Solubility Enhancement and Characterization of Tamoxifen Citrate Using Co-crystallization

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Abstract: *Tamoxifen citrate (TC) is one of the anti-estrogen agents which has low solubility in the water. As TC is still used as the main therapy in breast cancer treatment, modifications are still being made to increase the solubility of TC for a successful treatment. In this research, co-crystallization of TC was performed using Nicotinamide (NIC), Isonicotinamide (ISO), Saccharin (SAC), Aspartame (ASP), and Benzoic Acid (BNZ) as a coformer with the molar ratio of 1:1, 1:2, and 2:1. Co-crystal was prepared by solvent drop grinding (SDG) and solvent evaporation (SE) methods using methanol. The results of the solubility test showed that TC-NIC and TC-ISO co-crystals with a 1:2 molar ratio made using the SDG and SE methods gave the best results. Meanwhile, the best dissolution test results were shown by TC-ISO co-crystals with a ratio of 1:2. Based on the characterization of physical stability, the SDG method resulted in more stable TC co-crystals than the SE method. Therefore, in this case, the SDG method could be more advantageous to be used for development in the field of co-crystallization.*

Keywords: *tamoxifen citrate; co-crystallization; solubility*

■ INTRODUCTION

Since 2018, breast cancer cases in America have reached around 266,120 cases with a death rate of 72,590 [1]. Breast cancer is a disease diagnosed in women and ranks second as a cause of cancer death [2]. One of the treatments is chemotherapy using tamoxifen citrate (TC) [3]. TC (Fig. 1) is a salt form of tamoxifen consisting of tamoxifen and citric acid, a poorly soluble compound. Poor solubility based on physicochemical properties, namely solubility in water at a temperature of 20 °C (pH 3.0–3.5) reaching 0.3 mg/L and in HCl 0.02 M at 37 °C reaching 0.2 mg/mL, soluble in ethanol and methanol, and slightly soluble in acetone and chloroform. In addition, the bioavailability of tamoxifen citrate is in the range of 20–30% [4].

The success of a drug in achieving a therapeutic effect is influenced by its physicochemical properties, especially its solubility. Therefore, the solubility of the active substance greatly affects the performance of a drug [5]. Active substances with low solubility are a challenge in the pharmaceutical field in drug development and drug

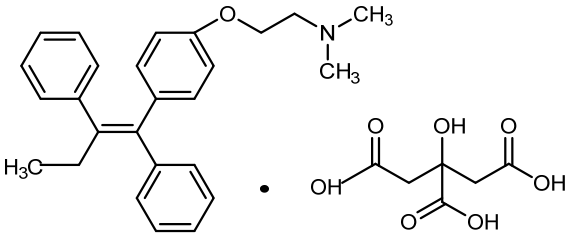


Fig 1. Structure of tamoxifen citrate (TC) [6]

dosage forms so that they can show a good profile in terms of solubility, dissolution, and bioavailability of a drug.

Previous studies reported efforts made to increase the efficiency of TC performance through the inclusion of TC complexes with cyclodextrins [6]. Subsequent studies observed PLGA nanoparticles containing TC as an anticancer drug [7], as well as increasing the solubility of TC through solid dispersion [2,4]. Some of the methods that have been carried out still have some drawbacks, such as the use of a lot of matrices, low drug loading, quite high process energy, and difficulties in the up-scaling process [8]. Thus, it is still possible to develop

methods with simple processes with considered results such as co-crystals.

Co-crystal is an approach that can be used to improve the physicochemical properties of an active substance (such as solubility, dissolution, bioavailability, and stability) without affecting the pharmacological activity of the active substance [9-10]. Co-crystallization can also be applied to acidic, basic, neutral, and ionic compounds [11], so it can be used on TC to increase its solubility. Several methods are used to produce co-crystals, namely solution-based crystallization (addition of solids to a crystallized solution, cooling crystallization, and solvent evaporation) and milling (dry or wet conditions) [9].

To achieve good results, the co-crystallization technique relies heavily on accuracy in coformer selection. The coformers used must have a synthon supramolecular group in order to interact with the active pharmaceutical ingredients to form hydrogen bonds. Requirements coformers must be inert and have low toxicity [12]. Some examples of commonly used coformers that successfully form co-crystals with active drug substances are nicotinamide–trimethoprim [12], isoniotinamide–furosemide [13], saccharin–ibuprofen [9], aspartame–glibencalamide [14], and benzoic acid–theophylline [15]. The chemical structure of TC, which has four hydrogen bond donors and nine hydrogen bond acceptors, opens the opportunity for co-crystal formation when interacting with co-crystal coformers, which also have synthon supramolecular groups. The interaction of co-crystal synthons with coformers can be predicted using a computational method approach (in-silico) [16].

So far, not much has been reported regarding the co-crystallization approach to increase the solubility of TC. Therefore, this study was carried out to increase the solubility of TC by using a co-crystallization approach using nicotinamide, isonicotinamide, saccharin, aspartame, and benzoic acid as coformers using solvent drop grinding and solvent evaporation methods, followed by evaluation of physicochemical properties and characterization using Fourier Transform Infrared Spectrophotometry (FTIR), Differential Scanning Calorimetry (DSC), Powder X-Ray Diffraction (PXRD), and Scanning Electron Microscopy (SEM).

■ EXPERIMENTAL SECTION

Materials

The materials for the experiment include Tamoxifen citrate (TC) (Pharmaceutical Industries Ltd., China), ethanol pro analysis (Merck), nicotinamide (Sigma Aldrich), isonicotinamide (Sigma Aldrich), benzoic acid (Sigma Aldrich), saccharin (Sigma Aldrich), aspartame (Sigma Aldrich), distilled water, and potassium bromide (pro analysis (brand).

