Formulation and evaluation of voriconazole ophthalmic solid lipid nanoparticles *in situ* gel

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Abstract

In the present investigation, solid lipid nanoparticles (SLNs)-loaded *in situ* gel with voriconazole drug was formulated. Further, the formulation was characterized for pH, gelling capacity, entrapment efficiency, *in vitro* drug release, drug content, and viscosity. Voriconazole is an antifungal drug used to treat various infections caused by yeast or other types of fungi. Film hydration technique was used to prepared SLNs from lecithin and cholesterol. Based on the entrapment efficiency 67.2-97.3% and drug release, the optimized formulation NF1 of SLNs was incorporated into *in situ* gels. The *in situ* gels were prepared using viscosity-enhancing polymers such as Carbopol and (hydroxypropyl) methyl cellulose (HPMC). Formulated SLN *in situ* gel formulations were characterized, which showed pH 4.9-7.1, drug content 65.69-96.3%, and viscosity (100 rpm) 120-620 cps. From the characterizations given above, F6 was optimized and evaluated for microbial assay and ocular irritation studies. Microbial assay was conducted by the cup-plate method using *Candida albicans* as the test organism. An ocular irritation study was conducted on albino rabbits. The results revealed that there was no ocular damage to the cornea, conjunctiva, or iris. Stability studies were carried out on the F6 formulation for 3 months, which showed that the formulation had good stability. These results indicate that the studied SLNs-loaded *in situ* gel is a promising vehicle for ocular delivery.

Key words: *In situ* gel, ophthalmic, solid lipid nanoparticles (SLNs), voriconazole

INTRODUCTION

Ocular dosage forms are designed to be instilled onto the external surface of the eye, administered inside or adjacent to the eye. Ideal ophthalmic drug delivery must be able to sustain the drug release and to remain in the vicinity of the front of the eye for a prolonged period of time.[1] Solid lipid nanoparticles (SLNs) are microscopic particles whose size is measured in nanometers (nm). The size of a nanoparticle is 1-100 nm.[2,3] SLNs are incorporated into *in situ* gels for sustained release of the drug, to prolong the residence time, and to increase the bioavailability of the drug.

This new concept (*in situ* delivery) of producing a gel *in situ* was suggested for the first time in the early 1980s. "*In situ*" is a Latin phrase translated literally as "in position." *In situ* gel-forming systems can be described as low-viscosity solutions that undergo phase transition in the conjunctival *cul-de-sac* to form viscoelastic gels due to conformational changes of polymers in response to change in a specific physicochemical parameter such as ionic strength, pH, or temperature. Gel dosage forms are successfully used as drug delivery systems, considering their ability to prolong drug release.[4] *In situ* formulations prolong the precorneal resident time and improve ocular bioavailability of the drug. The *in situ* formulation exhibited good viscosity, drug content, and sustained drug release.[5,6] Conventional liquid ophthalmic formulations demonstrate low bioavailability because of a constant lacrimal drainage in the eye.[7,8]

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Solution-to-gel phase transition may occur due to:

a. Physical stimuli: It includes change in temperature, electric field, and light;
b. Chemical stimuli: It includes changes in pH and ion activation from biological fluid; or
c. Biochemical stimuli: It includes changes in glucose level.[9,10]

Voriconazole is an antifungal drug used for treatment of various conditions caused by yeast or fungi. Voriconazole binds and inhibits ergosterol synthesis by inhibiting cytochrome P450-mediated 14 alpha-lanosterol demethylation, an essential step in fungal ergosterol biosynthesis. The accumulation of 14 alpha-methyl sterols correlates with the subsequent loss of ergosterol in the fungal cell wall and may be responsible for the antifungal activity. Voriconazole is available as powder for infusion, oral suspension, and tablet on the market.

In the present investigation, the main aim was to develop a solid lipid-loaded *in situ* gel formulation for ophthalmic drug delivery. The objective of this work was to improve precorneal retention time, thereby increasing therapeutic activity in a controlled-release manner. Reducing the frequency of dosage will improve patient compliance.

