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# Improving the antifungal activity of itraconazole by solid dispersion

Aymen Mohammed BASEET1\*, Ghaidaa S. HAMEED1, Dalya HANNA2

- 1 Mustansiriyah University, College of Pharmacy, Department of Pharmaceutics, Baghdad, Iraq
- 2 Mustansiriyah University, College of Pharmacy, Department of Clinical Laboratory Science, Baghdad, Iraq

#### ABSTRACT

Itraconazole (ITZ), a potent antifungal agent, suffers from poor water solubility and limited bioavailability, hindering its therapeutic effectiveness, particularly in the treatment of vaginal candidiasis. This study aimed to enhance the solubility, dissolution rate, and antifungal efficacy of ITZ through the formulation of solid dispersions (SDs) using hydrophilic polymers (Polyvinylpyrrolidone {PVP} K30, hydroxypropyl methylcellulose E5 {HPMC E5}, and Soluplus) via physical mixing and solvent evaporation techniques. Characterization was done by Differential Scanning Calorimetry, Powder X-ray Diffraction, and Fourier Transform Infrared Spectroscopy, which confirmed reduced crystallinity and improved drug-polymer interactions, especially in the ITZ-PVP K30 system prepared by solvent evaporation (RE). Furthermore, saturated solubility, dissolution study, and antifungal activity were performed. Then the optimized formulation was further developed into PEG-based vaginal suppositories with full evaluation test. Among all formulations, ITZ-PVP K30 RE exhibited the highest solubility (527.34 µg/mL), superior dissolution (91.18% at 120 minutes), and the greatest antifungal activity (25 mm inhibition zone) against Candida albicans. Evaluation demonstrated acceptable physicochemical properties, optimal drug release (96.4% with 10% glycerin), and enhanced antifungal efficacy (30 mm inhibition zone). This novel ITZ-PVP K30 mixture suppository formulation presents a promising alternative for the

Aymen Mohammed BASEET: 0009-0006-7902-8509 Ghaidaa S. HAMEED: 0000-0003-1470-6808 Dalya HANNA: 0000-0003-3201-4303 (Received 6 Jun 2025, Accepted 18 Aug 2025)

<sup>\*</sup> Corresponding authors: Aymen Mohammed BASEET E-mail: ayman muhammad@uomustansiriyah.edu.iq

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localized treatment of vaginal candidiasis with improved efficacy and patient compliance.

Keywords: itraconazole, Candida albicans, solid dispersion, vaginal candidiasis

#### INTRODUCTION

Itraconazole, a common antifungal, has limited water solubility and bioavailability, making medication distribution difficult (Figure 1). Oral medication delivery is the most convenient, safest, and cost-effective, but its efficacy depends on the active pharmaceutical ingredient's solubility and permeability. The Biopharmaceutical Classification System (BCS) classifies drugs by properties, with Class II drugs like itraconazole having low solubility (1 ng/mL) and high permeability, resulting in poor bioavailability. Their bioavailability and pharmacological action depend on their dissolving rate, which limits their therapeutic efficacy<sup>1,2</sup>. Itraconazole is a broad-spectrum triazole antifungal agent used to prevent and treat fungal infections. It is a fungistatic antifungal agent, hinders ergosterol synthesis in fungal cell membranes, and displays broad-spectrum activity3. Itraconazole is commercially available as an oral and intravenous formulation; however, its administration via these routes is associated with various side effects, such as hepatotoxicity, peripheral neuropathy, cardiac dysrhythmia, and hearing loss<sup>4</sup>. One of the most prevalent and widely used methods for improving solubility and dissolving rate is the formation of solid dispersions, which has been defined as 'the dispersion of one or more active pharmaceutical ingredients (API) dispersed in an inert matrix. The dissolving rate and apparent solubility of a medicine can be improved by distributing it within a carrier, especially at the molecular level (solid dispersion), and by preventing phase transitions from the solubilized to crystalline form. The carrier may be crystalline or amorphous. To improve the solubility and rate of dissolution of medications that are weakly water-soluble, the carrier is dissolved when exposed to water, releasing the drug as tiny colloidal particles<sup>5</sup>.

Multiple researchers have proposed SD dissolving rate increases. Polymeric carriers dispersing molecularly structured medications may improve surface area and decrease particle size, speeding solubility. Adjacent hydrophilic carriers boost drug solubility and wettability without energy because their crystal lattice breaks down passively. Co-milling, kneading, supercritical fluid technique, and ultra-rapid freezing are employed to make solid dispersion, while HME and solvent evaporation are the most popular. Itraconazole's solubility and bioavailability were improved by reducing particle size, but Visakhapatnam et al. found that micronised ITZ material has higher antifungal activity against Candida albicans and Aspergillus niger than coarser ITZ material<sup>6</sup>.

Francois et al. were prepared topical cyclodextrin-based, emulsified wax cream containing 1% ITZ was shown to be effective in treatment of vaginal candidiasis where the clinical cure was reported in about 77% of patients<sup>7</sup>. Development of itraconazole loaded deformable liposomes in the presence of HPBCD, the amount of itraconazole in subcutaneous, deeper skin layers and receptor fluid was enhanced. ITZ-loaded liposomes remain active against Candida albicans according to research done by Alomrani et al8.

