Journal of Pharmaceutical Sciences 106 (2017) 1384-1390

Contents lists available at ScienceDirect

Journal of Pharmaceutical Sciences

journal homepage: www.jpharmsci.org

Pharmaceutics, Drug Delivery and Pharmaceutical Technology

Pharmaceutical Co-Crystal of Flufenamic Acid: Synthesis and Characterization of Two Novel Drug-Drug Co-Crystal

Sunil Kumar Nechipadappu, Venkatadri Tekuri, Darshak R. Trivedi*

Department of Chemistry, Supramolecular Chemistry Laboratory, National Institute of Technology, Karnataka (NITK), Srinivas Nagar, Surathkal, Mangaluru, Karnataka 575025, India

ARTICLE INFO

Article history:
Received 20 October 2016
Revised 10 January 2017
Accepted 24 January 2017
Available online 7 February 2017

Keywords:
pharmaceutical co-crystal
co-crystal
crystal engineering
hygroscopicity
stability
solubility

ABSTRACT

Two novel pharmaceutical co-crystals of anti-inflammatory drug flufenamic acid (FFA) with 2-chloro-4-nitrobenzoic acid (CNB) and ethenzamide (ETZ) have been synthesized by solvent evaporation method as well as by solvent drop-assisted grinding method. The synthesized co-crystals were characterized thoroughly by various spectroscopic methods and crystal structures were determined by single-crystal x-ray diffraction technique. In FFA-CNB co-crystal, robust supramolecular acid-acid homosynthon was observed. FFA-ETZ co-crystal is formed via robust supramolecular acid-amide heterosynthon. In FTIR spectra, a significant shift in the carbonyl stretching frequency was observed for the co-crystals due to the presence of intermolecular hydrogen bond. ^{1}H nuclear magnetic resonance study suggests the presence of hydrogen bond in the solution state of FFA-ETZ co-crystal; however, it was absent for FFA-CNB co-crystal. Solubility study in Millipore water revealed that the solubility of FFA is increased by 2-fold when it is in the form of FFA-CNB co-crystal and no increment in the solubility of FFA was observed in FFA-ETZ co-crystal. About 5-fold increment in the solubility of FFA was observed in both the co-crystals in 0.1 N HCl (pH 1) solution. The synthesized co-crystals were found to be non-hygroscopic at ~75% relative humidity and stable for a period of 6 months at ambient temperature (~25°C).

Introduction

Pharmaceutical co-crystal can be defined as a single crystalline solid that incorporates 2 neutral molecules, one being an active pharmaceutical ingredient (API) and the other is a co-crystal former which is a solid at ambient temperature.[1-3] The field of pharmaceutical co-crystal has greater significance in the pharmaceutical field due to their ability to fine tune the physicochemical properties of the active ingredient without affecting the biological activity.[4,5] Pharmaceutical co-crystal offers a remarkable enhancement in solubility, dissolution rate, bioavailability, and physical stability of drug molecules.[6,7] Furthermore, co-crystallization helps in improving the chemical stability, flowability, compressibility, tabletability, and hygroscopicity of drug molecules.[8-12] Owing to the above advantages, the field of pharmaceutical co-crystal may have a key role in the drug development at pre-formulation stages.

In this study, pharmaceutical co-crystal of non-steroidal anti-inflammatory drug flufenamic acid (FFA) with 2 co-formers, namely 2-chloro-4-nitrobenzoic acid (CNB) and ethenzamide (ETZ) (Scheme 1), are reported. Co-crystallization experiment in this study with other co-formers, namely isonicotinamide, picolinamide, 4-aminobenzoic acid, and *p*-aminosalicylic acid did not yield co-crystal with FFA. FFA is an anthranilic acid derivative with analgesic, anti-inflammatory, and antipyretic properties.[13] FFA along with meclofenamic acid is a widely used drug for the treatment of lower back pain and is administered orally and topically.[14] FFA belongs to the class II drug according to the biopharmaceutical classification system because of its low aqueous solubility (9.09 mg/L) and high permeability (log $p = 5.25$).[15] Research has been carried out to increase the solubility of FFA drug by numerous techniques like amorphization using inorganic carrier[16] and forming a dispersion with polyvinyl pyrrolidine.[17] FFA exists in 9 polymorphic forms,[18,19] out of which only form I and form III are commercially available.[20] CNB and ETZ used in this study as co-formers are active drug ingredients. Study on pharmaceutical co-crystal with 2 active ingredients has been reported in the literature with enhanced physicochemical properties.[21-24] CNB is used as a novel potential therapy for immunodeficiency deceases such as anti-viral and anti-cancer agent.[25,26] It exists as dimorphs in the

