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Research Article

Characterization and efficacy evaluation of itraconazole microemulsion-based gels for treatment of dermal fungal infections

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ABSTRACT

This study aimed to formulate, characterize, and efficacy evaluate itraconazole microemulsion-based gels (ITZ-MBGs). Three blank microemulsions (MEs) were selected from the ME region of a system containing clove oil, polysorbate 80, and a 1:1 mixture of water and polyethylene glycol 400. Polyvinyl butyral at the concentration of 17% w/w was used to convert liquid MEs to semisolid MBGs. Simple ITZ-gel was formulated for comparison. ITZ was incorporated at 1% w/w in drug-loaded samples. All prepared formulations were characterized for physicochemical properties when being kept at 4°C, 25°C, and 40°C for three months. *In vitro* skin permeation efficacy was evaluated using shed snakeskin as model membrane. Antifungal activities against *C. albicans* and *T. rubrum* were assessed by agar diffusion method. A commercial emulgel was used for comparison in the skin permeation and antifungal activity studies. ITZ-MBGs were transparent yellow gels with water-in-oil type. They had pH in the range of 5.44-5.92. Their viscosity with pseudoplastic flow, gel strength, and adhesiveness were in direct relationship while spreadability was in inverse relationship. No interaction between ITZ and formulation ingredients was found in FTIR spectra. Although ITZ-gel provided high antifungal activity, it was too acidic for skin application. ITZ-MBGs showed more favorable skin permeation efficacy and larger fungal inhibition zones than a commercial emulgel. They were chemically stable under the investigated storage conditions. The findings revealed that ITZ-MBGs could be used as topical delivery systems for treatment of dermal fungal infections since they provided efficient antifungal activity and localization of ITZ in skin membrane.

Keywords:
Microemulsion-based gel; Itraconazole; Dermal delivery; Antifungal activity

1. INTRODUCTION

Dermal fungal diseases are superficial infections possibly caused by both dermatophytes (e.g. *Trichophyton rubrum*) and non-dermatophytes (e.g. *Candida albicans*)[1,2]. Although the diseases are not life-threatening, they adversely affect the quality of patients' life and substantial financial losses. Thus, effective and convenient therapy is required.

Itraconazole (ITZ) is a broad-spectrum antifungal triazole. Its antifungal mechanism is inhibition of cytochrome P450-dependent enzymes interfering with ergosterol synthesis in the fungal cell membrane[3]. Usually, an antifungal drug can be administered via both oral and topical routes. Topical drug delivery systems are attractive because they can avoid systemic adverse effects, first-pass metabolism, and drug interaction with foods, enzymes, or other drugs. Additionally, topical formulations have been acceptable for improving patient compliance. However, ITZ has an extremely low aqueous solubility (< 1 µg/mL) and high molecular weight (705.64 g/mol)[4]. These properties result in difficulty

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in formulation development and skin permeation. Therefore, the dermal fungal infections can be treated more effectively if ITZ is incorporated into effective topical formulations which can offer high skin accommodation to inhibit the mycoses at the target site. Moreover, clove oil (*Syzygium aromaticum*) could provide a synergistic effect on treatment of dermal fungal diseases due to the antifungal activity against *C. albicans*, *Aspergillus* sp., and *T. rubrum* of eugenol, its major component[5,6]. Additionally, ITZ could be highly dissolved in clove oil[7]. Hence, clove oil is interesting to be used as a component in antifungal formulations.

One of the widely used topical delivery systems is microemulsion (ME), defined as transparent, optically isotropic, and thermodynamically stable dispersion mainly consisting of oil, water, and surfactant with internal droplets in the nano-size range. ME is of interest for topical delivery of various drugs because of its spontaneous formation, high solubilization capacity, and skin permeability enhancement[8]. Moreover, mixing a gelling agent into ME in liquid form to be microemulsion-based gel (MBG) in semisolid form is also interesting due to improvement of skin adherence which could prevent drug loss by draining out of the application areas and prolong drug permeation.

ITZ microemulsions (ITZ-MEs) composed of 1% w/w ITZ as an active drug, clove oil as oil phase, polysorbate 80 (P80) as a nonionic surfactant, and 1:1 mixture of water and polyethylene glycol 400 (PEG400) as an aqueous phase were previously reported that they exhibited acceptable physicochemical stability, favorable dermal delivery, and efficient antifungal activity[7]. Therefore, these liquid ITZ-MEs were interesting to be converted to semisolid form as ITZ microemulsion-based gels (ITZ-MBGs). It was reported that ITZ in semisolid form could be effectively used to treat fungal infections in various body sites such as oral cavity[9] and skin[10]. ITZ-loaded nanoemulgel, a gel incorporated with ITZ nanoemulsions, was previously developed and proved to be potential for effective topical delivery[10]. However, it is generally known that preparation of nanoemulsions requires higher energy input than that of MEs[8]. In the current work, polyvinyl butyral (PVB), which has not been reported as a gelling agent in MBGs, was used for preparation of MBGs. PVB was previously formulated in ITZ-loaded film-forming solutions[11]. Although PVB has widely been used as a film-forming agent, it can be acted as a gelling agent in topical gel formulations[12].

