International Journal of Pharmaceutics 602 (2021) 120623

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Thermosensitive and mucoadhesive *in situ* ocular gel for effective local delivery and antifungal activity of itraconazole nanocrystal in the treatment of fungal keratitis

Andi Dian Permana [a,*], Rifka Nurul Utami [a], Patricia Layadi [a], Achmad Himawan [a,b], Nana Juniarti [c], Qonita Kurnia Anjani [a,b], Emilia Utomo [b], Sandra Aulia Mardikasari [a], Andi Arjuna [a], Ryan F. Donnelly [b]

[a] Department of Pharmaceutics, Faculty of Pharmacy, Universitas Hasanuddin, Makassar 90245, Indonesia
[b] School of Pharmacy, Queen's University Belfast, Belfast BT9 7BL, UK
[c] Department of Pharmaceutical Microbiolgy, Faculty of Pharmacy, Universitas Hasanuddin, Makassar 90245, Indonesia

ARTICLE INFO

Keywords:
Itraconazole
Nanocrystals
Thermosensitive *in situ* ocular gel
Fungal keratitis
Mucoadhesive
Antifungal

ABSTRACT

Itraconazole is a lipophilic drug, which limits its absorption for ocular administration. This study focused on the incorporation of itraconazole into nanocrystalline carrier system with stabilizer Pluronic® F127 and was further formulated into thermosensitive *in situ* ocular gel. Itraconazole nanocrystals (ITZ-NCs) were fabricated using media milling method with ultra-small-scale device. The obtained nanocrystals were observed to have a better *in vitro* activity against *C. albicans* (CA) compared to free itraconazole suspension in water. Furthermore, the optimization of the thermosensitive ocular gel formula was carried out with a central composite design, using three types of polymers, namely Pluronic® F127, Pluronic® F68, and hydroxypropyl methylcellulose (HPMC). After being dispersed into the optimized thermosensitive gel base, ITZ-NCs did not alter in terms of physical characteristics. *Ex vivo* ocularkinetic studies on infected porcine eye models showed a better profile of the optimized formula of thermosensitive *in situ* ocular gel when compared to standard gel base. Importantly, the *ex vivo* antifungal activity of these preparations was also increased, with a 93% decrease in the CA population observed after 48 h in infected porcine eye model. Altogether, this work has provided evidence of a novel approach in developing more advanced treatments for fungal keratitis.

1. Introduction

Fungal keratitis is an ocular inflammation, especially in the cornea, caused by fungal infection. Fungal keratitis has become one of serious eye diseases because it can lead to blindness and total loss of vision. Fungal keratitis may affect both male and female in either developing or developed countries. In developed country such as the United States, the use of contact lenses becomes the major cause of this condition. Conversely, in developing countries, ocular trauma caused by soil-contaminated-object or plants has been reported as the main factor inducing this disease (Acharya et al., 2017; Ansari et al., 2013). Most fungal keratitis is associated with the infection of *Fusarium, Candida, Aspergillus*. This condition remains a challenge to clinicians due to delay in diagnosis and difficulty in treatment which could aggravate the corneal ulcer (Anutarapongpan and O'Brien, 2014).

One of antifungal agents that has been widely used for the treatment of fungal keratitis is itraconazole (ITZ). This drug shows a wide spectrum antifungal activity and has been used at the dose of 200–400 mg/day (orally) and 1% (topically) to treat fungal keratitis (Miller and Alfonso, 2014). However, systemic therapy with ITZ is usually linked to some adverse effects, such as gastrointestinal upset, headache, transient skin reaction and rare case of hepatitis (Gupta et al., 2002; Lestner and Hope, 2013). Because of that, topical delivery of ITZ directly into the eye is more preferable. Still, ITZ has very low solubility in water (1 ng/mL) and in acidic solution (4 μg/mL), which results in poor bioavailability and limits the number of ITZ topical preparations available in the market (Permana et al., 2020b; Sahay et al., 2019).

To overcome those problems, nanocrystal (NC) approach can be utilized. NCs are nanoparticles in the form of nanosized crystalline (200 to 500 nm) and composed of pure drug with stabilizers on the surface.

* Corresponding author.
E-mail address: andi.dian.permana@farmasi.unhas.ac.id (A.D. Permana).

https://doi.org/10.1016/j.ijpharm.2021.120623
Received 16 February 2021; Received in revised form 23 March 2021; Accepted 18 April 2021
Available online 21 April 2021

Nanoparticle-based drug delivery provides several advantages, such as improved bioavailability, enhanced safety, higher drug loading, and most importantly increased solubility of poorly soluble drug, including ITZ (Sharma et al., 2016; Zhou et al., 2017). Previously, we have developed NC formulation of ITZ for improved efficacy in the *ex vivo* candidiasis skin infection model (Permana et al., 2020b). Bearing this in mind, formulating the drug into NC may help enhancing the efficacy following ocular administration.

Nevertheless, the anatomical and physiological structure of the eye may still hinder drug absorption. Not only that, the eye also has a tear turnover mechanism which results in rapid elimination of the drug (Macwan et al., 2016; Sharma et al., 2016). Previous studies had shown two ways in increasing the bioavailability of drug in the eye; by using permeation enhancer, such as cyclodextrin, ethylenediamine tetra acetic acid (EDTA), bile acid and its salts or by increasing the viscosity of the preparations such as in the form of hydrogel or *in situ* gel (Kaur and Smitha, 2002). *In situ* gel is a liquid preparation that will transform into gel when exposed to physiological conditions. The sol–gel transformation can be stimulated by body temperature, ionic strength of biological fluids, and physiological pH. This type of preparation provides numerous benefits, namely accurate dosing, control of drug release, and increased patient compliance due to less administration frequency required (Soliman et al., 2019; Wu et al., 2019).

The most frequently utilized thermosensitive polymers in pharmaceutical formulations, Poloxamers (known as Pluronics®), are amphiphilic synthetic copolymers comprising of polyoxypropylene oxide (PPO) block as hydrophobic part bounded by polyoxyethylene oxide (PEO) blocks as hydrophilic (Morsi et al., 2016). Because of their amphiphilic characteristic, Pluronics® can easily self-assemble to generate small micellar elements at low temperature. At higher temperature, they generate large micellar cross-linked network (Jiang et al., 2020). Importantly, in comparison with other thermosensitive polymers, such as polyester-based polymers, Pluronics® are reported to be more stable from degradation in *in vivo* environment (Russo and Villa, 2019).

This paper reports a formulation of ITZ-NC in a thermosensitive *in situ* ocular gel for increased efficacy in the therapy of fungal keratitis. First of all, ITZ was made into a nanocrystal system to increase its solubility. After that, we optimized the thermosensitive *in situ* gel formulation by taking into account various parameters related to its physicochemical characteristics. Finally, the optimized *in situ* gel formula was tested *ex vivo* to determine its ocularkinetic profile and antifungal activity.

2. Materials and methods

2.1. Materials

Itraconazole (ITZ) of analytical grade was obtained from Tokyo Chemical Industry (Tokyo, Japan) with the purity of $\geq$ 98%. Pluronic® F127 (PF127) and HPMC K100 were obtained from Sigma–Aldrich Pte Ltd, (Singapore, Singapore). Pluronic® F68 (PF68) was kindly gifted by BASF SE (Jakarta, Indonesia). All other chemicals used in this study were of pharmaceutical grade.

2.2. Preparation and characterization of itraconazole nanocrystals

Itraconazole nanocrystals (ITZ-NCs) were prepared by a milling method using the ultra-small-scale device according to method described in our previous study (Permana et al., 2020b). An amount of 10 mL of 1% w/v Pluronic® F127 and 0.2 g ITZ were mixed in a 12 mL glass vial. Afterwards, 8 g of zirconia beads were added into the vial. Then, the system was stirred with two magnetic bars (12 × 6 mm) on basic magnetic stirrer (Thermo Scientific, Massachusetts, USA) for 6 h at 1000 rpm. After that, in order to separate the zirconia beads and magnetic bars from the formulations, the slurry was filtered (mesh 200 sieve). Finally, the NCs were washed three times using distilled water at 14,000 rpm for 30 min.

Following preparation, the nanocrystals were characterized for the particle size and polydispersity index (PdI) by dynamic light scattering (DLS) using a Zetasizer (Malvern Zeta Sizer, Malvern Instruments, Malvern, UK). As a comparison, the characterization was also carried out after the NCs were dispersed in the thermosensitive *in situ* ocular gel formulation. All of the measurements were carried out at 25 °C with equilibration time of 3 min.

To understand the dissolution rate of ITZ from NC formulation, the *in vitro* release study was carried out using a dialysis method (Permana et al., 2019a; Permana et al., 2019b). Free-ITZ (10 mg) and ITZ-NCs which was equal to 10 mg of free-ITZ were dispersed into the dialysis membrane 12,000–14,000 MWCO (Spectra-Por®, Spectrum Medical Industries, Los Angeles, CA, USA). The dissolution study was conducted for 24 h at 100 rpm at 37C in 100 mL of PBS (pH 7.4). To ensure the sink condition, the dissolution media was 1% w/v of Tween 80. At predetermined time intervals, 1 mL was collected, and 1 mL of fresh dissolution media was added to release the media collected. The amount of ITZ released from ITZ was quantified using HPLC.

