Preparation and physicochemical characterization of sildenafil cocrystals

Sildenafil is a specific inhibitor of the phosphodiesterase type 5 (PDE-5) enzyme that protects cyclic guanosine monophosphate from breakdown by PDE-5. It is a biopharmaceutical categorization system Class II medication with low bioavailability because it is almost insoluble in water. The objectives of this study were to prepare sildenafil cocrystals with co-former molecules including aspirin (acetylsalicylic acid [ASA]), fumaric acid (FMA), and benzoic acid (BZA) to improve the water solubility of sildenafil. The cocrystals were prepared by antisolvent addition (AA) and slow solvent evaporation (SE) methods. The stoichiometric ratios of sildenafil and co-former molecules were varied. The obtained crystals were characterized by stereomicroscope, Fourier transformed infrared spectroscopy (FT-IR), nuclear magnetic resonance (NMR), and powder X-ray diffraction (PXRD). The water solubility of sildenafil cocrystals was compared with sildenafil base. In the AA method, the crystals only form in sildenafil-ASA reaction. These crystals were not cocrystals between sildenafil and ASA because they were formed to new substances that were confirmed by single-crystal X-ray diffraction. In the SE method, the cocrystals were successfully prepared in the reaction of sildenafil with ASA, FMA, and BZA which use acetone or ethyl acetate as a solvent. The obtained crystals are irregular shapes and their FT-IR, NMR, and PXRD results exhibited the characteristics of sildenafil and its co-former. The stoichiometric ratios of sildenafil and co-formers after cocrystallization were different from an initial of crystallization. The sildenafil cocrystals with ASA, FMA, and BZA by SE method had higher water solubility than sildenafil base. The sildenafil-FMA cocrystals had the highest water solubility and increased up to five times when compared with sildenafil base.

Key words: Aspirin, benzoic acid, cocrystal, fumaric acid, physicochemical properties, sildenafil, solubility

INTRODUCTION

Sildenafil [Figure 1] is a phosphodiesterase type 5 enzyme inhibitor that is selective. Sildenafil’s methods of action break down cyclic guanosine monophosphate, causing smooth muscle relaxation via the NO pathway. Sildenafil is a drug that is prescribed for erectile dysfunction, pulmonary hypertension, and premature ejaculation. Sildenafil is water insoluble
and has a high membrane permeability, making it a biopharmaceutical categorization system Class II substance. Despite the fact that the traditional sildenafil product was produced as a citrate salt to boost water solubility (4.1 mg/mL), it still has a low oral bioavailability (40%) and a slow onset of action.\textsuperscript{[4,6-9]}

One of the major issues facing the pharmaceutical industry right now is improving medication solubility. Solids that are structurally homogeneous crystalline single-phase materials with two or more components present in definite stoichiometric amounts that are neither solvates nor simple salts are known as cocrystals. The cocrystal components are discrete neutral molecular reactants which are solids at ambient temperature.\textsuperscript{[10,11]} A pharmaceutical cocrystal must have one active pharmaceutical ingredient (API) and a pharmacologically approved cocrystal former as its components. Pharmacologically, selected cocrystal formers should have no negative or harmful consequences.\textsuperscript{[11,12]} Cocrystal is a simple technique that can improve the physicochemical properties of the API, such as melting point, solubility, dissolution rate, bioavailability, stability, and mechanical properties.\textsuperscript{[13-15]}

Sildenafil contains a N-methylpiperazine fragment, and the N atom (attached to methyl group) is the most basic site (pK\textsubscript{a} = 8.7) and least hindered to form a hydrogen bond (in cocrystal) or ionic bond (in salt).\textsuperscript{[4,16]} The literature shows that sildenafil and sildenafil citrate are made cocrystals with carboxylic acid or dicarboxylic acid and enhance solubility or dissolution rate.\textsuperscript{[4,16]} The co-former molecules containing acidic moieties could increase the chance of cocrystallization with basic site by hydrogen bonding.\textsuperscript{[17]} The authors choose acetylsalicylic acid or aspirin (ASA), fumaric acid (FMA), and benzoic acid (BZA) [see chemical structure in Figure 2] as a co-former molecule due to they are pharmaceutical acceptance and also report successfully prepared cocrystals. Even though we use the same substances to form cocrystals according to previous literature reports, we prepare in a different way of cococrystallization methods. The objective of this study is to observe the cocrystals obtained from selected co-former molecules when prepared by several techniques with different stoichiometric ratios. The cocrystals are characterized by Fourier transform infrared (FT-IR), differential scanning calorimetry, powder X-ray diffraction (XRD), and solubility of cocrystals obtained.

