

Formation and Characterization of Sulfamethoxazole-Trimethoprim Cocrystal by Milling Process

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ARTICLE INFO

Article history:

Received on: 19/07/2017

Accepted on: 08/11/2017

Available online: 30/12/2017

Key words:

Co-crystal, trimethoprim, sulfamethoxazole, milling process.

ABSTRACT

The purpose of this study was to describe and characterize co-crystal formation of sulfamethoxazole and trimethoprim. The co-crystal formation was carried out by solid state milling process. Co-crystal by solution co-crystallization and physical mixture were also prepared as a comparison. The potential co-crystalline phase was characterized by powder X-ray Diffraction (PXRD), thermal analysis differential scanning calorimetry (DSC), scanning electron microscope (SEM), and Fourier transform Infrared (FT-IR) spectroscopy. Effect of milling time on formation of co-crystal sulfamethoxazole and trimethoprim was investigated by DSC and PXRD analysis. The study showed that the milling process of sulfamethoxazole and trimethoprim in equimolar yielded the co-crystal. Intact co-crystal was obtained by solution co-crystallization with methanol. Prolongation of milling time accelerated the formation of co-crystal. Physical characterization showed that sulfamethoxazole and trimethoprim co-crystal demonstrated a unique powder X-ray diffraction, thermal and spectroscopic properties. The study concludes that the milling process induces equimolar co-crystal formation between sulfamethoxazole and trimethoprim. The transformation to co-crystalline phase is affected by milling time.

INTRODUCTION

Sulfamethoxazole and trimethoprim are synthetic and broad-spectrum antimicrobial agents that have been used clinically in combination to prevent microbial resistance. They are a synergic combination and act as an inhibitor of bacterial dihydrofolate reductase. Though newer antibacterial and antimicrobial drugs have supplanted this combination, these drugs are still used due to their low cost relatively and ability to act against common bacterial diseases efficiently (Hughes *et al.*, 1974). Co-crystal is defined as a material which contains two or more discrete molecular entities in the crystal lattice (Kim *et al.*, 2013). Our previous study has proven that sulfamethoxazole and trimethoprim could interact to form a co-crystal by hydrogen

bonding in solid state by using solution co-crystallization with methanol. The 2-aminopyrimidine group of the trimethoprim is hydrogen bonded to the N(heteroatom)-C-NH(sulfonamide) moiety of sulfamethoxazole (Caira, 2007; Zaini *et al.*, 2010). The solid state form of drug substances significantly affects physical properties such as solubility, dissolution rate, stability and bioavailability (Huang and Tong, 2004). Pharmaceutical co-crystal has gained more interest from pharmaceutical industries because they offer multiple opportunities for modification of the chemical and or physical properties of drug substances without making or breaking any covalent bonds. Indeed, many studies have reported the evident advantages of physicochemical properties of pharmaceutical co-crystal from pure drugs (Hiendrawan *et al.*, 2016; Masuda *et al.*, 2012; Maeno *et al.*, 2014). Pharmaceutical co-crystal can be prepared by several methods. Recently, the preparation of co-crystal is mainly achieved by solution co-crystallization approach such as solvent evaporation and anti-solvent addition (Zhang *et al.*, 2007).

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In addition, crystallization from melting process and solid state grinding has also been employed (Berry *et al.*, 2008; Chadwick *et al.*, 2007). Shan *et al.* (2002) has modified this solid state grinding method by adding a small amount of appropriate solvent to wet the solids during grinding. This method is known with solvent-drop grinding and recognized as an alternative method to prepare the co-crystal.

The aims of the present study were to investigate the possibility of co-crystal formation between sulfamethoxazole and trimethoprim by milling process and to observe the effect of milling time on the formation of co-crystal. In addition, this study also carried out the formation of co-crystal by solution the co-crystallization method for comparative evaluation of co-crystal formation.

