Pharmaceutical Sciences Asia

Pharm Sci Asia 2024; 51(2), 164-172

DOI:10.29090/psa.2024.02.24.1498

Research Article

Formulation and evaluation of itraconazole-loaded film-forming solutions for treatment of onychomycosis

Saratsanan Promjan[1], Lai Wah Chan[2], Paul Wan Sia Heng[2], Prapaporn Boonme[1*]

[1] Department of Pharmaceutical Technology and Drug Delivery System Excellence Center, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Songkhla, Thailand

[2] GEA-NUS Pharmaceutical Processing Research Laboratory, Department of Pharmacy, National University of Singapore, 18, Science Drive 4, Singapore, Singapore

ABSTRACT

The formulations of film-forming solutions containing itraconazole (ITZ) were developed using clove oil, dichloromethane (DCM), isopropyl alcohol (IPA), polyvinyl butyral (PVB), polysorbate 80 (P80), and water as excipients for topical treatment of onychomycosis. Their physicochemical properties, stability, *in vitro* release, and antifungal activity were evaluated. The optimal formulations were transparent viscous solutions which formed translucent films within 10 min after application. Formulation components, especially PVB and P80, influenced on characteristics of the film-forming solutions and their resultant films. The stability data indicated that three formulations, i.e., F4, F7, and F8, were physically and chemically stable at room temperature (RT: 25°C) for three months. The data obtained from the stable formulations showed that they sustained drug release up to 24 h and kinetic profiles fitted to Korsmeyer and Peppas model. The synergistic antifungal activity of clove oil and ITZ against *C. albicans* and *T. rubrum* was found in all stable formulations. In summary, ITZ-loaded film-forming solutions have been proven to be good alternatives for treatment of onychomycosis.

Keywords:
Film-forming solution, Itraconazole, Onychomycosis, Ungual delivery

1. INTRODUCTION

Onychomycosis is a common fungal infection of finger and toe nails, resulting in nail discoloration, thickening, and separation from the bed. Approximately 10% of the general population is diagnosed with this infection and the prevalence rises to 20-50% in the elderly[1-2]. The risk of this infection in patients with diabetes is 1.9-2.8 times that of patients without diabetes[3]. Onychomycosis is commonly caused by dermatophytes, especially *Trichophyton rubrum* and *Trichophyton mentagrophytes*, the most predominant worldwide[4-5]. *Candida albicans* is also a dominant etiologic agent in some cases[4].

Itraconazole (ITZ) is a broad-spectrum lipophilic triazole antifungal drug approved for onychomycosis treatment[6]. It is well tolerated as compared to fluconazole, ravuconazole, and posaconazole. Its mode of action is attributed to inhibition of the cytochromes P450 enzyme lanosterol 14α-demethylase, which prevents ergosterol synthesis and impairs cell membrane function[7]. Nowadays, ITZ is commercially available as oral and parenteral dosage forms but enterally delivered ITZ may cause systemic side effects such as headache and gastrointestinal tract upset. Stevens-Johnson syndrome and hepatitis have been reported with continuous ITZ therapy[8]. Moreover, drug-drug interactions can occur with cytochrome P450 3A4 drugs[9]. Therefore, topical administration is preferred as the alternative treatment to avoid these problems.

Currently, the widespread use of ITZ has been associated with azole-resistant *T. rubrum* and *C. albicans*[10-11]. Hence, natural substances, especially essential oils with potential antifungal activity, have been studied as an attractive combination treatment for skin fungal infection. Clove oil is an essential oil derived from *Syzygium aromaticum*. It has been reported to possess broad-spectrum antimicrobial and anti-biofilm activities against some yeasts and bacteria with antioxidative and anti-inflammatory effects[12-14]. In addition, clove oil has also demonstrated

***Corresponding author:**
*Prapaporn Boonme Email: prapaporn.b@psu.ac.th

antifungal activity against dermatophytic fungi including *C. albicans*, *E. floccosum*, *M. audouinii*, *T. mentagrophytes*, and *T. rubrum*[15]. It was previously reported that clove oil in ITZ-loaded microemulsions could enhance solubility of the water-insoluble drug and significantly improve the antifungal activity compared to a commercial product[16]. Moreover, clove oil can act as a permeation enhancer by disrupting intercellular lipids of the stratum corneum and promoting drug penetration to the target site[17].

Drug delivery systems for treatment of nail fungal infections have been widely studied. Topical therapy for nail diseases using ungual delivery systems is highly desirable since it is non-invasive, avoids hepatotoxicity, delivers drug directly to the target site, reduces systemic adverse effects, and improves patient compliance. However, most conventional formulations such as gels, creams, or solutions have limitations of being removed by whipping, rubbing, and low adherence to infected nails[18]. Film-forming solutions can be employed to overcome these limitations.

Film-forming system is a non-solid dosage form that produces a film *in situ* after solvent evaporation[19]. If this system can exhibit appropriate adhesiveness and enhance drug release to the nails, it will be highly beneficial due to its ease of application and reduced application frequency. A film-forming solution consists of a polymer as a film-forming agent dispersed in at least a volatile solvent[20]. When applied to a bare nail surface and dried, the solution forms an adhering film that can be peeled off without the use of a polish remover. The drug is incorporated in the film-forming vehicle and the resultant film acts as a reservoir to control drug release[21].