MATERIALS AND METHODS

Materials
Voriconazole was obtained from FDC Ltd., Mumbai, Maharashtra. Carbopol 940 was purchased from CDH (P) Ltd., New Delhi. (hydroxypropyl) methyl cellulose (HPMC) K15M, ethylenediaminetetraacetic acid (EDTA), sodium chloride, and benzalkonium chloride were purchased from Loba Chem, Mumbai. All chemicals of analytical or pharmaceutical grade were used without further purification.

Method
Preparation of nanolipids
Nanolipids were prepared by film hydration technique.[11] The mixture of vesicle-forming ingredients such as lecithin and cholesterol was dissolved in a volatile organic solvent (dichloromethane and methanol) in a round-bottom flask. The rotary evaporator was rotated at 60°C for 45 min. Then the organic solvent was removed with gentle agitation and the organic solvent evaporated at 60°C, leaving a thin film of lipid on the wall of the rotary flash evaporator. The aqueous phase containing voriconazole drug was added slowly with intermittent shaking of the flask at room temperature and sonicated for 30 min. The obtained nanolipid solution was cooled by placing in the freezer. The composition of the nanolipid is presented in Table 1.

Formulation of nanolipid *in situ* *gel*
Nanolipid *in situ* gel was prepared on the basis of drug entrapment efficiency and morphology. The batch of nanolipid that gave maximum entrapment and good surface morphology was selected for preparation of *in situ* gel.[12] Appropriate

quantities of Carbopol 940 and HPMC K15M were sprinkled over nanolipid dispersion under constant stirring with a glass rod, taking care to avoid formation of lumps, and allowed to hydrate. Other ingredients such as benzalkonium chloride as preservative and sodium chloride to make gel formulations isotonic with tear fluid were added to the gel batches, incorporated in sufficient quantity to adjust the pH. The compositions of various nanolipid *in situ* gels prepared are presented in Table 2.

Preliminary studies
Drug polymer interaction studies
Each drug excipient's compatibility was determined using infrared (IR) spectrum recorded on Bruker Vertex 70/70v (Germany) FT-IR spectrophotometer. Samples of pure drug and physical mixtures of drug and excipients were scanned in the range of 400-4000 cm^{-1}.

Evaluation of SLNs
Vesicle shape and size analysis of SLNs
The size and shape of the vesicles were determined using optical microscopy and scanning electron microscopy (SEM) (SEM Jeol JSM)-5800 California, USA.[8]

Entrapment efficiency
Separation of unentrapped drug from the nanolipid formulation was done by the ultracentrifugation method. Here, centrifuging of nanolipid dispersion was carried out at 14000 rpm for 90 min. The clear supernatant from the resulting solution was diluted appropriately using pH 7.4 phosphate buffer and analyzed by ultraviolet (UV) spectrophotometric method.[9]

Drug release by diffusion
Nanolipids equivalent to 1 mg of voriconazole were accurately weighed and transferred to dialysis membrane. The membrane is

Table 1: Composition of nanolipid *in situ* gels

Ingredients (% w/v or v/v)	NF1	NF2	NF3	NF4	NF5	NF6
Drug	0.05	0.05	0.05	0.05	0.05	0.05
Lecithin	0.05	0.05	0.1	0.05	0.15	0.2
Cholesterol	0.05	0.1	0.05	0.15	0.1	0.05
Methanol	7.5	7.5	7.5	7.5	7.5	7.5
Water	10	10	10	10	10	10