Instrumentation

The instruments included UV-Vis spectrophotometer (Specord 205 Analytical Jena), USP dissolution apparatus type 2 (SotaX AT7 Smart dissolution tester), X-ray diffractometer (Pan-Analtycal), Differential Scanning Calorimetry (DSC) (Linseis PTA ST 1600), Fourier-Transformed Infrared Spectrophotometer (FTIR) (Specord 100), and Scanning Electron Microscope (SEM) (SU3500 SEM, HITACHI).

Procedure

Screening in-silico

Initial screening of co-crystals was carried out based on predicting hydrogen bond formation between TC and coformers. The two-dimensional structure of TC and coformers were obtained from PubChem Database and then converted into pdb format using Discovery Studio Visualizer v.16.1.0.15350. The interaction of two molecules (TC with each coformer) was observed using the AutoDockTools version 1.6 program. The parameters observed were the type of interaction such as hydrogen bonds, van der Walls bonds or π electron bonds and Gibbs free energy, and the distance of the bonds that occurred.

Polarizing microscope

Pure TC powder, pure coformer powder, and physical mixture were observed with a polarizing microscope. About 3 mg of TC powder and coformers (nicotinamide, isonicotinamide, saccharin, aspartame, and benzoic acid) were each placed in an object glass,

dropped with methanol, and left in contact until the methanol evaporated (supersaturated). The results of the interactions that occur are observed under a polarizing microscope equipped with a digital camera.

Co-crystal preparation

Before the co-crystallization process, a phase solubility test was carried out by making a series of ratios of active pharmaceutical ingredients (API) and coformers from 1:9 to 9:1 and then put into a vial containing 25 mL of distilled water. Then the sample was stirred using a mechanical stirrer at 25 °C for 24 h. The sample was then filtered, and dissolved API levels were measured and analyzed by UV-Vis spectrophotometry.

The phase solubility test was carried out to determine the ratio or the right number of coformers in the formation of co-crystals so that it is expected to change the physicochemical properties of the active substance, especially its solubility. The obtained phase solubility curves are used to describe the concentration of solutes in various concentrations of coformers. Thus, the solvent drop grinding and the solvent evaporation processes use a ratio of 1:1, 1:2, and 2:1.

Solvent drop grinding (SDG). TC and coformer were mixed with a molar ratio of 1:1, 1:2, and 2:1; and then grinded together in a mortar for 5–10 min while dripping with a suitable solvent (such as methanol), drop by drop until it looks wet during the grinding process. Then the obtained co-crystals were stored at room temperature for 24 h to obtain a dry crystal precipitate [17].

Solvent evaporation (SE). TC and coformer were mixed with a molar ratio of 1:1, 1:2, and 2:1; and dissolved in a suitable solvent such as methanol, shaken for 10 min, then evaporated in a water bath at 30 °C for 24 h to obtain a dry crystalline solid. Thus, the obtained co-crystals are stored at room temperature [18].

Co-crystal evaluation

Solubility test. Saturated solubility was measured by the shake flask method. 100 mg of co-crystal TC was mixed with 50 mL of distilled water in an Erlenmeyer flask. Then the sample was stirred using a mechanical stirrer at 25 °C for 24 h. The sample was then centrifuged at 5000 rpm for 10 min. The supernatant was then separated by filtering using filter paper, then diluted and analyzed using a UV-Vis spectrophotometry at a wavelength of 200–800 nm. After obtaining the maximum wavelength of TC, the concentration of dissolved in the filtrate is determined from the results of the solubility test by looking at the absorption of UV-Vis light in the sample solution.

Dissolution test. The dissolution test was carried out on the co-crystals formed and showed the best solubility results, using a type 2 dissolution test apparatus with 900 mL HCl pH 1.2 at a rotation speed of 50 rpm for 60 min at 37 °C. The sample was weighed carefully then compacted to form a tablet. The tablets were then put into the media, then 10 mL of the sample solution was taken periodically at intervals of 10, 20, 30, 45, and 60 min, and each intake was replaced with new media and the same volume. The sample was then filtered using filter paper and analyzed using UV spectrophotometer at a wavelength of 200–300 nm. Then the dissolved TC levels were calculated at each collection time interval with a sampling factor.

Characteristics of co-crystal

Powder X-ray diffraction (PXRD). Diffractogram patterns of co-crystal, physical mixture, and pure TC were obtained using an X-ray diffractometer with Cu Kα radiation (λ = 1.54 Å) and a voltage of 40 kV and a current of 30 mA. The slit width is 0.2 inches, with a scanning speed of 0.2–0.5°/min and a scanning distance of 2θ = 5–50° (organic compounds). Samples were measured then the results obtained were compared with the pure TC and TC co-crystals formed.

Differential scanning calorimetry (DSC). Thermal analysis of co-crystal, physical mixture, and pure TC were carried out with 3–5 mg of sample put into an aluminum pan, then measured on a programmed DSC device with a temperature range of 50–300 °C with a heating rate of 10°C/min. The thermogram results obtained will be compared with the pure TC thermogram to see any differences before and after treatment.

Fourier transform infrared (FT-IR) spectrophotometry. The IR spectra of co-crystal, physical mixture, and pure TC were analyzed at wavenumbers of 4000–400 cm^{-1}. 1 mg of sample was

mixed with 10 mg of KBr until homogeneous and then pellets were formed with a pressure of 20 psi using a KBr plate press. This procedure was carried out to see the functional groups of pure TC and TC co-crystals formed, the functional groups present in TC must also be present in TC co-crystals.

