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SOLVENT ASSISTED COCRYSTALLIZATION OF ITRACONAZOLE: SOLID-STATE PROPERTIES, DISSOLUTION, STABILITY, ANTIFUNGAL POTENTIAL, AND SOLUTION-MEDIATED PHASE TRANSFORMATION STUDIES

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ABSTRACT

The physical form of drugs significantly influences their effectiveness, particularly for BCS Class II compounds. The interplay between crystal structure and formulation is critical in determining bioavailability. Crystal engineering has emerged as an efficient tool to increase the solid-state characteristics of APIs while preserving their intrinsic structures. This study focused on itraconazole (ITZ), a poorly water-soluble drug, developing pharmaceutical cocrystals via the solvent drop grinding technique using various coformers to enhance solubility, dissolution rate, and bioavailability. The ITZ cocrystals were evaluated for Fourier transform infrared (FTIR), Differential scanning calorimetry (DSC), and X-ray diffractometry (XRD) analyses and evaluated for solubility, dissolution, and stability. In-vitro antifungal activity was assessed and compared with pure ITZ. Among the formulations, cocrystals developed with glycine (GLY) and serine (SER) exhibited stable forms, while those with benzoic acid (BENZ), proline (PRO), and succinic acid (SU) showed solution-mediated phase transformations. Nonetheless, all cocrystals demonstrated enhanced in vitro antifungal activity. Furthermore, stability studies confirmed that the cocrystals retained their properties after 45 days of storage at 40°C and 75% RH. These findings highlight the potential of ITZ cocrystals as effective candidates for improving solubility, dissolution, and antifungal activity. This crystal engineering-based strategy offers a valuable pathway to address challenges associated with poorly water-soluble drugs, enhancing therapeutic efficacy and advancing drug delivery systems for BCS Class II compounds.

Keywords: Cocrystallization, Itraconazole, Solvent Drop Grinding, Solubility, Stability, Solution-Mediated Phase Transformation.

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INTRODUCTION

Drug discovery is a complex and time-consuming process, often taking 10-15 years from initial discovery to becoming available for patient use. Unfortunately, the vast majority of molecules do not make it through the rigorous Research and Development (R&D) pipeline. Out of every 5,000-10,000 compounds entering R&D, only a few ultimately receive approval.[1,2] Crystal engineering, a concept introduced by Pepinsky in 1955, traditionally focused on the crystalline forms of Active Pharmaceutical Ingredients (APIs), including salts, polymorphs, and solvates (such as hydrates). Early research and development efforts aimed to discover new chemical entities with desired molecular structures and physical properties. However, identifying the optimal molecular structure of an API that balances therapeutic efficacy with suitable physical properties has remained a significant challenge. Many APIs exist in the solid state, but they often crystallize into several forms with unpredictable properties.[3] Solids can be modified through various approaches, including solvation, hydration, salting, and polymorphism. Cocrystallization has become a promising alternative approach to enhance the physical properties of drugs. Pharmaceutical cocrystals,

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which are nonionic supramolecular complexes, incorporate two neutral molecules: the API and a cocrystal former, which could be another drug or an excipient. These cocrystals can be used to alter properties such as solubility, stability, and bioavailability without affecting the APIs pharmacological and intrinsic properties. Cocrystallization, also known as molecular complexing, offers a promising approach to improve the rate of dissolution and solubility of APIs, as well as enhancing the physical properties of moisture-sensitive APIs.[4,5] Itraconazole (ITZ), a broad-spectrum antifungal agent classified under BCS Class II, has garnered significant attention in pharmaceutical research over the past two decades due to its potent activity against a wide range of fungal pathogens. However, its clinical application is limited by poor aqueous solubility despite high membrane permeability. Its solubility in body fluids and stability in the external environment are crucial for achieving the desired therapeutic effect. Various techniques, such as surfactants, inclusion complexation, and solid dispersion, have been employed to improve ITZ's solubility. Cocrystallization with excipients has gained popularity as a means of converting poorly water-soluble drugs like ITZ into multicomponent solids to improve dissolution. Techniques like dry and solvent-drop grinding have been utilized in cocrystal development, with the latter offering advantages like shorter formation time and the possibility of obtaining pure cocrystals.[6-13] The literature reveals various approaches to forming cocrystals of ITZ using different cocrystal formers, also called coformers.[14] Techniques employed include cogrinding with various amino acids, as well as creating micronized cocrystals with succinic acid or L-tartaric acid through jet milling for inhalation purposes. Another method involves gas antisolvent cocrystallization using L-malic acid and tetrahydrofuran. Additionally, the solvent evaporation technique has been applied with coformers such as 4-hydroxybenzoic acid, trans-cinnamic acid, suberic acid, and sebacic acid. Electrospray technology has also been utilized with fumaric and succinic acids in a 2:1 ratio. For inhalational therapy, rapid evaporation and spray drying methods with suberic acid have shown stability for up to one month at 60°C. One study specifically examined how the chain length of aliphatic carboxylic acids influences the cocrystallization process of ITZ. Additionally, co-milling of ITZ with malonic and succinic acids was performed using a planetary mill.[15-18] Solution-mediated phase transformation (SMPT) is a process in which one solid phase (e.g., a crystalline form) transforms into another solid phase through dissolution in a solvent and subsequent recrystallization.[19]