Table 2: Composition of nanolipid *in situ* gels

Ingredients (% w/v or v/v)	F1	F2	F3	F4	F5	F6
Drug	0.05	0.05	0.05	0.05	0.05	0.05
Lecithin	0.05	0.05	0.05	0.05	0.05	0.05
Cholesterol	0.05	0.05	0.05	0.05	0.05	0.05
Methanol	7.5	7.5	7.5	7.5	7.5	7.5
Water	10	10	10	10	10	10
HPMC	0.2	0.2	0.4	0.4	0.3	0.2
Carbopol	0.2	0.4	0.2	0.4	0.2	0.3
EDTA	0.1	0.1	0.1	0.1	0.1	0.1
Benzolkonium chloride	0.01	0.01	0.01	0.01	0.01	0.01
Sodium chloride	0.9	0.9	0.9	0.9	0.9	0.9
Phosphate buffer	100	100	100	100	100	100

tied with threads and placed in a 250 mL conical flask containing 100 mL phosphate buffer (pH 7.4). The flask was kept in an incubator at 37°C, 1 mL samples were withdrawn at regular intervals, and 1 mL of phosphate buffer was added to the release medium to replenish it. Similarly, the release of voriconazole from nanolipids was determined spectrophotometrically at 272 nm (Shimadzu 1800, Japan) after suitable dilution.[13]

Characterization of SLNs *in situ* gel
Visual appearance and pH
Visual appearance and clarity were observed for the presence of any particular matter. The pH was measured using pH meter. The pH was noted by bringing the electrode near the surface of the formulations and allowing it to equilibrate for 1 min.[14,15]

In vitro gelation study
The developed *in situ* gel was assessed for its gelling capacity. The gelling capacity was determined by placing a drop of the polymer solution in a vial containing 2 mL of freshly prepared simulated tear fluid (STF) equilibrated at 37°C. The time taken for gelation and dissolution by the developed gel was noted.[16-18]

In vitro drug release
In vitro release studies of voriconazole nanolipid *in situ* gel were carried out at 37°C using phosphate buffer (pH 7.4) as the release medium. Nanolipid *in situ* gel (5 mL) containing voriconazole was accurately weighed and transferred to the dialysis membrane. The gel was gently pushed down to the surface of the dialysis membrane to make contact with the membrane. Phosphate buffer (1 mL, pH 7.4) was added to the reservoir compartment to wet the gel; dialysis membrane was just immersed in the phosphate buffer, which acted as the receiving compartment. The receiving compartment was placed in magnetic stirrer (100 rpm, Remi, Mumbai, India) at 37°C. Samples (1 mL) were withdrawn from the receiving compartment at regular intervals, and the amount of voriconazole released from the nanolipid *in situ* gel was determined using a spectrophotometer at 272 nm (Shimadzu 1800). After each withdrawal of sample, an equal quantity of phosphate buffer was added to the receiving compartment to replenish it.[19]

Estimation of drug content
Nanolipid suspension equivalent to 50 mg was taken into a standard volumetric flask and mixed with 100 mL propan-1-ol by shaking. Then 0.1 mL of this solution was diluted to 10 mL with phosphate buffer (pH 7.4). The absorbance of the resulting solution was measured at 272 nm and the drug content was calculated from the calibration curve. Concentrations of drug were calculated from the standard calibration curve prepared in phosphate buffers (pH 7.4).[10,20]

Viscosity study
The viscosity of the gels was studied on Brookfield viscometer (DV-II+ Pro Viscometer, Middleboro, USA) by using CPE-42 spindle at 10 rpm, 20 rpm, 50 rpm, and 100 rpm.[21]

Antimicrobial activity
Antimicrobial study was carried out to check the antimicrobial efficiency of voriconazole *in situ* gel. The test organisms used were *Candida albicans*; the growth medium used was nutrient agar. The cup-plate method was used to carry out antimicrobial study. The method is based on the principle of diffusion of drug from vertical cup through solidified agar layer in Petri plate. Sterile solution of ciprofloxacin (on the market) eye drops was used as a standard. The standard solution and the developed formulations (test solution) were taken into separate cups bored into sterile nutrient agar previously seeded with *Candida albicans* organisms. The gels were allowed to diffuse for 2 h and then the plates were incubated for 24 h at 37°C. The zone of inhibition (ZOI) was compared with that of the standard.[22]

Ocular irritation studies
The optimized formulation was evaluated for *in vivo* performance in an animal model (albino rabbits). The protocol was approved by the Animal Ethics Committee (1722/PO/A/13/IAEC/CPCSEA EXP-030). Animals were housed at room temperature (27°C) and fed with standard diet and water. The albino rabbits, each weighing 2-3 kg, were placed in cages and the eyes were marked as test and control. The control eye received no sample and the test eye received the formulation (0.5 mL), and the eyes were observed at 1 h, 24 h, 48 h, 72 h, and 1 week after exposure. Ocular change was graded by a scoring system that includes rating any alterations to the eyelids, conjunctiva, cornea, and iris. Rabbits were observed periodically for redness, swelling, and watering of the eye.[8,23] Evaluation was carried out according to the Draize technique.

