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Research Article

Improved dissolution of ketoconazole by coprecipitation with nicotinamide using gas anti-solvent process

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ABSTRACT

The gas anti-solvent (GAS) process utilizing compressed carbon dioxide as an anti-solvent was applied to prepare coprecipitated particles between ketoconazole (KET), a poorly water-soluble drug substance, and nicotinamide (NIC), a water-soluble carrier. KET-NIC solid dispersion was also prepared by solvent evaporation and compared with the coprecipitated particles obtained from GAS process. DSC results indicated that KET formed eutectic with NIC at a weight ratio of 7:3. The results showed that the KET-NIC coprecipitated particles prepared by the GAS process, at an initial weight ratio of 1:1.5 in ethanolic solution, had suitable particle morphology and exhibited a remarkably higher dissolution than solid dispersion, physical mixture, and unprocessed KET. The formation of a simple eutectic mixture between KET and NIC in the coprecipitates prepared by both processes was confirmed by DSC, FTIR spectroscopy and powder X-ray diffraction. The enhanced dissolution of the GAS coprecipitated particles might be attributed to the eutectic formation, the improved wettability and hydrophilic microenvironment by the water-soluble carrier, the lower crystallinity, and the smaller size of the drug crystals.

Keywords:
Gas anti-solvent process, Coprecipitation, Ketoconazole, Nicotinamide, Eutectic mixture, Dissolution

1. INTRODUCTION

Oral drug delivery is the simplest and most widely available route of drug administration. Most drug substances are poorly water-soluble compounds, of which the absorption after oral administration is incomplete due to low drug dissolution[1]. Especially for biopharmaceutical classification system (BCS) class II drugs, low solubility distinctly determines the limitation in drug dissolution and subsequent oral absorption despite high permeability[2]. The dissolution of drugs from the oral dosage form is a critically important parameter attributed to oral bioavailability. The low oral bioavailability of poorly water-soluble drugs leads to high drug doses, multiple side effects, and low patient compliance[3]. Therefore, *in vitro* dissolution has been recognized as an important element in drug development, and thus, enhancing the dissolution rate of poorly water-soluble drugs is very challenging in the pharmaceutical industry.

Solid dispersion has been widely used to enhance the solubility and dissolution of poorly water-soluble drug substances. Solid dispersion comprises cocrystals, eutectic mixtures, solid solutions, and amorphous solid dispersion[1,4]. Traditional methods for preparing solid dispersions were melting and solvent evaporation methods. These processes possess some disadvantages, which are the main barriers for preparing solid dispersions. Because of high temperatures, several drugs, particularly thermo-labile substances, can be degraded by the melting method. The solvent evaporation method, in which the thermal decomposition of drug substances can be avoided, still has some drawbacks, including the use of large amounts of organic solvent, the high preparation cost, and the difficulties in completely removing the residual solvent.

Supercritical fluid techniques have emerged as an attractive process for the preparation of microparticles or composite particles with distinct characteristics. The supercritical fluid-based processes are recognized as green, sustainable, safe, and environmentally friendly[5]. These techniques utilize the properties of fluids in the

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vicinity of the critical point, which can be accessed in relatively mild conditions. Supercritical carbon dioxide ($scCO_2$) is widely utilized as a dense gas due to its low critical pressure (73.8 bar) and critical temperature (31.1°C). Additionally, carbon dioxide is non-toxic, non-flammable, chemically inert, and inexpensive, making it an attractive option for precipitating pharmaceutical active ingredients. The process of particle formation using $scCO_2$ can be classified into three main groups: a) precipitation from supercritical solutions composed of supercritical fluids and solutes; b) precipitation from solutions using supercritical fluids or compressed gases as anti-solvents; c) precipitation from gas-saturated solutions (PGSS) and related methods[5-6].

In this research, the $scCO_2$ technique based on the anti-solvent process, namely the gas anti-solvent (GAS) process, was exploited. In the GAS process, CO_2 is used in a gas, liquid, or supercritical state to supersaturate an organic solution containing dissolved solutes such as drugs and polymers, expand it, and induce the precipitation or coprecipitation of the solutes[5]. The GAS process involves initially dissolving the solutes of interest in a suitable primary organic solvent. Then, a compressed gas, in which the solutes are insoluble but either miscible or partially miscible with the organic phase, is gradually introduced into the chamber filled with the solution. The basis of this method depends on the ability of organic solvents to solubilize large amounts of gases. This solubilization produces a large volumetric expansion of the liquid phase (several folds) and a decrease in its density by up to a factor of 2. The volumetric expansion is accompanied by a decrease in the liquid solvent strength (solubilization power), leading to the precipitation of the solute of interest[5,7].

The GAS process is a promising technique for the preparation of microparticles with distinct characteristics. It is a versatile, relatively inexpensive, and mild process that can be used to prepare a wide variety of microparticles with different properties. The GAS process has been successfully used to prepare microparticles of a variety of pharmaceutical active ingredients, including ibuprofen, naproxen, and indomethacin. These microparticles have shown improved dissolution rates and bioavailability compared to the corresponding bulk materials.

Ketoconazole (KET) (Figure 1), which has a molecular weight of 531.43, is a synthetic imidazole derivative used to treat local and systemic fungal infections. KET is a weak dibasic compound with pKa of 6.51 and 2.94. The intrinsic aqueous solubility of KET is 4.5 µg/mL at 37°C[8]. It has a partition coefficient (log P) in octanol-water of 3.73. It is categorized as a BCS class II drug with low solubility and high permeability[9-10]. It is almost insoluble in water and exhibits strong pH-dependent solubility[9]. It has low solubility at a higher pH of the intestinal fluid but possesses high solubility at a lower pH of the gastric fluid. This results in erratic absorption and a broad range

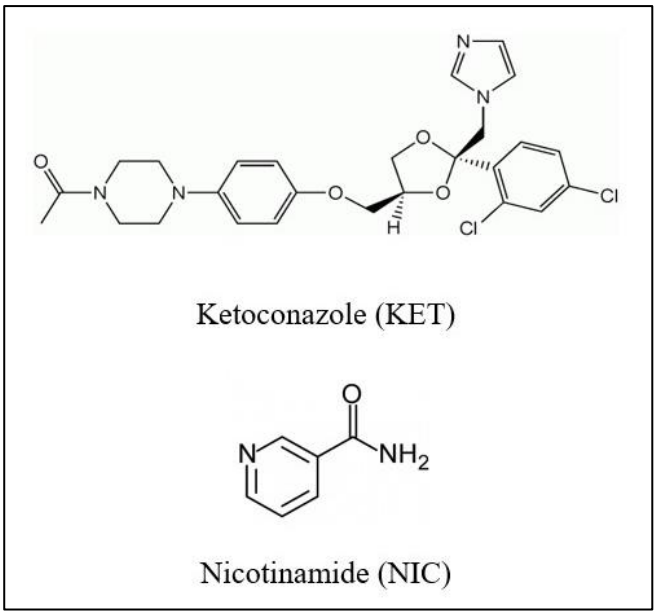

Figure 1. Chemical structure of ketoconazole (KET) and nicotinamide (NIC).

of bioavailability, ranging from 37% to 97%[11-13].