Polyvinyl butyral (PVB) is a useful film-forming resin due to its strong binding, optical clarity, adhesion to many surfaces, and flexibility. It is synthesized from polyvinyl alcohol by reaction with butyraldehyde. Its primary application is in laminated safety glass for automobile windshields and paints[22]. It is nowadays common for cosmetic products to contain PVB, such as nail (polish, strengthener, treatment), and eye (shadow, foundation) products. Moreover, PVB is classified as not bioaccumulative and not expected to be potentially toxic or harmful to organ systems according to the Environment Canada Domestic Substance List[23].

The aims of this study were to formulate, characterize the physicochemical properties, and evaluate the stability of ITZ-loaded film-forming solutions. *In vitro* drug release and antifungal activity of the stable formulations against *C. albicans* and *T. rubrum* were also investigated.

2. MATERIALS AND METHODS

2.1. Materials

ITZ was purchased from Acros Organics (Geel, Belgium). Polysorbate 80 (P80) was obtained from VWR International (Fontenay-sous-Bois, France). Clove oil was purchased from Tien Yuan Chemical (Singapore). Dichloromethane (DCM) and isopropyl alcohol (IPA) were obtained from Aik Moh Paints & Chemicals (Singapore). Polyvinyl butyral (PVB) was supplied by Eastman Chemical (Singapore). Acetonitrile, ethanol, and methanol were procured from RCI Labscan (Bangkok, Thailand). Sodium dihydrogen orthophosphate and disodium hydrogen orthophosphate anhydrous were purchased from Univar Australia Pty Ltd. (New South Wales, Australia) and used to prepare phosphate buffer solution pH 7.4 (PBS). All chemicals were of pharmaceutical or analytical grade and used as received without any modifications. Distilled water was produced in-house and used throughout the experiment. Standard grade regenerated cellulose (RC) dialysis membrane (Spectra/Por® 3 dialysis membrane, MWCO 3,500 Da) was purchased from Spectrum Laboratories (California, USA).

2.2. Preparation of ITZ-loaded film-forming solutions

ITZ-loaded film-forming solutions were prepared by a simple mixing method. The concentration of the drug was kept constant at 1% (w/w) ITZ in all formulations, which also contained 3% (w/w) clove oil and 20% (w/w) DCM to dissolve ITZ. The compositions of the tested formulations (F1-F8) which were obtained from the preliminary study are shown in Table 1. The drug was dissolved in clove oil and DCM using a magnetic stirrer.

Table 1. Composition of ITZ-loaded film-forming solutions.

Formulation	Ingredients (% w/w)						
	ITZ	Clove oil	DCM	IPA	P80	PVB	Water
F1	1	3	20	45	0	10	21
F2	1	3	20	55	0	10	11
F3	1	3	20	45	1	10	20
F4	1	3	20	55	1	10	10
F5	1	3	20	45	0	13	18
F6	1	3	20	55	0	13	8
F7	1	3	20	45	1	13	17
F8	1	3	20	55	1	13	7

Next, IPA and PVB were added and continuously mixed for 30 min. Finally, P80 and water were added and stirred until a solution formed.

2.3. Physicochemical characterization of ITZ-loaded film-forming solutions and the resultant films

2.3.1. Appearance observation of film-forming solutions and resultant films

The film-forming solutions obtained were visually inspected for homogeneity, transparency, and drug precipitation. The films were formed on a Teflon plate using a bird film applicator (Model No.3 Four-Side Coating Applicator Film Coater, CGoldenWall, China). Film formation was considered complete if no precipitation of drug or film-forming polymer was observed. In contrast, if precipitation was found, the film formation was classified as incomplete. The films obtained were characterized in terms of optical appearance (transparent, translucent, or opaque) and peelability. The thickness of each formed film obtained was measured at RT using a micrometer (Mitutoyo Corporation, Kanagawa, Japan) and measurement was triplicated.

2.3.2. Determination of drying time of film-forming solutions

A thin layer of each freshly prepared solution was spread out on a clean Teflon plate using the bird film applicator and observed at room temperature (RT: 25°C). The time taken to reach dry-to-touch condition at RT was determined with a stopwatch when the film was dry-to-touch, there should be no material transferred to a clean finger pressed on the film. Each determination was performed in triplicate.

2.3.3. Measurement of pH and viscosity of film-forming solutions

The freshly prepared solutions were tested for their pH values at RT using a pH meter (S20-K, Mettler Toledo, Switzerland). They were also characterized for their viscosity using a rheometer (Brookfield DV-III Ultra Programmable Rheometer, Brookfield Engineering Laboratories, Middleboro, USA) with LV spindle SC4-31 at five different rotation speeds with high %torque. All measurements were carried out at RT and in triplicate.

2.3.4. Evaluation of adhesiveness of film-forming solutions

The adhesiveness of the solutions was determined by the probe test method using a tensile tester (EZ Tester 100N, Shimadzu, Japan)[24]. The setup consisted of an acrylic probe with a contact surface area of 10.7467 cm^2 and a stainless-steel plate. A 10 mL solution was transferred onto the plate maintained at RT. The probe was slowly lowered into the solution to avoid air bubbles trapped, and left to rest for 30 s. The probe was then withdrawn from the solution at a speed of 150 mm/min to detach the probe from the solution. The force required to detach the probe was recorded. The adhesiveness of each film-forming solution was calculated from this force divided by the contact surface area of the probe. Ten replicates were performed.