This study aimed to formulate and characterize 1% w/w ITZ-MBGs. The efficacy of drug permeation via shed snakeskin and antifungal activity against *C. albicans* as well as *T. rubrum* of the formulated ITZ-MBGs were compared with a simple gel and a commercial emulgel. Stability of the prepared products was also evaluated after kept at 4°C, 25°C, and 40°C for three months.

2. MATERIALS AND METHODS

2.1. Materials

ITZ and PVB were purchased from Acros Organics (Geel, Belgium). P80 and PEG400 were obtained from PC Drug Center (Bangkok, Thailand). Clove oil was purchased from Union Chemical 1986 (Nonthaburi, Thailand). Acetonitrile, ethanol, and methanol were procured from RCI Labscan (Bangkok, Thailand). Sodium dihydrogen orthophosphate and disodium hydrogen orthophosphate anhydrous were purchased from Univar (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. A commercial emulgel containing 1% w/w ITZ was purchased from a drugstore in India for use in the *in vitro* skin permeation and antifungal activity studies. Shed snakeskin of *Naja kaouthia* was kindly supported by the Queen Saovabha Memorial Institute, Thai Red Cross Society, Bangkok, Thailand. It was collected without animal disturbance since snakes must naturally shed and discard their old skin to continue growing.

2.2. Preparation of ITZ-MBGs and ITZ-gel

The component ratios of three MEs were chosen from the ME region of the previous study[7]. Various concentrations of PVB (15, 17, 19, and 21% w/w) were preliminarily studied to form MBGs with the selected MEs. The obtained data showed that MBGs prepared from 17% w/w PVB were optically clear gels which could be easily spread on the smooth surface, leading to 17% w/w PVB be chosen as a gelling agent. Hence, three ITZ-MBGs (ITZ-MBG-1, ITZ-MBG-2, and ITZ-MBG-3) were composed of ITZ, clove oil, P80, 1:1 water: PEG400, and PVB while an ITZ-gel without surfactant and aqueous phase was prepared for comparison (Table 1). In preparation process of ITZ-MBGs, the PVB solution was prepared by mixing PVB, P80, and 75% of the total weight of clove oil at 50°C using a hot plate stirrer until PVB was dissolved and a clear solution was obtained. The PVB solution was then left to cool down at room temperature (25°C). In order to avoid thermal drug degradation, ITZ was dissolved in the rest 25% of the total weight of clove oil and then added to thoroughly mix with the cool down PVB solution. Finally, 1:1 mixture of water and PEG400 was added. The mixtures were blended until transparent MBGs were formed.

Table 1. The composition of ITZ-MBGs and ITZ-gel.

Formulation	ITZ (% w/w)	Clove oil (% w/w)	P80 (% w/w)	1:1 Water: PEG400 (% w/w)	PVB (% w/w)
ITZ-MBG-1	1.0	49.2	24.6	8.2	17.0
ITZ-MBG-2	1.0	41.0	32.8	8.2	17.0
ITZ-MBG-3	1.0	32.8	41.0	8.2	17.0
ITZ-gel	1.0	82.0	-	-	17.0

2.3. Characterization of ITZ-MBGs and ITZ-gel

2.3.1. Appearance observation and type determination of the samples

ITZ-MBGs and ITZ-gel were observed for visual appearance. ITZ-MBGs were also observed optically after being centrifuged at 6,000 rpm for 15 min by a universal centrifuge (Kubota 5922, Kubota Corporation, Japan). The conductivity values were measured using a conductivity meter (FiveEasy, Mettler Toledo, Switzerland) to identify for MBG type as oil-in-water (o/w) or water-in-oil (w/o).

2.3.2. Measurement of pH and texture properties of the samples

The pH values were measured using a pH meter (S20-K, Mettler Toledo, Switzerland). The viscosity values were measured by Rheometer Model DV-III (Brookfield DV III Ultra Programmable Rheometer, Brookfield Engineering Laboratories, Middleboro, USA) using LV spindle T-F at five different speeds, i.e., 1.0, 1.5, 2.0, 2.5, and 3.0 revolutions per minute (rpm). The rheological flow was determined by plotting the rheograms between viscosity values and spindle rotation speeds. The texture profiles of ITZ-MBGs and ITZ-gel were evaluated using a texture analyzer (TA.XTplus; Stable Micro System, Surrey, UK) and performed in a simple compression mode. Gel strength and adhesiveness were determined using the P/0.5R probe. The probe penetrated through each sample at a pre-test speed of 1.00 mm/s. The distance traveled by the probe was 20.00 mm and the test speed was 2.00 mm/s. The upper probe contacted each sample with a trigger force of 0.5 g and returned to the starting point (60.00 mm) with a post-test speed of 2.00 mm/s. The experienced force was operated by Stable Micro System software. The spreadability test was evaluated by placing 300 mg of each sample on a glass plate in a circle with a pre-marked diameter of 1 cm. The second glass plate was kept over it and a weight of 500 g was placed on the top for 5 min. The sample spreadability was noted from the increased diameter of the sample placed. All measurements were performed at 25°C in triplicate.