2.3. In vitro antifungal activity

2.3.1. Culture of Candida albicans (CA)

CA SC5314 (ATCC MYA-2876) purchased from (LGC Standards, Middlesex, UK) was maintained at 4 °C. The fungal cultivation was carried out at 37 °C and 100 rpm in Sabouraud Dextrose Broth (SDB) for 24 h. Fungal pellets were collected through centrifugation (3000 rpm for 30 min), then re-dispersed in fresh SDB. The optical density was set to equivalent to 1.5×10^7 CFU/ml at 550 nm prior to the antifungal study.

2.3.2. In vitro antifungal activity of ITZ-NCs

The antifungal activity study of ITZ-NCs, ITZ in dimethyl sulfoxide (DMSO), and ITZ in water against CA was carried out using the agar diffusion method. For ITZ in DMSO, initially, ITZ were prepared in DMSO. Afterwards, the work solution was prepared by diluting the ITZ-DMSO solution in water. The final concentration of DMSO in the work solution was 1% v/v. First of all, the growth medium of Sabouraud Dextrose Agar (SDA) was prepared with the addition of chloramphenicol (50 mg/mL) to prevent bacterial growth. Then, CA inoculum was spread all over the agar on the petri dish. An amount equal to 2.5 μg/mL of free ITZ of the test materials was placed on the paper disc. Afterward, the petri dishes were incubated for 48 h at 32 °C. The inhibitory zone formed around the paper discs was measured using digital vernier calipers.

2.3.3. Determination of minimum inhibitory concentration and minimum fungicidal concentration

Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) were evaluated using microtiter broth dilution method. Three different preparations of ITZ were studied, namely ITZ suspension in water, ITZ solution in DMSO, and TZ-NCs. The experiment was carried out as directed by the Clinical and Laboratory Standard Institute's Protocol. Briefly, fungal suspension in SDB (100 μL) with concentration of 1.5×10^7 CFU/ml was placed in each bottom of the microplates. Then, 100 μL of ITZ preparations in various concentration was added, corresponding to 7.5×10^6 CFU/mL of fungal concentration. After that, the plates were incubated at 37 °C for 24 h. MIC was determined based on the lowest concentration that inhibited fungal growth following incubation. Whereas, the MFC was determined by cultivating 20 μL of dilutions from MIC and concentration above the MIC wells into SDA plates and incubated at 37 °C for another 24 h. The concentration in which 99.9% of fungal death was observed was determined as the MFC.

2.3.4. Time kill assay

Time kill assay of ITZ and ITZ-NCs was performed three times according to previous method (Permana et al., 2021; Permana et al.,

Table 1
The experimental design of ITZ-NCs thermosensitive *in situ* ocular gel formulation.

Factors	Level		
	Low	Medium	High
Pluronic® F127 concentration (%w/v)	15.00	17.50	20.00
Pluronic® F68 concentration (%w/v)	0	3.75	7.50
HPMC K100 concentration (%w/v)	0.50	1.00	1.50

2020a) with modifications. Dilutions of ITZ and ITC-NCs with concentrations equivalent to MIC, 2x MIC, and 4x MIC were mixed with the CA fungal dispersion to form 7.5 × 10^6 CFU/ml. Following incubation at 37 °C for 24 h, 20 μL of culture media was collected at 0, 2, 4, 6, 8, 12, 18 and 24 h. The culture was then cultivated into fresh SDA plates and further incubated at 37 °C for 24 h. At last, the CFU on the SDA plates were counted and the log CFU/ml was plotted against time-kill to produce the curve.

2.4. Optimization of thermosensitive in situ ocular gel

A central composite design (CCD) using Design Expert Software ver. 11 (State-Ease, Minneapolis, MN, USA) was incorporated for the optimization of thermosensitive *in situ* ocular gel formulations. The variables chosen in this study were the concentrations of the gel-forming polymers, namely Pluronic® F127 (PF127), Pluronic® F68 (PF68), and HPMC (Table 1). The concentrations were analyzed at three different level (low, medium, high). The response was observed as the physicochemical characteristics of the resulting ocular gels, including sol–gel temperature, mucoadhesion strength, pH, drug content, and viscosity at 25 °C and 35 °C. The optimum formulation was determined as the *in situ gel* with sol–gel temperature at 35 °C.

2.5. Preparation of thermosensitive in situ ocular gel

The thermosensitive *in situ* ocular gel of ITZ-NCs was prepared with varied polymer concentrations based on the experimental design showed in Table 1. Firstly, specified amount of HPMC was dissolved in water and slowly stirred until clear solution was obtained. After that, Pluronic® F127 and F68 was added, and the mixture was placed in refrigerator overnight. The mixture was stirred continuously until the entire components fully dissolved without any visible lump. The amount of ITZ-NCs incorporated into the *in situ* gel was equal to 1% w/v of free ITZ.

2.6. Characterization of thermosensitive in situ ocular gel formulations

2.6.1. Sol-gel transition temperature measurement ($T_{sol\text{-}gel}$)

Sol-gel transition temperature was determined according to previous method (Khattab et al., 2019) by placing a 2 mL of *in situ* gel in a closed-tube vial and heated in the water bath at 20 °C. The vials were rotated 90° every 1 °C increment of the temperature until the bath temperature reached 65 °C. $T_{sol\text{-}gel}$ was determined as the temperature at which the gel did not flow while the vial being rotated. The test was performed in triplicate.

2.6.2. Mucoadhesion strength

Mucoadhesion strength was measured according to previously described physical balance method (Morsi et al., 2016). Conjunctiva membrane extracted from porcine eye was used as a model membrane. The membranes were fixed onto two separated glass vials with the mucosal side out. One vial was connected upside down to a balance, while the other was put on a height-adjustable pan. The gel was added onto the membrane of the first vial, and the height of the other vial was adjusted so that the two mucosal surfaces came into contact and held for 2 min. After that, weights were placed into the pan until the vials got separated. The minimum weight to separate the vials was then calculated to obtain the mucoadhesion strength using the equation (1) below:

$$Mucoadhesionstrength\left(dyne \cdot cm^2\right) = \frac{m \cdot g}{A} \quad (1)$$

where, m is the weight required for detachment (g), A is the area of membrane exposed (cm^2), g is the gravity acceleration (980 cm/s^2).

2.6.3. Viscosity

Rheological properties of *in situ* ocular gel of ITZ-NCs were observed using viscometer (Brookfield DV III). Each 0.5 mL of the formula was used and put on the lower plate of the apparatus, then, the viscometer was run with shear rate at 20 up to 500 s^{-1}. Rheology test was performed using spindle no. 52 and at various temperatures (4 ± 0.1 °C, 25 ± 0.1 °C and 35 ± 0.1 °C).

2.6.4. pH

pH of *in situ* ocular gel preparation is an important factor to ensure patient's comfort during administration. The pH was measured using digital pH meter (Horiba Scientific, Kyoto, Japan) and the test was run in triplicate.

2.6.5. Drug content

The amount of ITZ in the thermosensitive *in situ* ocular gel was determined by dissolving a 1 mL of the *in situ* gel (equal to 10 mg of ITZ) in a 2 mL of acetone, then the mixture was sonicated for 1 h in a bath sonicator. The mixture was then centrifuged for 15 min at 14,000 rpm. Afterwards, the supernatant was collected to be analyzed using High Performance Liquid Chromatography (HPLC). The percentage of drug loading was calculated using the following equation:

$$Drugloading(\%) = \frac{AmountofencapsulatedITZ}{Totalweight} x100\% \quad (2)$$

2.7. Ocularkinetic study and antifungal activity in ex vivo model of infection on porcine eye

2.7.1. Preparation of ex vivo model of infection on porcine eye

Preparation of infected cornea was started by wounding porcine eye with a scalpel (3 slashes both horizontally and vertically). Then on the corneoscleral button, a metal ring was placed to create a watertight seal. Next, 10^8 CA was added into the centre of the ring, or the organisms mentioned above were injected intrastromally (using a 26-gauge needle; Becton Dickinson, Oxford, UK) with the same number. Cornea that had been infecting were incubated at 37 °C for 24 h and were then homogenized and diluted serially until the colony could be enumerated onto agar plates.