Therefore, an attempt to improve the oral bioavailability of KET by enhancing drug dissolution has been made. Many strategies have been used to improve the dissolution of KET. The amorphous solid dispersion of KET with some polymers such as methacrylic acid ethyl acrylate copolymer (Eudragit L100-55)[10,14], and polyvinylpyrrolidone K25[15] exhibited improved dissolution. The cocrystals of KET with 4-amino benzoic acid[16], succinic acid, fumaric acid, and adipic acid[17] also showed enhanced dissolution of KET. In addition, a eutectic solid dispersion of KET with nicotinamide (NIC) (Figure 1) could improve drug dissolution[9]. Aggarwal and Jain[9] reported the preparation of the KET-NIC solid dispersion by the fusion method. The solubility of KET increased with increasing concentrations of NIC. The drug dissolved from the KET-NIC solid dispersion was 6-fold higher than the pure drug in pH 6.8 phosphate buffer. The DSC study indicated that KET and NIC formed a eutectic system at the KET-NIC ratio of 70:30, but the eutectic phase diagram was not reported.

In this study, it was the first time that the supercritical fluid-based process was employed to improve the dissolution of KET. The GAS process was applied to prepare the coprecipitated particles of KET with a water-soluble carrier, NIC. NIC is a hydrotropic agent known to increase the solubility of poorly water-soluble drug substances. It has two attractive advantages such as approved by the US Food and Drug Administration (FDA), and very low toxicity[9,18]. The eutectic phase diagram of KET and NIC was constructed. The coprecipitated particles prepared by the GAS process were characterized by scanning electron microscopy (SEM), differential scanning calorimetry (DSC), Fourier transform infrared (FTIR) spectroscopy, and powder X-ray powder diffractometry (PXRD). The

dissolution behavior of the KET-NIC coprecipitated particles was also investigated. The solid dispersion of KET with NIC was also prepared using the solvent evaporation method and compared with the GAS coprecipitated particles.

2. MATERIALS AND METHODS

2.1. Materials

Ketoconazole (KET) was purchased from Crosschem Intercontinental Company, Switzerland. Nicotinamide (NIC) was obtained from Fluka, Switzerland. Carbon dioxide (high purity grade) was purchased from Thailand Industrial Gas Co. Ltd., Thailand. Methanol (99.9%) was supplied by RCI Labscan Ltd., Thailand. Ethanol, absolute (99.9%) and acetone (99.8%) were purchased from Merck, Germany. Dimethyl sulfoxide (99.5%) was obtained from Riedel-de Haën, Germany. Triethylamine (HPLC grade) was purchased from Fisher Scientific, United Kingdom. Methanol (HPLC grade) for HPLC assay was obtained from RCI Labscan Ltd., Thailand. All other chemical reagents were analytical grade.

2.2. Determination of eutectic point

Binary phase diagram of KET and NIC was constructed by thermal analysis. Different weight ratios of KET and NIC of 10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9, and 0:10 were mixed using mortar and pestle. Each sample was subjected to DSC measurements to determine the eutectic formation. Approximately 2-3 mg of the mixture were accurately weighed into a standard aluminum pan and heated at a heating rate of 5°C/min under nitrogen gas flow of 20 mL/min using DSC instrument (DSC-7, PerkinElmer, Norwalk, Connecticut, USA). the onset melting and completion melting temperature were determined. In the phase diagram construction, the onset temperature of the first endothermic event was determined as the solidus point, and the end of the second endothermic event was used as the liquidus point.

2.3. Gas anti-solvent (GAS) process

The schematic diagram of the GAS apparatus was presented in Figure 2. The drug solution was transferred to a precipitation vessel which was immersed in a water bath to maintain temperature at 35°C. After that, the compressed CO_2 was pumped into the precipitation vessel (Jerguson sight gauge series No. 32, USA) using high pressure syringe pump (260D, ISCO, USA) through a 0.5 mm filter at the bottom of the vessel. The drug solution was pressurized to the desired pressure by compressed CO_2. After the end of the precipitation step, the $scCO_2$ was delivered into precipitation vessel to remove the residual solvent from the precipitated particles. Before harvesting the precipitated particles, the precipitation vessel was depressurized to atmospheric pressure.

2.4. Preparation of KET-NIC coprecipitated particles by the GAS process

2.4.1. Determination of apparent solubility in organic solvents

KET was added to a vial containing 1 mL of organic solvent. Methanol, ethanol, dimethyl sulfoxide (DMSO), or acetone. KET was added stepwise 2 mg to the vial until undissolved particles were observed. The vials were tightly closed and placed in a thermostatically shaking bath (SBD-50, HETO, Denmark) with a shaking frequency of 50 strokes/min at 35°C for 24 h. The maximum concentration at which KET was completely dissolved in the organic solvent, and the consecutive lower concentration at which KET powder remained in the bottle was recorded as a range of the solubility of KET in each organic solvent. In addition, apparent solubility of NIC was also conducted in the same manner as KET.

2.4.2. Investigation of threshold pressures (Cloud points)

The drug solution was prepared by dissolving it in each organic solvent at 25% and 75% of its maximum solubility. The 5 mL of solution was introduced into the precipitation vessel (Jerguson sight gauge series No.32)

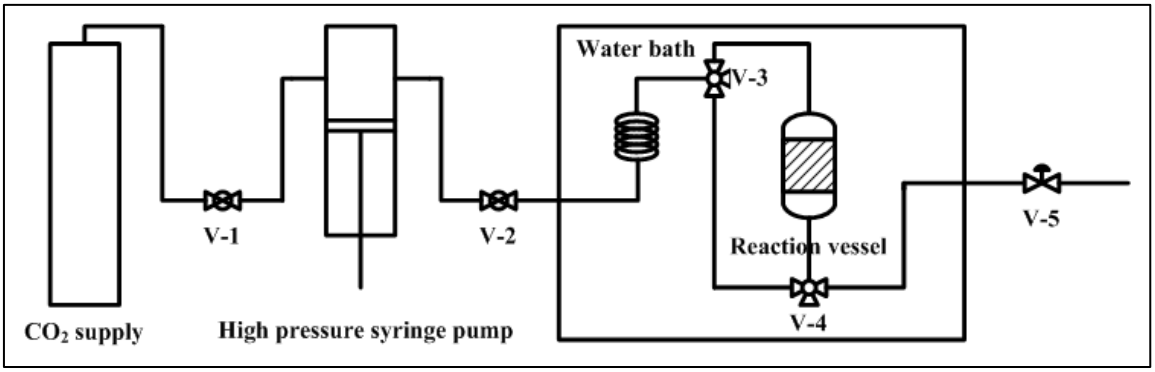

Figure 2. Schematic diagram of the experimental GAS process.

at 35°C. When the temperature was reached, the compressed carbon dioxide was fed at a constant flow rate of 10 mL/min. The minimum pressure at which solute particles commenced to precipitate was recorded as "threshold pressure".

The pressure was continuously increased over the threshold pressure of around 10-20 bar. The precipitated particles were washed with pure $scCO_2$ at that final pressure to remove the residual solvent. The pressure in the precipitation vessel was released to atmospheric pressure until the end of the washing period. Finally, the precipitated particles were collected on the frit filter (0.5 µm pore size) and stored in a cool and dry place.