## MATERIALS AND METHODS

### Materials

Sildenafil citrate and reference standard (potency 99.4% as is) were obtained from Smilax Laboratories Limited (Hyderabad, India). Aspirin and BZA were purchased from P. C. Drug Center Co. Ltd, Bangkok, Thailand. FMA and acetone were purchased from Merck KGaA, Germany. Acetonitrile, dimethyl sulfoxide, and ethanol were procured from Sigma-Aldrich, (St. Louis, MO, USA). Methanol, ethyl acetate, and all reagents were supplied by Labscan (Bangkok, Thailand). Potassium bromide and other excipients were purchased from PC Drug Center Co., Ltd. (Bangkok, Thailand). All chemical compounds and all reagents were used as received except sildenafil (base) was prepared in the laboratory.

### Crystallize sildenafil (base) from sildenafil citrate

Free base sildenafil was prepared by adaptation from sildenafil citrate according to a previous report.\textsuperscript{[4]} Briefly, sildenafil citrate in an excess amount was slurry in 0.05 M NaOH solutions for 2 days with stirring by magnetic stirrer. The free base sildenafil was crystallized from methanol as parallelogram plate crystals and compared the crystal appearance that described elsewhere.\textsuperscript{[18]} The sildenafil crystals are also confirmed by nuclear magnetic resonance (NMR) results.

### Preparation of sildenafil cocrystals

Sildenafil cocrystal with ASA, FMA, and BZA was prepared by two methods which included antisolvent addition and slow solvent evaporation methods.

#### Antisolvent addition method

Sildenafil solution 0.04 M in dimethyl sulfoxide (DMSO) was prepared. Co-former molecules were dissolved in several solvents (acetonitrile, acetonitrile, ethanol, methanol, and ethyl acetate) at a concentration of 0.1 and 0.2 M. The lower density solution between the sildenafil solution and the co-former molecule solution was gently poured into another one. The resulting solution was separated and stored in the reaction beaker at room temperature until crystals or cocrystals were formed [reaction numbers 1-10 and 26-31, Table1]. Crystals or cocrystals were kept in an airtightly glass container and stored in a desiccator until further analysis. Stoichiometry ratio of sildenafil and co-former was varied to optimize the preparation method.

#### Slow solvent evaporation method

Equimolar amounts of sildenafil and co-former molecules were dissolved in several solvents (acetonitrile, acetonitrile, ethanol, methanol, and ethyl acetate) [reaction number 11–25 and 32–61, Table 1] under heating and constant stirring until clear solution was obtained. The resulting solution was allowed to cool to room temperature before the solvent was allowed to evaporate, yielding crystals or cocrystals. The crystals or cocrystals were carefully handled and stored in an airtight desiccator awaiting further investigation. To improve the preparation procedure, the stoichiometry ratio of sildenafil and co-former was changed. All experiment reactions are summarized in Table 1 in the results and discussion section.
Morphology of crystals
Small amounts of samples were collected by crystal mount and loop and placed on a glass slide cover with mineral oil. Morphology of crystals or cocrystals was observed and photographed by a stereomicroscope (SMZ161, Motic Asia, Hong Kong).

Fourier-transform infrared spectroscopy
Before measuring the IR spectra with an FT-IR spectrometer (Bruker Tensor 27, Bruker Corporation, MA, USA) at ambient temperature, a limited amount of samples were sealed into KBr pellets using a hydraulic press. In the frequency range of 4000–400/cm, the functional groups of sildenafil and sildenafil citrate monohydrate were measured.