2.7.2. Ex vivo ocularkinetic study

Ex vivo ocularkinetic of thermosensitive *in situ* ocular gel of ITZ was examined using a Franz diffusion cells with method described previously with slight modification (Permana et al., 2020b). First, infected cornea membrane was connected using cyanoacrylate glue onto the donor compartment of the cell. The cell was then arranged so that the donor compartment connected to the acceptor part. Then, 1 mL of the *in situ* gel (corresponding to 10 mg of ITZ) was placed on top of the donor compartment. The cell was put on magnetic stirrer and stirred at 600 rpm while the temperature was maintained at 37 ± 1 °C. The cornea was removed at some predetermined time points (0.5, 1, 2, 3, 4, 5, 6, 8, 12, 24 and 48 h) and rinsed with sterile water three times. A 1.5 mL of chloroform was added into an Eppendorf tube along with the cornea to extract the ITZ. The sample was then vortexed for 15 min. Afterwards, the sample were centrifuged for 30 min at 14,000 rpm and the supernatant was analyzed using HPLC. The ocularkinetic profiles were evaluated using one-compartment open model of PKSolver (China Parmaceutical University, Nanjing, China) (Zhang et al., 2010). The

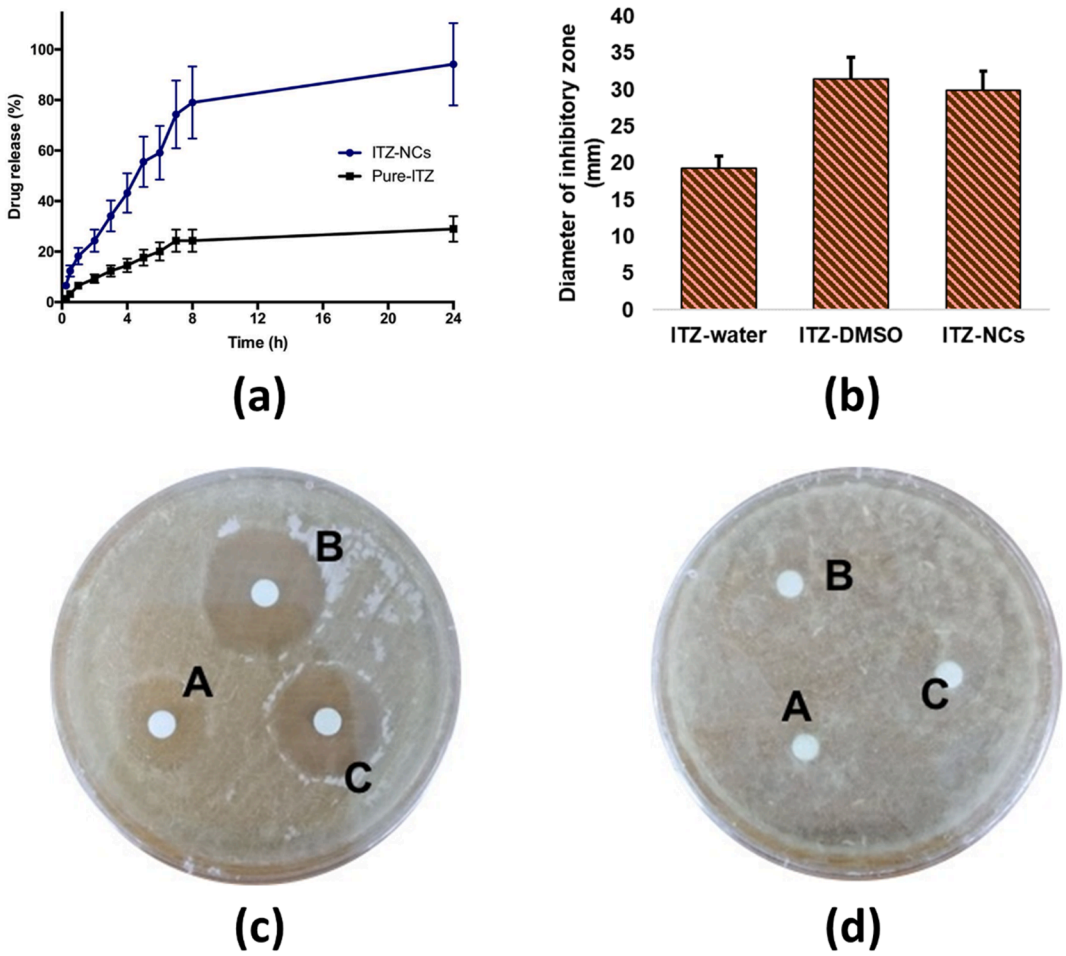

Fig. 1. (a) *In vitro* dissolution of ITZ-NCs compared to pure ITZ ; (b) Diameter of inhibitory zone formed against CA of three different forms of ITZ (mean ± SD, n = 3); (c) The inhibitory zone of (A) ITZ-water, (B) ITZ-DMSO, (C) ITZ-NCs; The inhibitory zone of controls.

maximum drug concentration (C_{max}), the time of maximum drug concentration (t_{max}), the drug concentration time curve from 0 to 72 h (AUC), the mean half-life ($t_{1/2}$) and the mean residence time (MRT) were all determined. As a comparison, non-infected cornea was also used in this study and treated with the same procedure.

2.7.3. *Antifungal activity in ex vivo model of infection on porcine eye*

Antifungal activity was studied using a modified method (Permana et al., 2020b). Supernatant collected from the ocularkinetic studies (24 and 48 h) was used to evaluate the antimicrobial activity of *in situ* ocular gel of ITZ-NCs against CA. A 20 µL of the supernatant was inoculated into TSA plate then, was incubated for 24 h at 37 °C. As a comparison, a non-infected eye was also used in this study and was treated same procedure as infected eye. At the end, the number of viable CFU was counted.

2.8. *HPLC analysis*

Samples were analyzed using HPLC at ambient temperature using a Phenomenex® Luna C18 (ODS1) column with a particle size of 5 µm and size of 150 mm × 4,6 mm. Samples were injected 50 µL, then analyzed using mobile phase of acetonitrile and 25 mM ammonium acetate buffer mixture with 65:35 v/v ratio of pH 5 solution. The mobile phase was run at a rate of 1 mL/min. Next, the samples were detected at 270 nm using UV detector. Analytical method was validated according to the International Committee on Harmonization (ICH) Guidelines 2005 (Permana et al., 2020b).

2.9. *Statistical analysis*

All data were represented as means ± standard deviation (SD) of the mean. SD of all results were calculated using Microsoft® Excel® 2016 (Microsoft Corporation, Redmond, USA). The statistical analysis was performed using GraphPad Prism® version 6 (GraphPad Software, San Diego, California, USA). The p value < 0.05 indicated a significant difference.

3. Results and discussion

3.1. *Characterization of ITZ-NCs*

The fabrication of ITZ-NCs in this present work was based on the optimization carried out in previous study by Permana, *et al.* (Permana et al., 2020b). To ensure the reproducibility of the utilized method and formulation, the ITZ-NCs were characterized for their particle size and PdI. The characterization results show particle size of the obtained NCs of 378 ± 31 nm with the PdI of 0.35 ± 0.03 (n = 3). Statistical analysis with previous report regarding this nanocrystal formulation shows no significant difference ($p > 0.05$), which suggests that the method and formulation were reproducible.

Fig. 1 shows the *in vitro* release performance of ITZ from NCs formulation compared to free ITZ. Based on this result, the dissolution rate of ITZ was significantly enhanced ($p < 0.05$) when formulated into NC form. After 24 h, 94.17 ± 16.32% of ITZ was released from NC formulation. On the other hand, the coarse ITZ dispersion was only release 28.98 ± 5.04% of ITZ after 24 h. The enhancement of the

Table 2
MIC and MFC of three different forms of ITZ against CA (n = 3).

	MIC (μg/ml)	MFC (μg/ml)
ITZ-water	1280	2560
ITZ-DMSO	2.5	5
ITZ-NCs	2.5	5

dissolution rate in NC formulation was caused by the enlargement of surface area of NC (Permana et al., 2020b).

3.2. In vitro antifungal activity

3.2.1. In vitro antifungal activity of ITZ-NCs

In this experiment, the antifungal activity of three different forms of ITZ against CA was studied, namely ITZ suspension in water (ITZ-water), ITZ solution in DMSO (ITZ-DMSO), and ITZ-NCs. The results of inhibitory zone of the different forms of ITZ are depicted in Fig. 1. The free suspension of ITZ in water had inhibitory zone diameter of 19.23 ± 1.76 mm. Remarkably, formulation of ITZ into NCs was able to enhance the antifungal activity, increasing the inhibitory zone diameter to 29.87 ± 2.65 mm. There were no inhibition zones found in water, 1% DMSO and stabilizer solution of NC formulation (vehicle control) (Fig. 1). Increased solubilization of ITZ in NCs form would allow the drug to diffuse to the medium and to have a more extensive penetration into the fungal cell membrane (Koks et al., 2002; Niwa et al., 2014). Additionally, the increase of inhibition zone of NC formulation might be also caused by the higher amount of free ITZ in comparison with the free ITZ of ITZ coarse dispersion in water. Accordingly, the inhibitory zone diameter of ITZ-DMSO did not differ significantly from ITZ-NCs ($p > 0.05$) with the value of 31.43 ± 2.98 mm. This also confirms that it is crucial for the drug to be present in soluble form in order to exhibit its antifungal activity.