Optimization of the poorly soluble medication itraconazole can be done by crystallization. Itraconazole crystallization with Para-Hydroxy Benzoic Acid (PHBA) was chosen as the co-former due to its recognized involvement in crystallization and possible solubility and stability advantages through noncovalent interactions. Compared to pure itraconazole, the optimized cocrystal formulation increased solubility by 2.4-fold in 0.1 N HCl and 25.77-fold in phosphate buffer (pH 6.8), after acidic medium, 40.12% drug release after 120 minutes was achieved, compared to 32.65% for pure itraconazole9.

Preparation of solid dispersion of ITZ (SD-ITZ) using PEG 6000, and PVP K-30 due to their high-water solubility and low toxicity by the solvent evaporation technique was successfully prepared by research done by Permana et al<sup>10</sup>. The inhibitory zone of free ITZ had a diameter of 19.23  $\pm$  1.76 mm. By enhancing ITZ solubility through solid dispersion, the inhibitory zone diameter was found to be 28.49  $\pm$  2.17 mm. Due to its greater solubility, SD-ITZ diffused to the growing medium and improved microbial cell permeability, resulting in stronger antibacterial action against candida albicans<sup>10</sup>.

The present study aimed to improve itraconazole's dissolving rate and antifungal efficacy by preparing a solid dispersion with HPMCE5, PVP K30, and Soluplus using solvent evaporation and examining its release and antifungal activity before and after vaginal suppository formulation.

Figure 1. Chemical structure of itraconazole

#### METHODOLOGY

#### **Materials**

Itraconazole (ITZ) was obtained from Hubei Widely Chemical Technology Co., Ltd. (China), PVP K30, HPMC E5, and Soluplus were sourced from Hangzhou Hyperchem (China). Ethanol, acetic acid, sodium acetate trihydrate, sodium lauryl sulphate supplied from Sama Al-Faihaa Pharmaceutical Industries (Iraq), PEG 6000, PEG 400, and glycerin supplied from Hangzhou Hyper Chemicals Limited (India).

#### Method

## Preparation of physical mixtures (PMs)

Utilizing a mortar and pestle, ITZ was combined at ambient temperature with each of the three polymers (PVP K30, HPMC E5, and Soluplus) individually for five minutes at a weight ratio of 1:2. The resultant mixtures were stored in a desiccator to prevent moisture absorption.

## Formation of solid dispersion of ITZ

A solid dispersion of ITZ was created via a solvent evaporation technique utilizing PVP K30, HPMC E5, and Soluplus polymers in a weight ratio of 1:2. The polymers were individually dispersed in 30 ml of ethanol using a magnetic stirrer until a homogeneous solution was achieved. ITZ was subsequently included, and agitation was maintained for 10 minutes. The solvent was subsequently evaporated using a rotary evaporator at 40°C for 2 hours. The resulting dried mass was pulverized to achieve uniform particle size, and after that kept in a desiccator for further analysis<sup>11</sup>.

# Differential scanning calorimetry (DSC)

ITZ melting points were assessed by DSC. A Shimadzu DSC 60 (Japan) was also used for ITZ S.E., PMs, and SDs.3 mg of accurately weighed samples were stored in aluminum pans that were crimp-sealed (5-6 mg). At a rate of 10°C/ min, the DSC was operated from 30 to 250°C12. The plotting of thermograms is with the endothermic points down and exothermic point up. Melting points are reported as peak temperature.

## Powder X-ray diffraction (PXRD)

The ITZ, ITZ S.E., PMs, and SDs were examined using a powder X-ray diffractometer from the Aires companies in the Netherlands. The PXRD was powered by 40 kV and 40 mA. Scan intensities in the 20 range of 5-60 degrees at a rate of 2°/min12.

## Fourier transform infrared spectroscopy (FTIR)

A Shimadzu 8300 Fourier transform infrared system (Japan) was used to evaluate the FTIR of ITZ, ITZ S.E., PMs, and SDs using the KBr disc method. Spectra between 4000 and 500 nm were examined<sup>13</sup>.

#### Determination of λ max

The stock solutions of itraconazole drug were prepared by dissolving 10 mg of itraconazole acetonide in 100 mL of acetate buffer that contains 1% SLS (pH 4.2), which is within the pH range of vaginal cavity and then left for 2 hours in sonicated bath for a complete dissolution of the drug. Afterwards, dilutions had been done to reach 30 µg/ml for itraconazole. After that, ITZ was scanned with a UV spectrophotometer from 200-400 nm, and the result was recorded 14.

#### Calibration curve

The calibration curve of the drug in acetate buffer that contains 1% SLS (pH 4.2) was constructed by preparing a series of dilute solutions with different concentrations from a stock solution containing 3 µg/ml of ITZ. The absorbance was then measured at the  $\lambda$  max of the drug. The measured absorbances were plotted against the respective concentrations<sup>15</sup>.

## **Determination of saturated solubility**

The saturation solubility of ITZ as received, ITZ RE, PMs, and SDs was determined in triplicate in acetate buffer at pH 4.2. An excess quantity of the medication was incorporated into 10 ml of buffer containing 1% SLS. Following 72 hours of agitation at 200 rpm and 37°C, centrifugation was performed, Subsequently, the supernatant portion was diluted and passed

through a syringe filter with a pore size of 0.45 µm, and analysed using UV spectrophotometry at 260 nm<sup>16</sup>.