2.4.3. Preparation of coprecipitated particles

The KET-NIC mixtures at the ratios of 1:1, 1:1.5, 1:2 and 1:4 (w/w) were dissolved in ethanol. The KET content was fixed at 75% of its solubility in ethanol, while NIC was varied in weight ratio with KET. The GAS process was carried out in the same manner as described in section 2.3. The 5 mL of KET-NIC ethanolic solution was employed in the GAS precipitation process. The coprecipitated particles were stored in a cool-dry place prior to characterization.

2.5. Preparation of KET-NIC solid dispersion by solvent evaporation

KET and NIC at the ratio of 1:1.5 (w/w) were dissolved in 50 mL of ethanol. Then the solvent was removed under vacuum (0.8 bar) to prepare solid dispersion. Then, the solid dispersion was sieved and stored in a silica gel desiccator at room temperature.

2.6. Preparation of KET-NIC physical mixture

KET and NIC were sieved and accurately weighed at predetermined ratios of KET to NIC, namely 10:90, 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, 80:20, 90:10, and 95:5 (w/w). The mixtures were mixed using mortar and pestle and stored in a desiccator at ambient temperature.

2.7. Solid-state characterization

2.7.1. Scanning electron microscopy (SEM)

Morphology of particles was examined using a scanning electron microscope (JSM-6300, Jeol, Tokyo, Japan)). The samples were fixed on a stub with conductive double-sided adhesive tape and then coated with gold in an argon atmosphere.

2.7.2. Differential scanning calorimetry (DSC)

Thermal properties of KET and its coprecipitated particles were evaluated using DSC equipment (DSC-7, PerkinElmer, Norwalk, Connecticut, USA). Approximately 2-3 mg samples were accurately weighed into a standard aluminum pan and heated from 50 to 200°C at a heating rate of 5°C/min under nitrogen gas purge of 20 mL/min.

2.7.3. Fourier transform infrared (FTIR) spectroscopy

A Nicolet FT-IR spectrometer equipped with a DLaTGS detector (Nicolet FT-IR 6700, Thermo Electron Scientific Instruments, Madison, Wisconsin, USA) was used for investigating molecular interaction. Samples were mixed and ground with dry KBr powder using mortar and pestle and then compressed into disks by means of a hydraulic press. The spectrum data were recorded in the transmission mode over the range of 400 to 4,000 cm^{-1} with a resolution of 4 cm^{-1} and analyzed by Omnic™ 8.0.342 software.

2.7.4. Powder X-ray diffraction (PXRD)

The PXRD patterns were measured using powder X-ray diffractometer (AXS D76181, Bruker, Karlsruhe, Germany). Samples were irradiated with monochromatized Cu Kα radiation (1.542 Å) and analyzed between 2° and 40° 2θ. The voltage and current used were 30 kV and 30 mA, respectively. The range and the chart speed were 2×10^3 CPS and 10 mm/degree 2θ, respectively.

2.8. Determination of drug content

The content of KET was determined using HPLC with UV/VIS detector. The HPLC system (Shimadzu, Japan) consisting of an auto injector equipped with an autosampler, high pressure pump (LC-10ATvp, Shimadzu, Japan), a UV detector (SPD-10Avp, Shimadzu, Japan) and a HPLC controller (SCL-10Avp, Shimadzu, Japan), and C18 reverse phase column (4.6 mm x 25cm) were used. The mobile phase was comprised of methanol (82% v/v) and 0.05 M triethylammonium phosphate buffer pH 7.0 (18% v/v). The conditions of analysis were as follows: 20 µL of injection volume, PDA detector at 230 nm, flow rate of 1 mL/min[19].

2.9. Determination of drug solubility

The solubility of KET was determined in three media: 0.1 N HCl, 0.05 M acetate buffer pH 4.5 and 0.05 M phosphate buffer pH 6.8. An excess amount of KET was added to test tubes containing 20 mL of medium and agitated 50 strokes/min at 37.0±0.5°C for 24 h. The supernatant was filtered through a 0.45 µm syringe filter. After that, the filtrate was assayed for KET using HPLC method as described in section 2.8.

2.10. *In vitro* dissolution study

Powder dissolution tests were performed using dissolution test apparatus II (SR 8 Plus, Hanson, Chatsworth, California, USA). The dissolution test was performed under sink conditions in 900 mL of 0.05 M phosphate buffer pH 6.8 with stirring speed of 50 rpm at 37.0±0.5℃. The samples were accurately weighed equivalent to 1.3 mg of KET. The KET content was determined using HPLC as described in section 2.8.

3. RESULTS AND DISCUSSION

3.1. Binary phase diagram of eutectic mixture

Binary phase diagram was constructed to determine the eutectic composition. DSC is a useful method for observing the phase transition behavior of a solid powder. The onset and completion of melting temperatures were obtained from DSC thermograms of the binary mixtures of KET-NIC with varying ratios of 10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9, and 0:10 (w/w). As shown in Figure 3, the onset of the first melting event was about 110℃. In addition, the minimum completion of the second melting event was 118.83℃ at a weight ratio of 70:30. This result suggests that the KET-NIC at a weight ratio of 70:30 (w/w) might be a eutectic point, which is consistent with the study by Aggarwal et al[9].

3.2. GAS process optimization

3.2.1. Apparent solubility of KET and NIC in organic solvents

Solubility is an important property for preparing the precipitated particles by the supercritical anti-solvent technique. Solubility data of KET and NIC in methanol, ethanol, DMSO, and acetone were presented in Table 1. The highest solubility of KET was 166 mg/mL in methanol and the lowest solubility of KET was 14 mg/mL in acetone. Whereas the highest solubility of NIC was 742 mg/mL in DMSO and the lowest solubility of NIC was 44 mg/mL in acetone.

3.2.2. Threshold pressures of KET and NIC in organic solvents

The threshold pressure is the minimum pressure at which solid particles begin to precipitate in a mixture of solute, solvent, and anti-solvent. It is a critical process parameter in the production of particles by the GAS process. The threshold pressures of KET and NIC at 25% and 75% of their maximum solubility in each organic solvent are shown in Figure 4a and Figure 4b, respectively.