3.2.2. Determination of minimum inhibitory concentration and minimum fungicidal concentration

The results for MIC and MFC are presented in Table 2. From the results, it is evident that the antifungal activity of ITZ-water is much lower than that of ITZ-DMSO and ITZ-NCs. The cause of this finding might be related to the hydrophobic nature of ITZ as explained previously. The antifungal mechanism of ITZ has been reported to be the inhibition of CYP450 enzyme on the fungal cell membrane. In order for the drug to exhibit its antifungal activity, it has to be able to penetrate to the cell membrane (Koks et al., 2002; Niwa et al., 2014). The penetration cannot occur if the drug presents as insoluble form, as per the case for ITZ suspension in water. On the contrary, ITZ-DMSO showed very high antifungal activity as a result of high solubility of ITZ in DMSO. Interestingly, similar results were observed for the ITZ-NCs. Indeed, this will uphold the previous hypothesis that the incorporation of ITZ into NCs will increase the solubilization. Consequently, this will result in enhanced antifungal activity of the drug. As for the MFC, all the obtained values are higher compared to the respective MIC. However, it is important to note that in all cases, the ratio between MFC and MIC was < 4, which suggests that ITZ exhibited fungicidal activity (Tato et al., 2014). Overall, these findings suggest that solubilization plays an important role in the antifungal activity of ITZ. Based on the observations, the antifungal activity of ITZ-NCs was comparable to that of free soluble ITZ in DMSO. This once again confirms that the formulation of ITZ into NCs in order to increase the solubility can be advantageous for any therapeutic means requiring this drug.

3.2.3. Time kill assay

The time kill curves of ITZ-DMSO and ITZ-NCs against CA are presented in Fig. 2. This experiment was performed to observe the time required for an antifungal agent to eradicate fungal growth completely. For the untreated group, the viable colony of CA reached 7.3 log CFU following 24 h of cultivation. It can also be seen from the result that at MIC, both ITZ solution in DMSO and ITZ-NCs could not kill CA 99.9%. At the concentration 2x MIC, no viable CA was observed after 18 h and 24 h for ITZ-DMSO and ITZ-NCs, respectively. Furthermore, shorter time kill was required with concentration 4x MIC, 12 h for ITZ-DMSO and 18 h for ITZ-NCs. From these observations, it can be stated that the fungicidal activity of ITZ is concentration- and time-dependent. The results also emphasized the importance of ITZ solubility in the fungal culture media to act as an antifungal agent.

3.3. Optimization of thermosensitive in situ ocular gel

Characteristics of an excellent thermosensitive *in situ* ocular gel can be determined based on several parameters to ensure the efficacy of the preparation to deliver drug to the eye. One of the most important factors in the formulation is the gel-forming polymer used. In this study,

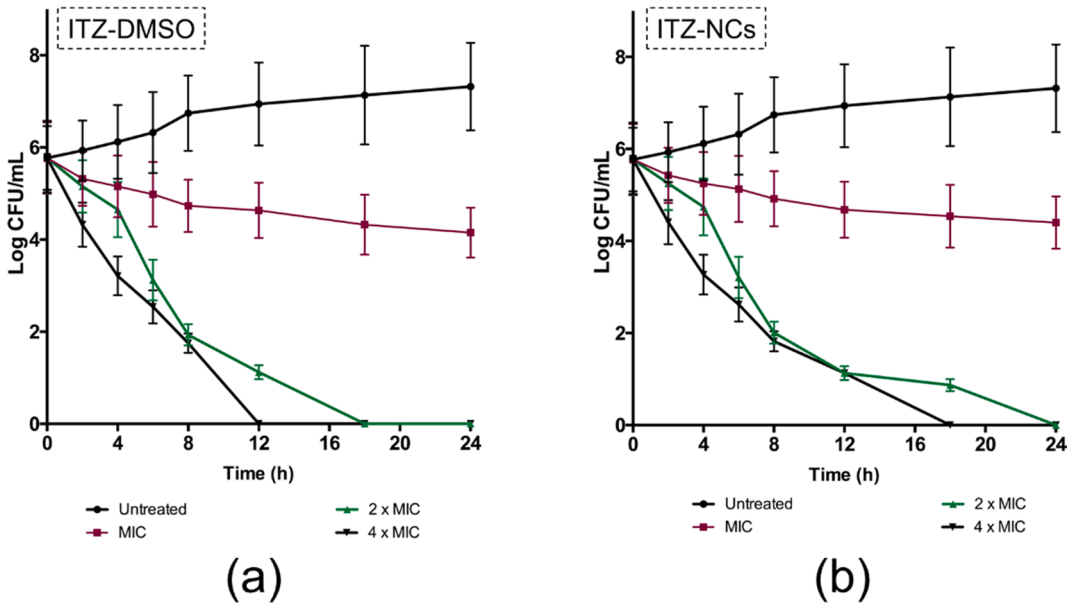

Fig. 2. Time kill assay of (a) ITZ-DMSO and (b) ITZ-NCs against CA (mean ± SD, n = 3).

Table 3
Variation of polymer concentration for the optimization of thermosensitive *in situ* ocular gel of ITZ-NCs.

Formula	ITZ-NCs (equal to free ITZ)	Polymer Concentration (% w/v)		
		PF127	PF68	HPMC
F1	1	17.5	3.75	1
F2	1	20	3.75	1
F3	1	17.5	0	1
F4	1	20	0	0.5
F5	1	17.5	7.5	1
F6	1	20	7.5	1.5
F7	1	15	7.5	0.5
F8	1	20	0	1.5
F9	1	15	0	1.5
F10	1	15	0	0.5
F11	1	15	3.75	1
F12	1	17.5	3.75	0.5
F13	1	17.5	3.75	1.5
F14	1	20	7.5	0.5
F15	1	15	7.5	1.5

combination of three polymers, namely Pluronic® F127, Pluronic® F68, and HPMC were utilized in the formulation of *in situ* ocular gel. Pluronic®, also known as poloxamer, is a triblock copolymer consists of hydrophilic ethylene oxide (EO) and hydrophobic propylene oxide (PO). Pluronic-based polymer, especially the type F127, has long been used for the formulation of thermosensitive gel. However, Pluronic® F127 possesses a number of drawbacks, such as short residence time once applied onto the ocular mucosal layer (Soliman et al., 2019). Therefore, other polymers are often added into the formulation, including Pluronic® F68 and HPMC (Khattab et al., 2019; Kurniawansyah et al., 2020; Morsi et al., 2016), to improve the gel characteristics. Since each polymer has distinct chemical properties, it is expected that different concentrations will affect the gel formation. For the formulation optimization, a central composite design was used with varying the polymer concentration. The polymer concentrations were tested on three level each; Pluronic® F127 (15%, 17.5%, 20%), Pluronic® F68 (0%, 3.75%, 7.5%), and HPMC (0.5%, 1%, 1.5%). The software suggested 15 formulas based on these compositions (Table 3). The response recorded for the characteristics of resulting *in situ* gel, including gelation temperature ($T_{sol\text{-}gel}$), mucoadhesion strength, viscosity, pH, and drug content.

3.3.1. *Effect of the polymer concentration on gelation temperature*

Gelation temperature ($T_{sol\text{-}gel}$) is one of important parameters for the formulation of thermosensitive gel. Formulation of thermosensitive *in situ* gel has to ensure that the preparation remains liquid at room temperature to provide accurate dosing (Soliman et al., 2019). The average temperature of mucosal layer in the eye is ~ 34 °C (Tan et al., 2010), thus the acceptable range of $T_{sol\text{-}gel}$ for thermosensitive *in situ* ocular gel is 30–35 °C (Khattab et al., 2019). $T_{sol\text{-}gel}$ values of the 15 formulations in the optimization range between 22.3 and 46.6 °C. The 3D graph representing the effect of polymer concentration on $T_{sol\text{-}gel}$ is shown in Fig. 3. Following the analysis, Pluronic® F127 and F68 both showed significant effect on the gelation temperature ($p < 0.05$). On the other hand, change in concentration of HPMC did not cause any significant