## In-vitro dissolution studies

For all pre-formulation mixtures, dissolution studies employed a paddle (USP Apparatus II) at 37°C. ITZ, ITZ S.E, PMs, and SDs were agitated at 100 rpm in 900 ml of pH 4.2 to simulate vaginal pH<sup>17,18</sup>. Acetate buffer with 1% SLS maintained sink state. The experiment was done three times. To maintain volume, following 15, 30, 45, 60, and 120 minutes, a 0.45 μm filter syringe was used to remove 5 ml of samples, and then a new warmed medium was used to replace the original one. A UV-visible spectrophotometer assessed samples at 260 nm. the selected  $\lambda$  max.

# *In-vitro* antifungal activity (inhibition zone determination)

Using the agar-well diffusion method, the antifungal activity was evaluated regarding Candida albicans using Sabouraud dextrose agar as the medium for the experiment<sup>10</sup>. Candida albicans was disseminated on the agar surface of the Petri dishes using sterile cotton swabs, after which wells were created in the agar with a sterile Pasteur pipette. Pure ITZ, PMs, and SDs solubilized in an acetate buffer with 1% SLS at pH 4.2. An equivalent of 0.2 mg/0.1 mL of ITZ solutions was dropped into the wells for all batches. A subsequent 48 hours were spent with the Petri dish at a temperature of 32°C.

## Formulation of itraconazole as suppositories

Suppositories were manufactured via the heat fusion technique of PEG 6000/400 as the suppository base in different ratios (1:1, 1.5:1, 2.5:1, 3.5:1, and 4.5:1)19,20. ITZ as received, ITZ-PVP k30 PM, and ITZ-PVP k30 RE were used to get the optimum formula. Each suppository (3.4 g) includes 100 mg of itraconazole (ITZ). The base was deposited in a beaker and heated with continuous agitation after the necessary quantity was determined. The therapeutic agent was subsequently incorporated into the molten base with continuous stirring to prevent the formation of bubbles and achieve a homogeneous mixture.

Near the congealing temperature, the mixture was poured into the mold that was lubricated with liquid glycerin for easy extraction of the suppositories<sup>21</sup>. The mold was kept at room temperature for solidification (about 1 hour), and finally, the suppositories were extracted and stored for further investigation.

# Evaluation of itraconazole suppositories

## Physical assessment

The physical inspection of produced suppositories included noting their surface roughness, color, and odor<sup>20</sup>.

# Weight variation

The average weight of 20 suppositories from each batch was calculated, which was followed by the determination of percentage variation from the average, which could not exceed 5%22.

#### Hardness

Hardness is the degree to which the suppository resists breaking or cracking. Ten suppositories were subjected to a Monsanto hardness test at room temperature (25°C). An indication of the suppository's hardness is the force that is required for it to fracture. An evaluation of the suppositories' tensile strength was carried out in order to determine whether the manufactured product could withstand the stresses of packaging and transit<sup>23</sup>. Suppositories should have a hardness of 1.8 to 2.0 kg24.

# Assessment of pH

The pH of the manufactured suppositories was measured using a digital pH meter. The suppositories were dissolved in 150 ml of warm distilled water at 50°C and then filtered using filter paper. An electrode was placed in the solution at 37°C, and the reading was recorded to find the pH of the filtrate<sup>25</sup>.

## Drug content percentage

The experiment was conducted by introducing one suppository into 1000 ml of acetate buffer at pH 4.2, keeping it at 37°C until fully melted. One milliliter of the sample was extracted and diluted to ten milliliters with acetate buffer. The ITZ content was quantified using a UV-visible spectrophotometer by measuring the absorbance of a diluted sample at 260 nm.

## *In-vitro* suppositories release

A rotational basket dissolution device was used to ascertain the rate of ITZ release from vaginal suppositories<sup>22</sup> at 100 rpm at 37°C in 500 milliliters of acetate buffer<sup>26</sup>. Suppositories were positioned in the basket and submerged in a dissolving medium. At regular intervals of 15 minutes, 5 ml samples were extracted, and an equivalent volume of fresh buffer was introduced. Following filtration via a 0.45 μm syringe filter, the appropriate λmax (260 nm) for each collected sample was ascertained utilizing a UV-Vis spectrophotometer.

# Antifungal activity (zone of inhibition determination) of suppositories

To achieve a concentration of 2 mg/ml of ITZ, one suppository from each patch was dissolved in 100 ml of acetate buffer using a vortex for complete dissolution; subsequently, the aliquot was filtered and cultured in the same medium for pre-formulation tests under identical incubation conditions. Following 24 hours of incubation at 37°C, the inhibition zone surrounding each well was evaluated for ITZ as received, ITZ-PVP K30 PM, and ITZ-PVP K30 RE suppositories.

# Statistical analysis

The results stored in an excel sheet and then transferred to SPSS for the statistical analysis. The numerical variables were expressed as mean  $\pm$  standard deviation (SD) and the statistical analysis included a one-way ANOVA of variance and a least-significant-differences (LSD) post hoc test. When (p<0.05), there was a statistically significant difference. However, when (p>0.05), the difference was not considered significant. We used SPSS 16.0, the statistical package for the social sciences, to accomplish all our math<sup>27,28</sup>.

