Scientific Foundation SPIROSKI, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2022 Jan 15; 10(A):141-145.
https://doi.org/10.3889/oamjms.2022.7920
eISSN: 1857-9655
Category: *A - Basic Sciences*
Section: *Pharmacology*

Multicomponent Crystal of Trimethoprim and Citric Acid: Solid State Characterization and Dissolution Rate Studies

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Edited by: Sinisa Stojanoski
Citation: Umar S, Farnandi R, Salsabila H, Zaini E. Multicomponent Crystal of Trimethoprim and Citric Acid: Solid State Characterization And Dissolution Rate Studies. Open Access Maced J Med Sci. 2022 Jan 15; 10(A):141-145. https://doi.org/10.3889/oamjms.2022.7920
Keywords: Trimethoprim; Citric acid; Multicomponent crystals; Solvent evaporation; Solubility; Dissolution rate
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Received: 11-Nov-2021
Revised: 02-Jan-2022
Accepted: 05-Jan-2022

Funding: This study was supported by the Faculty of Pharmacy, Universitas Andalas through the DIPA Research grant 2021 (contract no. 13/UN16.10.D/PJ.01/2012)
Competing Interests: The authors have declared that no competing interests

Abstract

BACKGROUND: Trimethoprim is a broad-spectrum antimicrobial agent with low solubility in water which causes low bioavailability in the systemic circulation.

AIM: This study aimed to increase the solubility and dissolution rate of trimethoprim by preparing multicomponent crystals of trimethoprim and citric acid.

MATERIALS AND METHODS: Multicomponent crystals were prepared by the solvent evaporation method. Characterizations of multicomponent crystalline solid phase properties were carried out by powder X-ray diffraction (PXRD) analysis, differential scanning calorimetry (DSC), FT-IR spectroscopy, scanning electron microscopy (SEM). Solubility and dissolution rate tests were carried out in an aqueous medium.

RESULTS: The PXRD characterization results showed a new X-ray diffraction pattern in the multicomponent crystal phase. DSC analysis showed the formation of a new endothermic peak. This indicates the formation of multicomponent crystal phase between trimethoprim and citric acid. The results of the SEM analysis indicate the formation of a new crystal habit. Solubility of multi-component crystal phase of trimethoprim increased seven times compared to intact trimethoprim. The dissolution of trimethoprim and multicomponent crystals in 0.1 N HCl medium at 60 min was 56.36% and 95.57% and the CO_2-free distilled water medium was 43.03% and 88.26%, respectively.

CONCLUSIONS: Based on the results of this study, it could be concluded that the novel multicomponent phase of trimethoprim crystals with citric acid successfully increases the solubility and dissolution rate of trimethoprim significantly.

Introduction

Solubility is one of the most important physicochemical properties of drug compounds in predicting drug absorption in the gastrointestinal tract. Drugs that have low solubility in water often show low bioavailability and dissolution rate is a determining step in the drug absorption process [1]. One of the active pharmaceutical ingredients with low solubility properties is trimethoprim. According to Biopharmaceutical Classification System, trimethoprim is classified under class II along with other drugs with low solubility and high permeability [2]. Trimethoprim acts by inhibiting enzyme dihydrofolate reductase in the reduction of dihydrofolate to tetrahydrofolate which causes bacteria to lack essential di- and tetrahydrofolic acids in their biosynthesis [3]. Trimethoprim is administered through various routes, including intravenous, intramuscular, and oral. However, due to its low solubility, oral solid preparation of trimethoprim has low bioavailability even though it has high gastrointestinal tolerability and low side effects [4]. Previous studies have reported several approaches in attempt to enhance solubility and dissolution rate of trimethoprim, including solid dispersion system with hydrophilic polymers, inclusion complexes formation with cyclodextrins, and spherical crystallization [2], [4], [5].

One of the recent strategies applied to enhance physicochemical properties of active pharmaceutical compounds is by forming a multi-component crystal phase with an inert and safe coformer. This approach has shown successful improvement in solubility, dissolution rate, physical and chemical stability, and compressibility [6], [7], [8], [9], [10]. Multicomponent crystal phase between active pharmaceutical compounds and coformers could be formed due to non-covalent intermolecular interactions such as van der Waals bonds and hydrogen bonds [11].

