of all formulations was found to be satisfactory. In addition the formulations were found stable for 3 month. Further, all the formulations showed sustained drug release for a period of 8 h, which satisfied to treat ocular disease. The developed *in situ* gels showed anti fungal activiy on *C. albicans*, *C. tropicalis*, *A. fumigatus* and *A. flavus*. In conclusion, this study showed that developed *in situ* gel formulations could be alternatively used as ocular delivery of voriconazole. The present study can open up a window for opthalmic application of *in situ* gels loaded with VCZ, they would be a better alternative to conventional eye drops in the treatment of fungal keratitis of the eye.

Table 4. Zone inhibition diameters of formulations

Groups/ Inhibition Zone (IZ) (cm±SD)	*A. flavus*	*A. fumigatus*	*C. albicans*	*C. tropicalis*
S	0±0	0±0	0±0	2.0±0.1
S1	5.7±0.5	4.8±0.3	2.1±0.2	3.1±0.1
S2	5.2±0.4	5.0±0.3	2.0±0.1	2.8±0.3
S3	5.8±0.1	4.9±0.2	1.9±0.1	3.3±0.1
S4	5.1±0.3	5.0±0.5	2.1±0.1	3.4±0.2

Declaration of Interest

The authors declare no conflict of interest.

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