2.3.3. Determination of component interaction in ITZ-MBGs

Fourier transform infrared (FTIR) spectroscopy was studied to investigate the presence of any chemical interactions between the drug and components. FTIR spectra of ITZ, PVB, P80, clove oil, and a representative ITZ-MBG were determined using Fourier Transform Infrared Spectrometer (VERTEX 70, Bruker, Germany). Each tested sample was freeze-dried before analysis. The samples were analyzed over the scanning wavelength range of 4,000-400 cm^{-1}.

2.4. *In vitro* antifungal activity evaluation

In vitro antifungal activity of the prepared ITZ-MBGs and ITZ-gel against *C. albicans* ATCC 10231 and *T. rubrum* DMST 30263 was compared with a commercial emulgel using agar diffusion method. An inoculum of *C. albicans* and that of *T. rubrum* were subcultured over the entire surface of Sabouraud-dextrose agar (SDA) media and incubated at 25°C for 48 h and seven days, respectively. The growth was harvested, adjusted to a 0.5 McFarland standard for *C. albicans* and 1.0 McFarland standard for *T. rubrum*, and eventually streaked onto SDA plates with sterilized cotton. A sterile 6 mm pipette tip was used to make wells on each plate. Subsequently, an equal amount of the studied samples and a commercial emulgel were dropped in each well. The plates containing *C. albicans* were incubated at 25°C for 48 h whereas those containing *T. rubrum* were incubated for seven days. The diameter of the inhibition zone was measured. The experiment was done in triplicate.

2.5. *In vitro* shed snakeskin permeation study

The skin permeation was determined using Franz diffusion cells (Hanson Model 57-6M, Hanson Research Corporation, CA, USA). Shed snakeskin of *Naja kaouthia* obtained from five different snakes was used as a model membrane. The shed snakeskin with an annular shape of 4 cm^2 was clamped between the donor and receptor chambers of the diffusion cells. The donor compartment with 1.77 cm^2 diffusion area (*A*) of shed snakeskin was placed with 1 g of each sample.

Parafilm was used to cover the donor compartment to prevent the evaporation of the components in the sample. The receptor compartment was contained with 12 mL of 70:30 PBS: ethanol as receptor fluid which was stirred continuously at 200 rpm by a magnetic bar on a magnetic stirrer. It was controlled at 37 ± 0.5°C by a circulating water jacket[7,13]. An aliquot of 0.5 mL was collected from the receptor fluid at intervals of 0.5, 1, 2, 4, 6, 8, 12, and 24 h. It was immediately replaced with an equal volume of the fresh receptor fluid to maintain a constant volume. The sampling receptor fluid was analyzed for ITZ concentration by the HPLC method. This study compared the amount of permeated ITZ between the prepared formulations and that of a commercial emulgel. For each sample, the experiment was performed in four or five replications.

The cumulative amount of permeated ITZ across the shed snakeskin into the receptor fluid (Q_t, μg) was calculated using Eq. 1.

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

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

The cumulative amount of ITZ permeated into receptor fluid per unit of the diffusion area (Q_t/A, μg/cm^2) was plotted as a function of time (t, h). The steady-state flux (J_{ss}) was calculated using linear regression from the slope of the linear portion. The lag time (T_{lag}) values were determined from the x-intercept of the slope at the steady state. Permeability coefficient (K_p) was calculated by dividing J_{ss} with the initial concentration of the drug in the donor chamber (C_d) following Eq. 2.

$$K_p = \frac{J_{ss}}{C_d} \qquad \text{(Eq. 2)}$$

At the end of *in vitro* shed snakeskin permeation study (24 h), all shed snakeskin membranes were extracted in order to analyze the content of ITZ retained in the skin. Each membrane was gently wiped with water-soaked cotton sheets to remove the residual formulation. The sample remained in the donor compartment and absorbed in the wiped cotton sheets was then extracted with methanol and analyzed to confirm the experimental reliability. The shed snakeskin was cut into small pieces, then 10 mL methanol was added and homogenized using a homogenizer (Polytron® PT 1200 E, Kinematica AG, Luzern, Switzerland) at 25°C for 2 min. It was subsequently sonicated for 10 min, filtered through a 0.45-μm nylon filter, and analyzed for drug accommodation by the HPLC method. Furthermore, the local accumulation efficiency index (LAEI) at 24 h was calculated according to Eq. 3[13].

$$\text{LAEI} = \frac{\text{Amount of drug deposited in the skin}}{\text{Amount of the permeated drug}} \qquad \text{(Eq. 3)}$$

2.6. Stability study

The prepared ITZ-MBGs and ITZ-gel were stored in well-closed glass containers and protected from light under three different storage temperatures, i.e., 4°C (in a refrigerator), 25°C (in the control of air conditioning system), and 40°C (in Constant Climate Chamber Model HPP260 (Memmert, Schwabach, Germany) at 75% relative humidity) for three months. All formulations were collected at predetermined times (at month 1, 2, and 3) to investigate physical and chemical stabilities. For physical stability, they were investigated for changing in texture characteristics in terms of viscosity, gel strength, and spreadability after storage for three months. For chemical stability, the drug contents in the samples were analyzed using HPLC technique every storage month and compared to those at the initial state. Briefly, 300 mg of each sample was extracted with methanol, diluted with methanol to the appropriate concentration, and filtered through a 0.45-μm nylon filter before HPLC analysis. The drug analysis was performed in triplicate at 25°C.