Scanning electron microscopy (SEM). SEM was used to study the morphology of co-crystals, physical mixtures, and pure TC. The light sample was sprinkled over an aluminum chamber and then covered with platinum 10 Å thick under an argon atmosphere using a mas module placed in a high vacuum evaporator. After that, the container containing the sample is placed on a scanning electron microscope.

Physical stability test

Physical stability testing was carried out on TC co-crystals which showed an increase in the solubility and dissolution profile and showed the typical characteristics of the co-crystals. Physical stability test was carried out at 40 °C and 75% relative humidity using a climatic chamber, by observing changes in the diffractogram pattern and comparing it with the initial storage conditions.

■ RESULTS AND DISCUSSION

Initial Characterization of TC

The diffraction pattern of tamoxifen citrate (TC) in Fig. 2 shows the form II polymorph. This is confirmed by the report of Gamberini et al. [19], with the characteristic high-intensity diffraction peak form II detected at 2θ = 5.69°, 13.15°, 14.05°, 21.01°, 24.15°, and 28.40°. TC, which is widely used as a pharmaceutical ingredient, exists in two main polymorphic forms, and the relatively stable polymorphic form is in form II.

Coformer Screening with *In-Silico*

TC has a structure containing functional groups as proton acceptors and donors. TC has nine functional groups that act as acceptors with acting atoms namely oxygen and nitrogen, and four functional groups that act as proton donors (Fig. 3).

The coformers selected for the formation of tamoxifen citrate co-crystals must be inert, have no pharmacological effects, and have hydrogen acceptor or donor groups that will form hydrogen bonds with TC. The coformers used were nicotinamide (NIC), isonicotinamide (ISO), saccharin (SAC), aspartame (ASP), and benzoic acid (BNZ). Table 1 shows the results of *in-silico* screening, which predicts the hydrogen bonds formed in the formation of co-crystals between the active substance and the coformer. Prediction results show that hydrogen bonds are only formed in the NIC and ISO coformers. The hydrogen bond formed between TC and NIC occurs between the amide group (–CO–NH_2) on the NIC with the ether group (–O–) on the TC, so the synthon formed N-H···O-C is called a monomer heterosynthon. Meanwhile, with ISO coformers, hydrogen bonds that occur between the amide group in ISO and the amine group (R_3N) in TC, so that the synthon

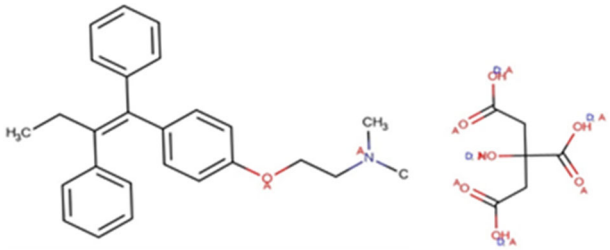


Fig 3. Donors and acceptors of TC

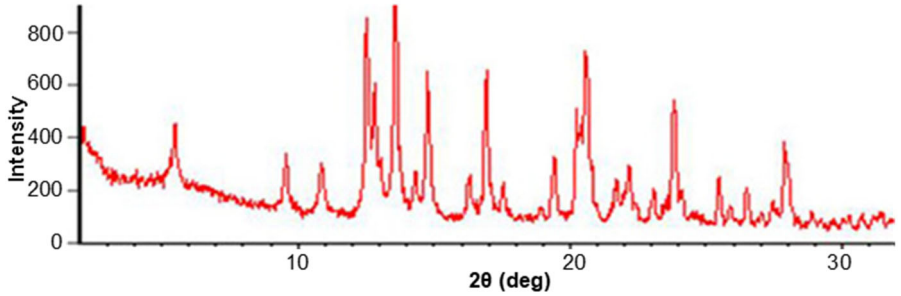


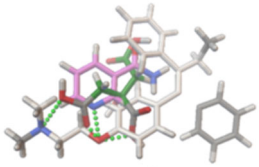
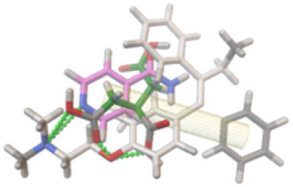
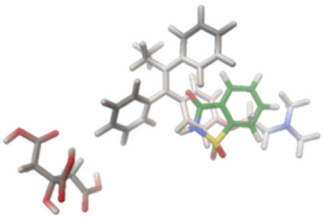
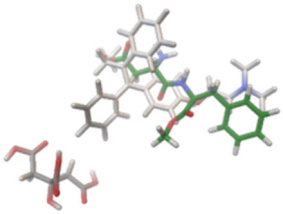
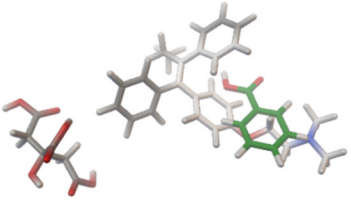
Fig 2. Diffractogram pattern of form II polymorphic form TC

formed N-H···N-C is called a homosynthon monomer [20].

The Gibbs free energy formed from the NIC and ISO coformer is -1.3 kcal/mole (Table 1), this indicates an interaction (hydrogen bond between coformers and TC), and the bond distance formed by each coformer is 2.34 and 2.20 Å, respectively. A good hydrogen bond has a distance of 2.8 Å. The number and distance of hydrogen bonds will affect the strength of the binding affinity between the ligand and the receptor [21]. Hydrogen bonds are said to be weak bonds, but in complex structures, these hydrogen bonds are strong enough to form supramolecular synthon in the co-crystal system and stabilize the molecular structure [10]. Hydrogen interactions between TC with NIC and ISO form parameters to increase the solubility of TC.