2.3.5. Determination of nonvolatile content of film-forming solutions

About 1 g of each freshly prepared solution was spread in a glass Petri dish and its precise weight (W_1) was recorded. The dish was placed in an oven at 80°C for 3 h before it was removed and allowed to cool down to RT. Its weight (W_2) was recorded as the nonvolatile content of the solution sample and calculated in term of percentage compared with W_1[25]. Each determination was performed in triplicate.

2.3.6. Evaluation of water absorption of films

A known amount of solution was applied on a Teflon plate and allowed to dry at RT. The film formed was peeled off and cut into pieces of 1 cm×1 cm. The weight (W_1) of each piece was determined before it was placed in a basket suspended in distilled water at 25±1°C for 180 min. The film was then removed from the basket and gently wiped with cotton sheets to remove the residual water droplets. Its weight was determined (W_2). The %water absorption was calculated from Eq. 1[26].

$$\text{The \%water absorption} = \frac{(W_2 - W_1)}{W_1} \times 100 \qquad \text{(Eq. 1)}$$

2.3.7. Determination of tensile strength and %elongation of films

The film formed was cut into the pieces of 7 cm length and 1 cm width. The tensile strength and %elongation values were determined using the tensile tester with rate of pull at 50 mm/min and gauge length of 80 cm. The maximum load force (F_{max}) that the film could withstand before breaking and the final length at break were recorded. The tensile strength (σ) and %elongation values were calculated using Eqs. 2 and 3, respectively[27]. Ten replicates were performed for each film formation.

$$\sigma = \frac{F_{max}}{A} \qquad \text{(Eq. 2)}$$

where F_{max} is the maximum loaded force (N) and A is the cross-section area (mm^2)

$$\%\text{elongation} = \frac{(L_f - L_0)}{L_0} \times 100 \qquad \text{(Eq. 3)}$$

where L_f is the fracture length (mm) and L_0 is the initial length (mm).

2.4. Stability study of ITZ-loaded film-forming solutions

The solutions were stored in tightly-closed glass containers and protected from light at RT and 4°C (in the refrigerator). The physical and chemical stabilities were investigated after storage at each temperature for three months. The appearance, pH, and viscosity of the solutions were assessed. The drug contents of the solutions before and after storage were also determined by extracting ITZ from 300 mg of each solution with methanol, diluted appropriately, and analyzed by high pressure liquid chromatography (HPLC; LC-2030 3D Shimadzu, Kyoto, Japan). All determinations were conducted in triplicate.

The ITZ analysis was performed with a validated HPLC method[28]. A reverse-phase C18 column (5 µm, 250×4.6 mm, C18-Phenomenex® Luna, USA) with a guard column was applied for separation using a mobile phase of 70:30 (v/v) acetonitrile:water at a flow rate of 1.5 mL/min. The injection volume was 10 µL and the drug was assayed at 263 nm.

2.5. *In vitro* release study of the samples

In vitro release study was performed using Franz diffusion cells (Hanson Model 57-6M, Hanson Research Corporation, CA, USA) with Spectra/Por® 3 dialysis membrane (Spectrum laboratories, USA). The membrane was pre-soaked for 30 min in PBS and mounted between the donor and receptor chambers. The receptor compartment was filled with 12 mL of 70:30 (v/v) PBS:ethanol as receptor fluid, which was stirred at constant speed of 300 rpm. The temperature of the diffusion cells was maintained at 37±0.5°C. The 1 g of each studied solution was applied on the surface of the membrane and it was optically observed that the solution in the donor chamber changed into a translucent film within 30 min. The donor compartment was covered with parafilm to prevent the evaporation of any ingredients from the sample. A 0.5 mL aliquot was taken from the receptor fluid in the receptor chamber at time intervals of 0.5, 1, 2, 4, 6, 8, 12, and 24 h with replacements of the same volume of fresh receptor fluid. The drug was assayed using HPLC method as previously described and the cumulative drug release was calculated. Five replicates were conducted for each sample. The cumulative amount of ITZ released (Q_t, µg/cm^2) was calculated by Eq. 4. The release profile was shown by the plot of cumulative amounts of ITZ per unit area of the membrane against time.

$$Q_t = V_r C_t + \sum_{i=0}^{t-1} V_s C_i \qquad \text{(Eq. 4)}$$

where C_t is the concentration of ITZ in the receptor fluid at each sampling time (t), C_i is the ITZ concentration of the i^{th} sample, and V_r and V_s are the volumes of the receptor fluid and the sample, respectively.

Each release profile was then analyzed by four different mathematical models, i.e., zero order, first order, Higuchi, and Korsmeyer-Peppas models as represented by Eqs. 5-8, respectively.

Zero order: $$Q_t = Q_0 - k_0 t \qquad \text{(Eq. 5)}$$

First order: $$\ln Q_t = \ln Q_0 - k_f t \qquad \text{(Eq. 6)}$$

Higuchi: $$Q_t = k_H t^{1/2} \qquad \text{(Eq. 7)}$$

Korsmeyer-Peppas: $$M_t/M_\infty = k_{kp} t^n \qquad \text{(Eq. 8)}$$

where Q_t is the cumulative amount of ITZ released in time *t*, Q_0 is the initial amount of ITZ in the studied film, and k_0, k_f, and k_H are release rate constants of zero order, first order, and Higuchi models, respectively. While M_t/M_∞ is the fraction of drug released at time (t), k_{kp} is the structural and geometrical constant, and n is the release exponent[29-30].