2.7. HPLC condition

The validated HPLC method was used to quantitatively determine ITZ[14]. The analysis was carried on a reversed-phase HPLC system (LC-2030 3D Shimadzu, Kyoto, Japan) equipped with a UV detector. A reverse-phase column C18 (5 μm, 250 × 4.6 mm, C18-Phenomenex® Luna, USA) with a guard column was used for sample separation. The mobile phase was a mixture of acetonitrile: water (70:30, v/v) at 1.5 mL/min flow rate. The UV detection wavelength was 263 nm. The injection volume was 10 μL. Calibration curves of ITZ concentrations in the range of 5 to 60 μg/mL were used for drug content analysis while those in the range of 0.5 to 8 μg/mL were used for *in vitro* shed snakeskin permeation study.

2.8. Data analysis

The characterization, antifungal, and stability data were reported as mean ± standard deviation (SD). For the *in vitro* shed snakeskin permeation 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, utilizing SPSS Statistics software version 22.0 (IBM Corp., Armonk, NY, USA).

Table 2. Characteristics of the prepared ITZ-MBGs and ITZ-gel after preparation (mean ± SD, n=3).

Formulation	Conductivity (µS/cm)	pH	Viscosity* (mPa.s)	Gel strength (g)	Adhesiveness (g.sec)	Spreadability (cm)
ITZ-MBG-1	0.39 ± 0.01	5.44 ± 0.02	164,092.71 ± 654.10	4.46 ± 0.02	-165.26 ± 2.81	4.40 ± 0.10
ITZ-MBG-2	0.49 ± 0.03	5.92 ± 0.02	221,879.74 ± 704.42	5.51 ± 0.03	-188.36 ± 8.21	4.20 ± 0.06
ITZ-MBG-3	0.60 ± 0.04	5.80 ± 0.03	295,015.18 ± 110.46	7.42 ± 0.11	-300.40 ± 9.79	4.00 ± 0.06
ITZ-gel	0.00 ± 0.00	1.24 ± 0.02	39,835.23 ± 883.67	2.53 ± 0.03	-66.46 ± 3.26	5.40 ± 0.10

*Viscosity measured by Rheometer Model DV-III (Brookfield DV III Ultra Programmable Rheometer, Brookfield Engineering Laboratories, Middleboro, USA) using LV spindle T-F at the representative spindle speed of 3.0 rpm.

3. RESULTS AND DISCUSSION

3.1. Characteristics of ITZ-MBGs and ITZ-gel

All obtained 1% w/w ITZ-MBGs and ITZ-gel were transparent yellow gels with smooth homogeneous texture. Visual appearance of ITZ-MBGs was similar to that of their blank counterparts. Moreover, neither turbidity nor phase separation of all ITZ-MBGs was found after the centrifugation, implying as physically stable gels[15]. Other characteristics (Table 2) showed that conductivity values of ITZ-MBGs were very low, indicating for w/o type. The type of ITZ-MBGs was similar to their ME counterparts since amounts of oil phase were higher than those of aqueous phase[7]. The conductivity value of ITZ-gel was zero due to the lack of water content in the formulation. The pH values of ITZ-MBGs were in the range of 5.44-5.92, which were considered as a suitable range for skin application. In contrast, too acidic pH values of ITZ-gel may cause skin irritation.

All formulations exhibited pseudoplastic flow. It has been generally known that formulations with shear-thinning behavior provide ease of skin application. The average viscosity values of ITZ-MBGs measured at the representative spindle speed of 3.0 rpm were in the range of 164,092.71-295,015.18 mPa.s whereas ITZ-gel had average viscosity of 39,835.23 mPa.s. It could be noted that ITZ-gel had very low viscosity compared with ITZ-MBGs. The viscosity values of ITZ-MBGs were directly related to P80 amounts similar to their original ITZ-MEs[7]. The experimental results showed that ITZ-MBG-3 had the highest gel strength and adhesiveness, followed by ITZ-MBG-2, ITZ-MBG-1, and ITZ-gel, respectively. ITZ-gel showed the largest area of spreading diameter, followed by ITZ-MBG-1, ITZ-MBG-2, and ITZ-MBG-3, respectively. It was noted that viscosity, gel strength, and adhesiveness of all samples were in direct relationship while the samples with lower viscosity provided larger size of spreading diameter as expected. The gel strength might be provided by hydrogen bonding between –OH of PVB chain and –OH of eugenol in clove oil. It was also possible that –OH of water in ITZ-MBGs might interact with –OH of PVB and thus act as strength to the network, leading to increased gel strength[16]. Additionally, gel strength-enhancing effect was observed with increasing P80 content in ITZ-MBGs. P80 participated in micellar gelation and its long chain length could increase the cross-linking area, resulting in higher-strength gels[17].