SMTP is often observed in polymorphic systems, where one polymorph converts to another that is thermodynamically more stable under the given conditions. This phenomenon can significantly impact drug stability, solubility, and bioavailability, making it an important consideration in pharmaceutical development. SMPT presents a major obstacle in the development of compounds with poor water solubility. The primary challenge of SMPT in developing poorly soluble drug compounds lies in its impact on drug dissolution and bioavailability.[20] SMPT can lead to the conversion of a metastable phase, which often exhibits better solubility, into a more thermodynamically stable but less soluble phase. This transformation reduces the dissolution rate, hindering drug absorption and therapeutic efficacy. Moreover, SMPT can complicate formulation design, requiring careful control of manufacturing processes and storage conditions to maintain the desired phase. The unpredictability of nucleation and growth rates during SMPT adds further complexity, making it a critical issue in the development of poorly soluble drugs.[21]

This current research work focuses on the development of cocrystals of itraconazole to address critical challenges associated with its poor physicochemical properties, such as limited solubility, low dissolution rate, and reduced stability.

The novelty of the present work consists of addressing the multifaceted hurdles of itraconazole formulation through the development of cocrystals using solvent-assisted cocrystallization. Unlike conventional methods, this study not only improves the physicochemical properties of itraconazole, such as solubility, dissolution rate, and stability, but also focuses on overcoming the critical issue of SMPT. SMPT often reverses the benefits of metastable forms, yet it is rarely addressed comprehensively in similar studies. Additionally, the work evaluates the antifungal efficacy of the cocrystals, ensuring that the improved physicochemical properties translate into enhanced therapeutic potential. This holistic investigation, combining solid-state characterization, dissolution performance, stability analysis, and antifungal testing, represents a significant advancement in itraconazole formulation science, making it a pioneering effort in optimizing poorly soluble drugs, marking a significant step forward in optimizing itraconazole formulations for clinical application.

EXPERIMENTAL

Materials and Methods

ITZ was acquired as a complimentary sample from Glenmark Pvt. Ltd., based in Mumbai, India. The coformers utilized in the study were sourced from Loba Chemie Pvt. Ltd. All chemicals and reagents utilized throughout the research were of analytical grade.

Preparation of Cocrystals

The combination of ITZ and coformers, namely proline (COPRO), serine (COSER), glycine (COGLY), succinic acid (COSU), and benzoic acid (COBENZ), was meticulously weighed in a 1:1 ratio to achieve 1 g of each sample. To conduct the cogrinding experiments, a mortar and pestle were employed, and the process was facilitated by adding acetone at a rate of 20 µl per 100 mg of powder for 45 minutes. As for the physical mixture, they were manufactured by gently blending ITZ and coformers at a 1:1 molar ratio using spatulation. The samples were stored in desiccators until further use.

Solid-State Characterization

The FTIR analysis was performed on a Shimadzu FTIR 83006 spectrophotometer, with the recorded spectrum falling within the wavelength range of 4000 to 400 cm^{-1}. The PXRD analysis was performed on a Bruker AXS equipped with a LYNXEYE™ detector. The DSC analysis was done using a DSC 60A calorimeter from PerkinElmer, USA. The temperature scanning was maintained at 10°C per minute, ranging from 0°C to 350°C.

Evaluation of Prepared Cocrystals

Saturation Solubility

A surplus amount of both ITZ and ITZ cocrystals was carefully added to 10 ml of simulated gastric fluid (SGF). The suspensions underwent sonication for 15 minutes and were then placed on a rotary orbital shaker and operated at 100 rpm and constant temperature. Following a period of 72 hours, small portions (aliquots) were drawn from the suspensions and passed through 0.45 µm membrane filters. Subsequently, appropriate dilutions were made using SGF. The samples were analyzed using UV spectroscopy at 254 nm.[22]

Dissolution Study

A dissolution study was conducted (n=3) in a USP Type II dissolution test apparatus, specifically the DBK Dissolution Test Apparatus. Cocrystals equivalent to 100 mg ITZ were weighed accurately and used for the dissolution study in 900 ml of different dissolution media, including SGF, 0.1N HCL, PBS pH 6.8, and 7.4 at 37 ± 0.5°C. To prevent powder aggregation upon contact with the dissolution medium, a rotation speed of 100 rpm was employed in the USP type II apparatus. The dissolution test samples were removed at specific time intervals, and an equal volume of fresh dissolution media was added to achieve sink conditions. The samples were filtered, followed by dilution, and subsequently analyzed at 254 nm. These measures were taken to ensure accurate and consistent results throughout the dissolution studies.

Stability Study

The stability of the cocrystals was evaluated by exposing them to RT. For a comprehensive evaluation of their chemical and physical stability, accelerated storage conditions were employed, involving storage at 40°C and 75 ± 5% RH. The prepared cocrystals were kept upright for 30 days. At designated time points, samples were withdrawn and subjected to analyses, including drug content assessment, FTIR spectroscopy, DSC studies, and observations of any changes in color, odor, and appearance. These assessments aimed to gain insights into the cocrystals' stability and potential alterations over the storage duration.[22]

In Vitro Antifungal Activity

Pure ITZ as well as cocrystals were evaluated for antifungal activity using agar diffusion technique against *Aspergillus niger* and *Candida albicans* strains. Initially, compounds were dissolved in dimethyl sulfoxide and used for further processing. A fresh suspension of test organisms was prepared in 1 mL of sterile normal saline and adjusted spectrophotometrically to a standardized concentration of 10^7 CFU/mL. Sabouraud's Dextrose agar plates were prepared for the experiment. A 100 ml suspension of the organisms was inoculated on each culture plate. Subsequently, one cavity well was created on each agar plate using a

sterilized cork borer. Subsequently, 1 mL of the compound solutions at different concentrations (1, 3, and 5 mg/mL) was individually introduced into the prepared wells. Additionally, ITZ, as standard, was tested at 100 mg/mL. To serve as controls, pure dimethyl sulfoxide was inoculated into the wells for each fungal strain. The agar plates were then incubated at a temperature of 34°C, and after 48-72 hours, the zones of inhibition were measured.[23] These evaluations aimed to determine the efficacy of the compounds against the tested fungal strains.