Nuclear magnetic resonance
To obtain solutions for measurement, all of the investigated compounds were dissolved in DMSO and magnetically agitated for 4–6 h at room temperature. NMR spectrometer (Ascend 500/Avance Neo, Bruker Corporation, MA, USA) was used to record the characteristics of the $^1$H NMR chemical shifts of materials.

Powder X-ray diffraction
The powder X-ray diffractometry (PXRD) was undertaken to investigate the crystalline nature of sildenafil, aspirin, FMA, BZA, and prepared cocrystals. Powder XRD (XtaLAB Supernova, Rigaku Corporation, Tokyo, Japan) analysis was performed at room temperature (298 K) and Cu, Kα radiation at voltage 20 KV, 0.05 mA. The analysis was performed on the range of 2 theta of 0°C–70°C.

Saturation solubility of sildenafil cocrystals
Excess amounts of pure drug and cocrystals were dissolved in 10-mL vials containing water to determine the solubility of the resulting cocrystals. The vials were agitated on a rotary shaker for 24 h before being allowed to stand for equilibrations. According to a prior study, the materials were filtered after 24 h, diluted with mobile phase, and evaluated by high-performance liquid chromatography with a ultraviolet detector at 240 nm.\[^{[19]}\]

RESULTS AND DISCUSSION

Sildenafil base preparation
Although sildenafil citrate can be prepared cocrystals as reported in the literature,\(^ { [ 3 ] }\) salt form of sildenafil may be hard to form hydrogen bonding with co-former molecules. Therefore, the sildenafil base was prepared by crystallization from sildenafil citrate and used to cocrytallize with ASA, FMA, and BZA.

Figure 3 shows the difference of sildenafil citrate [Figure 3a] and sildenafil base [Figure 3b] powder collected from the elimination of citrate salt by reaction with NaOH. Sildenafil crystalline powder is off-white color with a particle size about 0.5 mm determined by an optical microscope. The $^1$H NMR spectrum of sildenafil citrate shows eight signals [Table 2] including of signal I: δ(ppm) 0.95 (−CH$_3$,
### Table 1: Full list of experimental methods and results