#### RESULTS and DISCUSSION

# Differential scanning calorimetry (DSC)

Figure 2 displays the DSC thermograms of ITZ as received, PMs, and SDs. ITZ (as received) shows a sharp endothermic melting peak at 171°C, which is in accordance with data reported previously by Piccinni et al<sup>29</sup>. Indicating the crystalline nature of ITZ. After processing by rotary evaporator (ITZ RE), it showed in Figure 2 the presence of the melting peak at the same magnitude that mean no change in the polymorphic form. In the case of PM and RE with HPMC, both of the processing techniques, the melting peaks remained in the same regions, but there was a mild reduction in the intensity of the peaks, which mean that there is a reduction in the crystallinity. Broadening the peaks with a moderate reduction in intensity and shifting to a reduced value at 166°C were noticed in the ITZ-PVP K 30 polymer in PM and RE meaning increase in amorphous content. Finally, in Figure 2 the use of Soluplus polymer in the PM method resulted in a significant reduction in peak intensity, while the RE method saw a nearly complete disappearance of the melting peak, with a noticeable decrease in value to 162°C indicating the highest amorphous content of this mix.

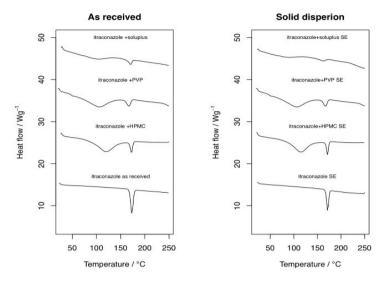


Figure 2. DSC thermogram of ITZ as received, ITZ when mixed with HPMC E5, PVP K30, and Soluplus before and after solvent evaporation.

# Powder X-ray diffraction (PXRD)

Figure 3 displays the formulas' PXRD pattern. The Braggs peaks at 2θ angles of pure ITZ, confirming it is a crystalline character as previously reported by Sriamornsak and Burapapadh<sup>30</sup>. After solvent evaporation, ITZ RE shows a very small change in 18 and 21.9 peaks which is compatable with DSC data indicating no polymorphic change, while in the case of ITZ HPMC PM and RE, a difference in peak pattern was seen with reduction in the intensity of the Braggs peaks indicating reduction in the crystallinity in accordance with the DSC results. However, in ITZ PVP K 30 PM and RE, there was a noticeable alteration in a manner with a reduction of the intensity of the comparable peaks which is an indicate of higher amorphous content than the HPMC mix. Finally, ITZ Soluplus PM and RE exhibited a significant modification in sharpness with the expansion of the peaks which is harmonize with the DSC in increasing the amorphous content to the highest level among other mixes.

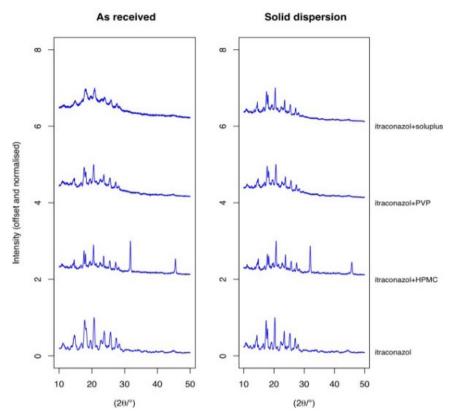


Figure 3. PXRD pattern of ITZ as received, ITZ when mixed with HPMC E5, PVP K30, and Soluplus before and after solvent evaporation.

# Fourier transform infrared spectroscopy (FTIR)

As seen in Figure 4 and Table 1, the FTIR spectra of ITZ show aromatic C-H stretching at 3134.43 cm<sup>-1</sup>, aliphatic C-H stretching at 2968 cm<sup>-1</sup>, a sharp band at 1699.34 cm<sup>-1</sup> is due to N-C=O (amide), and a C-O band at 1269.2 cm<sup>-1</sup>. Researchers had reported a similar ITZ spectral pattern<sup>31,32</sup>.

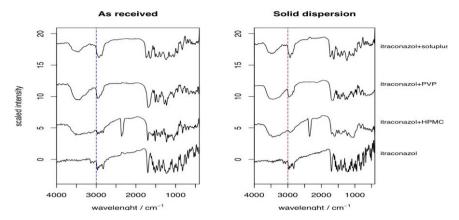


Figure 4. FTIR analysis of ITZ as received, ITZ when mixed with HPMC E5, PVP K30, and Soluplus before and after solvent evaporation.

Table 1. All itraconazole-containing formulas' FTIR spectra

As received (PMs)					
Group	Pure ITZ	ITZ- sol PM	ITZ-HPMC PM	ITZ- PVP K30 PM	
C=0	1699.34	1633.76	1699.34	1691.63	
Aliphatic C-H	2968.55	2931.9	2968.55	2960.83	
C-0	1269.2	1234.48	1271.13	1278.85	
Aromatic C-H	3134.43	3130.57	3244.38	3147.93	
Rotary evaporated (SDs)					
Group	ITZ RE	ITZ- sol RE	ITZ-HPMC RE	ITZ- PVP K30 RE	
C=0	1697.41	1629.9	1693.56	1658.84	
Aliphatic C-H	2966.62	2931.9	2928.04	2962.76	
C-0	1294.28	1236.41	1228.7	1290.42	
Aromatic C-H	3136.36	3124.79	3252.09	3348.54	

## **Determination of λ max**

Scanning of itraconazole in acetate buffer pH (4.2) contain 1% SLS by UV spectrophotometer showed that the maximum absorbance ( $\lambda$  max) is 260 nm. This result was in agreement with the reported data<sup>14</sup>.