Solution-Mediated Phase Transformation Study (SMPT)

The investigation of SMPT for both ITZ and its cocrystals was performed utilizing 8 8-station USP type II dissolution test apparatus at the specified conditions mentioned above. SMPT assessments were carried out in 900 mL of SGF. The undissolved portions of the samples and ITZ were withdrawn at 15 minutes and 45 minutes, respectively. These samples were subsequently characterized using Differential Scanning Calorimetry (DSC) conducted with a DSC-60A calorimeter (Shimadzu, Tokyo, Japan). [24-26]

RESULTS AND DISCUSSION

Formulation of Cocrystals

ITZ cocrystals were successfully developed using the solvent drop grinding (SDG) technique, utilizing COPRO, COSER, COGLY, COSU, and COBENZ as coformers in equimolar ratios (1:1). The SDG method is known for its simplicity, cost-effectiveness, and practical applicability, and proved to be an efficient approach for the development of ITZ cocrystals.

Evaluation of Prepared Cocrystals

Saturation Solubility

Table-1 illustrates a significant improvement in the solubility of ITZ after formation of cocrystals in SGF. Pure ITZ showed solubility of 9.9 µg/ml, while more than 3 times the solubility was found in the case of cocrystals in SGF.

Table-1: Comparative Solubility of Cocrystals in SGF

Sample	Solvent (ml)	Solubility (µg/ml)
ITZ	10 ml	9.9±0.66
COPRO	10 ml	34.2±0.86
COSER	10 ml	30.23±0.33
COGLY	10 ml	32.12±0.31
COSU	10 ml	37.2±0.83
COBENZ	10 ml	30.45±0.52

Among the cocrystals, COSU exhibited the highest solubility (37.2 ± 0.83 µg/mL), followed by COPRO (34.2 ± 0.86 µg/mL), COGLY (32.12 ± 0.31 µg/mL), COSER (30.23 ± 0.33 µg/mL), and COBENZ (30.45 ± 0.52 µg/mL), whereas pure ITZ showed the lowest solubility (9.9 ± 0.66 µg/mL). These observations are in line with already reported studies of ITZ cocrystals, which were formulated by different methods, including liquid-assisted grinding and solvent evaporation, with coformers like 4-aminobenzoic acid (4AmBA) and 4-hydroxybenzamide (4OHBZA), demonstrating solubility enhancements.[27] The enhanced solubility can be attributed to alterations in the crystal lattice and enhanced molecular interactions with the dissolution medium facilitated by the coformer. Although SDG is favored for its simplicity and cost-effectiveness, alternative techniques such as hot-melt extrusion have also been employed for the preparation of ITZ cocrystals, demonstrating comparable enhancements in solubility and dissolution profiles.[28] This comparison highlights the versatility of coformers and techniques in optimizing the physicochemical properties of ITZ cocrystals.

Fourier Transform Infrared (FTIR)

Potential interactions between ITR and the cocrystal formers were investigated using FTIR spectroscopy (Figure 1). In COPRO, it was observed that the cocrystal of proline contains aromatic CH and NH stretching absorption shows at 2878 cm^{-1} and 2964 cm^{-1}. It also contains a carboxylic acid moiety, and secondary amine prominent peaks were observed at 1698 cm^{-1}. The C-N stretch absorption 1422 cm^{-1}, 1451 $cm^{-1,}$ and 1510 cm^{-1}. Also, there are peaks observed at 1228 $cm^{-1,}$ which are indicative of the ether functional group.

The C-Cl groups are observed at 736 cm^{-1}. In COSER, it was observed that there are some prominent, very strong peaks at 2823 cm^{-1} and 2964 $cm^{-1,}$ which indicate aromatic N-H stretching. Cocrystals of Serine contain a carboxylic acid moiety, and secondary amine prominent peaks were observed at 1698 cm^{-1}. The absorption at 1451 cm^{-1} indicates C-N stretch. Also, there are peaks observed at 1228 cm^{-1} and 1272 cm^{-1}which are indicative of the ether functional group. The absorption band at 724 cm^{-1} indicates the presence of C-Cl groups. In COGLY, it was observed that cocrystals of glycine show aromatic C-H, aromatic N-H stretching peaks at 2822 cm^{-1} and 2962 cm^{-1}. Absorption at 1698 cm^{-1} indicates the presence of the CO-NH group. The C-N stretch and C-O-H plane bending are observed at 1451 cm^{-1}. CH_3 group observed at 1330 cm-1 and 1382 cm^{-1}. Similarly, there are some peaks present at 1228 cm^{-1}which is indicative of the ether functional group. The C-Cl groups are observed at 736 cm^{-1}. In COSU, Peaks at 2825 cm^{-1} and 2974 cm-1 indicate the aromatic N-H stretching. Absorption at 1695 cm^{-1} indicates the presence of C=O stretch. Likewise, the C-N stretch is observed at 1454 cm^{-1}. Peak at 1325 cm^{-1} indicating the presence of the CH_3 group. Also, there are peaks observed at 1219 cm^{-1}which are indicative of the ether functional group. The absorption at 731 cm^{-1} confirms the C-Cl group. In COBENZ it was observed very sharp peaks were observed at 2819 cm^{-1} and 2972 $cm^{-1,}$ which are at aromatic N-H stretching. Absorption at 1695 cm^{-1} indicates the presence of C=O. It also shows C-N stretch at 1454 cm^{-1}. CH_3groupshowsabsorption at 1332 $cm^{-1}$and 1381 cm^{-1}. Also, there are peaks observed at 1045 cm^{-1}, 1145 cm^{-1}, 1188 cm^{-1}, 1220 $cm^{-1,}$ and 1265 cm^{-1}which are indicative of the ether functional group. The C-Cl and O-benzoic groups are observed at 731 cm^{-1}. In the case of cocrystal, there was a lowering of the absorption wavelength and an increase in intensity of the peak as compared to ITZ, which shows strong hydrogen bonding due to the presence of the coformer.