Accelerated stability studies
The optimized nanolipid dispersion with the highest entrapment efficiency was placed in vials and sealed with aluminum foil for a short-term accelerated stability study at 25 ± 2°C/60 ± 5% relative humidity (RH) as per modified International Conference on Harmonisation (ICH) guidelines. Samples were analyzed every 90 days for drug content.[24]

RESULTS AND DISCUSSION

Drug polymer interaction was studied using the IR spectrum. The Fourier transform (FT)-IR spectra of the pure drug and the mixture of drug and polymers are shown in Figures 1 and 2. From the spectral study [Tables 3 and 4], it was observed that there was no significant change in the peaks of pure drug and of drug
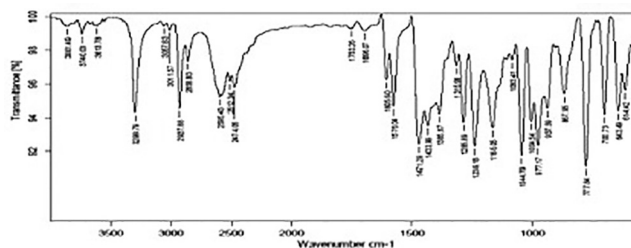

Figure 1: IR spectrum of drug

polymer mixture. Hence, no specific interaction was observed between the drug and the polymers used in the formulations.

SEM images showed that most of the vesicles formed were spherical in shape [Figures 3 and 4]. The nature of lipids played a major role in drug entrapment efficiency. The entrapment efficiencies were within the range of 67.2-97.3% [Table 5]. NF1 had shown the highest entrapment efficiency of 97.3%. NF5 showed the lowest entrapment efficiency of 67.2%. The other formulations NF2, NF3, NF4, and NF6 showed 87%, 69%, 77.56%, and 89.63% entrapment efficiency, respectively [Figure 5].

The drug release from nanolipids occurred for 10 h in pH 7.4 buffer. The drug release of nanolipids was within the range of 79.8-99.0%. From the drug release data, it was found that NF1 released maximum drug (99.0%) compare to other formulations [Figure 6]. NF1 was selected as the best SLN formulation and incorporated into *in situ* gel for further studies. An equal ratio of lecithin and cholesterol was responsible for good entrapment efficiency and drug release.

Visual appearance and clarity were observed for the presence of any particular matter. The pH of nanoparticles *in situ* gel was detected by using digital pH meter. Nanolipid *in situ* gels' pH range was pH 5-7.4 [Table 6]. Nanolipid *in situ* gel showed maximum pH value (7.1) for F6 formulation. The pH of the reported formulations was nonirritable to the eye. This reflects that the gel will be nonirritant to the eye surface.

The gelling capacity was determined by freshly prepared simulated tear fluid (STF). Gelation study revealed that the formulations F1 and F3 gelled slowly and dissolved rapidly within 1 h. Formulations F2 and F5 showed immediate gelation and remained for a few hours. Formulations F4 and F6 exhibited immediate gelation and remained for 2-4 h [Table 7].