## Calibration curve

Figure 5 shows the standard curve for ITZ at 260 nm. Plotting the absorbance against concentrations produced a straight line, indicating that, for the range of concentrations utilized, the calibration curve complies with Beer's law.

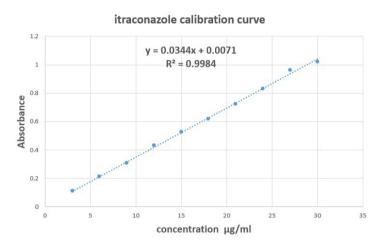


Figure 5. Standard calibration curve of itraconazole in acetate buffer pH 4.2 at 260nm

# Saturated solubility (phase solubility study)

In comparison to pure ITZ and PMs, ITZ RE and SDs had a greater solubility, as demonstrated in Table 2. When it came to saturated solubility, ITZ-PVP K30 RE was at the top.

**Table 2.** Acetate buffer saturation solubility tests with various water-soluble polymer ratios (1:2) for pure ITZ, ITZ when mixed with HPMC E5, PVP K30, and Soluplus before and after solvent evaporation.

Variables	ITZ Conc. μg / ml (Mean ± SD)	
ITZ as received	402.702 ± 0.88	
ITZ RE	409.672 ± 0.9	
ITZ-HPMC E5 PM	415.05 ± 1.1	
ITZ-HPMC E5 RE	425.658 ± 1.6	
ITZ-soluplus PM	420.07 ± 0.7	
ITZ-soluplus RE	467.933 ± 1.13	
ITZ-PVP K30 PM	430.237 ± 0.99	
ITZ-PVP K30 RE	527.342 ± 1.01	

(Means  $\pm$  SD, n=3) is how the data is presented.

# In vitro dissolution studies for the pre-formulation mixtures

As seen in Figure 6 and Figure 7, 55.32% of ITZ as received was dissolved in 120 minutes. When ITZ was mixed physically at the same time interval, there was an improvement in solubility in the following order: ITZ-HPMC (67.43%), ITZ-Soluplus (80.2%), and ITZ-PVP (82.28%). After the solvent evaporation technique, the solubility order was ITZ RE (61.89%), ITZ-HPMC RE (75.56%), ITZ-Soluplus RE (86.3%), ITZ-PVP RE (91.18%), p<0.05 (Significant). The PVP K30 RE formulation demonstrates the most pronounced enhancement among all groups, suggesting that this formulation is the most effective in improving drug release at 120 minutes.

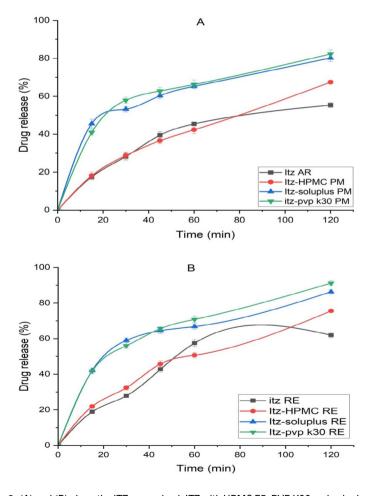


Figure 6. (A) and (B) show the ITZ as received, ITZ with HPMC E5, PVP K30 and soluplus release in acetate buffer versus time before and after the solvent evaporation process, respectively. (Means  $\pm$  SD, n=3) is how the data is presented.

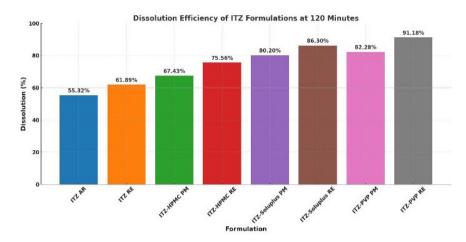


Figure 7. Dissolution efficiency of ITZ formulations at 120 minutes

# *In-vitro* antifungal activity (determination of inhibition zone)

As seen in Figure 8 and Figure 9, the maximum zone of inhibition was 25 mm in the case of ITZ-PVP K30 RE.

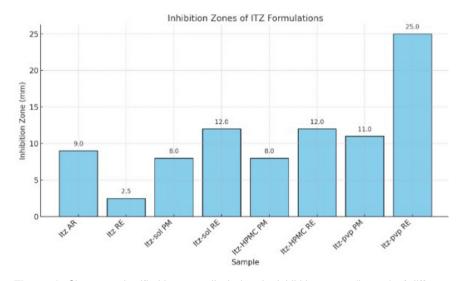


Figure 8. Shows a scientific histogram displaying the inhibition zones (in mm) of different ITZ (Itraconazole) formulations. Each bar represents the antimicrobial activity of a specific formulation, with Itz-PVP RE showing the highest inhibition zone (25 mm), indicating the strongest antimicrobial effect among the tested groups.

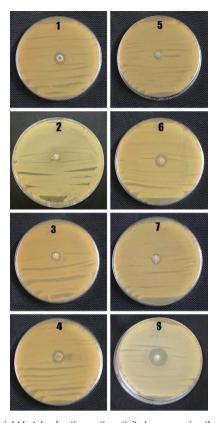


Figure 9. Evaluation of eight batches' antimycotic activity by measuring the region of inhibition (mm)

## Selection of the best formula

The best formula was selected based on the solubility study, dissolution profile, DSC, FTIR, and in vitro antifungal activity, with ITZ-PVP K30 RE being the best formula.