The FTIR spectrum of pure ITZ (Figure 1a) showed the characteristic peaks at 2824-2967 cm^{-1} (C-H stretching), 3070 cm^{-1} (the stretching of aromatic rings), 3442 cm^{-1} (N-H stretching of amide), 1045-1227 cm^{-1} (C-O stretching), 1045-1185 cm^{-1} (C-N stretching of the alkyl group), 1700 cm^{-1} (C=O stretching), and 597-824 cm^{-1} (C-Cl stretching). The FTIR spectrum of pure PVB (Figure 1b) exhibited the characteristic peaks at 3445 cm^{-1} (O-H group), 2854-2959 cm^{-1} (C-H stretching), 1243 cm^{-1} (ester group), 1012-1243 cm^{-1} (acetal group), and 1640 cm^{-1} (carbonyl group). In the case of P80 (Figure 1c), the FTIR peaks were found around at 3400 cm^{-1} (O-H group), 2900 cm^{-1} (CH2 stretching), 1734 cm^{-1} (C=O stretching), and 1600 cm^{-1} (HOH bending). Clove oil (Figure 1d) showed its signature FTIR peaks of eugenol in 720-1250 cm^{-1} corresponding to C=C. Besides, sharp peaks at 1638, 1607, and 1510 cm^{-1} were observed which could be due to C=C stretching of the eugenol aromatic moiety. No additional peaks or peak position shifts were observed in the FTIR spectrum of ITZ-MBG-3, the representative for ITZ-MBG formulations (Figure 1e), indicating the absence of any interactions between the drug and all formulation excipients.

3.2. *In vitro* antifungal activity

The studied formulations illustrated the potential to inhibit *C. albicans* and *T. rubrum* as evidenced by inhibition zones (Table 3). ITZ-gel provided the largest inhibition zone, followed by ITZ-MBG-1, ITZ-MBG-2, and ITZ-MBG-3, respectively. The antifungal data showed an invert relationship between viscosity and size of inhibition zone since the higher viscosity influenced the slower drug diffusion through the agar. In addition, blank MBGs and gel also showed antifungal activities against both *C. albicans* and *T. rubrum*; however, their potential was significantly lower than their ITZ-loaded counterparts ($p < 0.05$). Eugenol in clove oil can destroy the fungal cell wall and inner mitochondrial membranes, resulting in synergistic effect