2.6. *In vitro* antifungal activity study of the samples

C. albicans ATCC 10231 and *T. rubrum* DMST 30263 were selected for testing antifungal activity of the prepared formulations using agar well diffusion method[16]. The fungal culture was maintained on Sabouraud-dextrose agar (SDA) media and incubated at 25°C, 48 h for *C. albicans* and seven days for *T. rubrum*. The growth was harvested and adjusted to a 0.5 (*C. albicans*) and 1.0 (*T. rubrum*) McFarland standard, then streaked onto SDA plates with sterilized cotton. Sterile pipette tips (8 mm diameter) were punched into the agar to make wells and filled with 100 µL of each prepared solution. The flowable solution formed solid sample in the punched hole within 10 min after filling and the plates were then incubated at 25°C for 48 h (*C. albicans*) and seven days (*T. rubrum*). After incubation, the zone of inhibition was measured. All measurements were performed in triplicate.

2.7. Data analysis

The characterization and stability data were reported as mean±standard deviation (SD). For the *in vitro* release study, mean±standard error of mean (SEM) was computed. One-way ANOVA followed by Tukey's post hoc multiple comparison analysis was employed to analyze the data, and *p-value*<0.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1. Characteristics of film-forming solutions and resultant films

The visual appearance of freshly prepared formulations, F1 and F3 exhibited turbidity and non-homogeneity. In contrast, the other formulations (F2 and F4-F8) showed good homogeneity and clear viscous solutions, without precipitated particles (data not shown). The turbidity observed corresponded with higher water contents of 20% (w/w) in F3 and 21% (w/w) in F1 which could be attributed to precipitation of PVB, a water insoluble polymer. Consequently, F2 and F4-F8 were selected for further studies. The selected formulations showed good film-forming ability at RT due to evaporation of DCM. Very thin, peelable, and translucent films without precipitation of drug or film-forming polymer were produced (Figure 1). All films had non-sticky smooth surfaces. As delineated in Table 2, the drying times for all studied

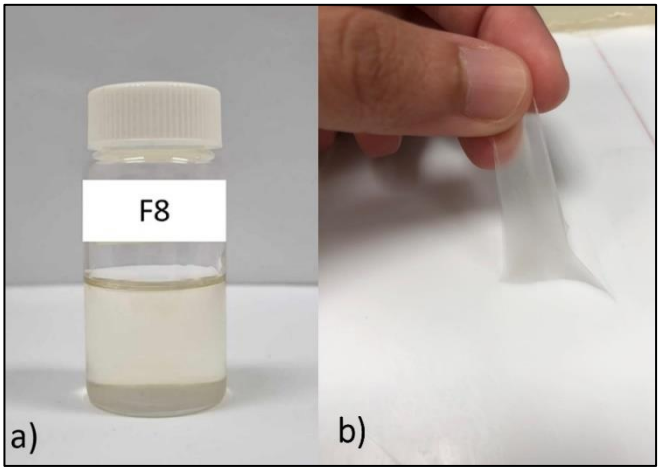

Figure 1. Appearances of (a) F8 as transparent ITZ-loaded film-forming solution and (b) its resultant thin, peelable, and translucent film.

formulations were in the range of 5 to 8 min. The ideal drying time of film-forming formulations should be less than 10 min, depending on the volatility characteristic of the solvents[25]. Hence, based on the criterion, F2 and F4-F8 had acceptable drying times. The results revealed that PVB concentration affected the drying time as an increase in PVB concentration increased the drying time. Additionally, it could be seen from F5-F6 that a decrease in the content of IPA, which corresponded with an increase in the water content, resulted in a much longer drying time. Similar trend was also found in F7-F8. It seemed reasonable to assume that the low amount of IPA and correspondingly more water slowed the evaporation rate of the film-forming solutions. Moreover, the use of P80 as a surfactant and permeation enhancer also increased the drying time since its viscous property may retard the volatility of DCM. It could be inferred that PVB, IPA, water, and P80 concentrations had significant impact on drying time of the film-forming solutions.

All selected formulations had pH values between 6.75±0.05 and 7.26±0.04 as shown in Table 2. The pH values of developed formulations were close to pH of washing cleanser in tap water which was reported as 7.90[31]. It could be attributed that the pH in the range of 6.75-7.26, which is slightly acidic-neutral and therefore unlikely to be harmful to the nail. The viscosity values of the film-forming solutions were between 109.35±1.12 and 366.34±1.04 mPa.s as presented in Table 2 and their rheological pattern was Newtonian flow (data not shown). A film-forming solution with Newtonian flow could be expected for ease of production and application processes. The results revealed that F5-F8 containing 13% (w/w) PVB had significantly ($p<0.05$) higher viscosity than F2 and F4 containing 10% (w/w) PVB. In contrast, F6 and F8 containing 55% (w/w) IPA had significantly ($p<0.05$) lower viscosity than F5 and F7 containing 45% (w/w) IPA. Additionally, the addition of 1% (w/w) P80 increased the viscosity owing to the intrinsic property of P80. It could be inferred that PVB, IPA, and P80 had significant influence on the viscosity of the film-forming solutions.

The adhesiveness of the solutions (Table 2) was found to be in the range of 0.21±0.01 to 0.80±0.03 N/cm^2. The adhesiveness of formulations containing 10% PVB (F2 and F4) was found to be significantly lower ($p<0.05$) than that of others containing 13% PVB. The former formulations were also observed to have lower viscosity. The results seemed to show that the increase in adhesiveness was related to the increase in PVB concentration and formulation viscosity.