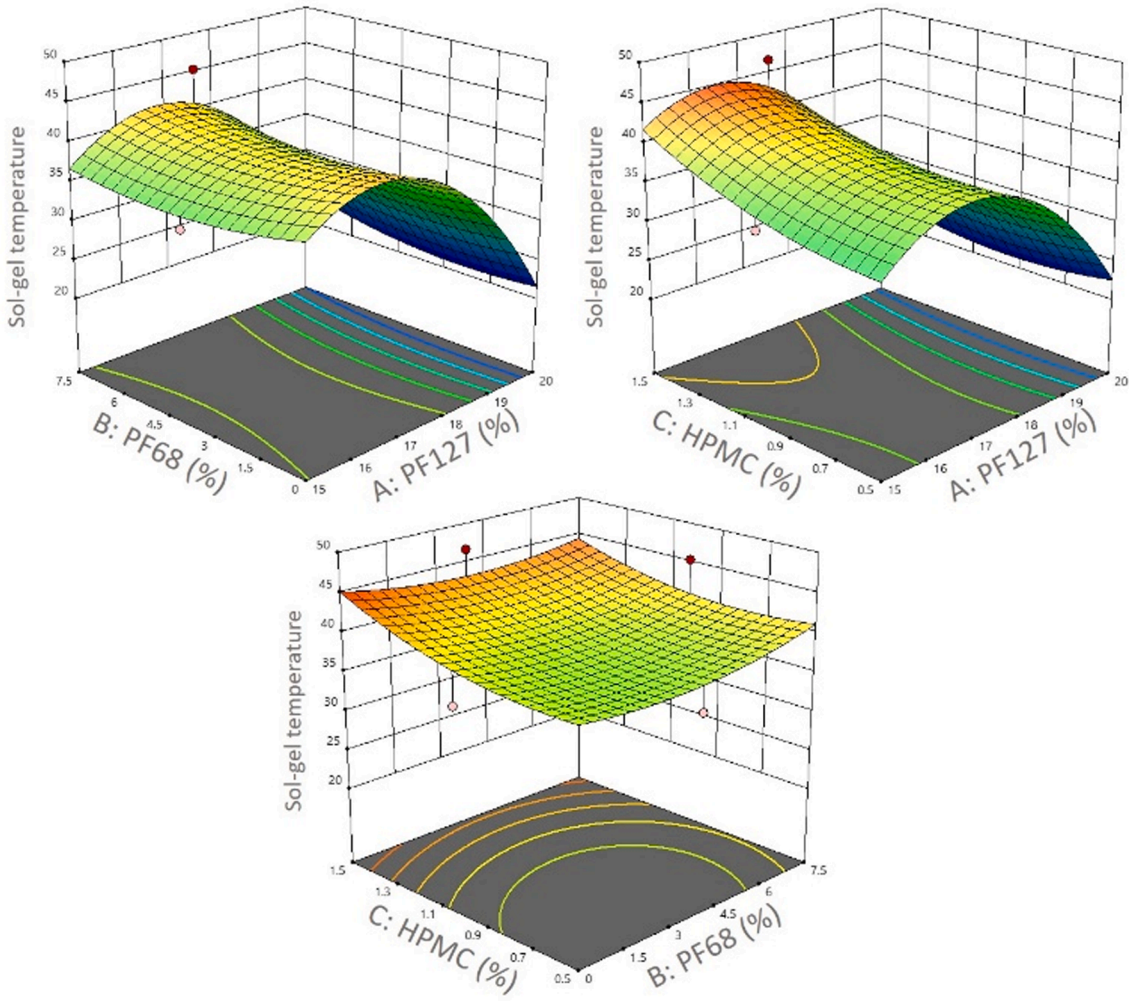

Fig. 3. Representation response surface plots describing the effect of polymer concentration on the sol–gel temperature of ITZ-NCs thermosensitive *in situ* ocular gel.

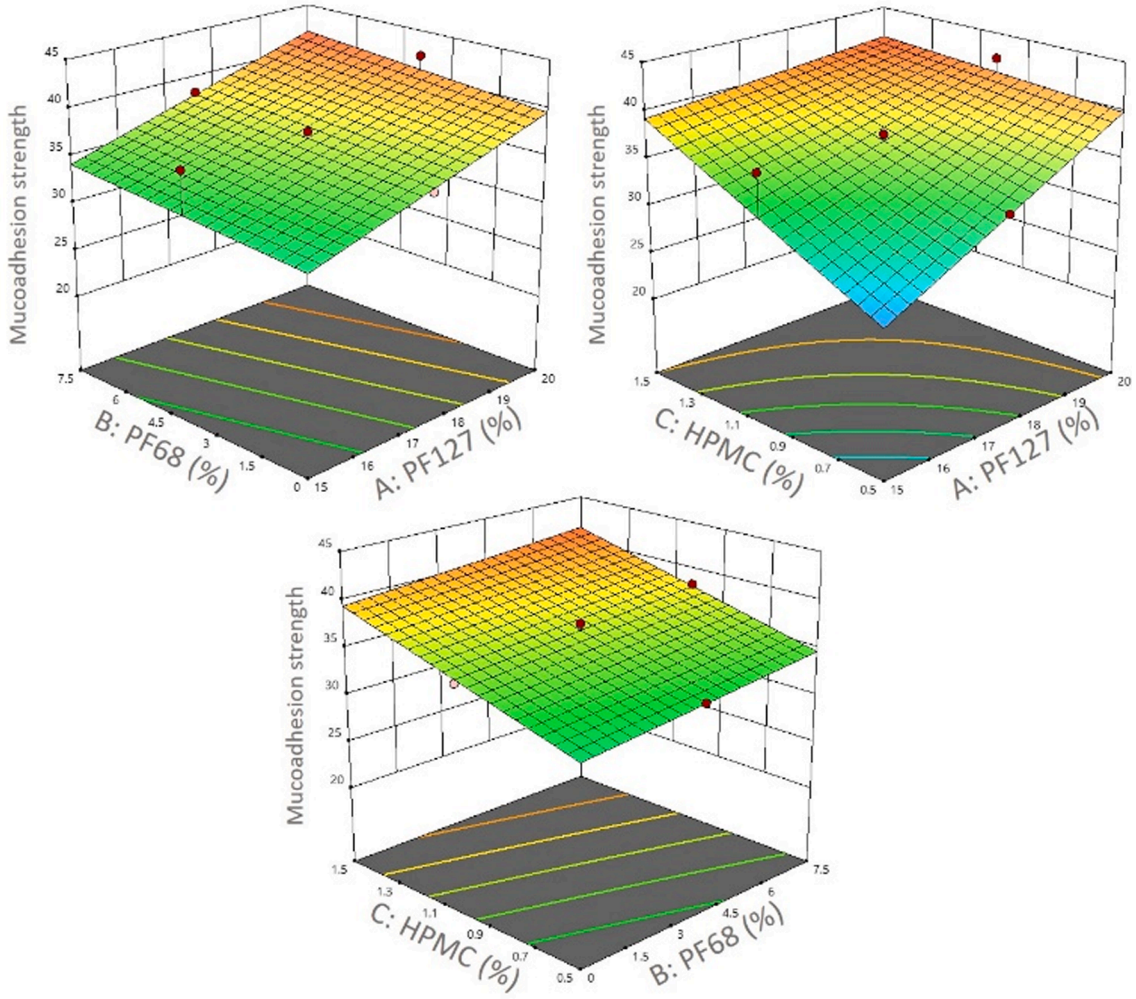

Fig. 4. Representation response surface plots describing the effect of polymer concentration on the mucoadhesion strength of ITZ-NCs thermosensitive *in situ* ocular gel.

alteration on the $T_{sol\text{-}gel}$. Interestingly, the effect of both types of Pluronic® F127 and F68 on the gelation temperature are the opposite of one another. Khattab *et al.* also found that the addition of Pluronic® F68 into F127 was able to increase the gelation temperature of the formulation (Khattab et al., 2019). Increase in the concentration of Pluronic® F127 lead to decrease in $T_{sol\text{-}gel}$ of resulting gel preparations. Conversely, the gelation temperature increased following the increasing concentration of Pluronic® F68. This phenomenon is considered to be due to the hydrophilic-hydrophobic structure difference between Pluronic® F127 and F68. The basic structure of Pluronic® is one PPO block as the center, flanked by two PEO blocks. The length of the blocks determines the type and physicochemical properties of the Pluronic® (Kozlov et al., 2000). The hydrophobic and hydrophilic portion ratios of Pluronic® F127 and F68 were 3:7 and 2:8, respectively, causing Pluronic® F127 to be more hydrophobic (Zhang et al., 2017). The gelation process of Pluronic involves dehydration of the hydrophobic PPO block, and hydration of the hydrophilic PEO block resulting in the polymer to swell at certain concentration and temperature. It is generally known that different PPO/PEO ratios will affect the gelation temperature of the resulting gel. Hydrophobic PPO block will cause a decrease in the $T_{sol\text{-}gel}$, whereas hydrophilic PEO will cause the opposite (Asasutjarit et al., 2011; Krtalić et al., 2018; M.A. Fathalla et al., 2017).

3.3.2. *Effect of the polymer concentration on mucoadhesion strength*

The contact time between the preparation and the ocular mucosal membrane also greatly determines the bioavailability of the drug administered to the eye. Therefore, the characteristic of a good ocular *in situ* gel is that it has sufficient mucoadhesion strength (Subrizi et al., 2019). The effect of the polymer on the mucoadhesion strength of the thermosensitive *in situ* ocular gel in this study is depicted on the 3D graphs in Fig. 4. Of the three polymers that were varied in concentration, only Pluronic® F127 and HPMC had a significant effect on the mucoadhesive properties of the gel formulations ($p < 0.05$). Generally, polymers with excellent mucoadhesive property are hydrophilic polymers, due to their ability to form hydrogen bond with mucin (Andrews et al., 2009). Cellulose derivatives, including HPMC, have been widely reported to have good mucoadhesive properties (Chowhan and Giri, 2020). The optimization results showed a positive relationship between the increase in the HPMC concentration in the formulation and the resulting mucoadhesion strength. Statistical analysis also showed a similar result with the increase of Pluronic® F127 concentration, although not at the same extent as that of HPMC. Nevertheless, various reports have shown that the use of Pluronic® F127 alone could not provide adequate mucoadhesion (Kurniawansyah et al., 2020; Morsi et al., 2016; Üstündağ Okur et al., 2019). Thus, the addition of other polymers such as HPMC is crucial for prolonging the residence time of the dosage form on the ocular surface. Ocular preparations with desirable mucoadhesive properties will help to avoid rapid drug elimination due to lacrimal drainage (Wu et al., 2019). This will consequently lead to enhanced efficacy of preparations given through ocular route.