As seen in Table 3 all the suppositories were odorless, and the color of the ITZ-PVP K30 suppositories was light yellowish (this color is formed when the polymer was melted), while those that contained just ITZ were white in color.

**Table 3.** pH, content percentage, hardness, shape and organoleptic characteristics of the formulated suppositories

рН	4.8 – 6.3		
Content	96 – 105%		
Hardness	1:1 (less than 1 kg), 1.5:1(1.2 kg), 2.5:1(2.6 kg), 3.5:1(1 kg), 4.5:1(less than 1 kg)		
Color	AR (white), PM, and RE (light yellowish)		
Surface texture	1:1(soft),1.5:1(mild soft ), 2.5:1(Homogenous, smooth), 3.5:1(mild crack), 4.5:1(mild crack and brittle )		
Shape	Torpedo-shaped		
Odor	Odorless		

The surface texture depended on the PEG ratios; the most suitable was 2.5:1 (a consistent and smooth surface that helps reduce vaginal discomfort while using suppositories); when a high amount of PEG 6000 was used, the suppositories became brittle with cracks on the surface see Table 4.

# In-vitro release of ITZ from the suppository base

As seen in Figure 10, after 60 minutes, the maximum release was in PEG 6000: PEG 400 (1:1) and (1.5:1) in all three patches (ITZ as received, PM, and RE). The ITZ release started to decline in all patches in ratios of (3.5:1 and 4.5:1). In the case of the 2.5:1 ratio, the release was in the following order: RE>PM>AR (36.06>30.15>18.32 %). Formula 2.5:1 RE was chosen for further study. To maximize the release of ITZ, glycerin was chosen in 10% w/w according to the co-solvency principle<sup>33</sup>.

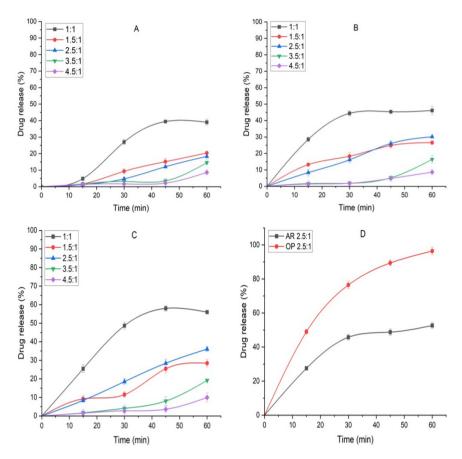


Figure 10. In vitro release of suppositories (A) ITZ as received, (B) ITZ PM, (C) ITZ RE, and (D) the selected suppository (as received and optimum formula) with 10% glycerin in acetate buffer (pH=4.2). (Means  $\pm$  SD, n=3) This is how the data is presented.

In order to get the ideal dosage form as vaginal suppositories, PEG 6000: 400 was chosen<sup>20</sup> as the suppository base at five different ratios for (AR, PM, and RE) as mentioned in Table 4, and the final decision was to choose 2.5:1 ratio to be the optimum formula that could be subjected to further improvement as it with maximum strength, a homogeneous and smooth, crack-free surface that made it suitable for vaginal delivery.

**Table 4.** The three variables of formulation (AR, PM, with RE) and five different PEG ratios with the selected ratio of the final formula

Variables	PEG 6000: PEG 400 ratios	
	1:1	
	1.5:1	
AR, PM, RE	2.5:1	
	3.3:1	
	4.5:1	
AR, RE optimum formula.+ 10% Glycerin	2.5:1	

# Antifungal activity of suppositories

As illustrated in Figure 11 and Figure 12, the 2.5:1 ratio (AR, RE, and PM) yields a nearly similar ZOI (21 mm). As the PEG 6000 ratio increased, API release decreased. The addition of 10% glycerin resulted in an ITZ release of 96.4%. This made the inhibition zone bigger (30 mm) because of maximum release resulted in high antifungal activity on cultured vaginal candida albicans. The 2.5:1 ratio appears optimal for balance across formulation types, yielding consistent antimicrobial activity. Higher PEG 6000 ratios can hinder drug release due to matrix effects. The incorporation of glycerin significantly boosts ITZ release and antimicrobial efficacy, as evidenced by the largest inhibition zone observed. This means the optimized formulation of the drug effectively diffused into the agar and inhibited fungal growth in its vicinity. The absence of inhibition in the controls (J.B and Buffer) validates that the observed zone around O.F is due to the active pharmaceutical ingredient and not the excipients or vehicle, and they do not interfere with the result, see Figure 13.