The nonvolatile content values for the tested formulations are shown in Table 2. There was approximately 70-85% (w/w) weight loss, which could be attributed to the evaporation of the DCM, IPA, and water. The addition of P80 into the film-forming solutions resulted in increased nonvolatile content in comparison to those without P80. It could be noted that when compared F5 with F7 and F6 with F8 that the addition of 1% P80 increased nonvolatile content by around 1.9%. This was possibly explained by that P80 could retain some water, but not DCM and IPA which are highly volatile. It was observed that the nonvolatile content was consistent with the drying time required. The thickness of resultant film was found in the range between 0.039±0.001 and 0.064±0.000 mm (Table 2). The film obtained from F8 containing 13% (w/w) PVB and 1% (w/w) P80 was the thickest. The thickness of the film was found to be consistent with the content of PVB and nonvolatile content. The values obtained in this study indicated that the resultant films had a suitable thickness for nail application.

The degree of resistance of a film towards water can be indicated by its ability to absorb water. It could be seen that an increase in the concentration of PVB resulted in an increase in the water resistance of the film (Table 2). F5 and F6 did not show any water absorption and could

Table 2. Physical properties of freshly formulated ITZ-loaded film-forming solutions and resultant films (mean±SD, n=3).

Formulation	Appearance of solution	Film formation	Drying time (min)	pH	Viscosity* (mPa.s)	Adhesiveness (N/cm^2)	Nonvolatile content (% w/w)	Thickness (mm)	Water absorption (% w/w)
F1	Turbid	-	-	-	-	-	-	-	-
F2	Transparent	Complete and peelable	5.43±0.15	6.76±0.02	109.35±1.12	0.21±0.01	14.92±0.21	0.039±0.001	3.13±0.00
F3	Turbid	-	-	-	-	-	-	-	-
F4	Transparent	Complete and peelable	5.92±0.36	7.11±0.01	114.31±1.06	0.31±0.03	17.56±0.32	0.050±0.001	10.38±1.10
F5	Transparent	Complete and peelable	6.43±0.07	6.75±0.05	330.93±1.00	0.74±0.03	24.64±0.14	0.056±0.001	0.00±0.00
F6	Transparent	Complete and peelable	6.29±0.09	7.14±0.02	277.74±0.42	0.53±0.01	24.46±0.38	0.057±0.001	0.00±0.00
F7	Transparent	Complete and peelable	7.33±0.10	6.83±0.01	366.34±1.04	0.80±0.03	26.60±0.24	0.060±0.000	5.17±1.50
F8	Transparent	Complete and peelable	6.94±0.32	7.26±0.04	294.06±0.57	0.68±0.04	26.38±0.50	0.064±0.000	6.97±0.00

*at representative spindle rotation speed of 50 rpm

Table 3. Mechanical properties of the formed films (mean±SD, n=10).

Formulation	Tensile strength (N/mm^2)	%Elongation
F1	-	-
F2	2.85±0.22	141.85±2.42
F3	-	-
F4	1.82±0.31	166.22±4.07
F5	5.08±0.14	95.73±4.07
F6	4.36±0.28	104.22±4.76
F7	3.73±0.12	121.64±3.97
F8	3.03±0.15	130.99±0.97

Table 4. pH, viscosity, and drug remaining in ITZ-loaded film-forming solutions during 3 months (mean±SD, n=3).

Formulation#	Temperature	pH			Viscosity* (mPa.s)			ITZ remaining (%)		
		Time (month)								
		1	2	3	1	2	3	1	2	3
F4	RT	7.02±0.02	6.98±0.01	6.87±0.01	131.47±2.20	132.60±1.21	154.84±1.30	100.86±0.10	100.38±1.03	99.62±0.50
F5	RT	6.55±0.01	6.47±0.01	DP	440.26±1.93	515.07±1.25	DP	100.99±0.78	100.96±0.70	DP
F6	RT	7.02±0.01	6.99±0.01	DP	386.04±0.87	402.87±0.60	DP	100.54±1.87	100.36±1.20	DP
F7	RT	6.75±0.02	6.69±0.01	6.56±0.01	516.06±1.44	563.08±2.96	551.78±6.15	100.55±0.12	100.24±0.24	99.40±0.53
F8	RT	7.14±0.02	7.09±0.01	6.99±0.02	333.30±0.65	413.91±0.86	464.76±6.12	100.97±0.92	99.84±0.69	99.98±0.54

* at representative spindle rotation speed of 50 rpm
# F2 was excluded from the stability study due to drug precipitation after 1 month of storage
DP, drug precipitation

be classified as waterproof. Interestingly, a high %water absorption was found in the formulations with 1% (w/w) P80. This could be attributed to P80 which is a hydrophilic surfactant capable of water absorption. In a previous study, the hydrophilic surfactant polysorbate 20 was found to improve the wettability and water vapor permeability of films, which corroborated the effect of P80[32].

The tensile strength and %elongation of the films were shown in Table 3. The tensile strength values were between 1.82±0.31 and 5.08±0.14 N/mm^2. F2 and F4 with 10% (w/w) PVB exhibited lower tensile strength as compared to F5-F8 with 13% (w/w) PVB. The results revealed that tensile strength values increased with the concentration of PVB. The %elongation represents film flexibility. F5 showed the highest tensile strength but the lowest %elongation. Similar observation was made in another study where strong films cracked easily[33]. The mechanical properties of F5 might be attributed to its high content of PVB and lack of P80. The addition of P80 reduced the tensile strength but increased %elongation of the film. As reported in another study, it appears that the surfactant could increase chain mobility and enhance the initial plastic effect since its small molecules could stay between polymer chains[32].