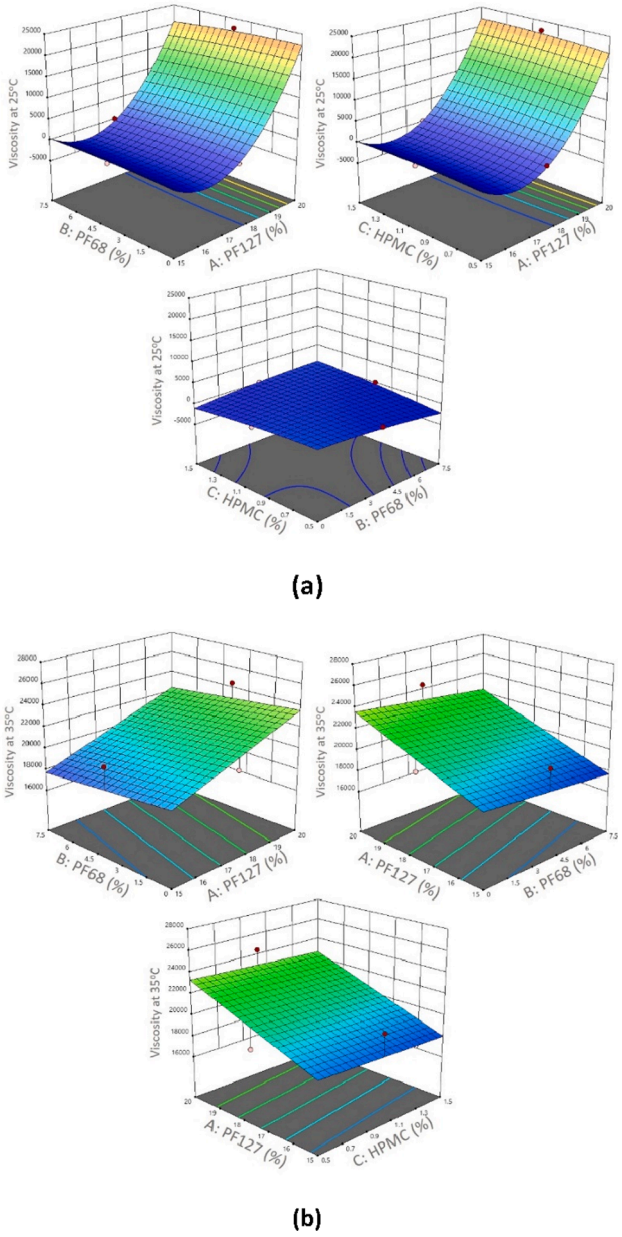

Fig. 5. Representation response surface plots describing the effect of polymer concentration on the viscosity of ITZ-NCs thermosensitive *in situ* ocular gel at 25 °C (a) and 35 °C (b).

3.3.3. Effect of the polymer concentration on pH and drug content

For ophthalmic preparations, pH is a factor that is closely related to patient compliance. Ideally, the pH of a preparation that is very well received by the eye is in the 6.5–8.5 range (Üstündağ Okur et al., 2019). pH that is too acidic or too alkaline will stimulate tears during application, thereby reducing therapeutic efficacy (Irimia et al., 2018). The results of this study indicate that of the 15 optimized *in situ* ocular gel formulations, all the measured pH meets the requirement, with a value of 7.22–7.51. Statistical analysis showed that out of the three polymers, only Pluronic® F127 had a significant effect on the pH of the preparation. The measurement results showed that the pH will increase with the increase in the concentration of Pluronic® F127. On the other hand, the type and concentration of the gelling polymer used had no significant effect on the drug content. The value of the drug content recorded was 98.08–100.21%. Incorporation of ITZ into NCs is believed to increase its dispersibility, including in aqueous polymeric solutions. The drug content measurement data also shows that the *in situ* ocular gel preparations produced have uniform drug content, thus ensuring the dosage accuracy when given to the patients.

Table 4
Predicted and actual value of the parameters following characterisation of the optimised formulation of ITZ-NCs thermosensitive *in situ* ocular gel.

Parameters	Predicted Value	Actual Value	Bias (%)
Sol-gel temperature (°C)	35.00	34.97 ± 0.39	−0.086
Mucoadhesion strength (dyne · cm^2)	43.657	43.91 ± 3.12	0.580
pH	7.38	7.43 ± 1.21	0.678
Drug content (%)	99.76	99.87 ± 1.02	0.110
Viscosity at 25 °C (cps)	126.161	127.91 ± 11.21	1.386
Viscosity at 35 °C (cps)	21584.431	22012.11 ± 1121.09	1.981

3.3.4. Effect of the polymer concentration on gel viscosity

One of the preferred attributes of *in situ* gel preparations is that they are in the form of a solution at room temperature, but are able to form gel following contact with physiological conditions. The solution form of the preparation during administration will help ensuring the dosage accuracy. Therefore, it is important to compare the viscosity of the gel *in situ* preparation at the storage temperature and the physiological temperature of the ocular membrane. Here, the viscosity of the formulations was measured at 25° and 35 °C. 3D graphs of the effect of polymer concentration on the viscosity are presented in Fig. 5. At 25 °C, only the concentration of Pluronic® F127 has a significant effect on viscosity, where the increase in polymer concentration also increases the viscosity of the preparation. In fact, formulations with a high Pluronic® F127 concentration (20% w/v) already formed gel at room temperature. Meanwhile, observations at 35 °C indicated that the concentration of Pluronic® F127 and F68, but not HPMC, had significant effect on the viscosity. Interestingly, the effects caused by the two types of Pluronic were the opposite of each other. These results are consistent with the measurement of gelation temperature, where a higher concentration of Pluronic® F127 caused the gel to form at lower temperatures. The explanation for this observation can be brought back to the nature of the Pluronic® F127 with higher hydrophobic chain ratio than that of Pluronic® F68. The hydrophobic PPO chain will become dehydrated upon contact with water. A higher extent of dehydration was found in Pluronic® F127 due to the longer PPO chain, making this polymer more easily entangled, so that a gel structure can form at lower temperatures (Morsi et al., 2016). Meanwhile, as previously described, Pluronic® F68 having a lower hydrophobic/hydrophilic ratio is able to modify the gelation temperature of the *in situ* gel formulation (Mahboobian et al., 2020; Morsi et al., 2016). Once again, this confirms that the combination of Pluronic® F127 and F68 is very advantageous for *in situ* gel formulations to obtain desirable viscosity, both at the time of storage and use of the preparations.

3.3.5. Characterization of the optimized thermosensitive in situ ocular gel formulation

Following the optimization and data analysis process using the software, an optimized formula was obtained. The *in situ* gel formulation was designed in such a way as to have gelation temperature at 35 °C, adequate mucoadhesion strength, suitable pH, high drug content and desirable viscosity at 25° and 35 °C. The optimum polymer concentrations as suggested by the software were 15.166 (% w/v), 5.629 (% w/v), and 1.105 (% w/v) for Pluronic® F127, Pluronic® F68, and HPMC, respectively. As for the predictions and actual results of the characterization of this optimal formula are presented in Table 4. Based on the test results, none of the optimal formula characterizations deviates > 15% from the predictions. Therefore, this optimal formula was used for further investigation in this study.

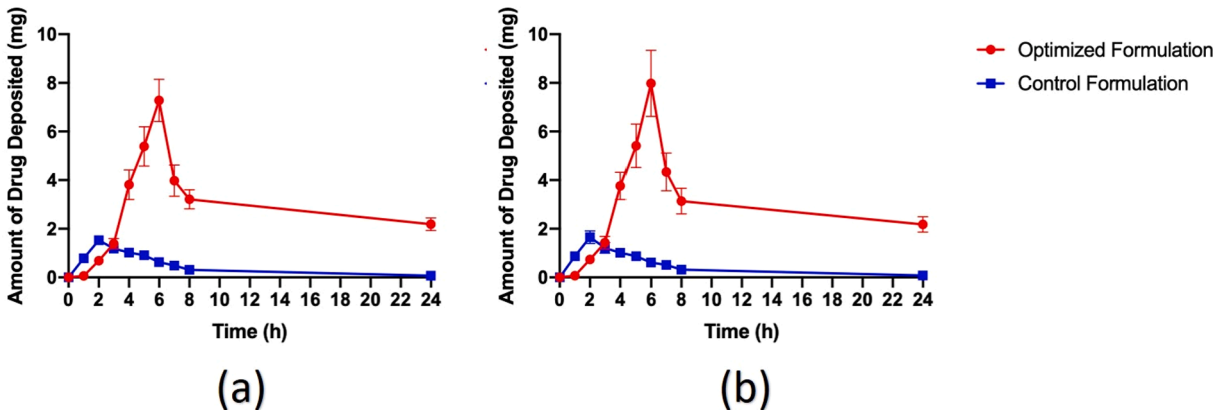

Fig. 6. Amount of ITZ deposited in (a) CA infected and (b) normal porcine eyes (mean ± SD, nn = n3).