In general, the higher concentration (75% of solubility) had a lower threshold pressure than the lower concentration (25% of solubility). This means that less

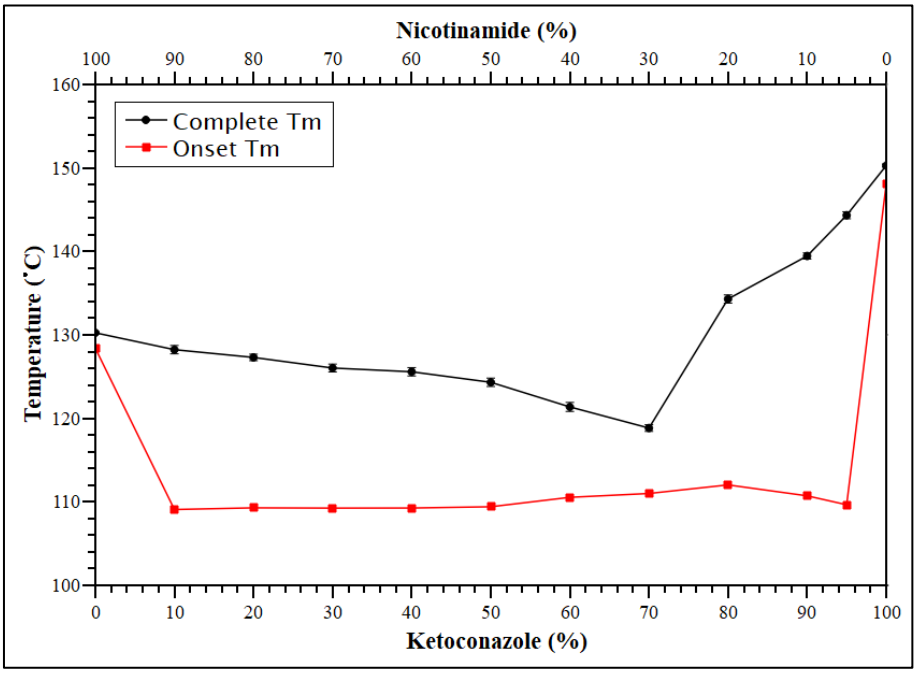

Figure 3. Eutectic phase diagram of ketoconazole-nicotinamide (KET-NIC) binary mixture (n=3).

Table 1. Solubility data of ketoconazole (KET) and nicotinamide (NIC) in organic solvents at 35°C.

	Apparent solubility (mg/mL)			
	Methanol	**Ethanol**	**DMSO**	**Acetone**
KET	166	36	44	14
NIC	360	154	742	44

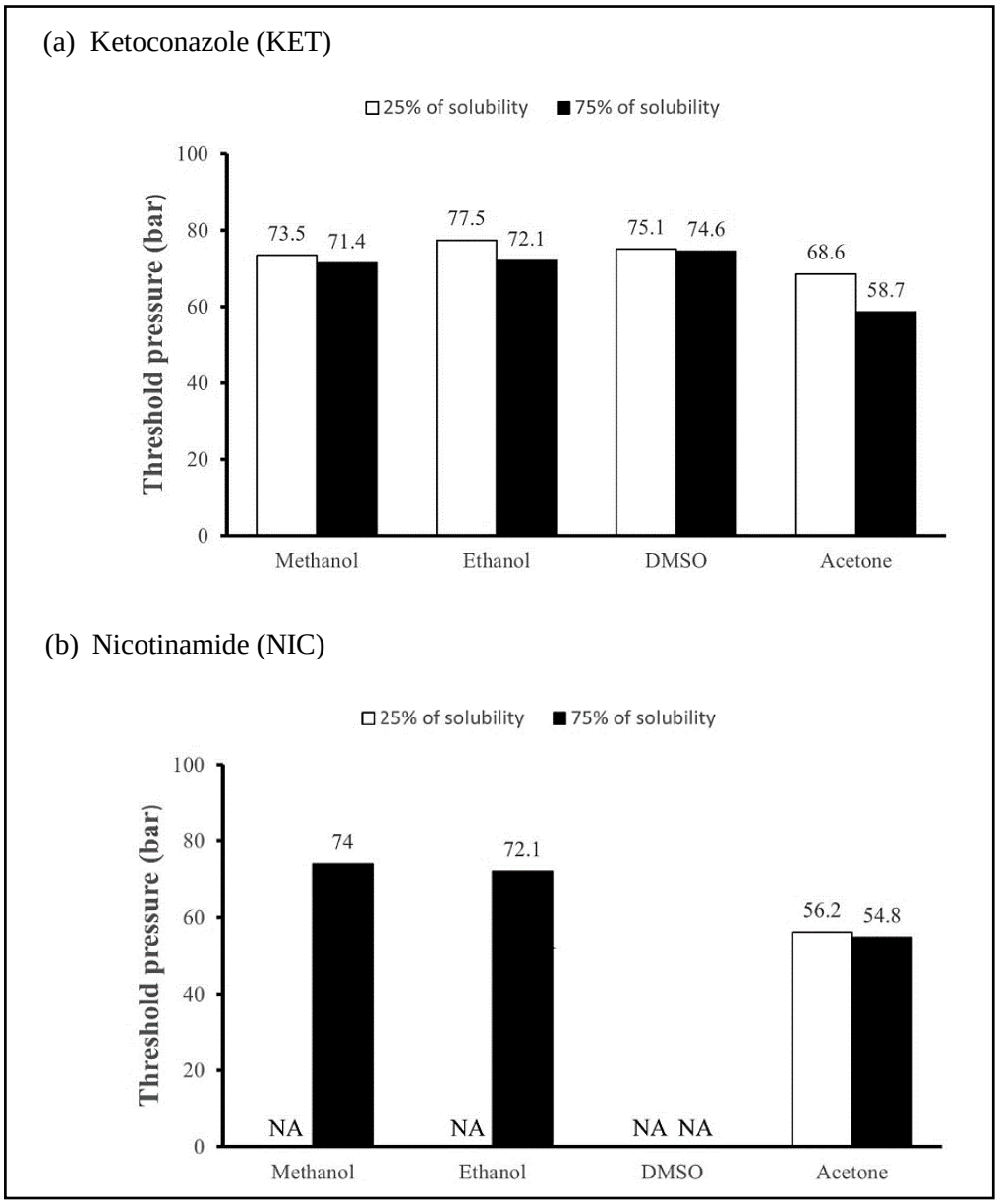

Figure 4. Threshold pressure of (a) ketoconazole (KET) and (b) nicotinamide (NIC) in organic solvents at 35°C.

$scCO_2$ is needed to generate the supersaturation of KET. This can be explained by the fact that less carbon dioxide is consumed to precipitate KET in the supersaturated solution. This result is consistent with the findings of Yeo et al.[20].

For NIC, the threshold pressure could not be observed at the concentration of 25% of maximum solubility in methanol and ethanol. However, coprecipitation occurred after the pressure was over 90 bar during the washing period. It is possible that it was difficult to observe the tiny nuclei in the three-phase mixture. In the case of NIC in DMSO, NIC is highly soluble. This may be due to the formation of a solvato-complex of NIC-DMSO, which is difficult to precipitate by anti-solvent molecules[21].

Ethanol was selected as a solvent for the preparation of KET-NIC coprecipitated particles by the GAS process due to its high production yield, desirable particle appearance, and ability to promote simultaneous coprecipitation. The ethanolic solution was prepared by fixing the KET concentration at 75% of its maximum solubility and varying the NIC concentration in different weight ratios.

3.3. Solid-state characterization of GAS-processed coprecipitated particles

3.3.1. Particle morphology

The unprocessed KET particles were irregular and highly agglomerated (Figure 5a). The unprocessed NIC particles were of irregular, distinct facets (Figure 5b). The KET-NIC solid dispersion was irregular and highly agglomerated, as shown in Figure 5c.