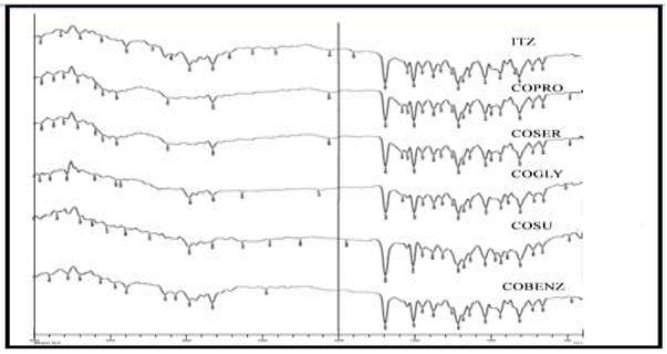

Fig.-1: FTIR Spectra of Pure ITZ and Cocrystal with Proline (COPRO), Serine (COSER), Glycine (COGLY), Succinic Acid (COSU), and Benzoic Acid (COBENZ)

Differential Scanning Calorimetry (DSC)

DSC thermograms of pure ITZ and its cocrystal formulations are shown in Table-2 and Fig.-2. DSC is a highly informative technique for identifying and characterizing eutectic phases.[29] DSC analysis of pure ITZ exhibited a distinct endothermic peak at 167°C. DSC of all cocrystals except benzoic acid showed a melting point shift lower than the drug and coformer.

The cocrystals exhibited a reduction in melting point and distinct thermal peaks compared to the pure drug and cocrystal formers, confirming the formation of a new crystalline phase. In the case of COBENZ, there is no peak found. It reveals that the ITZ with benzoic acid forms an amorphous cocrystal. The ΔH value represents the enthalpy of a system. A positive ΔH, indicating a value greater than zero, signifies that the system has absorbed heat.[30] Conversely, a negative ΔH, with a value less than zero, indicates that the system has released heat. There is a lowering of the melting point due to milling leads to breaking, recognition, and making of new bonds.[30]

The cocrystals exhibited a lower melting point compared to the drug and coformer, suggesting the potential involvement of hydrogen bonding, which facilitates cocrystal formation. In this work, PM of PRO, SER, GLY, and SU showed melting points lower than pure ITZ and pure coformer. In case of PM lowering of melting point than ITZ due to molecule in PM could become non-randomly organized upon heating lowers the melting point, and in case of cocrystals, lowering of melting point depends upon cogrinding.

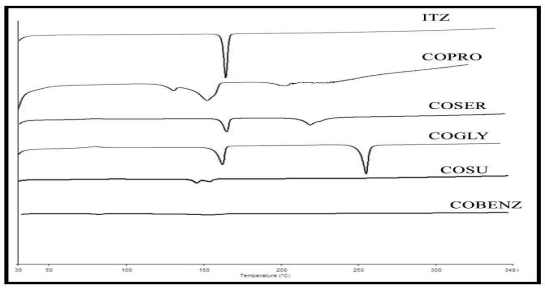

Fig.-2: A DSC Spectrum of Pure ITZ, Cocrystal with Proline (COPRO), Serine (COSER), Glycine (COGLY), Succinic Acid (COSU), and Benzoic Acid (COBENZ)

Table-2: Results of DSC Analysis of ITZ and Cocrystals

S. No.	Sample	Cocrystal M.P.			
		I (ºC)	ΔH (J/g)	II (ºC)	ΔH (J/g)
1	ITZ	167.76	111.77	-	-
2	COPRO	135.38	4.15	158.53	44.53
3	COSER	165.64	61.58	219.83	27.09
4	COGLY	164.64	76.72	258.61	110.41
5	COSU	146.33	17.24	154.67	14.84
6	COBENZ	-	-	-	-

Powder X-ray Diffractometry (PXRD)

The XRD pattern of pure ITZ exhibited sharp crystalline peaks at 10.71°, 14.38°, 17.40°, 20.31°, 23.40°, 25.22°, and 27.00° (2θ), with corresponding peak intensities of 538, 1023, 1537, 1947, 1278, 1102, and 899, respectively, confirming crystalline structure. In contrast, XRD patterns of formulated cocrystals showed the same 2θ values with reduced peak intensity, along with additional peaks corresponding to the coformer. The crystallinity of the cocrystals was assessed by comparing the heights of characteristic peaks in their diffraction patterns with those of the pure compound. Relative degrees of crystallinity (RDC) for ITZ in the cocrystals were determined, and the RDC and crystallinity index (CI) values for ITZ cocrystals with proline, glycine, serine, succinic acid, and benzoic acid at specific 2θ angles are summarized in Fig.-3 and Table-3.