Along with that, the ITZ-NCs were characterized following dispersion in the optimized thermosensitive ocular gel formulation. Encapsulation of ITZ into a nanocrystalline carrier aims to increase its solubility. Therefore, it is important to ensure that the characteristics of the NCs do not change after being dispersed into the polymeric gel base. After being dispersed to the optimized *in situ* ocular gel base, the characterization results obtained were 389 ± 37 nm for particle size with PdI 0.37 ± 0.03 (n = 3). From statistical analysis, it was found that there was no significant difference from the initial fabrication of the nanocrystals ($p > 0.05$). From these results, it can be concluded that the incorporation of NCs containing ITZ into an *in situ* ocular gel base consisting of a mixture of Pluronic® F127, Pluronic® F68, and HPMC polymers did not alter the physical characteristics of these NCs.

3.4. Ex vivo ocularkinetic studies

The main objective of ITZ formulation into thermosensitive *in situ* ocular gel preparation is to increase the efficacy of therapy following administration. Hence, it is imperative to assess the ocularkinetic properties of this preparation. The *ex vivo* ocularkinetic study of the thermosensitive *in situ* ocular gel was carried out on porcine eye model infected with CA. Experiments were carried out by comparing ITZ in NC formulation that was dispersed in thermosensitive gel and conventional eye drop preparation as a negative control. In general, it was observed that the thermosensitive gel formulation was able to increase ITZ absorption after ocular administration. The peak concentration (C_{max}) of ITZ measured after administration of the thermosensitive gel and control was 6.67 mg and 1.30 mg, respectively. The time required to reach the peak concentration (t_{max}) was 9.26 h for the thermosensitive gel and 2.26 h for control, with mean residence time (MRT) of 18.62 and 4.53 h, respectively. These three results prove the ability of the thermosensitive *in situ* ocular gel formulation to enhance the ocularkinetic profile of ITZ. Indeed, this enhancement could be related to the ability of thermosensitive gel to increase residence time on ocular surface. Al-Khateb, *et al.* reported that Pluronic® F127-based thermosensitive gel was able to form *in situ* gel with even coverage and reside longer on the corneal surface following application (Al Khateb et al., 2016). The longer contact time consequently increases the amount of drug that was deposited into the deeper eye tissues (Fig. 6). This was also evidenced by the significant difference ($p < 0.05$) of AUC of the drug measured in the optimized and control, which were 85.94 mg·h and 7.98 mg·h, respectively. Overall measurement of ocularkinetic parameters was also performed in normal eyes where all values were not significantly different from the infected eye. It has been reported that ITZ solubility was pH dependent, in which enhanced solubility was found at low pH (Yin et al., 2015). However, pH of tear fluid in mycotic keratitis was found to be around 7.15 (Norn, 1988), which is similar to normal tear fluid. Accordingly, the dissolution ability of ITZ in both cases might be similar, resulting in similar penetration profiles into cornea tissues.

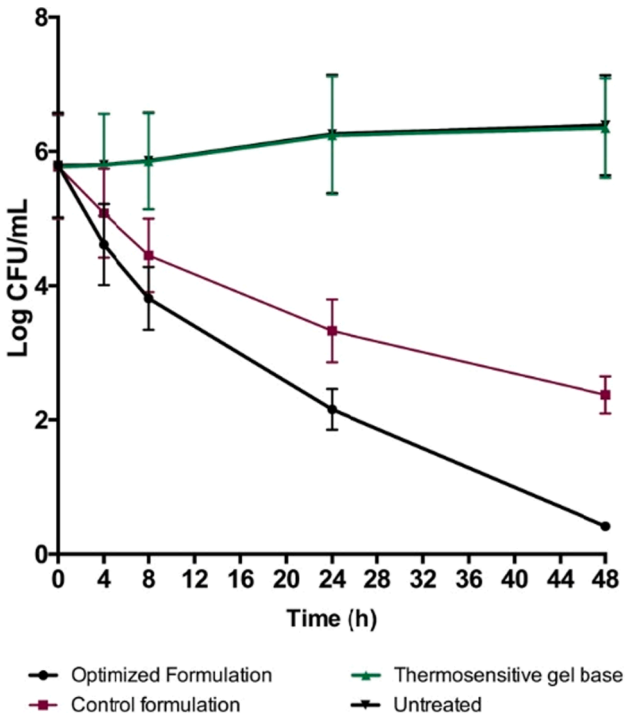

Fig. 7. The results of ex vivo antifungal activity studies expressed in log CFU/ml and (means ± SD, n = 3).

3.5. Antifungal activity in ex vivo infection model on porcine eye

Finally, to prove the ability of this preparation as a therapeutic candidate for fungal keratitis, antifungal activity study was carried out on CA cultures. The bioburden fungal observations carried out after 48 h showed that the incorporation of ITZ-NCs into the thermosensitive *in situ* ocular gel possessed greater antifungal activity. The fungal burden observed in untreated cultures increased from 5.8 log CFU to 6.4 log CFU. Based on the data shown in Fig. 7, administration of a thermosensitive *in situ* gel base (without ITZ) did not reduce the fungal bioburden at all after 48 h of incubation. In the administration of the control conventional eye drop preparation, a decrease in the number of fungal populations was observed, but not statistically significant, where the percentage decline in the population of CA was only 59% after 48 h. Meanwhile, application of thermosensitive *in situ* ocular gel containing ITZ-NCs showed impressive results, where the percentage of the decline

in the fungal population reached 63% after 24 h. After 48 h, a higher extent of reduction was observed of 93% (0.4 log CFU).

From these findings, it is apparent that the optimized formulation of thermosensitive *in situ* ocular gel of ITZ-NCs could enhance the delivery of the drug into the eye. The combination of NC carrier and thermosensitive gel system provide better ocularkinetic profile of ITZ which consequently increase the antifungal activity of the drug. This novel preparation evidently possesses distinguished characteristic for the development of fungal keratitis treatment compared to conventional eye drop. As an important future action, suitable animal model for *in vivo* study should be carefully considered.

4. Conclusion

Altogether, the experimental results from this present work indicate that the formulation of ITZ into NCs administered using a thermosensitive *in situ* ocular gel system shows superiority when compared to conventional eye drop. Incorporation of ITZ into the NCs increased the solubility of the drug, while further formulation into an optimized thermosensitive *in situ* ocular gel was found to increase residence time in the eye. This of course proves that this preparation can be a good candidate for the treatment of fungal keratitis. Further *in vivo* studies are highly recommended to test the effectiveness of this therapy in more depth as an effort to develop more advanced treatments for fungal keratitis.

CRediT authorship contribution statement

Andi Dian Permana: Conceptualization, Project administration, Funding acquisition, Validation, Supervision. **Rifka Nurul Utami:** Methodology, Writing - original draft. **Patricia Layadi:** Methodology, Investigation, Data curation. **Achmad Himawan:** Conceptualization, Supervision. **Nana Juniarti:** Methodology. **Qonita Kurnia Anjani:** Data curation. **Emilia Utomo:** Data curation. **Sandra Aulia Mardikasari:** Data curation. **Andi Arjuna:** Data curation. **Ryan F. Donnelly:** .

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

The authors thank Mrs. Hasliah and Mrs. Sumiati for their helps with the experiments. The authors also thank BASF SE (Jakarta, Indonesia) for providing Pluronic® F68.