MATERIALS AND METHODS

Materials

Sulfamethoxazole (SMZ) and trimethoprim (TMP) were kindly donated by PT. Pyridam (imported from Virchow Lab. India, batch no. 09150307 and Shouguang Fukang Pharm. Co. Ltd. batch no. 200703342, respectively). Potassium bromide crystal and methanol were produced by Merck, Germany. All materials were used as received

Methods

Preparation of co-crystal of sulfamethoxazole-trimethoprim

Co-crystal of sulfamethoxazole and trimethoprim were prepared in equimolar ratio by solid state milling method. Another co-crystal prepared by solution co-crystallization (SC) and the physical mixture (PM) of SMZ-TMP were used as comparison. Co-crystal produced by solid state milling was prepared by using *Retsch Mühle* apparatus (Germany) for 10, 20 and 30 minutes. Meanwhile, co-crystal by SC method was produced by dissolving in small amount of methanol at ambient temperature and then slowly evaporated at room temperature during 24 hours to promote co-crystallization. The physical mixture was prepared in a glass vial and underwent vortex mixing for 5 minutes. All samples were kept in a desiccator.

Powder X-ray diffraction (PXRD)

The PXRD patterns of intact materials, physical mixture and co-crystal were examined using Philips PW 1710 diffractometer (Philips, Holland). The experiments were conducted as following condition: CuK α as radiation source, current at 30 mA and generator voltage 40 kV. The instrument was set over range 2θ of 5 – 40° with a step size of 0.01, and 0.5 sec/step with receiving slit 0.2 mm.

Differential Scanning Calorimetry (DSC)

Approximately 1-4 mg of intact materials, physical mixture and co-crystal were placed in a pin-holed aluminum crimped pan to perform thermal analysis (Shimadzu Instrument Inc. DSC-60, Japan). Prior to measurement, the heating rate was

maintained at 10° C/min and temperature range was set at 30-200°C. Samples were purged with nitrogen gas at 60 mL/min throughout the analysis.

Scanning electron microscopy (SEM)

The micrographs of crystal habit from intact materials, physical mixture and co-crystal were taken using SEM apparatus (JEOL model JSM-6360LA, Tokyo, Japan). The specimen was mounted on a metal stub with double sided adhesive tape and coated under vacuum with gold-palladium (Au 80% and Pd 20%) prior to observation.

Fourier transform infrared (FT-IR) spectroscopy

The infrared spectra of intact materials, physical mixture and co-crystal were recorded by FT-IR spectrophotometer (JASCO model FT-IR-4200 type A) using potassium bromide pellet over a range of 400 – 4000 cm⁻¹.

RESULTS

Powder X-ray diffraction (PXRD) analysis

X-ray powder diffraction patterns of co-crystallization reaction products of intact sulfamethoxazole and trimethoprim can be seen in Figure 1A-B, while the diffractogram of its physical mixture is shown in Figure 2C. The diffraction pattern of co-crystal of sulfamethoxazole and trimethoprim shows some new and typical diffraction peaks at 2θ : 7.32; 11.5; 16.90; 19.15 and 24° (Figure 1D). On the other hand, diffraction pattern of the physical mixture after the milling process with variation time of 10, 20 and 30 minutes at 2θ are at 7.2; 9.58; 14.40; 16.77; 19.10; and 23.00° which demonstrates similar pattern with co-crystal phase from slow co-crystallization (Figure 2.).