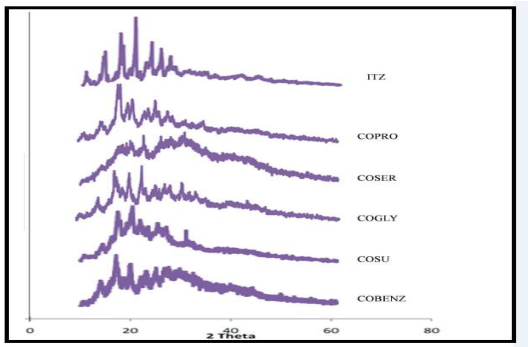

Fig.-3: PXRD Spectra of ITZ and Cocrystal with Proline (COPRO), Serine (COSER), Glycine (COGLY), Succinic Acid (COSU), and Benzoic Acid (COBENZ)

Table-3: Results of RDC Cocrystals by PXRD

S. No.	Cocrystals	Angle (2θ)	RDC	CI (%)
1	ITZ	20.31	1	100
		17.42	1	100
2	COPRO	20.31	0.46	46
		17.42	0.67	67
3	COSER	20.31	0.45	45
		17.42	0.46	46
4	COGLY	20.31	0.69	69
		17.42	0.41	41

5	COSU	25.33	0.32	32
		17.45	0.50	50
6	COBENZ	25.06	0.23	23
		17.14	0.28	28

Dissolution Study

The dissolution pattern of ITZ and cocrystals exhibited significant differences across various media, considering the 120-minute time point (Table-4). Pure ITZ exhibits relatively low dissolution rates, with the lowest 33.72% ± 0.45 in SGF and the highest 41.55% ± 0.47 in PBS pH 7.4, reflecting its poor aqueous solubility. The cocrystals, however, show improved dissolution rates in all media. For instance, COGLY showed enhanced dissolution, reaching 40.00% ± 0.45 in SGF, to 48.93% ± 0.58 in PBS pH 7.4. Similarly, COBENZ showed a superior dissolution rate in acidic conditions, achieving the highest dissolution in PBS pH 7.4 **(**54.59% ± 0.76**),** indicating its efficacy in enhancing solubility in such environments. COPRO and COSER also showed marked improvement, with dissolution values in PBS 7.4 at 49.95±0.42 and 45.37±0.76**,** respectively, and consistent performance in neutral and basic media. Interestingly, COSU displays moderate enhancement across all media, with values ranging from 37.74% ± 0.63 in SGF to 45.21% ± 0.83 in PBS pH 7.4, indicating its stability and moderate solubility enhancement properties. Overall, the data highlights the potential of cocrystal formulations to significantly improve the dissolution profile of ITZ, with variations influenced by the coformers and dissolution medium, making them promising candidates for enhancing bioavailability [31]

Table-4: Comparative Dissolution of Pure ITZ and Cocrystals in Different Media at the 120 min Time Point

Sample	SGF	0.1N HCl	PBS 6.8	PBS 7.4
ITZ	33.72±0.45	33.25±0.81	39.71±0.54	41.55±0.47
COGLY	40.00±0.45	40.73±0.37	45.21±0.74	48.93±0.58
COBENZ	41.14±0.53	52.43±0.73	47.13±0.66	54.59±0.76
COPRO	41.05±0.48	45.45±0.43	45.24±0.56	49.95±0.42
COSU	37.74±0.63	37.86±0.49	40.61±0.38	45.21±0.83
COSER	45.37±0.55	40.63±0.88	44.45±0.47	45.37±0.76

The solubility and dissolution profiles of ITZ cocrystals were significantly influenced by the type of coformer and pH conditions of the dissolution media, as evident from the rank analysis in various media (Table-5). In SGF, COSU showed the highest solubility while COSER demonstrated superior dissolution rates, suggesting that acidic conditions helped to enhance the interaction between the ITZ cocrystals and the medium. In 0.1N HCl, a similar type of trend was observed, where coformers like COBENZ and COSER significantly improved the solubility as well as dissolution of ITZ, likely due to the effect of protonation, which enhanced solute-molecule interactions. Dissolution and solubility ranking were found to be varied in PBS at pH 6.8 and 7.4. COSER and COPRO performed better in neutral and slightly basic media. This enhancement might be due to the ionization of the coformers, which leads to improved wetting and dissolution at higher pH levels. Adding surfactants, such as 1% SLS, further enhanced the solubility of COSER and COPRO in SGF and 0.1N HCl, possibly due to micelle formation, which improved the solubilization of hydrophobic drugs like ITZ.