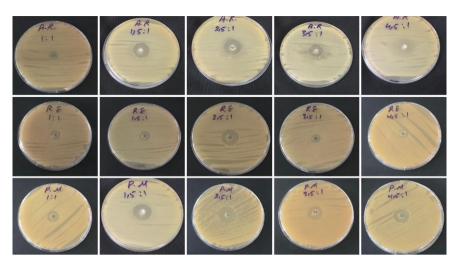


Figure 11. The zone of inhibition of suppositories of ITZ as received (AR), ITZ-PVP K30 rotary evaporated (RE), and ITZ-PVP K30 physical mix (PM) at different PEG 6000 PEG 400 ratios against candida albicans

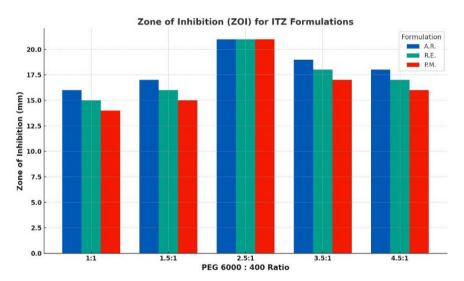


Figure 12. The scientific histogram showing the antimicrobial activity of ITZ (Itraconazole) formulations at different PEG 6000:400 ratios. AR (As Received) blue bars, RE (Rotary Evaporation) gray bars, and PM (Physical Mixtures) red bars.



Figure 13. Zone of inhibition of O.F (optimum formula) located at the center, J.B (just base; polyethylene glycol), and Buffer (acetate buffer)

From DSC results, the melting peak of ITZ as received indicates the crystalline solid long-range ordered nature of ITZ, and the powder was pure, while due to no polymer being added for ITZ, RE gave the same melting magnitude. Due to the polymer dilution effect, there was a mild reduction in the intensity of breaks for ITZ-HPMC in PM and RE. Broadening the peaks with a moderate reduction in intensity and shifting to a reduced value at 166°C were noticed in ITZ-PVP K 30 polymer in PM and RE that might represent good API-polymer miscibility, and the polymer PVP k30 might disorder the packed nature of ITZ but to an extent not reached to the short-range ordered amorphous state, which is in agreement with previously published data<sup>34</sup>. The complete amorphization was not the result, and there might be a need to increase the polymer-drug ratio to enough amount to stabilize the drug in an amorphous form, as in the previous study for the preparation of carvedilol SD with PVP K30, which showed that as the drug-polymer ratio increased, the solubility improved<sup>35</sup>. In PM and RE, PVP K30 shows a broad endothermic peak at 94.2°C which as reported previously might be attributed to the loss of water upon heating from the hygroscopic polymer<sup>36</sup>.

## Left Panel: "As received"

These patterns show sharp peaks, particularly for the bottom sample (itraconazole alone), indicating a highly crystalline form. As we move up through itraconazole-HPMC, itraconazole-PVP, and itraconazole-Soluplus, there is still noticeable crystallinity, although the intensity of peaks may vary slightly due to interactions with the polymer or dilution effects.

Right Panel: "Solid dispersion"

Here, all formulations including itraconazole with Soluplus, PVP, and HPMC show broad humps instead of sharp peaks. This is indicative of ITZ lost its crystalline structure.

The presence of sharp peaks of ITZ-Soluplus SD compared to ITZ-Soluplus PM might be due to incomplete evaporation of the solvent that resulted in recrystallizing of the mixture or humidity effect. PVP K30 is an amorphous polymer. ITZ-PVP RE retains the diffraction peaks but with less intensity of ITZ as received, which indicates the semi-crystalline nature of the mixture, even though of the evaporation<sup>37</sup>.

In the "As received" panel, the characteristic ITZ peaks are retained with minimal shifts, suggesting no significant interaction occurs in the physical mixtures and peak intensities may slightly decrease or broaden due to dilution effects or simple mixing. The C=O and C-O stretching peaks remain largely unchanged, indicating that no strong chemical interaction or bond formation occurs between ITZ and the polymers in physical mixtures.

In the "Solid dispersion" panel, peak intensity reduction and broadening is observed, particularly in the C=O (~1697 cm<sup>-1</sup>) and C-O (~1290 cm<sup>-1</sup>) regions. Aliphatic C-H peaks (~2966 cm<sup>-1</sup>) show minor shifts and broadening (highlighted by the red dashed line), suggesting hydrophobic interactions or physical dispersion. Disappearance or shift of peaks in some samples (notably with Soluplus and PVP) indicates possible hydrogen bonding or molecular interactions between ITZ and the polymers. Solid dispersions show noticeable spectral shifts and peak broadening, particularly in functional groups associated with hydrogen bonding (C=O, C-O)<sup>37</sup>. These spectral modifications support the formation of molecular-level interactions, loss of crystallinity and molecular dispersion of ITZ within the polymer matrix, which contribute to the amorphization of ITZ and correlate with improved solubility, as observed in the solubility data.

The saturated solubility test results in acetate buffer demonstrate a significant enhancement in the aqueous solubility of ITZ when formulated with hydrophilic polymers, particularly in the form of solid dispersions prepared via the RE method.

Pure ITZ exhibited a baseline solubility of 402.70 ± 0.88 µg/mL, which is consistent with its known poor aqueous solubility. Slight improvement was observed in the ITZ RE sample (409.67  $\pm$  0.90  $\mu$ g/mL), likely due to partial amorphization or reduction in crystallinity as a result of processing.

Among the polymer-based formulations: PMs with HPMC, Soluplus, and PVP K30 led to modest increases in solubility compared to pure ITZ, with PVP K<sub>30</sub> PM showing the highest among PMs ( $430.24 \pm 0.99 \,\mu\text{g/mL}$ ). This can be attributed to improved wettability and partial solubilization due to polymer interaction, but the crystalline nature of ITZ is likely preserved in PMs, limiting solubility gains.