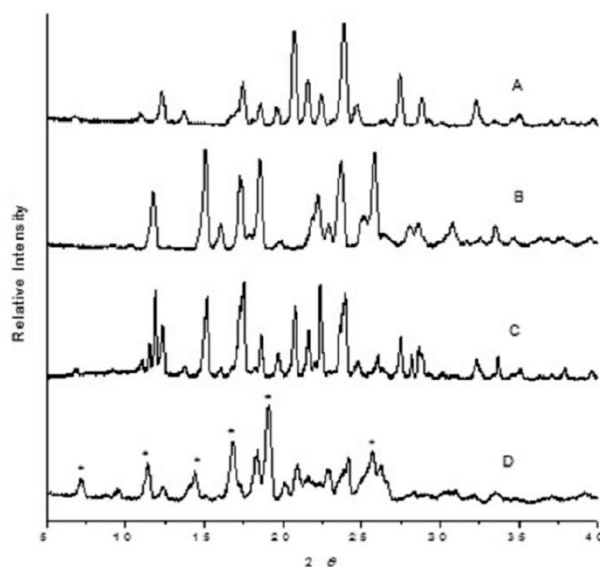


Fig. 1: Powder X-ray Diffractogram A) intact sulfamethoxazole, B) intact trimethoprim, C) physical mixture equimolar of sulfamethoxazole and trimethoprim and D) co-crystal of sulfamethoxazole and trimethoprim equimolar by cocrystallization technique from methanol.

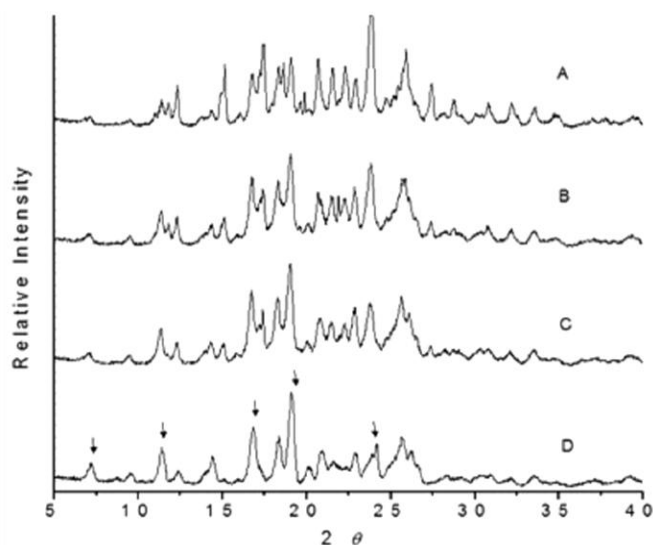


Fig. 2: Powder X-ray Diffractogram A) co-crystal by solid state milling for 10 min, B) 20 min, C) 30 min and D) co-crystal of sulfamethoxazole and trimethoprim equimolar by cocrystallization technique from methanol.

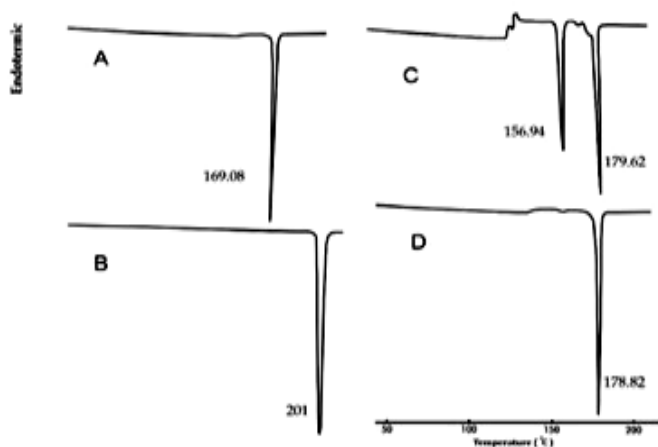


Fig. 3: DSC thermogram A) intact sulfamethoxazole, B) intact trimethoprim, C) physical mixture equimolar of sulfamethoxazole and trimethoprim and, D) co-crystal by co-crystallization technique from methanol.

Differential scanning calorimetry (DSC) analysis

Changes in thermal behavior of co-crystal of sulfamethoxazole and trimethoprim were shown in Figure 3 and 4. Intact sulfamethoxazole and trimethoprim crystals showed endothermic peaks due to the fusion at 169.8 °C and 201 °C, respectively (Figure 3A-B). The physical mixture shows three endothermic peaks at 138, 156.94 and 179.62 °C. The DSC thermogram of co-crystal from solution co-crystallization with methanol shows only one endothermic peak at 178.82 °C (Figure 3D). The mixture of sulfamethoxazole and trimethoprim milled in different duration induced the formation of a new co-crystalline phase. This formation is indicated by endothermic peak at 178.53 in DSC thermogram (Figure 4).