| Reaction number | Co-former molecules | Experimental conditions | Product obtained |
|-----------------|---------------------|-------------------------|------------------|
| 1               | ASA                 | 0.1 mM/acetonitrile/aa  | Needle crystals  |
| 2               | ASA                 | 0.2 mM/acetonitrile/aa  | Needle crystals  |
| 3               | ASA                 | 0.1 mM/ethanol/aa       | Plate crystals   |
| 4               | ASA                 | 0.2 mM/ethanol/aa       | Plate crystals   |
| 5               | ASA                 | 0.1 mM/methanol/aa      | Plate crystals   |
| 6               | ASA                 | 0.2 mM/methanol/aa      | Plate crystals   |
| 7               | ASA                 | 0.1 mM/ethyl acetate/aa | No crystal observed |
| 8               | ASA                 | 0.2 mM/ethyl acetate/aa | No crystal observed |
| 9               | ASA                 | 0.1 mM/isopropanol/aa   | Needle shape crystals |
| 10              | ASA                 | 0.2 mM/isopropanol/aa   | Needle shape crystals |
| 11              | ASA                 | 1:1/acetonitrile/RT/se  | No crystal observed |
| 12              | ASA                 | 1:2/acetonitrile/RT/se  | No crystal observed |
| 13              | ASA                 | 1:3/acetonitrile/RT/se  | No crystal observed |
| 14              | ASA                 | 1:1/ethanol/RT/se       | No crystal observed |
| 15              | ASA                 | 1:2/ethanol/RT/se       | No crystal observed |
| 16              | ASA                 | 1:3/ethanol/RT/se       | No crystal observed |
| 17              | ASA                 | 1:1/methanol/RT/se      | No crystal observed |
| 18              | ASA                 | 1:2/methanol/RT/se      | No crystal observed |
| 19              | ASA                 | 1:3/methanol/RT/se      | No crystal observed |
| 20              | ASA                 | 1:1/acetone/RT/se       | Irregular crystals |
| 21              | ASA                 | 1:2/acetone/RT/se       | Irregular crystals |
| 22              | ASA                 | 1:3/acetone/RT/se       | Irregular crystals |
| 23              | ASA                 | 1:1/ethyl acetate/RT/se | Irregular crystals |
| 24              | ASA                 | 1:2/ethyl acetate/RT/se | Irregular crystals |
| 25              | ASA                 | 1:3/ethyl acetate/RT/se | Irregular crystals |
| 26              | FMA                 | 0.001 mM/ethyl acetate/aa | No crystal observed |
| 27              | FMA                 | 0.002 mM/ethyl acetate/aa | No crystal observed |
| 28              | FMA                 | 0.003 mM/ethyl acetate/aa | No crystal observed |
| 29              | FMA                 | 0.002 mM/acetone/aa     | No crystal observed |
| 30              | FMA                 | 0.0032 mM/acetone/aa    | No crystal observed |
| 31              | FMA                 | 0.0048 mM/acetone/aa    | No crystal observed |
| 32              | FMA                 | 1:1/acetonitrile, se    | No crystal observed |
| 33              | FMA                 | 1:2/acetonitrile, se    | No crystal observed |
| 34              | FMA                 | 1:3/acetonitrile, se    | No crystal observed |
| 35              | FMA                 | 1:1/ethanol, se         | No crystal observed |
| 36              | FMA                 | 1:2/ethanol, se         | No crystal observed |
| 37              | FMA                 | 1:3/ethanol, se         | No crystal observed |
| 38              | FMA                 | 1:1/methanol, se        | No crystal observed |
| 39              | FMA                 | 1:2/methanol, se        | No crystal observed |
| 40              | FMA                 | 1:3/methanol, se        | No crystal observed |
| 41              | FMA                 | 1:1/acetone, se         | Irregular crystals |
| 42              | FMA                 | 1:2/acetone, se         | Irregular crystals |
| 43              | FMA                 | 1:3/acetone, se         | Irregular crystals |
| 44              | FMA                 | 1:1/ethyl acetate, se   | Irregular crystals |
| 45              | FMA                 | 1:2/ethyl acetate, se   | Irregular crystals |
| 46              | FMA                 | 1:3/ethyl acetate, se   | Irregular crystals |
| 47              | BZA                 | 1:1/acetonitrile, se    | No crystal observed |
| 48              | BZA                 | 1:2/acetonitrile, se    | No crystal observed |
| 49              | BZA                 | 1:3/acetonitrile, se    | No crystal observed |
| 50              | BZA                 | 1:1/ethanol, se         | No crystal observed |
| 51              | BZA                 | 1:2/ethanol, se         | No crystal observed |
| 52              | BZA                 | 1:3/ethanol, se         | No crystal observed |

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broad t, 3H), signal II: \( \delta \text{ppm} 1.19 \) (–CH\(_2\), broad t, 2H), signal III: \( \delta \text{ppm} 1.54 \) (–CH\(_2\), broad sext, 2H), signal IV: \( \delta \text{ppm} 2.86 \) (–CH\(_2\), broad t, 2H), signal V: \( \delta \text{ppm} 3.75 \) (–CH\(_3\), broad s, 3H), signal VI: \( \delta \text{ppm} 7.11 \) (ring C, broad dd, 1H), signal VII: \( \delta \text{ppm} 7.34 \) (ring C, broad s, 1H), and signal VIII: \( \delta \text{ppm} 7.42 \) (ring C, broad s, 1H). The \( ^1H \) NMR spectrum of sildenafil citrate and sildenafil base is shown in Figure 4 and the chemical shift values are indicated in Table 2. The \( ^1H \)-NMR spectrum of the obtained powder [Figure 3b] did not exhibit the signals of citric acid at chemical shifts around 2.5. In addition, signal V of sildenafil base (\( \delta \text{ppm} 3.72 \)) shifted from signal V of sildenafil citrate (\( \delta \text{ppm} 3.75 \)). This position is –CH\(_3\) attached with an N atom that can form salt with citrate. The absence citric acid signals and the shifting of signal V in sildenafil base sample confirmed that sildenafil was separated from citrate salt.