The drug release studies of nanolipid *in situ* gel with voriconazole were performed for 24 h in pH 7.4 phosphate buffer. From the

Table 3: IR spectral data of voricanazole

Group	Frequency	
	Expected	Obtained
C=N	1630-1690	1644
C=C	1450-1600	1451
C-F	1000-1400	1270
C-N	1000-1400	1112

Table 4: IR spectral data of nanolipid *in situ* gel

Group	Frequency	
	Expected	Obtained
C=N	1630-1690	1696
C=C	1450-1600	1433
C-F	1000-1400	1285
C-N	1000-1400	1165

Table 5: Entrapment efficiency of nanolipid *in situ* gels

Formulation	Entrapment efficiency (%)[*]
NF1	97.30±0.57
NF2	87.00±0.84
NF3	69.00±0.39
NF4	77.56±0.86
NF5	67.20±0.29
NF6	89.63±0.25

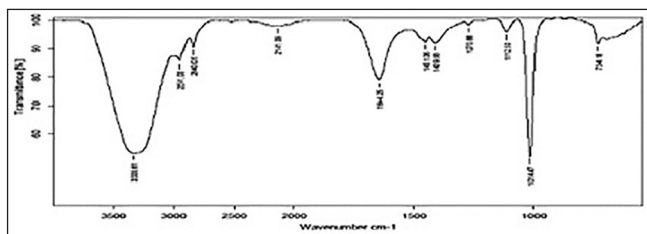[*]Values expressed as mean ± SD, *n* = 3, SD = Standard deviation

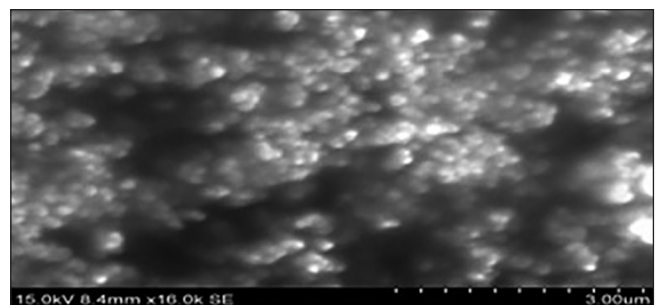**Figure 2:** IR spectrum of drug and excipients

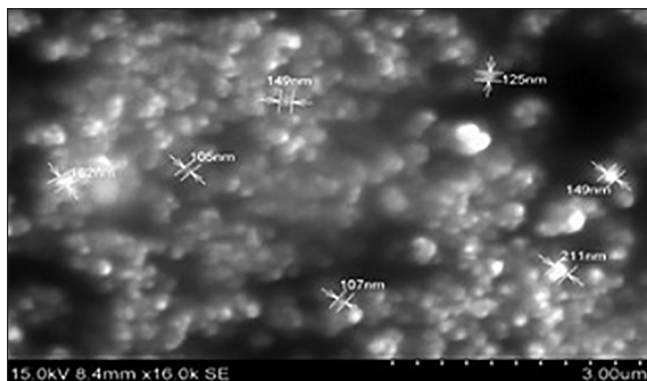**Figure 3:** SEM image of voriconazole

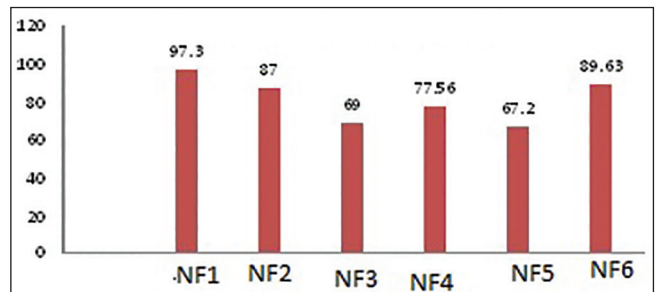
Figure 4: SEM image of voriconazole

Figure 5: Comparative entrapment efficiency of nanolipids

release data, it was found that drug release from the formulation F6 was 91.24% for 24 h. The cumulative percentages of drug released from various SLN formulations are shown in Figure 7. Formulation F6 showed steadier drug release than other formulations; moreover, release of the drug was in a controlled manner. Release pattern of the drug was mainly influenced by the polymer ratio and the viscosity of the gel. Hence, F6 was selected as the best SLNs *in situ* gel formulation.

Formulations were analyzed for drug content spectrophotometrically at 272 nm. All the formulations exhibited fairly uniform drug content. The drug contents of all formulations were in a range of 87.03-96.36% [Table 8]. Out of several tested formulations, F6 showed the highest drug content (96.36%).