Abbreviations used: API, active pharmaceutical ingredient; CNB, 2-chloro-4-nitrobenzoic acid; DSC, differential scanning calorimetry; ETZ, ethenzamide; FFA, flufenamic acid; NMR, nuclear magnetic resonance; PXRD, powder x-ray diffraction; RH, relative humidity; SC-XRD, single-crystal X-ray diffraction.

This article contains supplementary material available from the authors by request or via the Internet at http://dx.doi.org/10.1016/j.xphs.2017.01.033.

* *Correspondence to:* Darshak R. Trivedi (Telephone: +91-824-2473205; Fax: +91-8242474033).

E-mail address: darshak_rtrivedi@yahoo.co.in (D.R. Trivedi).

http://dx.doi.org/10.1016/j.xphs.2017.01.033
0022-3549/

solid state.[27] ETZ is a non-steroidal anti-inflammatory drug largely used in combination with drugs such as aspirin, dipyrone, allyl isopropyl acetyl urea, caffeine, and ibuprofen for the treatment of mild to moderate pain.[28-32]

Pharmaceutical co-crystals of FFA have been reported recently with generally recognized as safe and non-generally recognized as safe molecules. Reported co-crystals include nicotinamide,[33] theophylline,[10] 2-pyridone,[10] and 4,4′-bipyridine.[10,34] Remarkable increment in the solubility of FFA was observed in FFA-nicotinamide and FFA-theophylline co-crystal.[10] In all the reported co-crystals, robust supramolecular acid-pyridine heterosynthon was observed. Conformational polymorphism in FFA molecule was described by Delaney et al.[35] Calcium and barium salts of FFA were reported in recent year by Mohamed et al.[36] In continuation of research on the development of new crystal forms of FFA with improved physical properties, co-crystallization experiment has been carried out and resulted in 2 drug-drug co-crystal of FFA. Owing to the importance and the development of combination drugs, physicochemical properties of the synthesized co-crystals were studied and evaluated in this study. Advantages of drug-drug co-crystal includes reducing the pill burden to patients with improved medications; ability to combine profiles like pharmacokinetics, effects, and adverse effects of a combination drug in a single product; and the drug-drug co-crystals can be protected by patents. The main disadvantage of a drug-drug co-crystal is the probable adverse side reaction that may occur so intriguingly, leading to the difficulty in finding the responsible drug molecule after administration. Numerous reports are available in the literature on drug-drug co-crystal for any particular API; however, drug-drug co-crystal of FFA is found to be scarce. Therefore, this study on pharmaceutical co-crystal of FFA drug may have more significance in the future for the development of combination drug of FFA.

Materials and Methods

FFA and CNB were purchased from Alfa Aesar and Sigma-Aldrich, respectively, and it was used as such without any further purification. ETZ was synthesized in-house as per reported procedure[37] and its structure was confirmed by infrared (IR) and nuclear magnetic resonance (NMR) spectroscopy. Analytical grade solvents were used for all the crystallization experiments. FFA sample obtained from the commercial vendor confirms to form I by differential scanning calorimetry (DSC) and powder x-ray diffraction (PXRD; Supporting Information Fig. S14) techniques. It is observed from the literature that form I can easily be converted to form III during the experimentation, where form III is found to be the most stable crystal form of FFA. Both solvent evaporation and solvent drop grinding approach were performed for the synthesis of co-crystals. The advantage of solvent drop grinding method over solvent evaporation method is that the amount of solvent required for the synthesis can be decreased drastically; however, the purification of co-crystal is difficult by grinding approach. Furthermore, the rate of co-crystallization will be faster by solvent drop-assisted grinding method due to the mechanical force. Immediate co-crystal formation with color change from colorless to orange was observed for FFA-CNB co-crystal. However, no color change was observed for FFA-ETZ co-crystal. After grinding, solvent evaporation method is followed in this study in order to get the crystals as the grinding approach decreases the size of the crystal.