### Cocrystals obtained from various techniques

Table 1 shows the full experimental results from the crystallization process. Reaction number 1–10 and 26–31 crystallized from an antisolvent method and reaction number 11–25 and 32–61 crystallized from slow solvent evaporation (SE).

### Antisolvent method

The crystals formed only in the reaction between sildenafil and ASA observed with a stereomicroscope. The crystals of reaction number 1, 2, 8, and 9 obtained needle shape and number 3–6 obtained plate shape. The crystals from reaction number 2 were the highest stable and they were selected to investigate by single-crystal XRD. The XRD result [Figure 5] showed that the crystals from reaction number 2 were not cocrystals between sildenafil and ASA because these crystals were formed to unknown substances by some parts of sildenafil and ASA. Figure 5a shows the hydrogen bonding that is intermolecular interaction between the molecules, and Figure 5b expands the structure of this new molecule. This molecule will be studied in greater depth in the future. In the antisolvent method, DMSO was used to dissolve sildenafil. This solvent might react with sildenafil

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**Table 1: Contd...**

| Reaction number | Co‑former molecules | Experimental conditions | Product obtained |
|-----------------|---------------------|------------------------|------------------|
| 53              | BZA                 | 1:1/methanol, se       | No crystal observed |
| 54              | BZA                 | 1:2/methanol, se       | No crystal observed |
| 55              | BZA                 | 1:3/methanol, se       | No crystal observed |
| 56              | BZA                 | 1:1/acetone, se        | Irregular crystals |
| 57              | BZA                 | 1:2/acetone, se        | Irregular crystals |
| 58              | BZA                 | 1:3/acetone, se        | Irregular crystals |
| 59              | BZA                 | 1:1/ethyl acetate, se  | Irregular crystals |
| 60              | BZA                 | 1:2/ethyl acetate, se  | Irregular crystals |
| 61              | BZA                 | 1:3/ethyl acetate, se  | Irregular crystals |

**aa=Antisolvent addition method. se=Slow solvent evaporation method, RT=Room temperature (25°C–32°C), ASA: Acetylsalicylic acid or aspirin, FMA: Fumaric acid, BZA: Benzoic acid**

**Table 2: \(^1H\) chemical shift (\( \delta \text{ppm} \)) of sildenafil citrate and sildenafil base in dimethyl sulfoxide-d\(_6\)**

| Signal number | Functional group | Position number | \( \delta \text{ppm} \) |
|---------------|-----------------|----------------|-----------------|
| I             | –CH\(_3\)        | 13             | 0.95            |
| II            | –CH\(_2\)        | 21             | 1.18            |
| IV            | –CH\(_2\)        | 12             | 1.54            |
| IV            | –CH\(_2\)        | 11             | 2.84            |
| V             | –CH\(_3\)        | 10             | 3.72            |
| VI            | Ring C           | Ring C         | 7.07            |
| VII           | Ring C           | Ring C         | 7.30            |
| VII           | Ring C           | Ring C         | 7.40            |
| DMSO          | -                | -              | 2.50            |

\( \text{DMSO: Dimethyl sulfoxide} \)

**Figure 5: The chemical structure simulation using single‑crystal XRD result that obtained new crystal from reaction number 2; new crystal molecule with hydrogen bonding (a), single molecule (b)**
and ASA and could modify their structures that caused the formation of unknown substances, but the chemical reaction is not clear. The cocrystals did not form in the reactions of sildenafil-FMA and sildenafil-BZA because the

Figure 6: Crystals photographed with stereomicroscopes of sildenafil react with co‑former molecules by various crystallization condition techniques.
amount of solvent and solute might not be appropriate for supersaturated condition. This condition occurred when the solvent molecules discharged interaction from solute molecules to bind with antisolvent molecules. If the amount of solvents was too high or the amount of solute was too low, the supersaturated condition would not occur and the cocrystals would not be formed.