The coprecipitated particles from a KET-NIC ethanolic solution (1:1 w/w) appeared to have a dramatically wide size distribution and tended to aggregate into massive particles. (Figure 5d). The coprecipitated particles

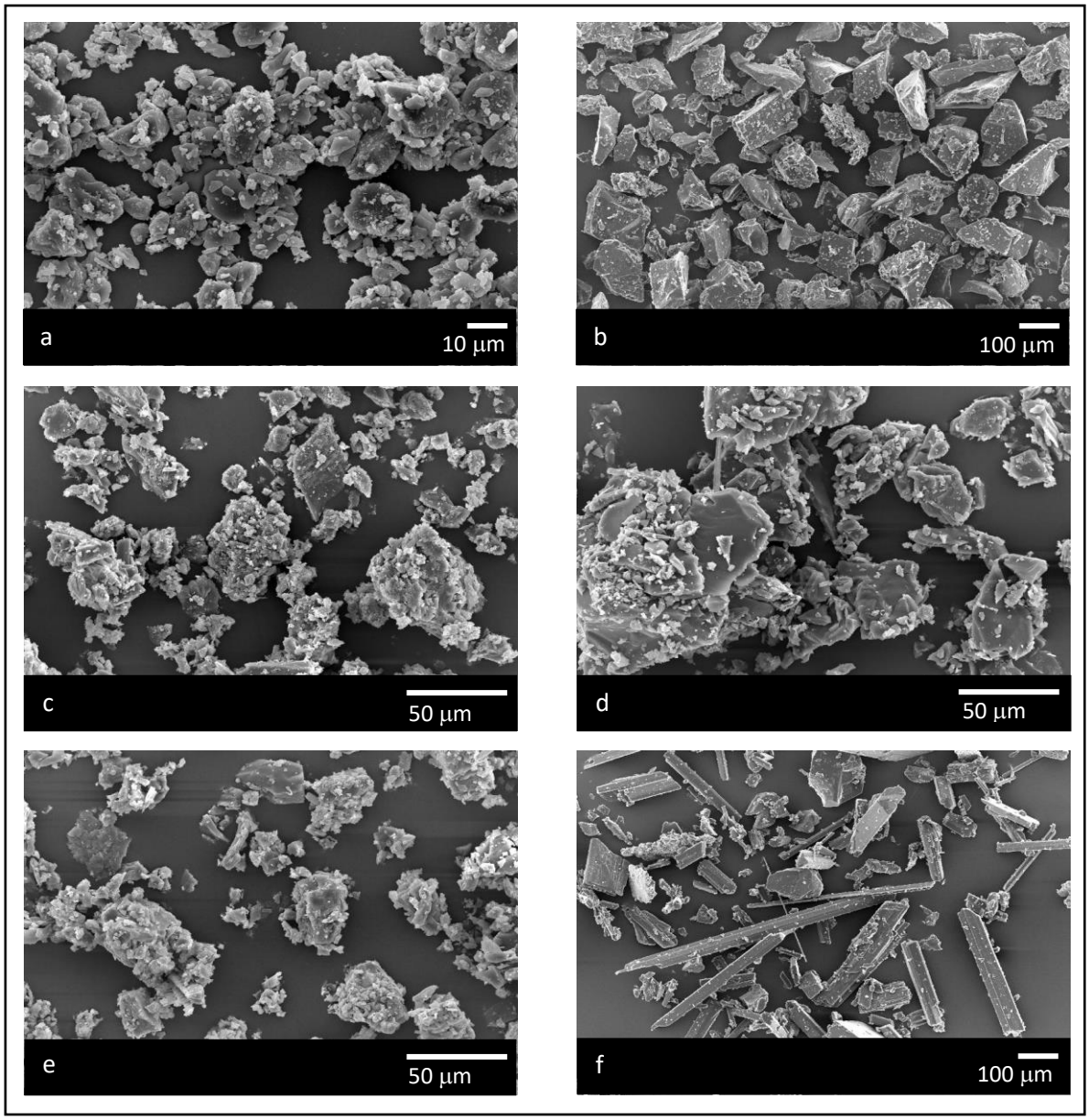

Figure 5. SEM photomicrographs of (a) ketoconazole (KET); (b) nicotinamide (NIC); (c) KET-NIC solid dispersion prepared by solvent evaporation; and KET-NIC coprecipitated particles prepared by GAS process at weight ratios of (d) 1:1, (e) 1:1.5, and (f) 1:2.

from a KET-NIC ethanolic solution (1:1.5 w/w) were irregular particles surrounded by smaller particles (Figure 5e). Additionally, the KET-NIC coprecipitated particles at weight ratios of 1:2 and 1:4 showed two different shapes: caking particles and long needle-shaped particles (Figure 5f). The long needle-like crystals are attributed to the excess amount of NIC, as they are similar to the particles obtained from the precipitation of NIC alone. Since NIC provides potential intermolecular hydrogen bonding between the hydrogen atom and oxygen atom in the amide group, the crystal growth of NIC inclines in one crystallographic direction, resulting in acicular crystals[22].

3.3.2. Thermal behavior

Figure 6 shows the DSC thermograms of KET-NIC coprecipitated particles prepared by the GAS process. The unprocessed KET and NIC showed an onset temperature of the melting peaks at 149.35°C (Figure 6a) and 130.01°C (Figure 6b), respectively. At a KET-NIC weight ratio of 85:15 (w/w), the onset temperatures of the melting peaks of the physical mixture and the solid dispersion were observed at 110.83°C and 108.87°C, respectively (Figure 6c-d). These onset temperatures were lower than those of unprocessed KET and NIC. These results are consistent with the binary phase diagram of KET-NIC, indicating a eutectic temperature of about 110°C.

The DSC thermogram of the GAS-processed coprecipitated particles, at a weight ratio of 1:1, showed a single endothermic peak with an onset of 147.33°C (Figure 6e). This onset temperature is attributed to the melting point of KET. It is indicated that at this condition only KET was precipitated, while NIC was completely extracted by the mixture of compressed carbon dioxide and ethanol.

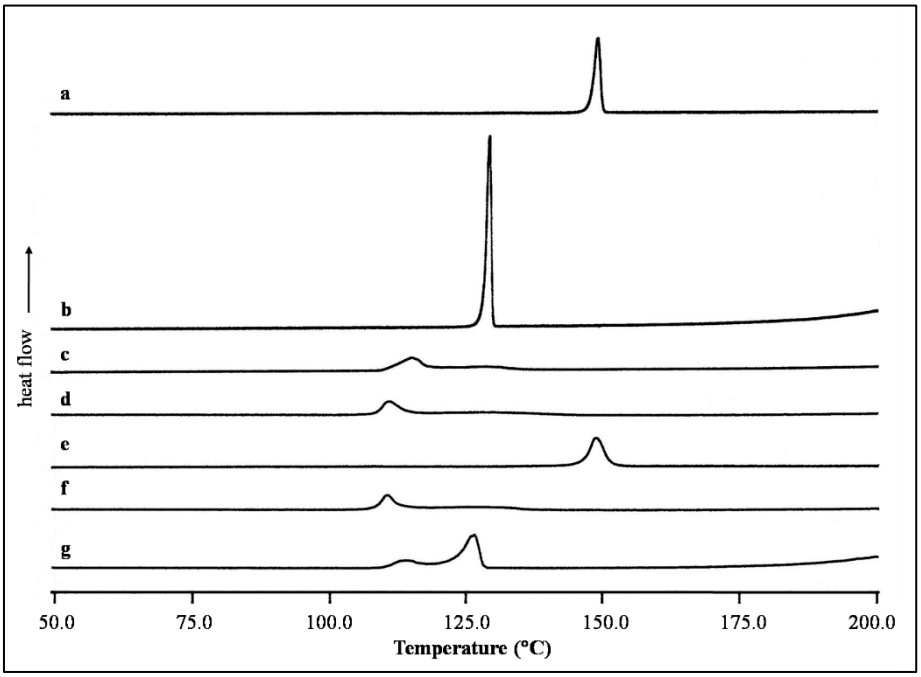

Figure 6. DSC thermograms of (a) ketoconazole (KET); (b) nicotinamide (NIC); (c) KET-NIC physical mixture (85:15 w/w); (d) KET-NIC solid dispersion (85:15 w/w). prepared by solvent evaporation; and KET-NIC coprecipitated particles prepared by the GAS process at weight ratios of (e) 1:1, (f) 1:1.5, and (g) 1:2.