References

Acharya, Y., Acharya, B., Karki, P., 2017. Fungal keratitis: study of increasing trend and common determinants. Nepal J Epidemiol 7, 685–693.
Al Khateb, K., Ozhmukhametova, E.K., Mussin, M.N., Seilkhanov, S.K., Rakhypbekov, T. K., Lau, W.M., Khutoryanskiy, V.V., 2016. In situ gelling systems based on Pluronic F127/Pluronic F68 formulations for ocular drug delivery. International Journal of Pharmaceutics 502, 70–79.
Andrews, G.P., Laverty, T.P., Jones, D.S., 2009. Mucoadhesive polymeric platforms for controlled drug delivery. European Journal of Pharmaceutics and Biopharmaceutics 71, 505–518.
Ansari, Z., Miller, D., Galor, A., 2013. Current Thoughts in Fungal Keratitis: Diagnosis and Treatment. Curr Fungal Infect Rep 7, 209–218.
Anutarapongpan, O., O'Brien, T.P., 2014. Update on Management of Fungal Keratitis. Clinical Microbiology: Open. Access 3.
Asasutjarit, R., Thanasanchokpibull, S., Fuongfuchat, A., Veeranondha, S., 2011. Optimization and evaluation of thermoresponsive diclofenac sodium ophthalmic in situ gels. International Journal of Pharmaceutics 411, 128–135.
Chowhan, A., Giri, T.K., 2020. Polysaccharide as renewable responsive biopolymer for in situ gel in the delivery of drug through ocular route. International Journal of Biological Macromolecules 150, 559–572.
Gupta, A.K., Chwetzoff, E., Del Rosso, J., Baran, R., 2002. Hepatic safety of itraconazole. Journal of cutaneous medicine and surgery 6, 210–213.
Irimia, T., Ghica, M.V., Popa, L., Anuta, V., Arsene, A.L., Dinu-Pirvu, C.E., 2018. Strategies for Improving Ocular Drug Bioavailability and Corneal Wound Healing with Chitosan-Based Delivery Systems. Polymers Basel) 10.
Jiang, Q., Zhang, P., Li, J., 2020. Elucidation of Colloid Performances of Thermosensitive In Situ-Forming Ophthalmic Gel Formed by Poloxamer 407 for Loading Drugs. Journal of Pharmaceutical Sciences 109, 1703–1713.
Kaur, I.P., Smitha, R., 2002. Penetration Enhancers and Ocular Bioadhesives: Two New Avenues for Ophthalmic Drug Delivery. Drug Development and Industrial Pharmacy 28, 353–369.
Khattab, A., Marzok, S., Ibrahim, M., 2019. Development of optimized mucoadhesive thermosensitive pluronic based in situ gel for controlled delivery of Latanoprost: Antiglaucoma efficacy and stability approaches. Journal of Drug Delivery Science and Technology 53, 101134.
Koks, C.H.W., Meenhorst, P.L., Bult, A., Beijnen, J.H., 2002. Itraconazole Solution: Summary of Pharmacokinetic Features and Review of Activity in The Treatment of Fluconazole-Resistant Oral Candidosis in HIV-Infected Persons. Pharmacological Research 46, 195–201.
Kozlov, M.Y., Melik-Nubarov, N.S., Batrakova, E.V., Kabanov, A.V., 2000. Relationship between Pluronic Block Copolymer Structure, Critical Micellization Concentration and Partitioning Coefficients of Low Molecular Mass Solutes. Macromolecules 33, 3305–3313.
Krtalić, I., Radošević, S., Hafner, A., Grassi, M., Nenadić, M., Cetina-Čižmek, B., Filipović-Grčić, J., Pepić, I., Lovrić, J., 2018. D-Optimal Design in the Development of Rheologically Improved In Situ Forming Ophthalmic Gel. Journal of Pharmaceutical Sciences 107, 1562–1571.
Kurniawansyah, I.S., Rusdiana, T., Sopyan, I., Ramoko, H., Wahab, H.A., Subarnas, A., 2020. In situ ophthalmic gel forming systems of poloxamer 407 and hydroxypropyl methyl cellulose mixtures for sustained ocular delivery of chloramphenicole: optimization study by factorial design. Heliyon 6, e05365.
Lestner, J., Hope, W.W., 2013. Itraconazole: an update on pharmacology and clinical use for treatment of invasive and allergic fungal infections. Expert opinion on drug metabolism & toxicology 9, 911–926.
M.A. Fathalla, Z., Vangala, A., Longman, M., Khaled, K.A., Hussein, A.K., El-Garhy, O.H., Alany, R.G., 2017. Poloxamer-based thermoresponsive ketorolac tromethamine in situ gel preparations: Design, characterisation, toxicity and transcorneal permeation studies. European Journal of Pharmaceutics and Biopharmaceutics 114, 119-134.
Macwan, J.S., Hirani, A., Pathak, Y., 2016. Challenges in Ocular Pharmacokinetics and Drug Delivery. In: Pathak, Y., Sutariya, V., Hirani, A.A. (Eds.), Nano-Biomaterials For Ophthalmic Drug Delivery. Springer International Publishing, Cham, pp. 593–611.
Mahboobian, M.M., Mohammadi, M., Mansouri, Z., 2020. Development of thermosensitive in situ gel nanoemulsions for ocular delivery of acyclovir. Journal of Drug Delivery Science and Technology 55, 101400.
Miller, D., Alfonso, E.C., 2014. Management of fungal keratitis: Topical or Systemic therapy? Vision Pan-America, The Pan-American. Journal of Ophthalmology 13.
Morsi, N., Ghorab, D., Refai, H., Teba, H., 2016. Ketoroloac tromethamine loaded nanodispersion incorporated into thermosensitive in situ gel for prolonged ocular delivery. International Journal of Pharmaceutics 506, 57–67.
Niwa, T., Imagawa, Y., Yamazaki, H., 2014. Drug interactions between nine antifungal agents and drugs metabolized by human cytochromes P450. Current drug metabolism 15, 651–679.
Norn, M.S., 1988. Tear fluid pH in normals, contact lens wearers, and pathological cases. Acta ophthalmologica 66, 485–489.
Permana, A.D., Anjani, Q.K., Sartini, Utomo, E., Volpe-Zanutto, F., Paredes, A.J., Evary, Y.M., Mardikasari, S.A., Pratama, M.R., Tuany, I.N., Donnelly, R.F., 2021. Selective delivery of silver nanoparticles for improved treatment of biofilm skin infection using bacteria-responsive microparticles loaded into dissolving microneedles. Materials Science and Engineering: C 120, 111786.
Permana, A.D., McCrudden, M.T.C., Donnelly, R.F., 2019a. Enhanced Intradermal Delivery of Nanosuspensions of Antifilariasis Drugs Using Dissolving Microneedles: A Proof of Concept Study. Pharmaceutics 11.
Permana, A.D., Mir, M., Utomo, E., Donnelly, R.F., 2020a. Bacterially sensitive nanoparticle-based dissolving microneedles of doxycycline for enhanced treatment of bacterial biofilm skin infection: A proof of concept study. International Journal of Pharmaceutics: X 2, 100047.
Permana, A.D., Paredes, A.J., Volpe-Zanutto, F., Anjani, Q.K., Utomo, E., Donnelly, R.F., 2020b. Dissolving microneedle-mediated dermal delivery of itraconazole nanocrystals for improved treatment of cutaneous candidiasis. European Journal of Pharmaceutics and Biopharmaceutics 154, 50–61.
Permana, A.D., Tekko, I.A., McCrudden, M.T.C., Anjani, Q.K., Ramadon, D., McCarthy, H.O., Donnelly, R.F., 2019b. Solid lipid nanoparticle-based dissolving microneedles: A promising intradermal lymph targeting drug delivery system with potential for enhanced treatment of lymphatic filariasis. Journal of Controlled Release 316, 34–52.
Russo, E., Villa, C., 2019. Poloxamer Hydrogels for Biomedical Applications. Pharmaceutics 11.
Sahay, P., Singhal, D., Nagpal, R., Maharana, P.K., Farid, M., Gelman, R., Sinha, R., Agarwal, T., Titiyal, J.S., Sharma, N., 2019. Pharmacologic therapy of mycotic keratitis. Survey of ophthalmology 64, 380–400.
Sharma, O.P., Patel, V., Mehta, T., 2016. Nanocrystal for ocular drug delivery: hope or hype. Drug delivery and translational research 6, 399–413.
Soliman, K.A., Ullah, K., Shah, A., Jones, D.S., Singh, T.R.R., 2019. Poloxamer-based in situ gelling thermoresponsive systems for ocular drug delivery applications. Drug Discovery Today 24, 1575–1586.
Subrizi, A., del Amo, E.M., Korzhikov-Vlakh, V., Tennikova, T., Ruponen, M., Urtti, A., 2019. Design principles of ocular drug delivery systems: importance of drug payload, release rate, and material properties. Drug Discovery Today 24, 1446–1457.

Tan, J.-H., Ng, E.Y.K., Rajendra Acharya, U., Chee, C., 2010. Study of normal ocular thermogram using textural parameters. Infrared Physics & Technology 53, 120–126.
Tato, M., López, Y., Morosini, M.I., Moreno-Bofarull, A., Garcia-Alonso, F., Gargallo-Viola, D., Vila, J., Cantón, R., 2014. Characterization of variables that may influence ozenoxacin in susceptibility testing, including MIC and MBC values. Diagnostic Microbiology and Infectious Disease 78, 263–267.
Üstündağ Okur, N., Yozgatlı, V., Okur, M.E., Yoltaş, A., Siafaka, P.I., 2019. Improving therapeutic efficacy of voriconazole against fungal keratitis: Thermo-sensitive in situ gels as ophthalmic drug carriers. Journal of Drug Delivery Science and Technology 49, 323–333.
Wu, Y., Liu, Y., Li, X., Kebebe, D., Zhang, B., Ren, J., Lu, J., Li, J., Du, S., Liu, Z., 2019. Research progress of in-situ gelling ophthalmic drug delivery system. Asian Journal of Pharmaceutical Sciences 14, 1–15.
Yin, X., Daintree, L.S., Ding, S., Ledger, D.M., Wang, B., Zhao, W., Qi, J., Wu, W., Han, J., 2015. Itraconazole solid dispersion prepared by a supercritical fluid technique: preparation, in vitro characterization, and bioavailability in beagle dogs. Drug Des Devel Ther 9, 2801–2810.
Zhang, M., Yang, B., Liu, W., Li, S., 2017. Influence of hydroxypropyl methylcellulose, methylcellulose, gelatin, poloxamer 407 and poloxamer 188 on the formation and stability of soybean oil-in-water emulsions. Asian Journal of Pharmaceutical Sciences 12, 521–531.
Zhang, Y., Huo, M., Zhou, J., Xie, S., 2010. PKSolver: An add-in program for pharmacokinetic and pharmacodynamic data analysis in Microsoft Excel. Computer Methods and Programs in Biomedicine 99, 306–314.
Zhou, Y., Du, J., Wang, L., Wang, Y., 2017. Nanocrystals Technology for Improving Bioavailability of Poorly Soluble Drugs: A Mini-Review. Journal of Nanoscience and Nanotechnology 17, 18–28.