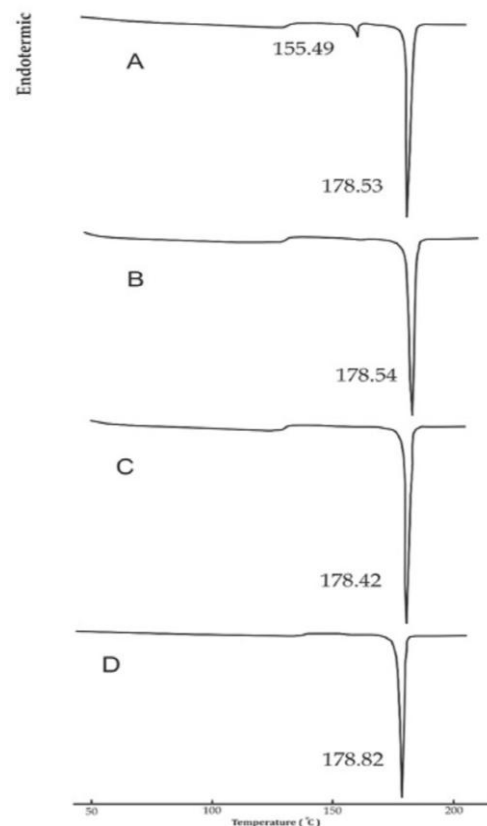


Fig. 4: DSC thermogram A) co-crystal by solid state milling for 10 min, B) 20 min, C) 30 min and D) co-crystal of sulfamethoxazole and trimethoprim equimolar by cocrystallization technique from methanol.

Scanning electron microscopy (SEM) analysis

SEM micrographs (Figure 5. A-B) of intact sulfamethoxazole and trimethoprim shows different particle size of those materials. Figure 5C shows the habit of co-crystal prepared by milling process for 30 minutes, while Figure 5D shows the habit of co-crystal prepared by solution co-crystallization obtained within 24 hours that appears as needle-shaped crystals.

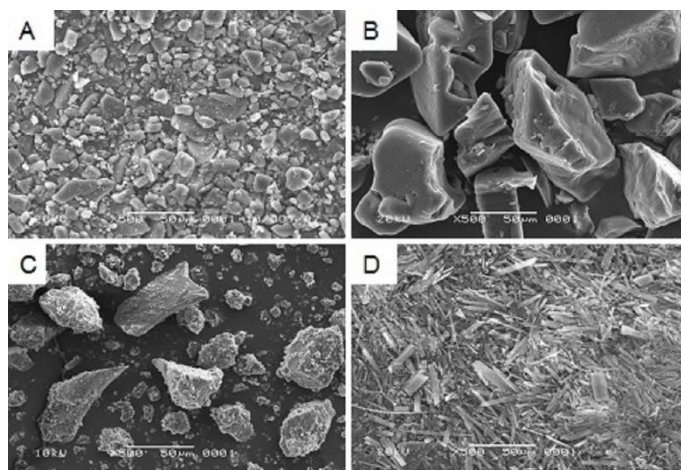


Fig. 5: Scanning Electron Micrographs A) intact sulfamethoxazole, B) intact trimethoprim, C) co-crystal sulfamethoxazole and trimethoprim by solid state milling and D) co-crystal by cocrystallization technique from methanol.

Fourier transform infrared (FT-IR) spectroscopy analysis

Figure 6 shows molecular interaction between sulfamethoxazole and trimethoprim in milled mixture was investigated by FT-IR spectroscopy. The spectral differences between the co-crystal and intact sulfamethoxazole and intact trimethoprim are seen at wave number around 3000 to 4000 cm^{-1} . This spectrum is particularly noticed at wave numbers 3400 cm^{-1} , in which the N-H strain is shifted to a lower wave number.