**Slow solvent evaporation method**

The crystal successfully formed in reaction number 20–25, 41–46, and 56–61 which used acetone or ethyl acetate as solvents. The crystals formed in every ratio (1:1, 1:2, and 1:3) of sildenafil and each conformer (ASA, FMA, and BZA). The crystal shapes were observed by stereomicroscope and showed irregular shape. The crystals did not form in reactions which solvents were acetonitrile, methanol, and ethanol (reaction number 11–19, 32–40, and 47–55). In contrast, the previous studies of Žegarac et al., 2014, showed that the cocrystals between sildenafil and ASA could form in reaction which they used acetonitrile as a solvent. There are many parameters which interfere the crystal growth such as temperature, evaporation rate of solvent, and similarity of structure between solute and solvent. In our study, the amount of solvent and temperature conditions for each reaction was not determined. Sildenafil and co-formers were dissolved until they became clear solutions at room temperature for all reactions. The evaporation rate in reaction of acetonitrile, methanol, and ethanol might not be proper for crystal formation. The suitable amount of solvent and temperature conditions is necessary because they are factors to control the evaporation rate of solvents that cause supersaturated condition for crystal formation. The cocrystals of reaction number 20–25, 41–46, and 56–61 were selected to characterize by FT-IR, 1H NMR, and PXRD.

**Cocrystal characterization**

**Fourier transformed infrared results**

The FT-IR spectrum of sildenafil, co-former molecules, and cocrystals is shown in Figure 7. Sildenafil spectrum exhibited N-H stretching at 3334/cm, C–H stretching at 2959/cm, C = C aromatic at 1588/cm, C–N stretching at 1325/cm, and SO\(^2\) symmetric at 1194/cm. IR-spectrum peak of ASA exhibited C = O stretching ester at 1756/cm, C = O stretching acid at 1690/cm, C = C stretching at 1605/cm, C–O acid stretching at 1306/cm, and C–H bending at 1188/cm. The FMA spectrum exhibited = C–H stretching at 3083/cm, C = O stretching at 1675/cm, COO– stretching symmetric at 1424/cm, and C–H bending at 1271/cm. The BZA spectrum exhibited aromatic C–H stretching at 3069/cm, O–H stretching at 3010/cm, C = O stretching at 1687/cm, aromatic C = C stretching at 1604/cm, C–O–H bending at 1424/cm, and C–O stretching at 1294/cm.

The wave number (per cm) of cocrystals (reaction number 20–25, 41–46, and 56–61) is shown in Table 3. The FT-IR spectrum of reaction number 20 and 23 exhibited the signals of sildenafil functional groups but did not exhibit the signal of ASA functional groups. The reaction number 21, 22, 24, and 25 exhibited C = O peak at 1690–1755/cm which was carboxyl functional group of ASA. The reaction number 41–46 exhibited the signal of sildenafil function groups and the C–H bending of FMA at 1271–1275/cm. Only the reaction number 45 and 46 were found the C = O signal of FMA at 1756/cm.
The reaction number 56–61 only exhibited the signals of sildenafil functional groups and not exhibited the signals of BZA functional groups. The reaction number 59–61 exhibited the C–O stretching of BZA at 3071–3072/cm. The obtained cocrystals from reaction number 20–25, 41–46, and 56–61 were confirmed their functional groups with $^1$H NMR spectroscopy.

$^1$H nuclear magnetic resonance results
The $^1$H NMR spectrum of sildenafil, ASA, FMA, and BZA is shown in Figures 8, and the $^1$H NMR spectrum of cocrystals from reaction number 20–25, 41–46, and 56–61 is shown in Figures 9-11. The cocrystals of all reactions exhibited both the signals of sildenafil and its co-formers. In reaction number 22, 25, 43, 46, 57, and 61, the $^1$H NMR signals of sildenafil and its co-former were integrated including signal