Several multi-component crystal phases of trimethoprim include cocrystals, some of which have been reported including salts with sulfamethoxazole, mefenamic acid, cinnamic acid, formic acid, acetic acid, malic acid, and maleic acid [8], [12], [13], [14], [15], [16], [17]. However, so far there has been no report of multicomponent crystal phases of trimethoprim with citric acid. Citric acid is a safe excipient categorized by the food and drug administration as generally recognized as safe. This study aimed to prepare a multicomponent crystal phase of trimethoprim with citric acid by the solvent

evaporation method. Solid-state properties were characterized by powder X-ray diffraction (PXRD), differential scanning calorimetry (DSC), scanning electron microscopy (SEM), and FT-IR spectroscopy. Solubility and dissolution rate were evaluated in aqueous medium, compared to intact trimethoprim.

Material and Methods

Materials

Materials used include trimethoprim (Wako Pure Chemical Industries, Japan), citric acid (TCI-EP, Tokyo Japan), ethanol (Merck, Germany), methanol (Merck, Germany), hydrochloric Acid (Bratachem, Indonesia), and CO_2-free distilled water (Brataco).

Preparation of multicomponent crystal phase

Multi-component crystals of trimethoprim and citric acid were prepared by solvent evaporation method with an equimolar ratio (0.290 g: 0.192 g). Then trimethoprim was dissolved with methanol and citric acid was dissolved in ethanol, then the two materials were quickly mixed at 85 rpm in a magnetic stirrer. After that, it was dried in a desiccator to form a crystalline solid.

PXRD analysis

Analyses were carried out on trimethoprim, citric acid, and multi-component crystals. X-ray diffraction analyses of the samples were performed at room temperature using an X-ray diffractometer (Philips X'Pert Powder, The Netherland) with Cu K radiation (λ = 1.54178Å), current 40 mA, voltage 40 kV. Samples were measured in reflection mode at 0.05 theta with an angle range of 5°–40° theta at a scan speed of 5°/min.

Analysis of DSC

Thermal analyses on trimethoprim, citric acid, and multicomponent crystal compounds were carried out using a temperature-calibrated DSC tool. Samples were placed in a closed aluminum pan. The DSC device (DSC-60 Plus Shimadzu, Japan), is programmed in a temperature range of 30–250°C, heating speed of 10°C/min, under nitrogen gas flow of about 30 Psi.

FT-IR spectroscopic analysis

Analysis was carried out on trimethoprim, citric acid, and multi-component crystals. A small amount of sample (2–3 mg) was mixed with KBr after which it was placed in the sample holder of the FT-IR spectroscopic instrument (IRT Racer-100 Shimadzu, Japan) and the samples were analyzed at room temperature. The spectrum was measured in the range of 4500–500 cm^{-1} wavenumber.

SEM analysis

SEM (Hitachi Type S-3400N, Japan) analysis was performed on trimethoprim, citric acid, and multi-component crystals. The samples were coated with a thin layer of palladium-gold prior to analysis. SEM works using a beam speed of 30 kV.

Solubility test

In the solubility test, an excess amount of trimethoprim and multicomponent crystals were added to 100 ml of CO2-free distilled water, stirred at a speed of 150 rpm for 24 hours at room temperature, and then filtered using filter paper. Concentration of trimethoprim was determined from the absorbance measurement at 287 nm using ultraviolet-visible light (UV-Vis) spectrophotometer.

Dissolution rate profile study

The dissolution rate study of trimethoprim and multicomponent crystals used the paddle method (Hanson Research SR08, USA) at 37 ± 0.5°C at a speed of 100 rpm for 60 min with two mediums namely 0.1 N HCl and CO_2-free distilled water. Five mL of each dissolution medium was pipetted at 5, 10, 15, 30, 45, 60 min. The absorbance of the solution that had been pipetted from the dissolution medium was measured using UV-visible spectrophotometer (at 287 nm) to determine the amount of trimethoprim dissolved.

Results and Discussion

X-ray diffraction analysis is a technique to characterize the solid properties of active pharmaceutical compounds. Changes in the X-ray diffraction pattern in solids resulting from intramolecular interactions indicate the formation of new crystalline phases such as cocrystals or salts [18]. Figure 1 presents the X-ray diffraction pattern of trimethoprim, citric acid, and the multi-component crystalline phase. Figure 1a is an X-ray diffraction pattern of trimethoprim showing typical diffraction peaks at 2 theta = 11.62; 12.86; 16.12; 25.46 and 28.45. Figure 1b, is an X-ray diffraction pattern of citric acid which has a specific diffraction peak at 2 theta = 14.10; 17.89; and 20.08. Figure 1c shows the X-ray diffraction pattern of the multi-component crystalline