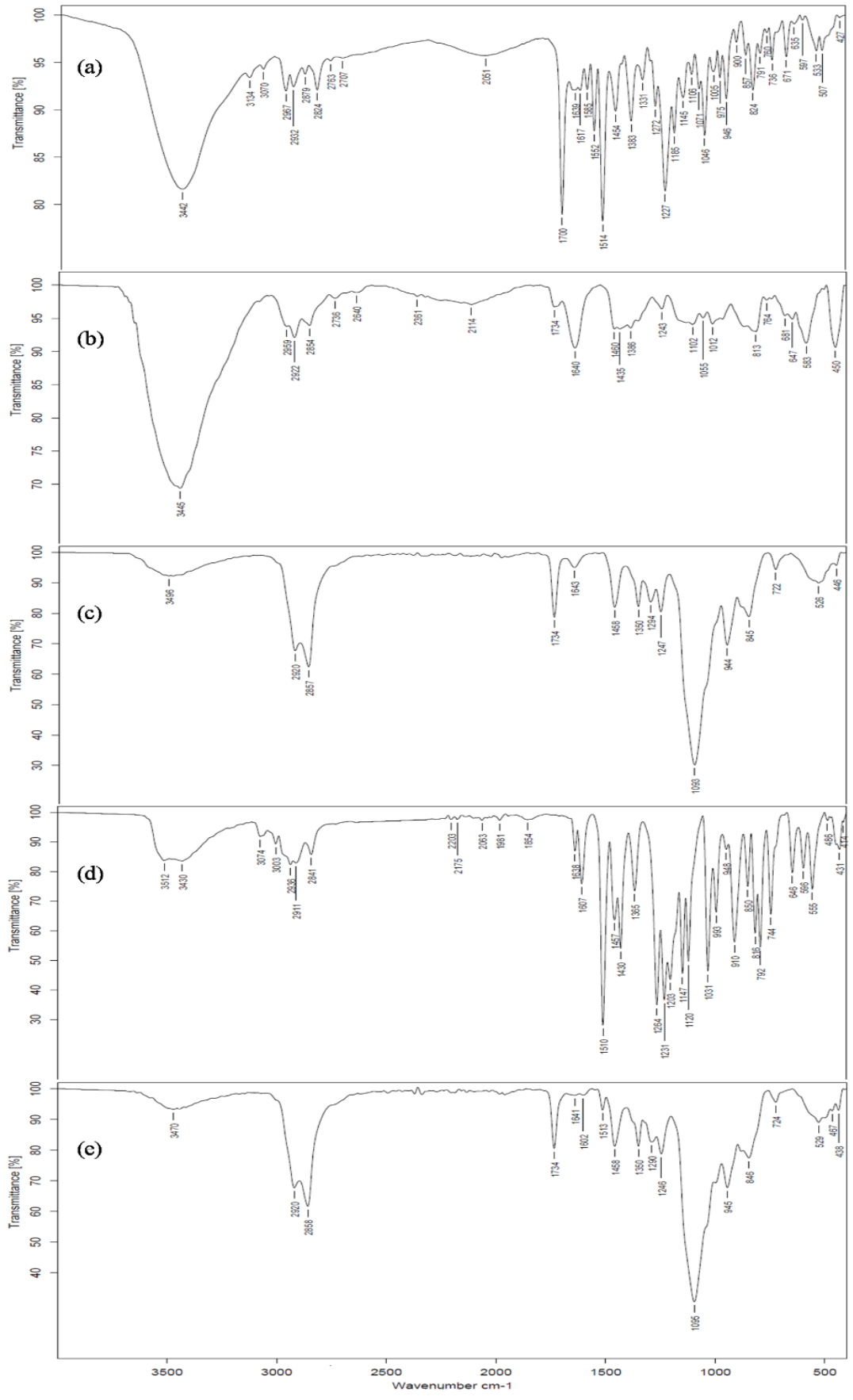

Figure 1. FTIR spectra of (a) ITZ, (b) PVB, (c) P80, (d) clove oil, and (e) ITZ-MBG-3.

Table 3. Antifungal activity against *C. albicans* and *T. rubrum* of the studied formulations (mean ± SD, n=3).

Formulation	Inhibition zone (mm)	
	C. albicans	***T. rubrum***
Blank MBG-1	22.47 ± 0.23	23.10 ± 0.75
Blank MBG-2	21.43 ± 0.06	22.33 ± 0.50
Blank MBG-3	21.30 ± 0.10	21.93 ± 0.58
Blank gel	21.60 ± 0.17	23.45 ± 0.07
ITZ-MBG-1	25.70 ± 0.30*	26.53 ± 0.21*
ITZ-MBG-2	24.37 ± 0.32*	25.80 ± 0.40*
ITZ-MBG-3	23.60 ± 0.10*	25.47 ± 0.12*
ITZ-gel	26.20 ± 0.00*	28.47 ± 0.31*
Commercial emulgel	15.93 ± 0.23	19.10 ± 0.46

*$p < 0.05$; statistically significant differences (One-way ANOVA) from the results of commercial emulgel and their blank counterparts.

on antifungal activity[5,6]. The results of this study were in accordance with the previous research which demonstrated that eugenol exhibited synergistic interactions with antifungal drugs against *C. albicans*[18]. The interesting data showed that the inhibition zone of a commercial emulgel against the tested pathogenic fungi was the smallest and significant difference ($p < 0.05$) from ITZ-MBGs and ITZ-gel. It might be possible that high affinity between ITZ and the internal oil phase of the o/w commercial emulgel caused the delay in drug release and diffusion through agar.

3.3. *In vitro* shed snakeskin permeation

The permeation profiles (Figure 2) and parameters (Table 4) of the studied samples presented the surprising outcome that the drug from the commercial emulgel was not detected in receptor fluid at all interval times except at 24 h. It could be explained that lipophilic ITZ in the commercial product was entrapped in the internal phase of o/w emulgel, resulting in slow diffusion out of the formulation. Although inactive ingredients of the commercial emulgel were not disclosed by the manufacturer, it might be possible that this product did not contain any permeation enhancers. Moreover, the commercial emulgel exhibited the highest LAEI due to its very low permeated drug amount at 24 h. Even though high LAEI is preferable for drug delivery to the dermal site, the undetectable permeated drug before 24 h of the commercial emulgel may provide too delayed action. A trend of continually increasing ITZ permeated from the prepared MBGs and gel across shed snakeskin into receptor fluid was found. However, the cumulative amount of drug permeated from ITZ-gel was likely to decrease slowly after 4 h. For the experimental evidence on ITZ-MBGs, ITZ-MBG-1