3.2. Stability of film-forming solutions

After 3 months of storage at 4°C, it was found that drug precipitated in all the tested solutions, which were excluded from further study. In contrast, there were no changes in physical appearance and pH value of the solutions stored at RT, except for a significant (p<0.05) increase in viscosity (Table 4). The reason for increase in viscosity was unclear; however, it might be due to swelling of PVB during storage. Consequently, the concentration of PVB increased, leading to higher viscosity. Moreover, drug precipitation was found after 1 month for F2 but after 3 months for F5 and F6. The possible reason might be the lack of P80 as drug solubilizer in F2, F5, and F6. As drug precipitation was observed in the first month of storage for F2, it was excluded from the further stability study.

The drug remaining in the physically stable formulations (F4, F7, and F8) after 3 months of storage at RT was close to 100% (p<0.05), indicating excellent drug stability in these formulations. Therefore, these three formulations were selected for further evaluation. The results indicated that the ITZ-loaded film-forming solutions could be stored at RT in tightly closed glass containers and protected from light to preserve drug stability.

3.3. *In vitro* release properties of the samples

It was observed that 1 g of the freshly prepared solution was able to form a film within 30 min after application on the membrane. Consequently, the drug was found in the receptor fluid by ITZ release from the film formed. Drug release profiles from F4, F7, and F8 were generally more rapid in the first 30 min and the rate gradually decreased (Figure 2). ITZ release profile of F4 exhibited the highest cumulative amount of drug over 24 h (7.14± 0.67 µg/cm^2), followed by F8 (5.58±0.39 µg/cm^2), and F7 (5.39±0.89 µg/cm^2), respectively. This could be attributed to the lower PVB content and viscosity of F4, which allowed the drug to diffuse more readily through its resultant film. The results demonstrated the capability of sustained drug release up to 24 h and impacted on nail drug retention that could reduce drug application frequency. The model that best fitted the ITZ release profiles was the Korsmeyer and Peppas model (R^2=0.9952 for F4, R^2=0.9897 for F7, and R^2=0.9956 for F8). The evaluated data showed that values of n were 0.5678, 0.5275, and 0.5014 for F4, F7, and F8, respectively. These implied that the release mechanism was non-Fickian or anomalous transport, indicating the release of ITZ via a combination of drug diffusion from the swelling matrix and polymer erosion process[29-30]. Release rate constants of Korsmeyer and Peppas model (K_{kp}) for ITZ release from F4, F7, and F8 were 1.2125, 1.0611, and 1.1678 µg/cm^2/h^{-n}, respectively, indicating a quicker releasing rate of ITZ into the medium via a non-Fickian diffusion mechanism. Moreover, the K_{kp} values increased when the PVB content decreased and correlated with the higher drug release from the film.

3.4. *In vitro* antifungal activity of the samples

The results indicated that all stable ITZ-loaded formulations, i.e., F4, F7, and F8, as well as their blank counterparts without the active drug exhibited antifungal activity against the two types of the tested fungi (Table 5). The antifungal activity of the blank formulations could be attributed to the antifungal effect of clove oil and IPA while DCM showed no inhibition clear zone[15,34-36]. However, the significant antifungal activity of the drug-loaded solutions was largely due to ITZ. The results of this study were in accordance with a previous investigation which reported that microemulsions containing clove oil presented synergistic interaction with ITZ against *T. rubrum* and *C. albicans*[16]. Additionally, the viscosity values demonstrated a relationship with the inhibition zone. The lowest viscosity of F4 allowed the highest drug diffusion ability through the agar, resulting in the largest inhibition zone (p<0.05) compared to the others The inhibition zone size directly correlated with the *in vitro* drug release rate, i.e., F4 > F8 > F7.

4. CONCLUSION

The present study was designed to develop ITZ formulations for ungual delivery to treat onychomycosis. Film-forming solutions containing ITZ, clove oil, DCM,