Solvent Evaporation Method for Co-Crystallization

Pure co-crystals were synthesized by solvent evaporation method at room temperature. Experiments were performed with various solvents ranging from most polar to non-polar solvents.

FFA-CNB Co-Crystal

FFA (100 mg, 0.355 mmol) and CNB (71.55 mg, 0.355 mmol) were taken in equimolar ratio and dissolved in 10 mL ethyl acetate solvent at room temperature and left for slow evaporation at ambient condition. Flake-shaped orange colored crystals were obtained after 7 days with 1:1 stoichiometry in the co-crystal. Other solvents like methanol, ethanol, dichloromethane, acetonitrile, acetone, chloroform, and tetrahydrofuran did not yield co-crystal.

FFA-ETZ Co-Crystal

FFA (100 mg, 0.355 mmol) and ETZ (58.64 mg, 0.355 mmol) were taken in equimolar ratio and dissolved in 10 mL ethanol solvent at 60°C and left for slow evaporation at ambient condition. Flake-shaped colorless crystals were obtained after 4 days with 1:1 stoichiometry in the co-crystal. Other solvents like methanol, isopropanol/water yielded co-crystal with 1:1 stoichiometry as well. Polymorphism was not observed in this co-crystal.

Solvent Drop-Assisted Grinding Method

Solvent drop-assisted grinding method was performed to synthesize the co-crystal using mortar and pestle. An equimolar ratio of FFA and the co-former was taken, mixed together, and ground well for 2-3 min with the addition of 1-2 drops of solvent (ethyl acetate for FFA-CNB co-crystal and ethanol for FFA-ETZ co-crystal). This paste was then dissolved in respective solvents for crystallization by slow evaporation (ethyl acetate solvent [10 mL] for FFA-CNB co-crystal and ethanol solvent [10 mL] for FFA-ETZ co-crystal). Good quality crystal was obtained for both co-crystals.

Single-Crystal X-Ray Diffraction

X-ray diffraction data for both the co-crystals were collected on a Bruker Apex II Duo diffractometer with charge coupled device detector. Monochromatic molybdenum Kα radiation ($\lambda = 0.7107$ Å) was used as a source of radiation. Data collection was done at ambient condition (296 K). All the structures were solved by the direct method using SHELXL-2007/2014 software and refinement was carried out by full-matrix least squares technique.[38] Anisotropic displacement parameters were calculated for all non-hydrogen atoms. H atoms attached to the O atoms were located in a different Fourier density map and refined anisotropically. All the diagrams were prepared using Mercury 3.5.1 software.

Powder X-Ray Diffraction

PXRD analysis was carried out using Joel (JDX-8P) powder X-ray diffractometer with Cu Kα radiation ($\lambda = 1.54059$ Å). The voltage and current applied was 40 kV and 30 mA. For a typical experiment, samples were placed on the standard sample holder and then scanned continuously with a scan rate of 2°/min.[39] The diffraction patterns obtained from the co-crystal screening experiments were compared with that of parent API and the co-former. The co-crystal formation was confirmed by the appearance of new peaks in the diffraction pattern.

^{1}H NMR Spectroscopy

^{1}H NMR Spectra were recorded on a Bruker Biospin 400 MHz Spectrometer (Bruker, Billerica, MA). ^{1}H NMR was recorded in a dimethyl sulfoxide-d_6 solvent with tetramethylsilane as the internal reference standard. For the analysis, approximately 5-10 mg of the sample was dissolved in a dimethyl sulfoxide-d_6 solvent and the spectrum was recorded with 16 numbers of the scan.