Table-5: Rank of Dissolution Rate and Solubility Profile of Cocrystals in Various Media

S. No.	Media	Rank	
		Solubility	Dissolution
1	SGF	ITZ<COSER<COBENZ<COGLY<COPRO<COSU	ITZ<COSU<COPRO<COBENZ<COGLY<COSER
2	0.1N HCL	ITZ<COPRO<COGLY<COSER<COSU<COBENZ	ITZ<COSU<COBENZ<COPRO<COGLY<COSER
3	PBS pH 6.8	ITZ<COGLY<COBENZ<COSU<COPRO	ITZ<COPRO<COSU<COGLY<COBENZ<COSER
4	PBS pH 7.4	ITZ<COBENZ<COSU<COSER<COGLY<COPRO	ITZ<COBENZ<COSU<COPRO<COGLY<COSER
5	SGF + 1% SLS	ITZ<COSU<COBENZ<COGLY<COPRO<COSER	-

6	0.1 N HCL +1% SLS	ITZ<COBENZ<COGLY<COSU<COSER<COPRO	-
7	PBS pH7.4 + Methanol (6:4)	ITZ<COSU<COBENZ<COPRO<COSER<COGLY	-
8	D/W	ITZ<COSU<COBENZ<COPRO<COSER<COGLY	-

In PBS with methanol (6:4) and distilled water (D/W), COSER and COGLY showed excellent solubility, indicating that the polarity and hydrogen bonding capacity of the media play critical roles in dissolving the cocrystals. The differential behavior of cocrystals in various media underscores the importance of media composition in modulating solubility and dissolution, highlighting the role of pH, surfactants, and coformers' properties in optimizing the performance of ITZ cocrystals. Moreover, the increased dissolution rate of ITZ in cocrystal form with coformers can be attributed to changes in crystal lattice structure as well as solvation of the cocrystal component. Solubility and dissolution rates are influenced by crystal lattice strength and cocrystal solvation properties. The enhancement in dissolution rate may result from a reduction in lattice energy and an increase in solvent affinity, which is sustained by the formation of a cocrystal. In the case of hydrophobic drugs, solvation is the rate-determining step, and limitations arise due to solvent-solute interactions. The modification of physicochemical properties of the active pharmaceutical ingredient (API) by the coformer, along with a decrease in the interfacial barrier, could also contribute to the observed improvement in dissolution rate.

Stability Studies

Comparison of the FTIR spectrum of ITZ and its cocrystals, both before and after stability testing, revealed no significant changes (Fig.-4 and 5). This indicates that the drug, as well as cocrystals, remain stable at 40°C and 75% relative humidity (RH).

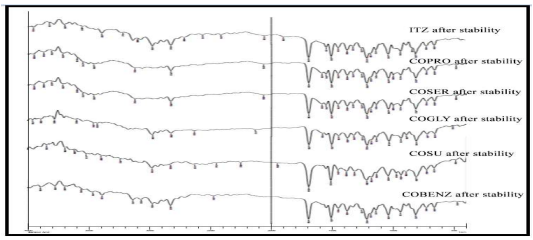

Fig.-4: FTIR Spectra of Pure ITZ and Cocrystals with Proline (COPRO), Serine (COSER), Glycine (COGLY), Succinic Acid (COSU), and Benzoic Acid (COBENZ) after Stability Studies

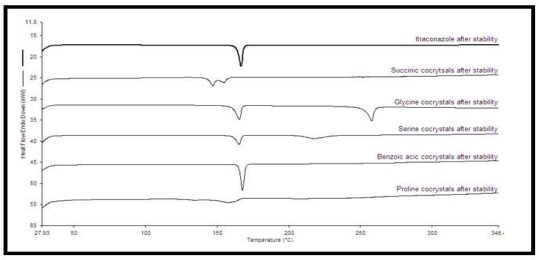

Fig.-5: DSC Spectra of ITZ and Cocrystals with Proline (COPRO), Serine (COSER), Glycine (COGLY), Succinic Acid (COSU), And Benzoic Acid (COBENZ) after Stability Studies

DSC studies showed slight changes in M.P. and ΔH values in comparison with cocrystals and ITZ before stability (Table-6). Cocrystals of ITZ with proline, serine, glycine, succinic acid, and benzoic acid were found stable at 40ºC and 75% RH for a period of 45 days.

Table-6: Results of DSC of ITZ and Cocrystals

S. No.	Sample	Cocrystal M.P.							
		Before stability				After stability			
		I (ºC)	ΔH (J/g)	II (ºC)	ΔH (J/g)	I (ºC)	ΔH (J/g)	II (ºC)	ΔH (J/g)
1	ITZ	167.76	111.77			167.76	111.77	-	-
2	COPRO	135.38	4.15	158.53	44.53	135.30	4.15	158.53	44.53

3	COSER	165.64	61.58	219.83	27.09	165.47	38.42	-	-
4	COGLY	164.64	76.72	258.61	110.41	164.45	85.65	257.78	96.24
5	COSU	146.33	17.24	154.67	14.84	146.97	22.92	155.00	9.08
6	COBENZ	-	-	-	-	166.93	96.48	-	-

In-vitro Antifungal Activity

Experimental results demonstrate that ITZ exhibits an increased rate of dissolution when combined with amino acids. This combination may also increase its permeability across fungal cell membranes. This enhanced permeability could explain the higher zone of inhibition observed with the cocrystals compared to pure ITZ, even at lower concentrations. The minimum inhibitory concentration (MIC) values against *Aspergillus* and *Candida* species are presented in Tables-7 and 8.

Table-7: Zone of Inhibition of ITZ and Formulations Against *Aspergillus niger*

Conc. (µg/ml)	Control	Std. (mm)	COPRO (mm)	COSU (mm)	COBENZ (mm)
1	0	12	14	17	16
3	0	14	16	20	19
5	0	20	21	23	22

Table-8: Zone of Inhibition of ITZ and Formulations against *Candida albicans*

Conc. (µg/ml)	Control	Std. (mm)	COPRO (mm)	COSU (mm)	COBENZ (mm)
1	0	11	15	12	14
3	0	15	19	16	18
5	0	22	24	22	23

Solution-Mediated Phase Transformation Study (SMPT)

The SMPT study revealed distinct behaviors among the cocrystals as shown in Fig.-6 and 7, and Table-9. COPRO showed a melting point (M.P.) similar to that of pure ITZ, indicating conversion back to the pure drug. Conversely, COGLY and COSER maintained a consistent melting point, signifying the preservation of their cocrystal integrity. COBENZ, initially in an amorphous form, transformed into pure ITZ upon exposure to the dissolution medium. COSU showed a slower transformation rate as compared to the other cocrystals.