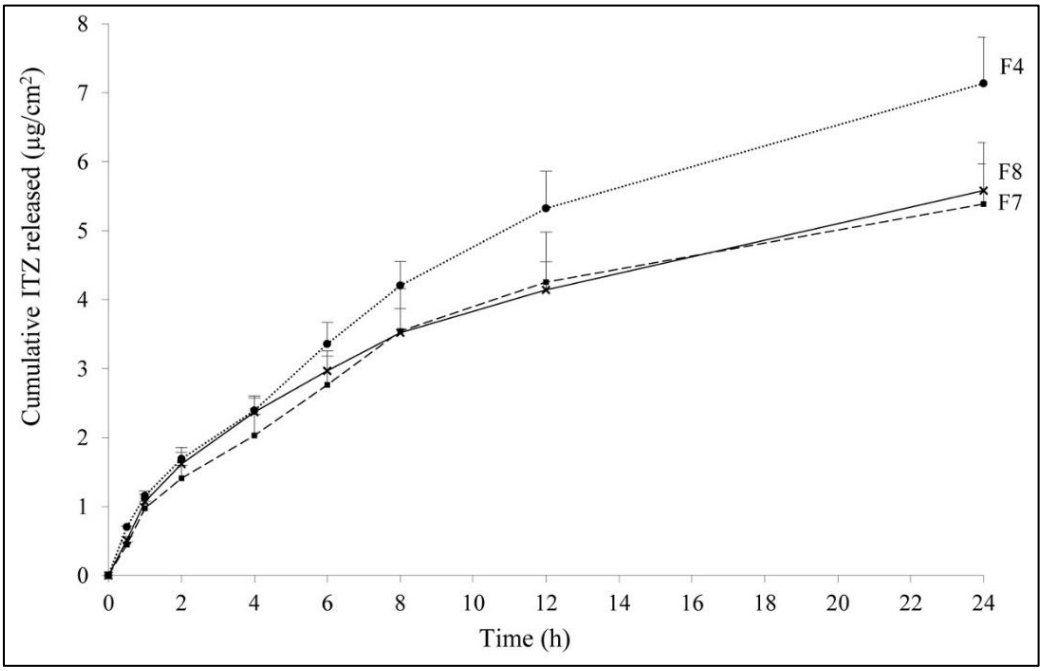

Figure 2. *In vitro* release profiles of ITZ from F4, F7, and F8 (mean±SEM, n=5).

Table 5. Antifungal activity of the stable blank and ITZ-loaded film-forming solutions against *T. rubrum* and *C. albicans* (mean±SD, n=3).

Formulation	Inhibition clear zone (mm)	
	T. rubrum	***C. albicans***
Blank F4	10.37±0.15	10.93±0.55
Blank F7	8.80±0.20	9.27±0.12
Blank F8	9.53±0.31	10.20±0.72
F4	27.47±0.12*	27.40±0.92*
F7	24.47±1.01*	25.00±0.40*
F8	25.20±0.60*	26.13±0.81*

*p<0.05; statistically significant differences (one-way ANOVA) from the results of their blank counterparts

IPA, PVB, P80, and water were successfully prepared. Their properties were dependent on the concentrations of components, especially PVB and P80. PVB affected drying time, viscosity, and adhesiveness of the prepared solutions and tensile strength of the resultant films. P80 affected water resistant properties. After 3 months of storage, the evaluation of F4, F7, and F8 revealed the stability at RT and excellent drug stability of samples with P80. These film-forming solutions exhibited not only sustained drug release but also antifungal activity. Clove oil could provide synergistic effect with ITZ on antifungal activity. Therefore, ITZ-loaded film-forming solutions containing clove oil have greater potential as an ungual drug delivery system for treating onychomycosis. Additionally, the medicated film-forming solutions could be easily applied and their resultant films could be simply peeled off, resulting in an improvement in patient acceptability and compliance. Among the tested formulations, F4 was the most appropriate formulation with regard to short drying time, good stability, sustained drug release, and high antifungal efficacy against *T. rubrum* and *C. albicans*. The water resistant, mechanical, and adhesive properties of its resultant film were also suitable.

5. ACKNOWLEDGEMENT

Sincere thanks to Natalia Veronica for helping with laboratory equipment and chemicals.

Conflict of interest

The authors declare no conflict of interest.

Funding

This research was supported by the Graduate School and the Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand. The first author gratefully acknowledges the supports provided by the Ph.D. overseas Thesis Research Scholarship.

Ethics approval

None to declare. This study did not involve human or animal specimens.

Article info:

Received December 28, 2023
Received in revised form February 29, 2024
Accepted March 5, 2024

Author contribution

Conceptualization: SP, LWC, PWSH, and PB. Performed research: SP. Analyzed data: SP. Original draft preparation: SP and PB. Review and editing: SP, LWC, PWSH, and PB.