Viscosity is an important parameter for characterizing the SLNs *in situ* gel formulations, as it affects the release of the drug. The highest viscosity, for gel F6 formulation, led to retarded drug release up to a considerable extent (97.24%) in 24 h when compared with other formulations. Low-viscosity formulations showed highest drug release. Intermediate viscosity of formulations has shown maximum retardation of drug release due to the viscous nature of the polymers. Carbopol 940 and HPMC as polymer system have contributed majorly toward building the viscosity of the formulation. Viscosities of the prepared SLNs *in situ* gel formulations were found to be in the range 120-915 cps [Table 9].

The optimized nanolipid *in situ* gel formulations F6 showed antimicrobial activity when tested microbiologically by the

cup-plate technique. The prepared nanolipid *in situ* gel formulation F6 inhibited the growth of *Candida albicans*. ZOI

Table 6: Visual appearance and pH

Formulations	Visual appearance	pH*
F1	Cloudy	5.9±0.070
F2	Clear	5.1±0.141
F3	Clear	6.2±0.749
F4	Cloudy	4.9±0.021
F5	Clear	6.1±0.728
F6	Clear	7.1±0.145

*Values expressed as mean ± SD, *n* = 3, SD = Standard deviation

Table 7: Gelling capacity of nanolipid *in situ* gel

Formulations	Gelation capacity
F1	+
F2	++
F3	+
F4	+++
F5	++
F6	+++

+Gelation slow and dissolves rapidly, ++Gelation immediate and remains for short period of time, +++Gelation immediate and remains for extended period of time

Table 8: Drug content estimation of nanolipid *in situ* gel

Formulations	Drug content (%)*
F1	87.03±0.34
F2	75.83±0.56
F3	65.69±0.48
F4	87.79±0.57
F5	80.00±0.46
F6	96.36±0.78

*Values expressed as mean ± SD, *n* = 3, SD = standard deviation

Table 9: Viscosity profile of nanolipid *in situ* gel

Angular velocity (rpm)	Viscosity (CPS)					
	F1	F2	F3	F4	F5	F6
10	427±1.992	672±1.557	695±1.192	803±0.603	732±0.564	915±1.798
20	365±1.894	445±1.119	654±0.213	798±1.921	675±0.589	827±0.690
50	243±1.669	332±1.995	364±0.7071	623±0.131	432±1.529	750±1.482
100	154±1.454	243±0.811	120±0.698	263±0.828	255±0.858	620±0.426

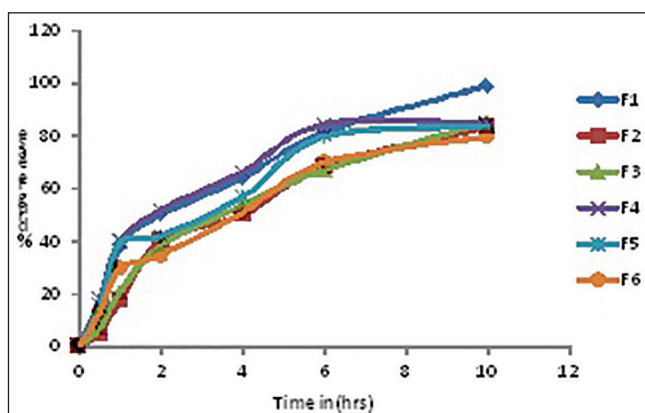*Values expressed as mean ± SD, *n* = 3, SD = Standard deviation

Figure 6: *In vitro* drug release of nanoparticles
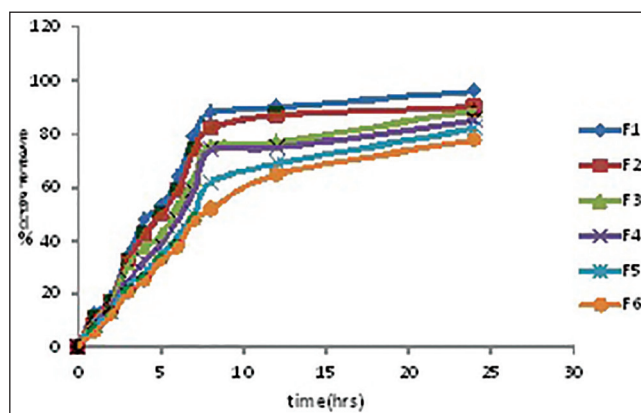