| Functional groups | Wave number (per cm) | Acetone | | | | | Ethyl acetate | | | |
|-------------------|---------------------|---------|---|---|---|---|---|---|---|---|---|
|                   | Sil:Co 1:1 | Sil:Co 1:2 | Sil:Co 1:3 | Sil:Co 1:1 | Sil:Co 1:2 | Sil:Co 1:3 | Sil:Co 1:1 | Sil:Co 1:2 | Sil:Co 1:3 | Sil:Co 1:3 |
| Sildenafil        | 3332 | 3334 | 3334 | 3336 | 3336 | 3335 | 3337 | 3337 | 3332 | 3332 |
| C–H stretching   | 2959 | 2960 | 2961 | 2959 | 2960 | 2960 | 2959 | 2960 | 2959 | 2960 |
| C=–C aromatic     | 1588 | 1588 | 1588 | 1587 | 1587 | -   | 1587 | 1587 | 1587 | 1587 |
| C–N stretching   | 1325 | -   | -   | 1324 | 1322 | -   | -   | -   | -   | -   |
| SO$_2$ symmetric | 1193 | 1190 | 1190 | 1193 | 1190 | 1189 | 1190 | 1189 | 1190 | 1189 |
| ASA               | -   | 1755 | 1754 | -   | 1753 | 1755 | -   | 1690 | 1690 | -   |
| C=O stretching, ester | -   | 1691 | 1691 | -   | 1690 | 1690 | -   | 1608 | 1608 | -   |
| C=O stretching, acid | -   | -   | -   | -   | -   | 1608 | -   | -   | 1307 | -   |
| C–O acid stretching | -   | 1308 | 1307 | -   | -   | 1307 | -   | -   | -   | -   |
| C–H bending       | -   | 1190 | 1190 | -   | 1190 | 1189 | -   | 1189 | -   | -   |
| FMA               | 3081 | -   | -   | -   | 3081 | 3081 | -   | -   | -   | -   |
| C–O                 | -   | -   | -   | -   | 1628 | 1629 | -   | -   | -   | -   |
| COO$^-$ symmetric | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   |
| C–H bending       | 1275 | 1271 | 1271 | 1272 | 1272 | 1272 | -   | -   | -   | -   |
| BZA               | -   | -   | -   | 3332 | 3336 | 3341 | -   | -   | -   | -   |
| O–H stretching (carboxylic) | -   | -   | -   | 2958 | 2960 | 2960 | -   | -   | -   | -   |
| C=O stretching   | -   | -   | -   | 1586 | -   | 1587 | -   | -   | -   | -   |
| Aromatic C=C stretching | -   | -   | -   | 1324 | 1325 | 1325 | -   | -   | -   | -   |
| C–O–H bending    | -   | -   | -   | 1192 | 1191 | 1191 | -   | -   | -   | -   |
| C–O stretching   | -   | -   | -   | 3071 | 3071 | 3072 | -   | -   | -   | -   |

Sil=Sildenafil, Co=Co-former
V of sildenafil (–CH₃, broad s, 3H), signal I of ASA (–CH₃, broad s, 3H), signal I of FMA (–CH₂, broad s, 1H), and signal III of BZA (–CH, broad dd, 1H). The integrated values and number of protons were used to calculate the ratios of sildenafil and co-former molecules. The results in Table 4 showed that the ratios of sildenafil and ASA in reaction number 22 and 25 were 1:1, ratios of sildenafil and FMA in reaction number 43 and 46 were 1:2, and ratios of sildenafil and BZA in reaction number 57 and 61 were 1:2.

**Powder X-ray diffraction results**

The PXRD pattern of sildenafil, ASA, FMA, BZA, and

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**Figure 8:** 500 MHz 1D ¹H nuclear magnetic resonance spectrum of sildenafil (a), acetylsalicylic acid (b), fumaric acid (c), and benzoic acid (d)

**Figure 9:** 500 MHz 1D ¹H nuclear magnetic resonance spectrum of cocrystals from reaction number 20 (a), 21 (b) 22 (c), 23 (d), 24 (e), and 25 (f)
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Sildenafil cocrystals (reaction number 20–25, 41–46, and 56–61) is shown in Figures 12 and 13. PXRD patterns of obtained cocrystals were sharp patterns that confirmed the crystal form, and they exhibited both the pattern of sildenafil and its co-former molecules. When considered with H¹ NMR spectrum [Figures 8-10] which shows both the signals of sildenafil and its co-former, they confirmed that the obtained crystals from reaction number 20–25, 41–46, and 56–61 were cocrystals between sildenafil and its co-former molecules.