In the other three coformers SAC, ASP, and BNZ, no hydrogen bonds are formed. However, it has a smaller Gibbs free energy value compared to the formation by the NIC and ISO coformers. The formation of hydrogen bonds is not caused by the distance between the atoms that are too far apart. The bond free energy indicates the ability of the ligands to interact. A compound is said to have a good interaction if the value of free energy is lower so that the bond that occurs is more stable [21].

Table 1. Simulation results of interaction of TC and coformers with *in-silico*

Compounds	2D interaction	Bond interaction	Gibbs energy (kcal/mol)	Bond distance
Tamoxifen citrate–nicotinamide (TC-NIC)		4 hydrogen interactions	-1.3	2.34 Å
Tamoxifen citrate–isonicotinamide (TC-ISO)		4 hydrogen interactions, and 1 π-π interaction	-1.3	2.20 Å
Tamoxifen citrate–saccharin (TC-SAC)		No hydrogen interaction	-1.7	–
Tamoxifen citrate–aspartame (TC-ASP)		No hydrogen interaction	-2.5	–
Tamoxifen citrate–benzoic acid (TC-BNZ)		No hydrogen interaction	-1.2	–

Polarizing Microscope

Fig. 4 is the result of polarization microscopy between the TC mixture and the five coformers, which shows the presence of a new crystal habit only in the NIC and ISO coformers, while the other three coformers (SAC, ASP, and BNZ) show mixing with TC, but do not produce new crystal habits. If the crystal habit obtained through recrystallization of the API mixture is significantly different from the single habit, then the mixture is indicated to show interaction. Differences in crystal habit and thermal behavior indicate a solid interaction between the two components of the active substance and the conformer.

Co-crystal Preparation

The results of the TC *in-silico* test with five coformers were then made *in-vitro* co-crystals using solvent drop grinding (SDG) and solvent evaporation (SE) methods with molar ratios of TC and coformers of 1:1, 1:2, and 2:1 (30 variations), which resulted in a solid powder according to provided that the co-crystal form is a solid powder at room temperature. Co-crystallization using SDG does not require a lot of solvent so this method is environmentally friendly [22]. The purpose of adding solvent (methanol) in this method is to accelerate the interaction of the two components, which will accelerate the achievement of an amorphous state in each component so that both components become more reactive.

Co-crystallization using SE method using the principle of dissolving API and coformer with a suitable solvent. Molecular interactions are expected to occur in the dissolution process of the two compounds so that hydrogen bonds are formed, where this interaction is expected to occur in the co-crystallization process. The SE

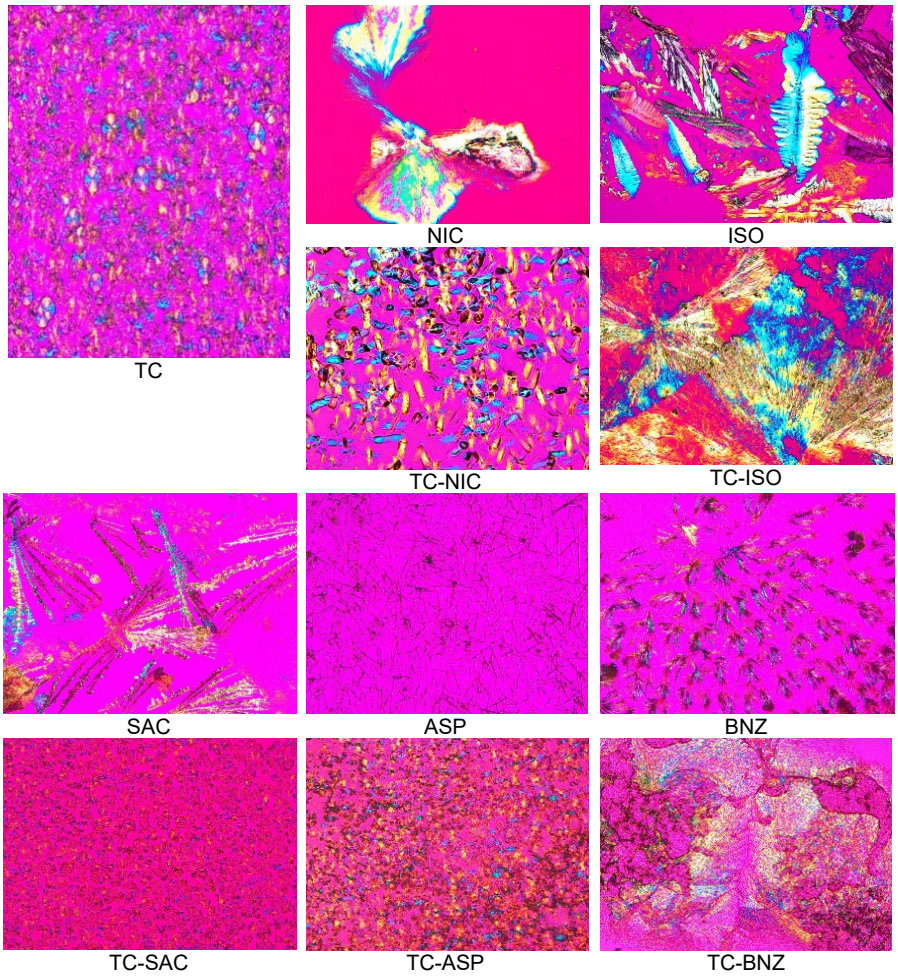


Fig 4. Polarizing microscopy of mixed TC and coformers

process aims to remove the solvent so as to form a crystalline solid that has different physicochemical properties from the pure substance. Methanol is used as a solvent, because each material is soluble in the solvent and the result is solid at room temperature. Dissolution with organic solvents can affect changes in the crystal structure of a substance. This change in crystal structure will affect changes in the physicochemical properties of the substance [23].