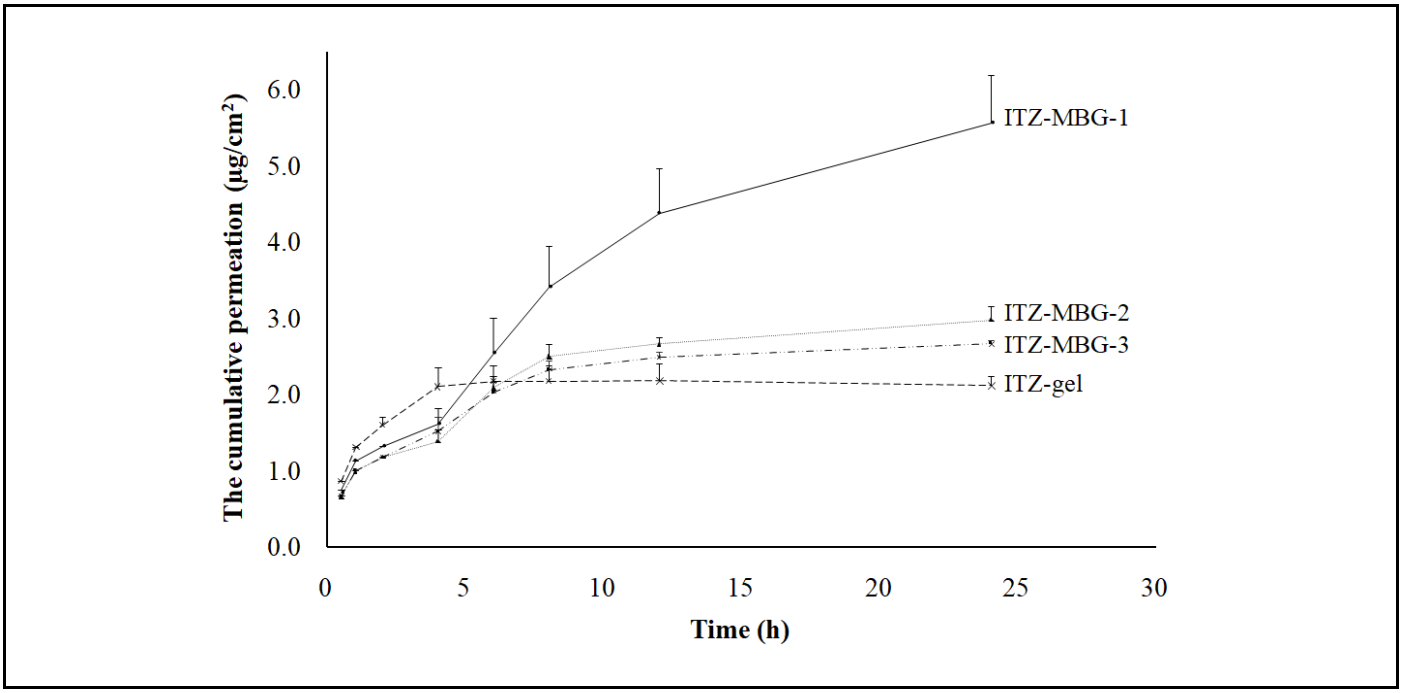

Figure 2. Permeation profiles of ITZ from the prepared formulations across shed snakeskin through the receptor fluid (mean ± SEM, n=4-5).

Table 4. Shed snakeskin permeation parameters of ITZ-MBGs, ITZ-gel, and commercial emulgel (mean ± SEM, n=4-5).

Formulation	J_{ss} (x10^{-2} µg/cm^2/h)	Q_{24} (µg/cm^2)	Skin retention (µg/cm^2)	T_{lag} (h)	K_p (x10^{-6} cm/h)	LAEI
ITZ-MBG-1	35.60 ± 6.31	5.57 ± 0.62*	19.02 ± 1.50	1.08 ± 0.09	34.23 ± 6.56	3.41*
ITZ-MBG-2	26.70 ± 3.82	2.98 ± 0.18*	20.43 ± 0.65	1.13 ± 0.05	25.15 ± 3.54	6.86*
ITZ-MBG-3	22.97 ± 1.23	2.68 ± 0.04*	23.21 ± 0.58	1.16 ± 0.06	21.00 ± 1.17	8.66*
ITZ-gel	20.61 ± 1.10	2.13 ± 0.12*	8.72 ± 1.49*	0.87 ± 0.05	19.50 ± 1.19	4.09*
Commercial emulgel	-	0.60 ± 0.17[a]	18.26 ± 2.56	-	-	30.43

*$p < 0.05$; statistically significant differences (One-way ANOVA) from commercial emulgel.
[a]The amount of ITZ permeated into receptor fluid was detected at only 24-h time point.

tended to provide higher amount of ITZ across shed snakeskin than others and presented the highest J_{ss} and K_p. ITZ-MBG-1 also showed a short T_{lag}; however, there was no significant difference among ITZ-MBGs ($p > 0.05$). According to ITZ-MBGs, the drug flux increased as concentrations of clove oil increased. Both P80 and clove oil could play a role as permeation enhancers by disturbing lipid structure and reducing the diffusion barrier of the stratum corneum[19,20]. The results of the present study indicated that ITZ-MBGs provided the same trend of permeation parameters with their original ITZ-MEs[7]. Thus, these results confirmed the association between clove oil and permeation-enhancing properties. Additionally, the aqueous phase of ITZ-MBGs could significantly improve ITZ skin permeation due to skin hydration. However, ITZ-gel did not contain P80, water, and PEG400, resulting in that the drug permeation occurred fast only in the first 4 h.

In comparison to a commercial emulgel, ITZ retained in shed snakeskin after 24 h increased by 1.04, 1.12, and 1.27-fold when applying ITZ-MBG-1, ITZ-MBG-2, and ITZ-MBG-3, respectively. In contrast, the retained drug from ITZ-gel was found to be 2-fold less than that from the commercial one. The skin retention study concerned drug accumulation in the skin for local fungal treatment. ITZ-MBGs showed higher drug localization in shed snakeskin than ITZ-gel and the commercial emulgel. These results were related to those of skin permeation profiles. However, the increase in skin accommodation presented by ITZ-MBGs was not significantly different compared to the commercial emulgel. One factor probably influenced on high skin accommodation of the commercial o/w emulgel might be related to skin hydration of water in the formulation. In contrast, ITZ-gel provided the lowest drug retention due to lack of skin barrier disruption by P80 and skin hydration by the aqueous phase when compared with ITZ-MBGs and the commercial emulgel.