**Saturation solubility of sildenafil cocrystals**
The cocrystals from reaction number 22, 25, 43, 45, 53, and 61 were selected to do this experiment and the results are shown in Table 5. Sildenafil cocrystal of ASA, FMA, and BZA improved 2–5 folds water solubility compared with sildenafil base. The water solubility of sildenafil-FMA cocrystal was 2.3944 μg/ml that was the highest water solubility compared with sildenafil-ASA (1.6298 μg/ml) and sildenafil-BZA (1.3439 μg/ml). The molecular weight of FMA, BZA, and ASA was 116.07, 122.12, and 180.16 g/mol, respectively. The LogP of FMA, ASA, and BZA was 0.46, 1.18, and 1.87, respectively. The water solubility of FMA, ASA, and BZA was 7.0, 4.6, and 3.4 g/L, respectively. FMA had the lowest molecular weight, lowest LogP, and highest water solubility when compared with ASA and BZA. In addition, FMA composed two H-bond donors and four H-bond acceptors that it had the most position for hydrogen binding with water molecules. While ASA had one H-bond

![Figure 10: 500 MHz 1D ¹H nuclear magnetic resonance spectrum of cocrystals from reaction number 41 (a), 42 (b) 43 (c), 44 (d), 45 (e), and 46 (f)](image)

**Table 4:** ¹H nuclear magnetic resonance peak integration and the ratios of sildenafil and co-former

| Reaction number | Co-former molecule/signal | Integrated value | Number of molecules⁴ | Ratios of sildenafil and co-former⁵ |
|-----------------|---------------------------|------------------|---------------------|-----------------------------------|
|                 | Sildenafil (Signal V, –CH₃) | Co-formers       | Sildenafil          | Co-formers                        |
| 22              | ASA/signal I (–CH₃)        | 3.92             | 3.92                | 1.30:1                            |
| 25              |                           | 3.02             | 3.27                | 1.01:1                            |
| 43              | FMA/signal I (–CH)        | 3.00             | 4.76                | 1.00:2                            |
| 46              |                           | 3.00             | 4.62                | 1.00:2                            |
| 57              | BZA/signal III (–CH)      | 3.00             | 3.38                | 1.00:2                            |
| 61              |                           | 3.00             | 3.85                | 1.00:2                            |

¹Number of molecules=Integrated value/number of protons at integrated chemical shift; ⁴Ratios of sildenafil and co-former=Number of sildenafil molecules: Number of co-former molecules. ASA: Acetylsalicylic acid or aspirin, FMA: Fumaric acid, BZA: Benzoic acid

![Table 4: ¹H nuclear magnetic resonance peak integration and the ratios of sildenafil and co-former](image)
donor and four H-bond acceptors and BZA had one H-bond donor and two H-bond acceptors. It was reasonable that the sildenafil-FMA cocrystals had the highest water solubility.

CONCLUSION

The antisolvent method failed to prepare cocrystals because the obtained crystals were not cocrystals, but they were new substances that were confirmed by single-crystal XRD technique. The sildenafil cocrystals were successfully prepared by slow SE method which used acetone or ethyl acetate as a solvent. Although in this method we did not evaluate the cocrystals by single-crystal XRD technique, the characteristics of cocrystals were confirmed by FT-IR, $^1$H NMR, and PXRD and their result related with characteristics of sildenafil and its co-former molecules. The integrated value of $^1$H NMR chemical shift demonstrated that the stoichiometric ratios of sildenafil and co-formers after cocrystallization were different from an initial of crystallization. ASA, FMA, and BZA improved the water solubility of sildenafil when they cocrystallized together. The cocrystals of sildenafil with FMA exhibited the highest water solubility of sildenafil. For further study, in vitro and in vivo pharmacokinetics and pharmacodynamics should be done to confirm the effectiveness and safety of these cocrystal molecules.

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