Co-crystal Evaluation

The TC co-crystal approach was carried out to improve the solubility properties and dissolution profile of TC. Therefore, solubility and dissolution tests were carried out to find the best co-crystal variation, in terms of molar ratio, type of coformer, and method of preparation.

Solubility test

Fig. 5 shows the solubility results of TC co-crystals from the SDG method. The increase in solubility in TC-ISO co-crystals reached 55–74% with a ratio of 1:1, 1:2, and 2:1, respectively 368.184, 367.448, and 327.807 mg/mL compared to the saturated solubility of pure TC (211.940 mg/mL). Theoretically, coformers that have high solubility in water will affect the solubility of the active ingredient, the higher the hydrophilic nature of a material, the co-crystals formed will show high solubility [24]. The SDG method shows that the increase in saturation solubility is relatively small compared to the SE method, this may be due to the less solvent catalytic process at the time of manufacture.

The increase in the saturation solubility of TC co-crystals from the SE method also occurred in TC-ISO co-crystals reaching 61-103% from the ratios of 1:1, 1:2, and 2:1, respectively, for 347.333, 430.666, and 342.175 mg/L (Fig. 6). The increase in solubility that occurs is due to the hydrogen bonds formed in the TC-ISO co-crystal. Besides being able to change the crystal lattice, it can also increase the solubility by attracting more solvent so that the interaction of TC with the solvent is more. The increase in solubility was due to the increase in solvent affinity for TC due to the presence of coformers. Coformers are compounds that are polar so they will be linear with affinity for water. With this, the NIC and ISO coformers provide effectiveness in increasing the solubility of TC through the co-crystallization technique. In another study, co-crystallization with NIC coformer was reported to increase trimethoprim [12]. In addition, ISO coformer was also used to increase the solubility of furosemide which showed an increase of 5.6 times higher than pure furosemide [13].

Dissolution test

The drug dissolution profile was studied for TC-NIC and TC-ISO co-crystals which showed increased solubility of TC. Fig. 7 shows the dissolution profile of the TC-NIC co-crystal drug. Increased dissolution occurred in TC-NIC 1:2 co-crystals prepared by the SE method. In the SDG method, the drug dissolution profile is not higher than pure TC. The choice of the molar ratio

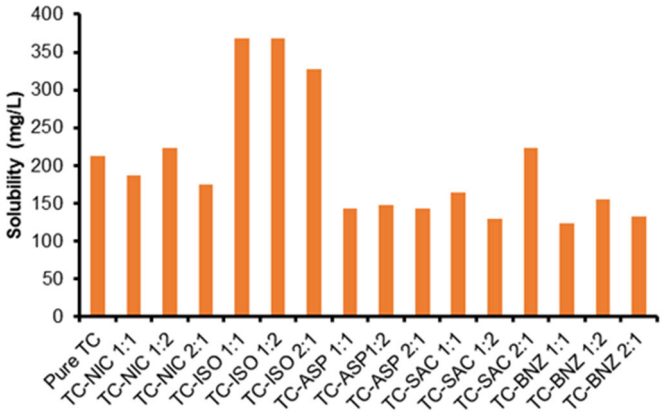


Fig 5. Comparison of solubility of TC co-crystal with SDG method

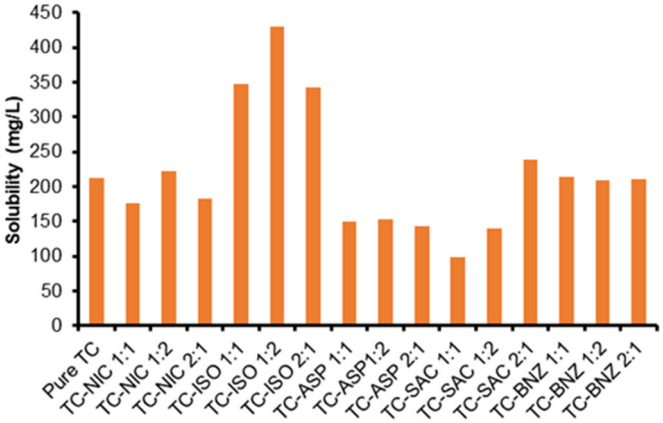


Fig 6. Comparison of solubility of co-crystal TC with SE method

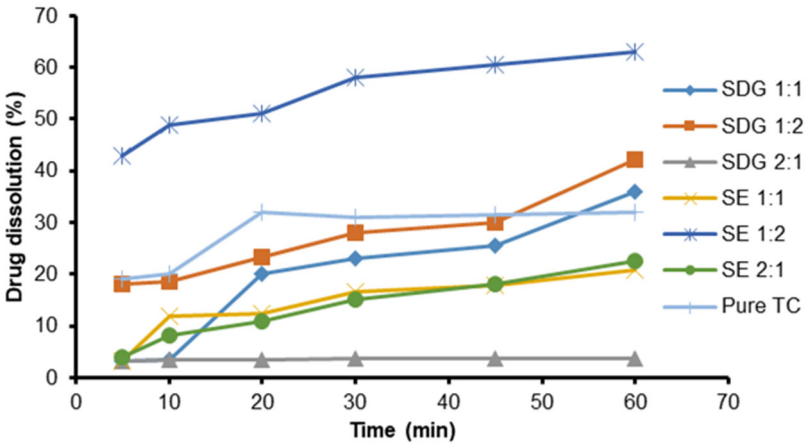


Fig 7. Dissolution profile of pure TC and TC-NIC co-crystal

also greatly determines the success of co-crystal formation. If the correct molar ratio is used, there will be an increase in solubility caused by several mechanisms, including the formation of a new co-crystal crystalline phase which can change the physicochemical properties (e.g., solubility). The increase in dissolution in the process with the NIC coformer occurs due to the formation of hydrogen bonds between the amide functional group and the nicotinamide carboxyl group. Therefore, in relation to TC, it increases the polarity of the hydro-carboxyl hydroxyl thereby increasing its solubility [25].