3.4. Stability

The texture characteristics of the prepared samples after being kept for three months at different temperatures (Table 5) showed that the viscosity of all formulations increased as storage time increased. This might be due to the realignment of PVB chains during storage. According to the sample preparation, PVB was gelatinized by heating in the presence of oil. It was probably

Table 5. Texture characteristics of the prepared formulations after storage for 3 months and percentage of ITZ remaining in the prepared formulations during storage period at various temperatures (mean ± SD, n=3).

Formulation	Temperature (°C)	Viscosity* (mPa.s) at 3rd month	Gel strength (g) at 3rd month	Spreadability (cm) at 3rd month	ITZ remaining (%)		
					1st month	2nd month	3rd month
ITZ-MBG-1	4	186,841.53 ± 441.85	5.89 ± 0.17	4.32 ± 0.06	100.40 ± 0.26	99.37 ± 0.44	99.65 ± 0.77
	25	184,075.31 ± 957.34	5.74 ± 0.03	4.30 ± 0.00	100.96 ± 0.33	99.90 ± 0.71	99.02 ± 0.68
	40	174,572.13 ± 773.23	4.99 ± 0.06	4.35 ± 0.06	100.98 ± 0.46	97.52 ± 0.39	96.85 ± 0.49
ITZ-MBG-2	4	305,065.11 ± 405.03	8.26 ± 0.06	3.90 ± 0.06	99.81 ±1.26	100.52 ± 0.36	100.06 ± 0.43
	25	286,839.84 ± 625.95	8.10 ± 0.82	4.10 ± 0.03	100.79 ± 1.09	100.49 ± 0.58	100.34 ± 0.84
	40	271,973.22 ± 1,325.54	8.00 ± 0.09	4.14 ± 0.03	100.12 ± 0.81	98.20 ± 0.52	98.02 ± 0.44
ITZ-MBG-3	4	373,676.67 ± 657.34	9.43 ± 0.47	3.41 ± 0.02	100.06 ± 0.21	100.37 ± 0.40	99.97 ± 0.32
	25	348,676.67 ± 1,357.02	9.10 ± 0.82	3.50 ± 0.04	100.74 ± 0.79	100.61 ± 0.49	100.31 ± 0.82
	40	327,013.56 ± 1,620.11	8.25 ± 0.72	3.60 ± 0.04	99.98 ± 0.98	98.42 ± 0.95	97.64 ± 0.79
ITZ-gel	4	61,080.72 ± 662.77	3.47 ± 0.02	4.80 ± 0.02	95.23 ± 0.10	95.89 ± 0.27	94.40 ± 0.08
	25	53,426.10 ± 441.85	3.01 ± 0.03	4.88 ± 0.04	96.44 ± 0.09	96.08 ± 0.10	95.36 ± 0.02
	40	45,927.70 ± 441.85	2.87 ± 0.06	5.20 ± 0.05	90.11 ± 0.07	83.79 ± 0.26	82.62 ± 0.25

*Viscosity measured by Rheometer Model DV-III (Brookfield DV III Ultra Programmable Rheometer, Brookfield Engineering Laboratories, Middleboro, USA) using LV spindle T-F at the representative spindle speed of 3.0 rpm.

that the disordered PVB chains might rearrange into a different ordered structure during storage period or cooling after gelatinization, which could affect the viscosity. The viscosity values were also directly related to the gel strength values. However, all samples still possessed good ability to spread. The chemical stability was evaluated in terms of ITZ remaining in the formulations (Table 5). ITZ-MBGs stored at all studied temperatures remained stable over three months with the drug contents higher than 90% compared to the initial, indicating good chemical stability. However, the samples stored at 40°C tend to have slightly less ITZ remaining than those kept at 4°C and 25°C. Although it has been reported that degradation of ITZ in solid form did not occur at high temperature (40°C, 75% RH)[21], storing samples at high temperature may decrease ITZ content in the solubilized form. ITZ-gel exhibited significantly lower drug remaining than ITZ-MBGs ($p < 0.05$) under all storage conditions during three months. It was due to ITZ degradation under acidic condition since the degradation of ITZ was found as 17.2% in acidic conditions and no peak of degradation product was observed in the chromatogram[22]. The results revealed that MBGs could improve the chemical stability of ITZ and storage at low temperatures was preferable.

4. CONCLUSIONS

The findings showed that the obtained 1% w/w ITZ-MBGs prepared from PVB were transparent spreadable gels with appropriate pH for skin application, efficient antifungal activity, suitable skin permeation parameters for dermal drug delivery, and high stability. No incompatibility between ITZ and other MBG components was found. Although ITZ-gel could also provide high antifungal activity, it was not suitable in real application since its acidic pH could cause skin irritation. Additionally, the chemical stability of ITZ-gel was lower than that of ITZ-MBGs. The key strengths of the present study were that the developed w/o ITZ-MBGs appeared to have better shed snakeskin permeation as well as retention and antifungal activity against *C. albicans* as well as *T. rubrum* when compared to the o/w commercial emulgel. Therefore, the developed ITZ-MBGs could be considered as favorable topical drug delivery systems to treat dermal fungal infections.

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Author contribution

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

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Conflict of interest (If any)

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