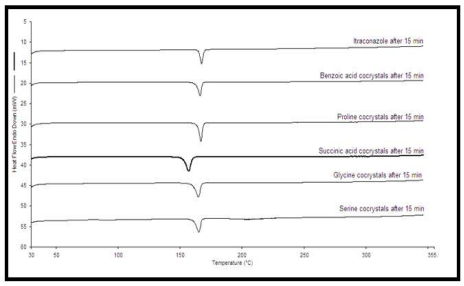

Fig.-6: DSC Spectra of ITZ, Cocrystals with Proline, Serine, Glycine, Succinic Acid, and Benzoic Acid after 15 minutes of solution-mediated phase transformation studies

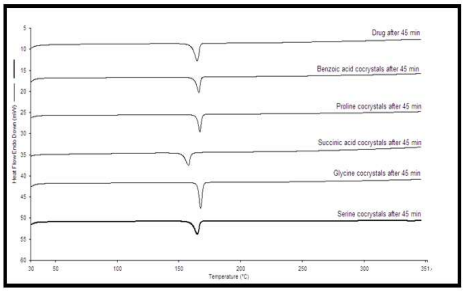

Fig.-7: DSC spectra of ITZ, Cocrystals with Proline, Serine, Glycine, Succinic Acid, and Benzoic Acid after 45 minutes of Solution-Mediated Phase Transformation Studies

The MPs of ITZ and its cocrystals after 15 and 45 minutes are presented in Table-9, highlighting the phase stability under the given conditions.

Table-9: Results of DSC Data Comparison of Cocrystals after 15 min and 45 minutes of Solution-Mediated Phase Transformation Studies

S. No.	Sample	Cocrystal M.P. (ºC)					
		Cocrystals	ΔH (J/g)	After 15 min. in dissolution media	ΔH (J/g)	After 45 min. in dissolution media	ΔH (J/g)
1	ITZ	167.76	111.77	167.47	60.46	167.59	115.69
2	COPRO	158.53	44.53	166.93	71.55	167.09	79.78
3	COSER	165.64	61.58	165.29	83.16	165.12	87.47
4	COGLY	164.64	76.72	164.89	96.60	164.93	122.46
5	COSU	154.67	14.84	157.10	91.84	157.79	77.56
6	COBENZ	-	-	166.34	74.00	166.29	81.42

CONCLUSION

We have successfully developed cocrystals of ITZ using the crystal engineering technique. Proline, serine, glycine, succinic acid, and benzoic acid were employed as coformers in the cocrystal development process, which was accomplished through the solvent drop grinding method. This technique proved to be very efficient, time-saving, and easy. Formulated cocrystals exhibited enhanced solubility as well as improved in vitro dissolution performance compared to pure ITZ. In this study, COGLY and COSER cocrystals demonstrated stable forms, while COBENZ, COPRO, and COSU exhibited solution-mediated phase transformation. Despite this, the formulated cocrystals displayed efficacy in the in vitro antifungal activity assessment. Furthermore, the cocrystals demonstrated stability after a 45-day storage period at 40°C and 75% RH. These observations indicated the potential of developed cocrystals as promising candidates for improved drug solubility, dissolution rate, and antifungal activity, thereby offering a valuable approach for improving the therapeutic potential of ITZ.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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REFERENCES

1. W. Chen, X. Liu, S. Zhang, S. Chen, *Molecular Therapy Nucleic Acids*, **31**, 691(2023), http://dx.doi.org/10.1016/j.omtn.2023.02.019
2. S.F. Zhou, W.Z. Zhong, *Molecules*, **22(2),** 279(2017), http://dx.doi.org/10.3390/molecules22020279
3. A.V. Mundhe, N.V. Kumar, K.R. Biyani. *Journal of Drug Delivery & Therapeutics*, **3(4),** 166(2013), http://dx.doi.org/10.22270/jddt.v3i4.555
4. P. Mounika, R.S. Vijaya, G. Divya, A. Gowramma, and G. Vijayamma, *International journal of innovative pharmaceutical research,* **6(1),** 58(2015).
5. M.R. Dhondale, P. Thakor, A.G. Nambiar, M. Singh, A.K. Agrawal, N.R. Shastri, D. Kumar, *Pharmaceutics*, **15(1)**, 189 (2023), http://dx.doi.org/10.3390/pharmaceutics15010189