The dissolution profile of the TC-ISO co-crystal drug is presented in Fig. 8. Like drug release in TC-NIC co-crystals, increased drug release occurred in TC-ISO 1:2 co-crystals using the SE method. However, the release that occurred was higher than that of TC-NIC co-crystals. ISO is reported to increase the dissolution rate because it forms complexes with drug compounds through a mechanism as a donor and acceptor in the formation of hydrogen bonds. It also causes an increase in solubility through the formation of co-crystal complexes [17]. ISO is a hydrophilic conformer, that causes increased wetting of hydrophobic drug particles with the dissolution medium and has a positive effect on the dissolution profile. The results of the dissolution test have a linear relationship with the saturated solubility test and *in-silico* test where there is an increase in the solubility and dissolution profile of the TC-NIC and TC-ISO co-crystals with a molar ratio of 1:2 due to hydrogen bond interactions between the TC and NIC and ISO coformers.

Co-crystal Characterization

TC-ISO co-crystals with a ratio of 1:2 made by SDG and SE methods showed that the best solubility and

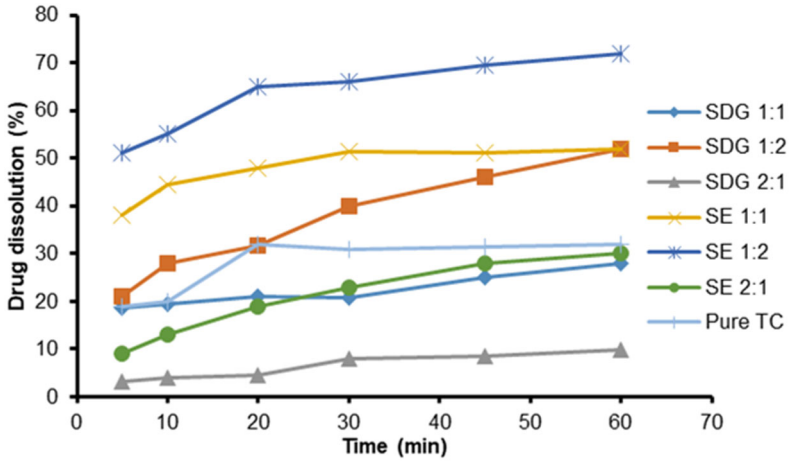


Fig 8. Dissolution Profile of pure TC and TC-ISO co-crystal

dissolution profiles were followed by characterization stages, namely PXRD, DSC, FTIR, and SEM.

Powder X-ray diffraction (PXRD)

The results of the TC, ISO, and TC-ISO co-crystal diffractograms with a ratio of 1:2 made by SE and SDG methods are presented in Fig. 9. The TC diffractogram shows the highest intensity at an angle of 2θ = 5.47°, 9.56°, 10.80°, 12.51°, 13.57°, 14.75°, 17.57°, 19.41°, 20.59°, 23.86°, and 28.90° indicates that TC is a crystal. Meanwhile, ISO shows the highest intensity at 2θ = 17.31°, 17.50°, 17.62°, 20.69°, 23.11°, 23.19°; 24,15°, 25,60°; 26.18°, 29.66°, 31.16°, and 32.16°. The diffraction pattern of TC-ISO 1:2 co-crystal with SDG has almost the same diffraction as the physical mix (PM), but there are several new peaks at 2θ = 11.08°, 13.05°, 13.84°, 15.00°, 17.03°, and 20.77° which indicates the new crystal phase of TC. Meanwhile, in the TC-ISO 1:2 co-crystal with SE method, several new peaks were seen at 2θ = 3.33°, 4.98°, 5.18°, 6.50°, 7.99°, and 8.38° which also shows the phase change of the formed crystals.

The phenomenon of decreasing the degree of crystallinity that occurs in co-crystals is also one of the causes of the increased solubility and dissolution of TC. Changes in the crystalline phase in each of these co-crystals will affect the physicochemical properties (such as solubility and dissolution) and mechanical properties. X-ray diffraction is a commonly used technique to confirm the shape of new solids from multicomponent crystals, because the crystal shape of each compound will give a distinctive characteristic to the diffractogram pattern [26].