REFERENCES

1. Thomas J, Jacobson GA, Narkowicz CK, Peterson GM, Burnet H, Sharpe C, et al. Toenail onychomycosis: an important global disease burden. J Clin Pharm Ther. 2010;35(5):497-519.
2. Gupta AK, Venkataraman M, Talukder M. Onychomycosis in older adults: Prevalence, diagnosis, and management. Drugs Aging. 2022;39(3):191-8.
3. Mayser P, Freund V, Budihardja D. Toenail onychomycosis in diabetic patients: Issues and management. Am. J Clin Dermatol. 2009;10(4):211-20.
4. Elewski BE. Onychomycosis: Pathogenesis, diagnosis, and management. Clin Microbiol Rev. 1998;11(3):415-29.
5. Hay R. Literature review. Onychomycosis. J Eur Acad Dermatol Venereol. 2005;19(Suppl 1):1-7.
6. Gupta AK, Sauder DN, Shear NH. Antifungal agents: An overview. Part II. J Eur Acad Dermatol Venereol. 1994;30(6):911-33.
7. Groll AH, Piscitelli SC, Walsh TJ. Clinical pharmacology of systemic antifungal agents: A comprehensive review of agents in clinical use, current investigational compounds, and putative targets for antifungal drug development. Adv Pharmacol. 1998; 44:343-500.
8. Baran R, Gupta AK, Piérard GE. Pharmacotherapy of onychomycosis. Expert Opin Pharmacother. 2005;6(4):609-24.
9. Niwa T, Imagawa Y, Yamazaki H. Drug interactions between nine antifungal agents and drugs metabolized by human cytochromes P450. Curr Drug Metab. 2014;15(7):651-79.
10. Hryncewicz-Gwóźdź A, Kalinowska K, Plomer-Niezgoda E, Bielecki J, Jagielski T. Increase in resistance to fluconazole and itraconazole in *Trichophyton rubrum* clinical isolates by sequential passages *in vitro* under drug pressure. Mycopathologia. 2013; 176(1-2):49-55.
11. Zarrinfar H, Kord Z, Fata A. High incidence of azole resistance among *Candida albicans* and *C. glabrata* isolates in Northeastern Iran. Curr Med Mycol. 2021;7(3):18-21.
12. Perry LM. Assessment report on *Syzygium aromaticum* (L.). London: European Medicines Agency; 2011.
13. Zhang Y, Wang Y, Zhu X, Cao P, Wei S, Lu Y. Antibacterial and antibiofilm activities of eugenol from essential oil of *Syzygium aromaticum* (L.) Merr. & L.M. Perry (clove) leaf against periodontal pathogen *Porphyromonas gingivalis*. Microb Pathog. 2017; 113:396-402.
14. Satthanakul P, Taweechaisupapong S, Luengpailin S, Khunkitti W. The antifungal efficacy of essential oils in combination with chlorhexidine against *Candida* spp. Songklanakarin J Sci Technol. 2019;41(1):144-50.
15. Chee HY, Lee MH. Antifungal activity of clove essential oil and its volatile vapour against dermatophytic fungi. Mycobiology. 2007;35(4):241-3.
16. Promjan S, Boonme P. Itraconazole-loaded microemulsions: Formulation, characterization, and dermal delivery using shed snakeskin as the model membrane. Pharm Dev Technol. 2023;28 (1):51-60.
17. Herman A, Herman AP. Essential oils for transdermal drug delivery. J Pharm Pharmacol. 2015;67(4):473-85.
18. Patel RP, Naik SA, Patel NA, Suthar AM. Drug delivery across human nail. Int J Curr Pharm Res. 2009;1:1-7.
19. Mcauley WJ, Caserta F, Hoboken NJ. Film-forming and heated systems. In: Donnelly RF, Sing TRR, editors. Novel delivery systems for transdermal and intradermal drug delivery. United States: John Wiley & Sons; 2015. p. 97-107.
20. Kathe K, Kathpalia H. Film forming systems for topical and transdermal drug delivery. Asian J Pharm Sci. 2017;12(6):487-97.
21. Vij NN, Saudagar RB. Formulation, development and evaluation of film-forming gel for prolonged dermal delivery of terbinafine hydrochloride. Int J Pharm Sci Res. 2014;5:537-54.
22. Hallensleben ML. Polyvinyl compounds, others, Ullmann's encyclopedia of industrial chemistry, 7th ed. Weinheim, German: Wiley-VCH; 2000. p. 605-21.
23. Environment Canada. Domestic substances list categorization. Ontario, Canada: Canadian Environmental Protection Act (CEPA), Environmental Registry; 2008.
24. Heng PWS, Wong TW, Wan LSC. A probe test method for determination of molten binder tack. Pharm Dev Technol. 1999;4(3): 457-60.
25. Chandra R, Kumar S, Aggarwal A. Evaluation of nail lacquer. Indo Glob J Pharm Sci. 2012;2(4):379-82.
26. Mithal BM, Saha RN. A handbook of cosmetics, 1st ed. Delhi: Vallabh prakashan; 2005. p. 176-82.
27. Heng Paul WS, Lucy SC Wan, Yvonne TF Tan. Relationship between aggregation of HPMC coated spheroids and tackiness/ viscosity/additives of the coating formulations. Int J Pharm. 1996; 138(1):57-66.
28. Promjan S, Boonme P. Validation of HPLC method for itraconazole determination in microemulsions and samples of shed snakeskin permeation study. Lat Am J Pharm. 2022;41(10):1955-61.
29. Korsmeyer RW, Gurny R, Doelker E, Buri P, Peppas NA. Mechanisms of solute release from porous hydrophilic polymers. Int J Pharm. 1983;15(1):25-35.
30. Peppas NA. Analysis of Fickian and non-Fickian drug release from polymers. Pharm ActaHelv. 1985;60(4):110-1.
31. Murdan S, Milcovich G, Goriparthi GS. An assessment of the human nail plate pH. Skin Pharmacol Physiol. 2011;24(4):175-81.
32. Rodríguez M, Osés J, Ziani K, Maté JI. Combined effect of plasticizers and surfactants on the physical properties of starch based edible films. Food Res Int. 2006;39(8):840-6.
33. Luangtana-Anan M, Limmatvapirat S, Nunthanid J, Wanawongthai C, Chalongsuk R, Puttipipatkhachorn S. Effect of salts and plasticizers on stability of shellac film. J Agric Food Chem. 2007; 55(3):687-92.
34. Lens C, Malet G, Cupferman S. Antimicrobial activity of butyl acetate, ethyl acetate and isopropyl alcohol on undesirable microorganisms in cosmetic products. Int J Cosmet Sci. 2016;38(5): 476-80.
35. Han G, Lee DG. Indole propionic acid induced Ca^{2+}-dependent apoptosis in *Candida albicans*. IUBMB Life. 2022;74(3):235-44.
36. Pipatphatsakorn M, Temkitthawon P, Teenawong C, Tanthasane R, Wongwiriya A, Damrongwanich O, et al. Antifungal activity and cytotoxicity to human gingival fibroblast of *Terminalia Bellirica* compounds in dichloromethane fraction. CM Dent J. 2023;39 (1):61-74.