6. A. Shevchenko, L.M. Bimbo MiroshnykI, J. Haarala, K. Jelinkova, K. Syrjänen, B. Veen, J. Kiesvaara, H.A. Santos, J. Yliruusi, *International Journal of Pharmaceutics*, **436(1-2)**, 403(2012), http://dx.doi.org/10.1016/j.ijpharm.2012.06.045
7. S. Kumar, O. Prakash, A. Gupta, S. Singh, *Current Organic Synthesis*, **16(3)**, 385(2019), http://dx.doi.org/10.2174/1570179416666190329194926
8. M. Solares-Briones, G. Coyote-Dotor, J.C. Páez-Franco, M.R. Zermeño-Ortega, C.M. de la O Contreras, D. Canseco-González, A. Avila-Sorrosa, D. Morales-Morales, J.M. Germán-Acacio, *Pharmaceutics*, **13(6)**, 790(2021), http://dx.doi.org/10.3390/pharmaceutics13060790
9. S. Sareen, G. Mathew, L. Joseph, *International Journal of Pharmaceutical Investigation,* **2(1)**, 12(2012), http://dx.doi.org/10.4103/2230-973X.96921
10. X. Yin, L.S. Daintree, S. Ding, D.M. Ledger, B. Wang, W. Zhao, J. Qi, W. Wu, J. Han, *Drug Design, Development and Therapy,* **9**, 2801(2015), http://dx.doi.org/10.2147/DDDT.S81253
11. R. Machado, T. Boleslavska, J. Beranek, E. Tieger, B. Twamley, M.J. Santos-Martinez, O. Dammer, L. Tajber, *Pharmaceutics*, **12(8)**,741(2020), http://dx.doi.org/10.3390/pharmaceutics12080741
12. CA. Ober, R.B. Gupta, *AAPS PharmSciTech*, **13(4)**, 1396(2012), http://dx.doi.org/10.1208/s12249-012-9866-4
13. S. Dwi, O.J. Rachel, I. Muhammad, P. Abhimata, Y. Helmy, L.L. Maria, *Tropical Journal of Pharmaceutical Research,* **17(6)**, 997(2018), http://dx.doi.org/10.4314/tjpr.v17i6.3
14. A. Butreddy, M. Almutairi, N. Komanduri, S. Bandari, F. Zhang, M. Repka, Journal of *Drug Delivery Science and Technology* **63**, 102529(2021), http://dx.doi.org/10.1016/j.jddst.2021.102529
15. S. Patil, V. Ujalambkar, A. Mahadik, *Journal of Drug Delivery Science and Technology*, **39**, 217(2017), http://dx.doi.org/10.1016/j.jddst.2017.04.001
16. J. Weng, S. Wong, X. Xu, B. Xuan, C. Wang, R. Chen, C. Sun, R. Lakerveld, P. Kwok, S. Chow, *Crystal Growth & Design,* **19**, 2736(2019), http://dx.doi.org/10.1021/acs.cgd.8b01873
17. A. Elshahat, A. Hasan, H. Elghamry, G. Balata, *Journal of Drug Delivery Science and Technology,* **100**, 106093(2024), http://dx.doi.org/10.1016/j.jddst.2024.106093
18. M. Alvarenga, N. Mendes, G. Parreira, R. Marreto, C. Silva, A. Fatima, F. Silva, Martins, *Journal of Molecular Structure,*(2024), http://dx.doi.org/10.1016/j.molstruc.2024.140217
19. A.A. Mohamed, N.Z. Kbah, *Al-Zahraa Journal for Health and Medical Sciences*, **1(3),** 11(2023).
20. A. Elkhabaz, S. Sarkar, G.J. Simpson, L.S. Taylor, *Pharmaceutical Research*, **36**, 1(2019), http://dx.doi.org/10.1007/s11095-019-2718-0
21. H. Bagavatula, S. Lankalapalli, V.K. Tenneti, N.M. Beeraka, B.T. Bulusu, *Advances in Pharmacology and Pharmacy,* **2**, 85(2014), http://dx.doi.org/10.13189/app.2014.020601
22. T. Tao, Y. Zhao, J. Wu, B. Zhou, *International Journal of Pharmacy*, **367**, 109(2009), http://dx.doi.org/1010.1016/j.ijpharm.2008.09.034
23. A. Shete, S. Murthy, S. Korpale, A. Yadav, S. Sajane, S. Sakhare, R. Doijad, *Journal of Drug Delivery Science and Technology*, **28**, 46(2015), http://dx.doi.org/10.1016/j.jddst.2015.05.006
24. C. Hansen, Hansen Solubility Parameter: A User's Handbook, Second edition, Tayler and Francis Group, pp.75-137(2007).
25. K. Greco, R. Bogner, *Journal of Pharmaceutical Sciences,* **101(9)**, 2997(2012), http://dx.doi.org/10.1002/jps.23025
26. G.G. Zhang, R.F. Henry, T.B. Borchardt, X. Lou, *Journal of Pharmaceutical Sciences,* **96(5)**, 990(2007), http://dx.doi.org/10.1002/jps.20949
27. N. A. Vasilev, A.O. Surov, A.P. Voronin, K.V. Drozd, G.L. Perlovich, *International Journal of Pharmaceutics*, **599**, 120441(2021), http://dx.doi.org/10.1016/j.ijpharm.2021.120441
28. B. Lang, J.W. McGinity, R.O. Williams III, *Molecular Pharmaceutics,* **11(1)**, 186(2014), http://dx.doi.org/10.1021/mp4003706
29. H. Fatahi, J. Claverie, S. Poncet, *Applied Sciences*, **12(23)**, 12019(2022), http://dx.doi.org/10.3390/app122312019
30. I. Dincer, Y.A. Cengel, *Entropy,* **3(3)**,116(2001), http://dx.doi.org/10.3390/e3030116
31. D.D. Chavan, V.M. Thorat, A.S. Shete, R.R. Bhosale, S.J. Patil, D.D. Tiwari, *Cureus*, **16(9)**, e70328(2024), http://dx.doi.org/10.7759/cureus.70328

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