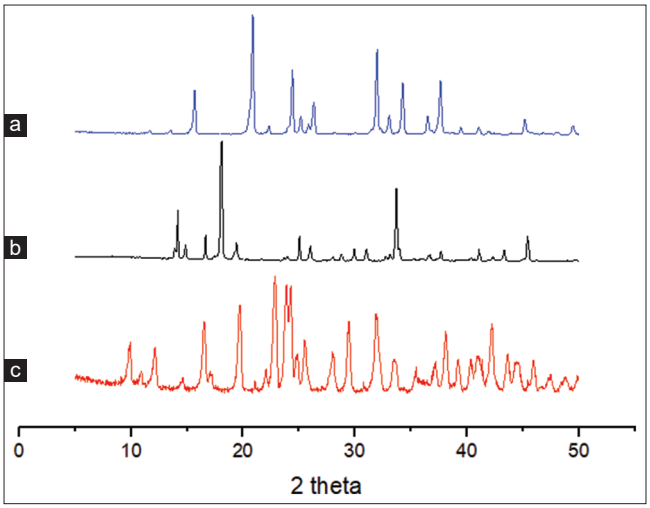

Figure 1: Powder X-ray diffraction diffractogram of (a) Trimethoprim, (b) Citric acid, and (c) Multicomponent crystals

phase which is unique and different from the X-ray diffraction pattern of trimethoprim and citric acid coformer compounds. There are several new peaks, namely at 2 theta = 13.13; 21.53 and 24.01. These results show the formation of a new crystalline phase as a consequence of the interaction between trimethoprim and citric acid. Multicomponent crystal phase between the two solid phases is very possible because of the presence of functional groups between the two molecules that can be bonded through weak non-covalent bonds. Based on the ΔpKa rule theory, if the pKa difference between the active pharmaceutical compound and the coformer is greater than (>) 3, then the interaction can form a salt type of multicomponent crystal [19], [20]. pKa difference between trimethoprim and citric acid of 4.17 makes it possible to form a salt-type multicomponent crystal.

Thermal analysis is an analysis used to evaluate the physical and chemical properties of a substance as a function of temperature. The existence of solid-state interactions is indicated by a change in the melting point between a binary mixture of active pharmaceutical ingredients and coformers.

Figure 2a displays a thermogram of trimethoprim which shows an endothermic peak at 202.82°C as the melting point of this solid compound. Citric acid thermogram (Figure 2b) shows a single sharp endothermic peak at 157.72°C, which is also a melting event of citric acid. Multicomponent crystals of trimethoprim-citric acid show several endothermic peaks of 90–120°C which is either loss of solvent or dehydration of the solid. While the sharp endothermic peak at a temperature of 171.40°C is the new melting point of the multi-component crystal phase. The results of the DSC thermal analysis supported the PXRD analysis, that between trimethoprim and citric acid, a new multi-component crystal phase was formed [21].

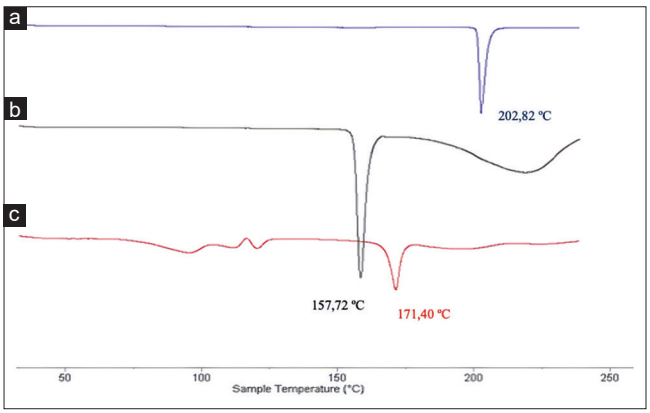

Figure 2: Differential scanning calorimetry thermogram of (a) Trimethoprim, (b) Citric acid, and (c) Multicomponent crystals

FT-IR spectroscopic analysis is one of the important techniques used to evaluate intramolecular interactions in multicomponent crystal systems. The presence of solid-state interactions between components in a crystalline multicomponent system is indicated by the presence of a new or a shifting transmittance peak. The presence of hydrogen bonds formed between the two solid substances is indicated by a shift in the wavenumber [21], [22].