Differential scanning calorimetric (DSC)

The decrease in the melting point of the co-crystal is directly correlated with the increase in the solubility value of the active substance in the co-crystal. The melting point of the co-crystal will be between or below the melting point of the active substance and its coformer so that the physicochemical properties of the co-crystal can be predicted from the polarity of the conformer. Based on Fig. 10, lower melting point values of pure TC and ISO are seen in TC-ISO 1:2 co-crystals with the SE method. The endothermic peak is in the temperature range of 81.71–104.05 °C with a sharp peak at 90.50 °C, which is the melting point. Meanwhile, TC-ISO 1:2 co-crystal with SDG method showed an endothermic peak in

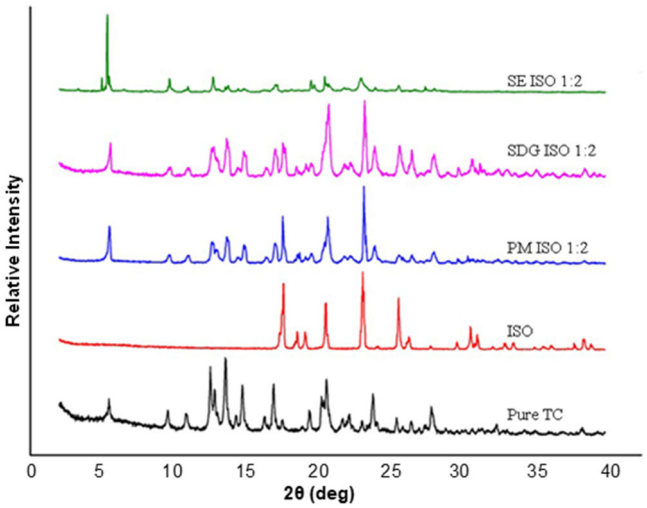


Fig 9. Comparison of pure TC and TC-ISO co-crystal diffractograms

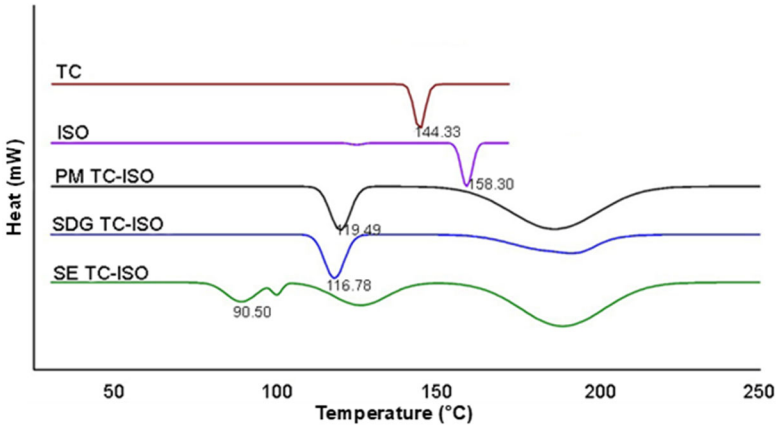


Fig 10. Thermogram profile of TC and TC-ISO 1:2 co-crystal

the range of 113.70–135.11 °C with a melting point of 116.78 °C, which was indicated by a sharp peak. Not much different from the physical mixture (PM), the endothermic peak is found at a temperature of 114.19–124.77 °C with a sharp peak as the melting point at 119.49 °C. TC-ISO co-crystal with PM and SDG method showed a similar thermogram profile. In the process, PM and SDG methods involve a mechanical process to mix the coformer and TC. In this case, it is possible in the PM mechanical process involving strong pressure, which results in the similarity of the thermogram profile. Meanwhile, the TC-ISO co-crystal with the SE method doesn't involve a mechanical process but uses a solvent to mix the coformer and TC as well as solvent evaporation. The solvent evaporation process is considered imperfect, resulting in a different shape of the thermogram profile.

When the melting point of the co-crystal solid mixture decreases, the TC-ISO 1:2 co-crystal has a higher solubility than pure TC. The endothermic peak that appears has the possibility of transforming in the co-crystals formed. The shift is caused by the interaction between TC and coformers, which changes the shape of the crystal lattice and forms a relatively different internal crystal structure, as shown in the diffractogram pattern [18]. The co-crystals formed have different melting point characteristics from the starting material.

Fourier transform infrared (FTIR) spectrophotometry

To confirm the formation of co-crystals, FTIR analysis was used to evaluate the changes in the vibrational frequencies of the specific functional groups of the crystals compared to their constituent components. Changes in hydrogen bonds at the intermolecular level provide a marked shift in the vibration frequency [27]. The FTIR spectrum of the TC crystal can be seen in Fig. 11. In the form II IR TC spectrum, it can be seen that there is a stretch of OH group at a wavenumber of 3400 cm^{-1}. In addition, the peaks at 2800 and 2600 cm^{-1} are due to the OH stretching of the citric acid carboxylic group forming two distinct hydrogen bonds. Peaks indicating C=O strain were also observed at 1738 cm^{-1} with new peaks at 1720 and 1703 cm^{-1}, indicating the presence of two carboxylic groups with different bonds.

ISO has an aromatic ring and an amide group in its molecular structure which gives it its characteristics in the IR spectrum (Fig. 11). The C-H stretching vibration on the aromatic ring is seen at 3160 cm^{-1}, and the C=C–C vibration on the aromatic ring is seen at 1618 cm^{-1}. The presence of nitrogen atoms in the aromatic ring gives a C–N vibration (stretch) at the peak of 1404 cm^{-1}. While the carbonyl group on the amide is shown at 1680 cm^{-1} with high intensity, and the strain vibration N–H is shown at 3364 cm^{-1} [25].