This is because the KET-NIC ethanolic solution, at a weight ratio of 1:1 (w/w), contained NIC at a concentration of 17.5% of its saturated solubility. NIC is very soluble in ethanol (154 mg/mL), so it may not have reached its maximum solubility when 100 bars of $scCO_2$ were added. This result is similar to the previous finding that, at 25% of its saturated solubility in ethanol, NIC could not be precipitated at 100 bars.

At a weight ratio of 1:1.5, the DSC thermogram of the KET-NIC coprecipitated particles prepared by the GAS method showed an onset temperature of eutectic melting at 108.65°C and a small broad endothermic peak at about 128°C (Figure 6f), which was attributed to the excess KET. The results of the drug assay showed that these particles contained 84.57±0.25% (n=3) of KET. The eutectic of KET and NIC was formed at weight ratio of 70:30 (Figure 3), therefore the excess KET crystals existed in these coprecipitated particles. It was seen that the GAS-processed coprecipitated particles at a weight ratio of 1:1.5 were highly close to the eutectic system, thus they were selected for further characterization and dissolution study.

In addition, the DSC thermogram of the GAS-processed coprecipitated particles, at a weight ratio of 1:2, showed two distinct overlapping endothermic peaks (Figure 6g). The first peak was the peak of the eutectic mixture, and the consecutive peak was attributable to the excess NIC. This finding correlated well with the two different morphologies of particle observed in SEM photomicrograph (Figure 5f).

As the dug content in GAS-coprecipitated particles at weight ratio of 1:1.5 was 84.57±0.25%, the weight ratio of 85:15 was selected to prepare the solid dispersion with equal amount of KET. The thermogram of KET-NIC solid dispersion, 85:15 (w/w), obtained from solvent evaporation showed a similar eutectic melting peak to that of KET-NIC coprecipitated particles, 1:1.5 (w/w), prepared by the GAS process (Figure 6d). In addition, the drug content in the solid dispersion was analyzed and found to be 84.66±0.35%.

3.3.3. FTIR spectroscopy

FTIR spectroscopy was used to detect the molecular interaction in coprecipitated samples. The characteristic absorption bands of KET (Figure 7a) are ν(C=O) at 1,646.8 cm^{-1} (amide group), ν(C=C) and ν(C=N) at 1,584.8 cm^{-1}, ν(C-N) at 1,372.5 cm^{-1}, and ν(C-O-C) at 1,244.0 cm^{-1} [23]. The dominant absorption bands of NIC (Figure 7b) are ν(N-H) at 3,366.9 and 3,163.0 cm^{-1}, ν(C=O) at 1,681.8, and ν(C-N) at 1,395.3 cm^{-1} [24].

The FTIR spectra of the solid dispersion contained a combination of absorption bands of KET and NIC (Figure 7d). The GAS-processed coprecipitated particles also showed similar spectra to those of solid dispersion (Figure 7e). The band positions of both solid dispersion and the coprecipitated particles were not significantly different from those of the physical mixture (Figure 7c). FTIR results indicated no molecular interaction between KET and NIC in the coprecipitated particles and solid dispersion. It is suggested that the intermolecular bonds between the compounds in the eutectic mixture are weak[25]. Therefore, FTIR spectroscopy could not clearly detect the molecular interaction between KET and NIC.

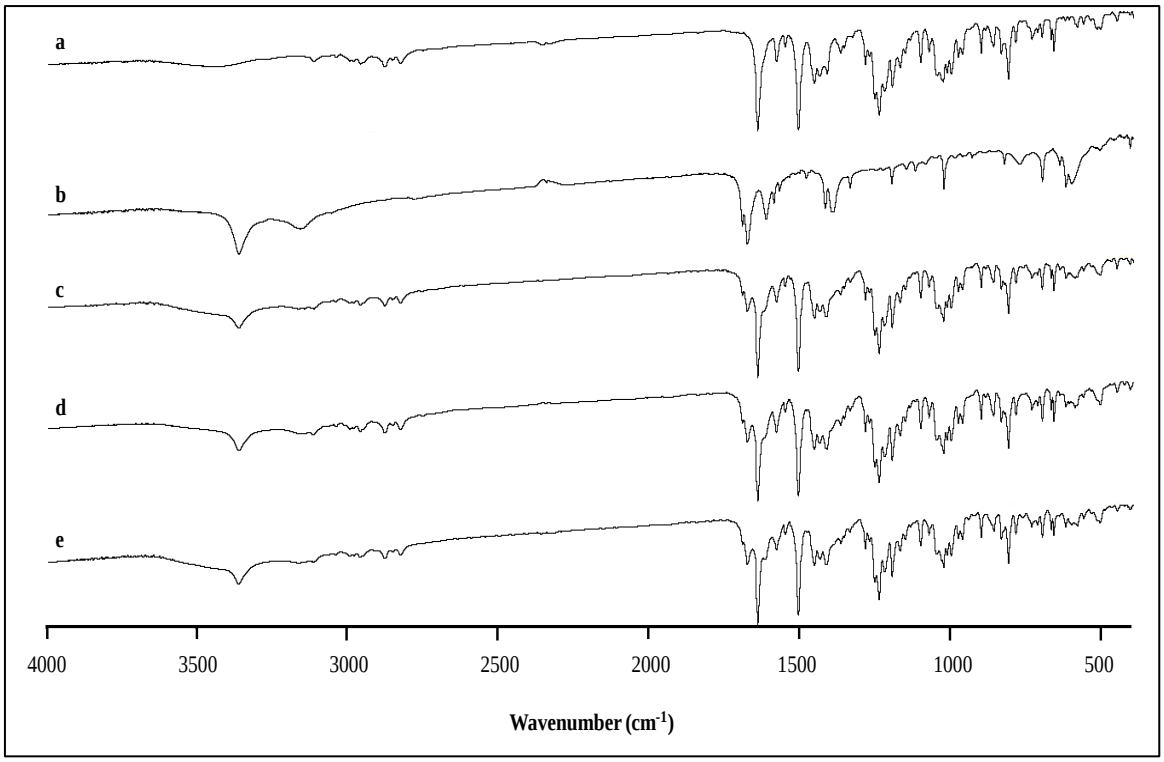

Figure 7. FTIR spectra of (a) ketoconazole (KET); (b) nicotinamide (NIC); (c) KET-NIC physical mixture (85:15 w/w); (d) KET-NIC solid dispersion (85:15 w/w) prepared by solvent evaporation; and (e) KET-NIC coprecipitated particles prepared by the GAS process at weight ratio of 1:1.5.