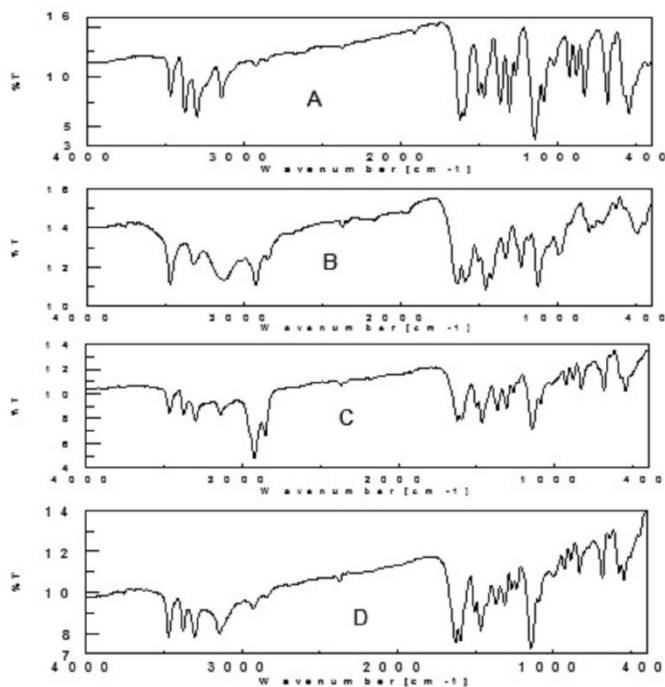


Fig. 6: FT-IR Spectra A) intact sulfamethoxazole, B) intact trimethoprim, C) physical mixture equimolar of sulfamethoxazole and trimethoprim and, D) co-crystal by solid state grinding during 30 min.

DISCUSSION

Milling is one of essential processes in manufacturing pharmaceutical preparations, especially to reduce particle size of active ingredients and excipients (Loh *et al.*, 2015). Co-milling of solids have been reported to cause solid phase reactions, either physically or chemically (Boldyrev, 2004; Hu *et al.*, 2014). Co-crystal phase formation between two crystalline solids by solid state milling method has been recognized as an alternative method (Friscic and Jones, 2009; Lin, 2016). In addition, milling can be conducted by mixing two components to form co-crystal, milling either manually with a mortar and pestle, or mechanically using a ball mill and vibratory mill. The milling process has been utilized to induce the co-crystal phase formation of carbamazepine with nicotinamide and saccharin (Chieng *et al.*, 2009; Jayasankar *et al.*, 2006).

PXRD is a foremost method to characterize the formation of a new crystalline phase in solid state. The diffraction pattern of co-crystal should be distinct from the superimposition of each compounds if a true co-crystal has been formed between two solid

phases. Our previous study showed that the co-crystal of sulfamethoxazole and trimethoprim had been successfully formed through solution co-crystallization (Zaini *et al.*, 2010). This co-crystal was used in the present study as a reference. The PXRD pattern of each intact drugs demonstrates a crystalline solid marked by typical interference peaks at various diffraction angles (2θ) shown in Fig. 1A-B. However, PXRD pattern of physical mixture (Fig 1C) shows all characteristic interference peaks of sulfamethoxazole and trimethoprim, which indicates a superimposition of the two components. The duration of milling time influenced the intensity of some interference peaks, particularly at 2θ : 16.77 and 19.10°. At these points, the interference peaks increase and broaden, which indicates the amorphization of partial solid phase interaction (Fig. 2A-C). The diffraction pattern of co-crystal shows some new and typical interference peaks at 2θ : 7.32; 11.5; 16.90; 19.15 and 24°. This indicated the physical interaction between sulfamethoxazole and trimethoprim and the formation of a new crystalline phase, known as co-crystalline phase (molecular compounds or molecular complexes) in material sciences (Masuda *et al.*, 2012; Zhang *et al.*, 2007).