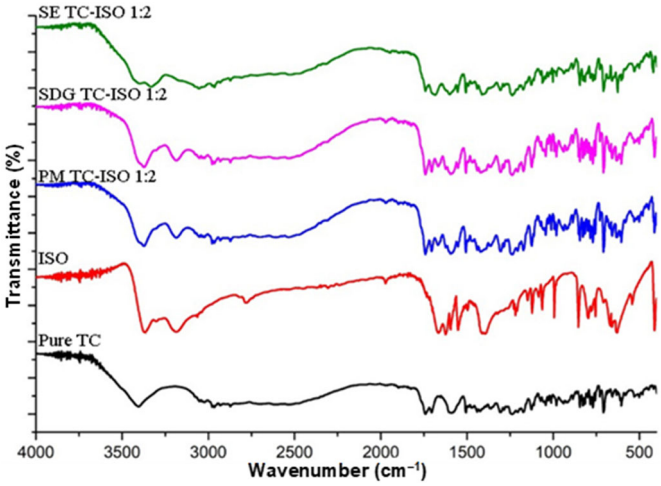


Fig 11. IR spectrum of pure TC and TC-ISO 1:2 co-crystal

The IR spectrum of the TC-ISO 1:2 co-crystal (Fig. 11) shows a similar pattern to the two constituent components. Based on the *in-silico* simulation, TC and ISO form hydrogen bonds between nitrogen atoms. In the spectrum, it can be seen that there is a shift in the N-N strain vibration in the TC-ISO 1:2 co-crystal. The hydrogen bonds formed were indicated by a shift in the vibration of the N atom from 3198 to 3329 cm^{-1} in the TC-ISO 1:2 co-crystal. Another characteristic pattern of the FTIR spectrum is a shift from the carbonyl group, which usually appears at wavenumber 1760 to 1620 cm^{-1}.

Scanning electron microscopy (SEM)

The morphology of the active ingredient includes changes in the crystal habit of an active ingredient so that it can affect the physicochemical properties and ultimately affect the performance of the preparations made. The results of the SEM analysis (Fig. 12) show a change in the crystal habit of the co-crystals of its constituent components. TC crystal habit has a beam-like

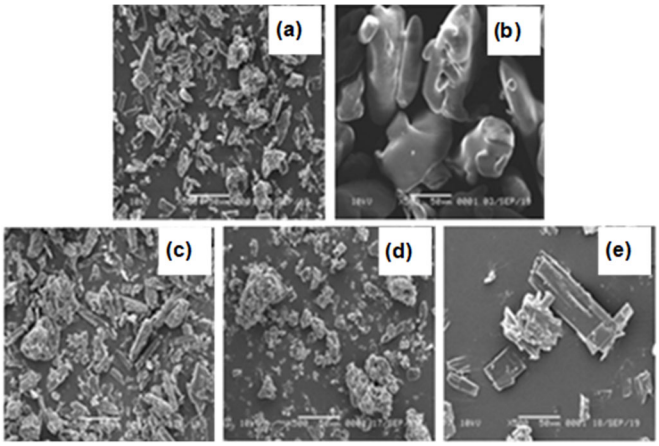


Fig 12. SEM Photomicrographs of (a) TC, (b) ISO, (c) PM TC-ISO 1:2, (d) SDG TC-ISO 1:2, and (e) SE TC-ISO 1:2

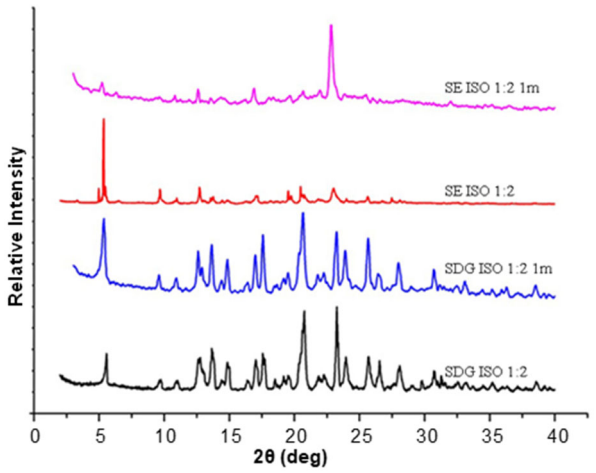


Fig 13. Comparison of diffractogram from before and after storage

shape at 500× magnification, while ISO forms irregular lumps. Morphological analysis of co-crystals showed a more compact structure with a higher density and combined to form larger molecules. This phenomenon is caused by the interaction of hydrogen bonds of TC and coformers in supramolecular synthons.

Physical Stability Test

Physical observations were made to observe the physical stability of the co-crystals before and after storage, resulting in a diffraction pattern (Fig. 13). TC-ISO co-crystal with drop grinding dissolution did not change from the aspect of peak number and peak shift at an angle of 2θ, as well as peak intensity. This indicates that the stability of the TC-ISO co-crystal is relatively stable. However, at co-crystal TC-ISO 1:2 with the SE method, changes are seen in the aspect of peak number and peak shift at an angle of 2θ. At angles of 3.33° and 4.98°, they were not seen after co-crystal storage for one month. Therefore, the SDG method is more stable than the SE method. Stability examination by x-ray diffraction is a very sensitive method to see changes in the three-dimensional structure of a molecule [18].

■ CONCLUSION

Increasing the solubility of TC through the co-crystallization approach in this study showed success using the NIC and ISO coformers. Through the in-silico approach, only the two coformers exhibit hydrogen interactions. However, based on the evaluation of solubility and dissolution tests, TC-ISO co-crystal showed the best results than TC-NIC at a ratio of 1:2. The formation of TC co-crystals will decrease the degree of crystallinity of TC, so that it becomes one of the causes of increased solubility and dissolution of TC. The increase in solubility was also caused by a decrease in the melting point value of TC after the formation of TC co-crystals. In addition, the morphology of TC co-crystals is more compact with higher density. In the physical stability test, the manufacture of co-crystals using the SDG method resulted in the stability of TC co-crystals which were more stable than the SE method.

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