3.3.4. Powder X-Ray diffraction

The PXRD technique was employed to investigate crystalline properties of KET-NIC particles. The PXRD patterns of KET, NIC, KET-NIC physical mixture, KET-NIC solid dispersion prepared by the solvent evaporation method and KET-NIC coprecipitated particles prepared by the GAS process were depicted in Figure 8. The PXRD patterns of KET-NIC coprecipitated particles prepared by the GAS process consisted of characteristic diffraction peaks of KET and NIC. Additionally, all crystalline peaks of GAS coprecipitated particles were similar to those of physical mixture and solid dispersion prepared by the solvent evaporation method. It was noted that both GAS coprecipitated particles and solid dispersion had diffraction peaks with relatively lower intensity than the physical mixture, except the peak of solid dispersion at 2θ=24°. The dominant peaks at 2θ=24° of solid dispersion might be due to the excess KET crystals that were not form eutectic mixture with NIC. As a result, the lower in peak intensity might be attributed to the relative low crystallinity of the eutectic coprecipitates from both methods. A random distribution of the molecules of one component into the crystal lattice of the other one occurs during eutectic mixture formation. This leads to minor changes in the diffraction pattern of the eutectic mixture[26]. Therefore, in a simple eutectic mixture the diffraction peaks of each crystalline component can be found in the diffraction patterns. Additionally, whether solid solution formation occurs, typical diffraction peak of a minor component should not be able to observe whereas the lattice parameters like diffraction peak angles of the solvent crystal can be either increased, unchanged, or decreased[27]. Corresponding to the DSC and FTIR results, KET-NIC binary system can be correctly indicated as a simple eutectic mixture by discarding solid solubility. The occurrence of new and distinct diffractogram is the evidence of the formation of a new crystal phase, what is not observed in eutectic mixtures. Similarly, all the characteristic peaks of the components were preserved in the diffraction pattern of diacerein/fumaric acid eutectic mixture with lower intensity than the physical mixture and this might be due to reduction in the crystallinity[28].

3.5. *In vitro* powder dissolution

Solubility of ketoconazole in 0.1 N HCl, pH 4.5 acetate buffer, and pH 6.8 phosphate buffer was 37.062, 0.032 and 7.697×10^{-3} mg/mL, respectively. The solubility of KET decreased with increasing pH of medium and exhibited strong pH-dependent solubility[12]. The solubility values obtained correlated relatively well with the previous reports. The intrinsic aqueous solubility of KET is 4.5 μg/mL at 37°C[8]. Kalantzi et al. reported that the solubility of KET in pH 6.5 phosphate buffer was 6.9 μg/mL[29]. In the European Pharmacopeia, sink conditions are defined as a volume of dissolution medium that is at least three to ten times the saturation volume[30]. Thus, the 900 ml of phosphate buffer pH 6.8 was considered as sink condition thorough this study.

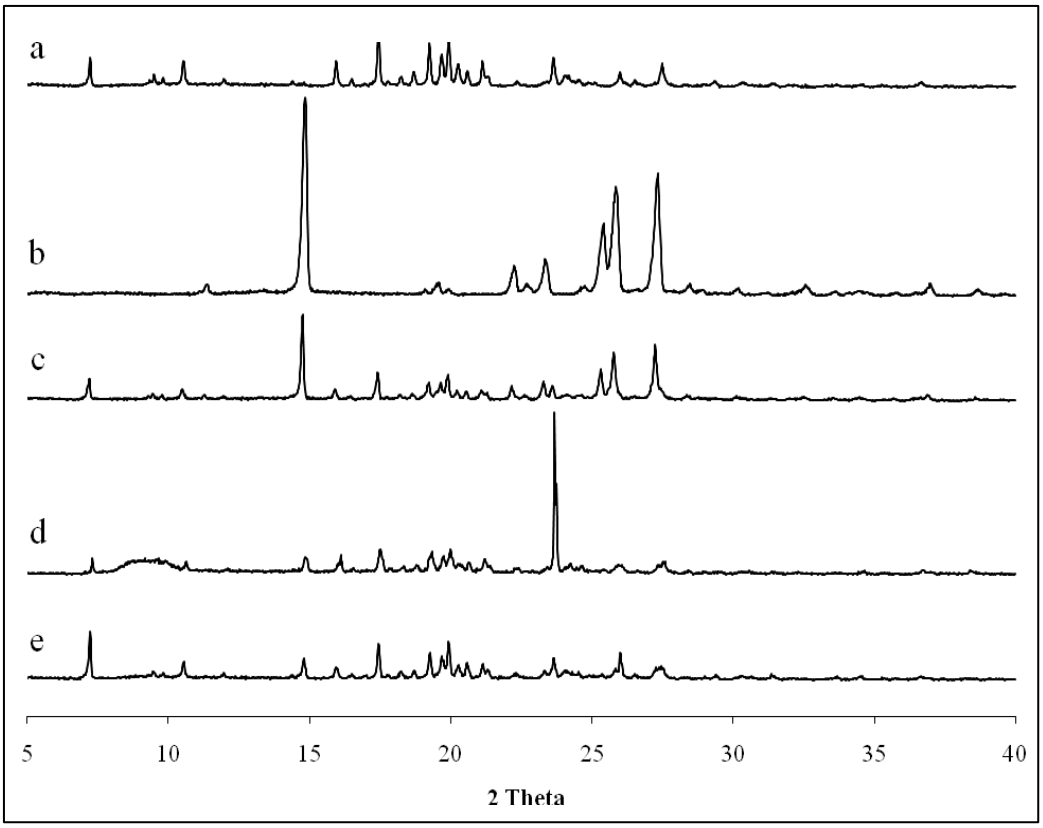

Figure 8. PXRD patterns of (a) ketoconazole (KET), (b) nicotinamide (NIC), (c) KET-NIC physical mixture (85:15 w/w); (d) KET-NIC solid dispersion (85:15 w/w) prepared by solvent evaporation, and (e) KET-NIC coprecipitated particles prepared by the GAS process at weight ratio of 1:1.5.