Figure 7: *In vitro* release profile of nanoparticles *in situ* gel

was measured by using zone reader in mm. The voriconazole retained its antimicrobial efficacy when formulated as an *in situ* gelling system [Table 10 and Figure 8]. F6 formulation displayed the maximum ZOI of 40 mm. This proves that the test formulation has better efficacy and has an excellent antimicrobial property.

The results of the ocular irritation studies indicate that the formulations have no average score (zero) according to the Draize scale [Table 2]. Excellent ocular tolerance was noted; no ocular damage or abnormal clinical signs in the cornea, iris, or conjunctiva were visible [Table 11].

The stability studies of SLNs *in situ* gel were performed at 5 ± 2°C and 25 ± 2°C/60 ± 5% RH for 3 months. The formulations were examined visually for precipitation. The drug content was determined every 30 days for 3 months. It was observed that there was no change in the physical appearance of the formulation. The drug content was analyzed and there were marginal differences between the formulations kept at different temperatures [Table 12]. SLNs *in situ* formulations retained good stability throughout the study.

Table 10: ZOI of microbial assay

Formulation (10 μg/mL)	Microorganism	ZOI
Nanolipid *in situ* gel	*Candida albicans*	40 mm
Marketed eye drops	*Candida albicans*	32 mm

Table 11: Ocular irritation study

Eye part	Cornea	Iris	Conjunctivae
Observation	Normal	Normal	Normal

Table 12: Accelerated stability data of optimized formulation

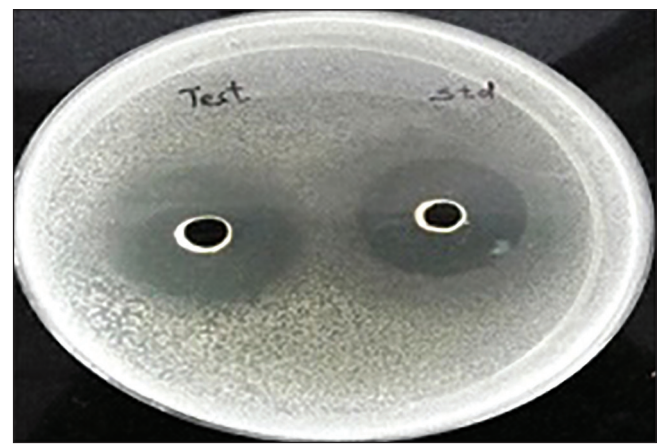| Conditions	Drug content (%)			
	Initial	1 month	2 months	3 months
5±2°C	96.36	96.31	96.27	96.15
25±2°C/60±5% RH	96.36	96.29	96.21	96.12

Figure 8: ZOI of voriconazole

CONCLUSION

It can been concluded that incorporation of voriconazole-loaded SLNs with *in situ* gel was formulated successfully. The SLNs *in situ* gel formulations was prepared using various polymers such as Carbopol 940 and HPMC, which release the drug in a sustained manner to decrease dosing frequency and to maintain prolonged therapeutic effect. The SLNs *in situ* gel formulation produced an excellent ZOI in microbial assay. Ocular irritation studies in rabbits showed no irritation. From the above study, it can be concluded that the use of SLNs-loaded *in situ* gel provides a number of advantages over the conventional ocular dosage forms. Sustained and prolonged release makes the delivery system more reliable and more acceptable to the patients, and increases patient compliance. The SLNs *in situ* gel formulation can be developed as an acceptable and excellent formulation for ocular drug delivery. However, various *in vivo* studies and clinical trials are required for it to be developed as an ocular dosage formulation.

Conflicts of interest
There are no conflicts of interest.

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