Figure 3 display the FT-IR spectra of trimethoprim, citric acid, and a multi-component crystal phase. The peak transmittance of trimethoprim at wave numbers (OH) 311.19 cm^{-1}, (NH) 3468.07 cm^{-1}, (CH) 2929.92 cm^{-1}, (C=N) 1634.70 cm^{-1} and (C=H) 1464,00 cm^{-1}. Citric acid at wave number (OH) 3493.15 cm^{-1}, (C=O) 17421.71 cm^{-1}, (C=H) 1424.45 cm^{-1} and the peak multicomponent crystal transmittance at wavenumber (OH) 3388.89 cm^{-1}, (NH) 3573.19 cm^{-1}, (CH3) 3132.45 cm^{-1}, (CH) 2843.12 cm^{-1}, (C=O) 1734.04 cm^{-1}, (C=N) 1589.37 cm^{-1}, and (C=H) 1322.23 cm^{-1}. The shift in the wavenumber of trimethoprim in the multicomponent crystal in the OH group from 3101.59 cm^{-1} to 3388.89 cm^{-1}, the NH group from 3468.07 cm^{-1} to 3573.19 cm^{-1} the CH group from 2929.92 cm^{-1} to 2843.12 cm^{-1}, the C=N group from 1636.70 cm^{-1} to 1589.37 cm^{-1} and the C=H group from 1464.00 to 1452.42 cm^{-1}.

The results of the analysis of trimethoprim, citric acid, and multi-component crystals by SEM can

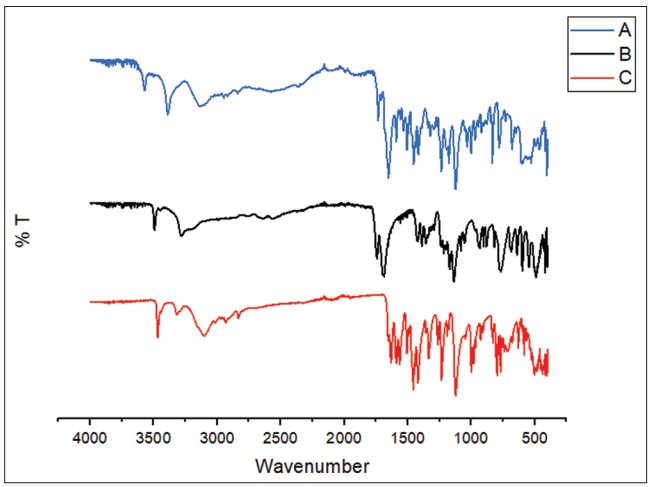

Figure 3: FT-IR Spectra of (a) trimethoprim, (b) citric acid, and (c) multicomponent crystals

be seen in Figure 4. Based on observations, it can be seen that trimethoprim crystals are irregular cubic crystals, citric acid is rigid prismatic crystals, while multicomponent crystals show a new crystal habit as long rod-shaped.

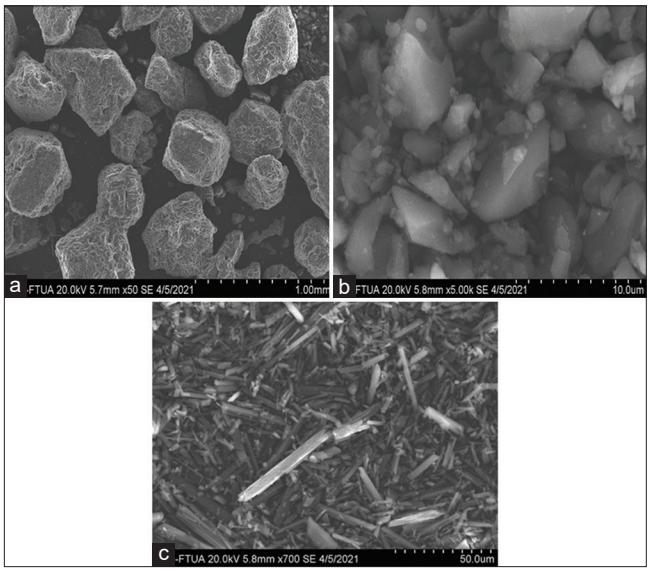

Figure 4: Scanning electron microscopy of (a) trimethoprim, (b) citric acid, and (c) multicomponent crystal

Orally administered drugs that are poorly soluble in water generally have limited bioavailability since solubility plays an important role in gastrointestinal absorption. The formation of multicomponent crystals with coformers could modify active pharmaceutical ingredients' properties and increase solubility and pharmacological effectiveness [1], [11]. The main advantage of this approach is the ability to maintain its thermodynamic stability and retain the drug in the solid crystalline phase. Moreover, this method does not change the pharmacophore structure of the active pharmaceutical ingredients [23].