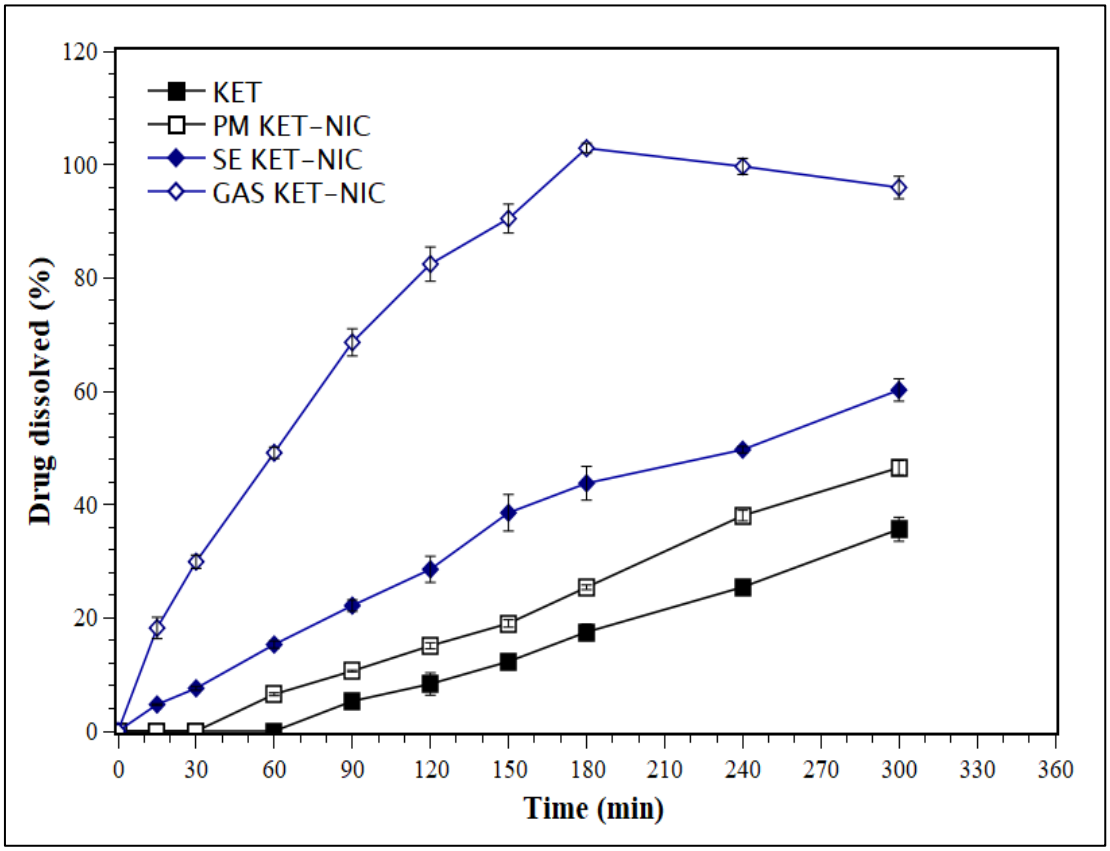

Figure 9. *In vitro* powder dissolution profiles of ketoconazole (KET); (b) KET-NIC physical mixture (PM) (85:15 w/w); (d) KET-NIC solid dispersion (85:15 w/w) prepared by solvent evaporation (SE); and (e) KET-NIC coprecipitated particles (1:1.5 w/w) prepared by the GAS process in pH 6.8 phosphate buffer (n=3).

The powder dissolution profiles of unprocessed KET, physical mixture, KET-NIC solid dispersion prepared by solvent evaporation and KET-NIC coprecipitated particles prepared by the GAS process are shown in Figure 9. The percentage of unprocessed KET dissolved in pH 6.8 phosphate buffer was low, approximately 35.62% at 300 min. In addition, the dissolution of physical mixture, KET-NIC solid dispersion prepared by solvent evaporation and KET-NIC coprecipitated particles prepared by the GAS process was 46.50%, 60.21%, and 95.95%, respectively. The percentage of drug dissolved from the coprecipitated particles by the GAS process was significantly higher than solid dispersion prepared by solvent evaporation, physical mixture, and unprocessed KET.

The higher dissolution of physical mixture compared to unprocessed KET is attributed to the solubilizing effect of NIC[9]. NIC is a hydrotropic agent used to enhance the aqueous solubility of a variety of drugs such as flurbiprofen, nimesulide, curcumin and felodipine[25]. The increase in the dissolution rate of physical mixture is attributed to a local solubilization effect, produced by the NIC in the diffusion layer immediately surrounding the drug particles. Furthermore, this effect is thought to be due to the ability of NIC to reduce the surface tension of the dissolution medium, which allows the drug particles to dissolve more easily.

The enhancement in dissolution of solid dispersion prepared by solvent evaporation over physical mixture is attributed to an increase in the surface area of the drug exposed to the dissolution medium. When exposed to the dissolution medium, the solid dispersion might produce smaller drug particles than physical mixture, resulting in a larger surface area for dissolution[27]. Despite comparable KET content in both GAS coprecipitated particles and solid dispersion, the dissolution of solid dispersion was remarkably lower than that of GAS coprecipitated particles. From the DSC results, the solid dispersion was composed of eutectic mixture and excess KET crystals. The lower dissolution of solid dispersion might be attributable to the higher fraction of excess KET in the coprecipitates. Furthermore, during solvent evaporation, the solubilities of KET and NIC in ethanol are quite different. As a result, the two substances may precipitate separately, leading to a dissolution profile that is not much better than that of physical mixture.

Meanwhile, the coprecipitated particles prepared by the GAS process completely dissolved in pH 6.8 phosphate buffer at 180 min. KET was completely released from the GAS coprecipitated particles at 180 min, however, a slightly decrease of the dissolved KET was then observed. It might be attributed to supersaturation of KET-NIC eutectic mixture and subsequent conversion to KET[31]. The GAS coprecipitates are eutectic mixture as confirmed by DSC, PXRD and FTIR in the previous section. Firstly, the dissolution enhancement of the GAS coprecipitated particles might be attributed to the size reduction of drug crystals during eutectic formation[9]. In addition, coprecipitation with water-soluble carrier may enhance the wettability and the hydrophilic microenvironment of KET crystals, leading to significantly increased drug dissolution. The SEM photomicrographs indicated that the KET-NIC (1:1.5 w/w) coprecipitated particles (Figure 5c) had smaller size than solid dispersion prepared by solvent evaporation (Figure 5e). The higher dissolution of the GAS coprecipitated particles might be due to lower relative crystallinity as observed in PXRD study (Figure 8e). When compared with the solvent evaporation method, it was suggested that more rapid and faster coprecipitation occurred in the GAS process, resulting in the coprecipitates with smaller drug crystals. When the GAS coprecipitated particles were exposed to dissolution medium, NIC rapidly dissolved and very fine drug crystals were released from the system. This led to a larger surface area for contacting with surrounding solution, resulting in higher dissolution when compared with the corresponding solid dispersion and physical mixture[32].

4. CONCLUSION

The KET-NIC coprecipitated particles with enhanced dissolution were successfully prepared by the GAS process. DSC study indicated KET formed eutectic mixture with NIC at a weight ratio of 7:3. The coprecipitated particles prepared by the GAS process exhibited significantly higher drug dissolution than solid dispersion prepared by solvent evaporation, physical mixture, and unprocessed drug. The solid-state characterization revealed that the eutectic mixture of KET with NIC was formed in the coprecipitates prepared by GAS process and solvent evaporation. It was suggested the eutectic formation, the improved wettability and hydrophilic microenvironment by water-soluble carrier, the lower crystallinity and the smaller drug crystals were the main factors attributed to the enhanced dissolution of GAS coprecipitated particles.

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Conflict of interest

None to declare.

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NC: Data curation, Validation, Formal analysis, Visualization, Writing – Review and Editing.
SP: Conceptualization, Methodology, Validation, Formal analysis, Writing – review and editing, Supervision, Resources, Project administration, Funding acquisition.

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