Thermal analysis is one of the most widely used methods for studying the solid state interaction of pharmaceutical substances. In particular, Differential Scanning Calorimetry (DSC) study is an important analytical tool in characterization of solid-state interaction between two or more drug substances. Among available methods, DSC is used for investigating the thermodynamic changes which occur while heating a substance. These include recrystallization, melting and de-solvation which can all show endothermic or exothermic peaks on the DSC curves (Giron, 2002). The present study showed that the milled sample in solid state for different duration caused the formation of co-crystal (Fig. 4A-C). The DSC thermogram of physical mixture shows three endothermic peaks, at 138 °C and 156.94 °C which are attributed to fusion of eutectic mixture, and at 179.62 °C due to the fusion of co-crystal. Meanwhile, the exothermic peaks at 140 °C is attributed to recrystallization of mixture from melting. On the other hand, the co-crystal by milling method induced the formation of a new co-crystalline phase, indicated by endothermic peak at 178.53 °C in DSC thermogram. The DSC peak area of eutectic fusion decreases by prolongation of grinding time, indicating the milling-induced progress of sulfamethoxazole-trimethoprim co-crystal formation (Chieng *et al.*, 2009). In similar, the DSC thermogram of co-crystal from solution co-crystallization of methanol also shows only one endothermic peak at 178.82 °C, which indicates the formation of new crystalline phase of co-crystal between sulfamethoxazole and trimethoprim (Zaini *et al.*, 2010).

Scanning Electron Microscopy (SEM) analysis depicts a decrease of particle size and drugs is embedded each other to form coherence particles. As a reference to compare the possibility of co-crystal formation between sulfamethoxazole and trimethoprim by solid state grinding, co-crystal was also prepared by solution co-crystallization technique by using methanol. The reaction

between the solid phases can be mediated by an intermediate liquid phase, or requires the destruction of the crystalline structure prior to the formation of a new crystalline phase. This condition is often observed in solid state reactions which is activated by mechanical energy. The molecular reactions of sulfamethoxazole and trimethoprim crystals may occur on the surface of the crystal or require molecular diffusion within or between crystal lattices. The mechanical energy generated by the grinding process of two or more solids will cause molecular diffusion along the crystal fracture and an increase on the surface area of the crystal, thereby allowing solid-solid interaction to occur (Braga *et al.*, 2005).

FT-IR spectroscopy analysis is used to characterize the complex of two compounds in solid state which can be linked by hydrogen bonding (Bugay, 2001). As seen in Figure 6, there is a shift of N-H strain which indicates the formation of hydrogen bond as a consequence of co-crystal phase formation between sulfamethoxazole and trimethoprim (Caira, 2007). The interaction between sulfamethoxazole and trimethoprim may occur as a result of pKa difference, in which a molecule of trimethoprim is bonded with one molecule of sulfamethoxazole. This bonding may present through hydrogen bond of 2-aminopyrimidine group in trimethoprim with group N (heteroatom) -C-NH (sulfonamide) of sulfamethoxazole (Bettinetti, G., and Giordano, F., 1988).

CONCLUSION

This study confirms that solid state milling method induce the formation of co-crystal from sulfamethoxazole and trimethoprim. The PXRD pattern of the co-crystal demonstrates a new crystalline phase. The co-crystal is formed due to mechanical stress-induced interaction in a solid state between the two substances.

ACKNOWLEDGEMENTS

We would like to thank Indonesia National Agency of Drug and Food Control for providing thermal DSC analysis.

Financial support and sponsorship: Nil.

Conflict of Interests: There are no conflicts of interest.

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How to cite this article:

Zaini E, Sumirtapura YC, Halim A, Fitriani L, Soewandhi SN. Formation and Characterization of Sulfamethoxazole-Trimethoprim Cocrystal by Milling Process. *J App Pharm Sci*, 2017; 7 (12): 169-173.