Table 1: Solubility of trimethoprim and its multicomponent with citric acid (n = 3)

Compound	Solubility ± SD (mg/100mL)
Trimethoprim	1.08 ± 0.233
Multicomponent Crystals	7.05 ± 0.110

Solubility studies (Table 1) show that the multicomponent crystals of trimethoprim are 7 times more soluble than intact trimethoprim alone. The dissolution rate profiles of trimethoprim and multicomponent crystals were performed by paddle method at 100 rpm for 60 min at 37 ± 0.5°C with 0.1 N HCl medium and CO_2-free distilled water. The increase in the rate profile of trimethoprim and multicomponent crystals of 0.1 N HCl medium can be seen in Figure 5. with an increase in dissolution efficiency of 1.46 times. Moreover, the increase in the rate profile of trimethoprim and multicomponent crystalline CO_2-free distilled water media can be seen in Figure 6 with an increase in dissolution efficiency of 2.45 times. The formation of salt has been proven to improve the physicochemical properties of drug, especially dissolution rate and solubility. Earlier studies have reported improved

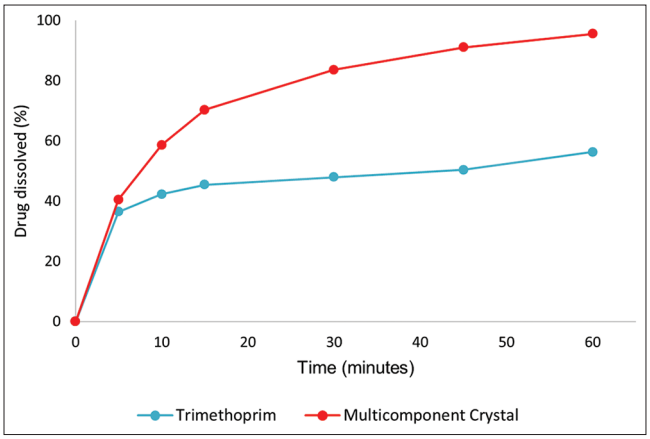

Figure 5: Dissolution rate profile of trimethoprim and multicomponent crystals in 0.1 N HCl medium

dissolution rate and solubility of trimethoprim through multicomponent crystalline phase with several excipients [12], [13], [14], [15], [16], [17], [24].

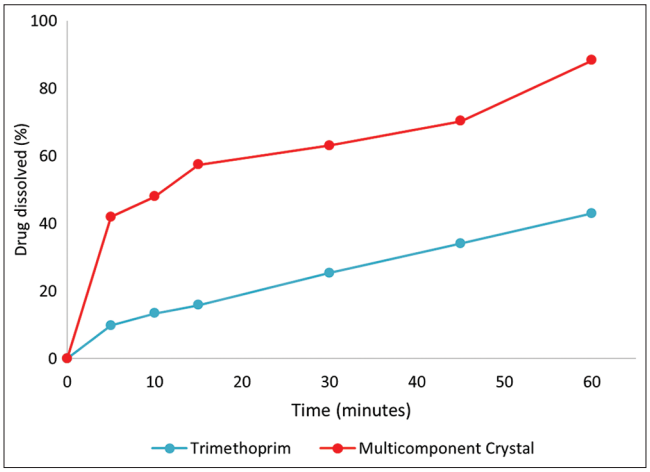

Figure 6: Dissolution rate profile of trimethoprim and multicomponent crystal in CO_2-free distilled water medium

Some factors may contribute to the increment of solubility and dissolution rate of multicomponent crystals of trimethoprim and citric acid. First, solid phase of multicomponent crystals is more hydrophilic so it has a better affinity for the aqueous medium. The salt form dissociates into cationic and anionic ions once in contact with aqueous media. Second, in terms of solid-state properties, lower melting point of the crystal phase and change in the crystal structure indicates weaker lattice energy that binds the molecules in the unit cell. Weaker lattice energy of the crystal phase leads to a faster dissolution rate [8], [19], [23].

Conclusions

In this current research, the salt-type multicomponent crystal of trimethoprim and citric acid were successfully prepared and characterized its solid-state properties. Novel trimethoprim – citric acid

multicomponent crystal phase significantly improves solubility and dissolution rate of trimethoprim in compared to intact trimethoprim.

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