The RE formulations demonstrated a more pronounced increase in solubility for all polymers, confirming the effectiveness of the technique in enhancing drug dissolution. This is likely due to: molecular dispersion of ITZ within the polymer and hydrogen bonding or miscibility between drug and polymer, reducing the lattice energy of the crystalline drug.

The improved saturated solubility of ITZ with PVP K30 in PM and RE might be PVP K30 can lower the surface tension between the API and solvent, making it easier for particles to mix with the solvent and this may be attributed to the hydrophilic characteristics of the carrier in which the drug was molecularly dispersed and transformation from crystalline to semi-crystalline.

The most notable solubility enhancement was observed with ITZ-PVP K30 RE, achieving 527.34  $\pm$  1.01  $\mu$ g/mL, representing a 30.9% increase compared to pure ITZ. This significant improvement (p<0.05) may be due to the high solubilizing capacity of PVP K30, its hydrophilic nature, and its ability to form hydrogen bonds with the drug.

Because of its superior solubility, ITZ-PVP K30 RE was able to penetrate into the growing medium and increase permeability to the microbial cells, leading to enhanced antimicrobial activity, similar to results obtained by Permana et al<sup>10</sup>.

To ensure that suppository medicine distribution was consistent and that batches were homogeneous, a weight variation test was performed. Inadequate mixing or improper filling of the suppository might result in air bubbles or axial cavities, which could cause the weight variation test to fail. Weighing 20 suppositories independently from each batch allowed us to calculate the mean value and then find the percentage variance (which should not be more than 5%)<sup>24</sup>.

The hardness of suppositories depends on different PEG ratios (PEG 400 has a plasticizing and softening effect)20, as seen in Table 3. The 2.5:1 ratio formula achieved the maximum hardness (2.5) kg that could endure the mechanical strains of handling and transit.

To avoid irritation of vaginal tissue, pH levels were assessed for all produced suppositories. The values ranged from 4.8 to 6.5 (acidic to slightly acidic), closely mirroring the results obtained in the prior investigation with the identical bases<sup>25</sup>. The pH values were close to physiological vaginal pH (3.8-5.0)38. The acidic pH plays a crucial role in defending the vaginal mucosa against harmful pathogens<sup>39</sup>. The drug content percentage was established to comply with the demands of the British Pharmacopeia (2007), and it was in the range of 96 to 105%40. The mean weights of PEG formulations ranged from 3.41 to 3.46 grams. The torpedo shape could successfully retain the suppositories within the vaginal cavity.

The maximum release was in PEG 6000: PEG 400 (1:1) and (1.5:1) in all three patches (ITZ as received, PM, and RE), owing to the low molecular weight of PEG 400 (which had a plasticizing and softening effect, with soft consistency)<sup>20</sup>. The ITZ release started to decline in all patches in ratios of (3.5:1 and 4.5:1), due to the high melting temperatures and solid PEG 6000 molecular weight, which made the suppositories resistant to dissolve when immersed in dissolution media i.e. increasing the PEG 6000 ratio beyond 2.5:1 resulted in a reduction in ITZ release, which was reflected by a decrease in the inhibition zone. This could be attributed to the increased viscosity and possible drug entrapment within the polymer matrix, limiting the diffusion of itraconazole into the surrounding media. Because ITZ is lipophilic and does not bind to PEG, a water-miscible base, the suppositories prepared from PEG with water-soluble glycerin had the fastest and highest release feasible<sup>26,41</sup>. Conversely, the addition of 10% glycerin significantly enhanced the release profile of ITZ, leading to a drug release of 96.4%. The presence of glycerin likely acts as a plasticizer, improving polymer flexibility and reducing matrix density, thereby facilitating drug diffusion. This enhancement in solubility and release translated into a substantially larger ZOI of 30 mm, indicating a stronger antimicrobial effect, as seen in Figure 10D.

Antifungal activity of suppositories at 2.5:1 resulted in a similar zone of inhibition, which may be attributed to the homogenous mixture and appropriate distribution of the API in PEG bases while the PEG 6000 ratio increase resulted in less API release and lower zone of inhibition since PEG 6000 has high molecular weight that restricts API release.

It was concluded that the using amorphous solid dispersion in the preformulation study, ITZ-PVP K30 RE showed a high dissolution rate in acetate buffer with improved solubility and antifungal activity against Candida albicans in Sabouraud dextrose agar. After that, ITZ-PVP K30 RE was successfully formulated as vaginal suppositories for the first time using the water-soluble base PEG 6000/PEG400 (2.5:1) ratio, devoid of holes and cracks, odorless, and with a light vellowish color. To maximize ITZ release, the addition of 10% glycerin made ITZ release up to 96.4% with higher antifungal activity demonstrated by a larger zone of inhibition.

#### STATEMENT OF ETHICS

No need for ethical approval for this study.

## CONFLICT OF INTEREST STATEMENT

No conflict of interest was declared by the authors.

## **AUTHOR CONTRIBUTIONS**

Design, GSH; acquisition of data, AMB; analysis of data, AMB, DH; drafting of the manuscript, AMB, DH; critical revision of the manuscript, GSH; statistical analysis, AMB, DH, GSH; technical or financial support, AMB, DH, GSH; supervision, AMB, DH.

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