Carbamazepine-Fumaric Acid Co-Crystal Screening Using Solution Based Method

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Abstract. Co-crystals is a multi-component system which connected by non-covalent interactions, present physically as a solid form under ambient conditions. Nowadays, co-crystal has becoming as an alternative approach to improve the bioavailability of poor water soluble drugs especially for a weakly ionisable groups or neutral compounds. In this study the co-crystal screening was carried out for carbamazepine (CBZ) and fumaric acid (FUM) co-crystal former (CCF) using non-stoichiometric method (addition of CBZ to CCF saturated solution) and stoichiometric method (evaporation of 1:1 molar ratio of CBZ to CCF) in acetonitrile, ethyl acetate, propanol, ethanol and formic acid solvent systems. The crystals produced from the screening were characterized using Powder X-ray Diffraction (PXRD), Differential Scanning Calorimetry (DSC) and Fourier Transform Infrared (FT-IR). The PXRD analysis had confirmed that the co-crystal was successfully formed in both methods for all of the solvent system studied with an exception to formic acid in the stoichiometric method where no crystal was found precipitate. The findings from this study revealed that Form A and Form B of CBZ-FUM co-crystal had been successfully formed from different solvent systems.

1 Introduction

In general, developers and regulatory authorities in pharmaceutical industry are favouring the pharmaceutical crystal because it provides high purity products that are superior with regard to scalability and reproducibility [1]. The pharmaceutical industry deeply wanted to meet patient's therapeutically needs with the invention of co-crystal where the active pharmaceutical ingredient (API) and co-crystal former (CCF) are playing a key role in formulation development of co-crystal [2].

The pharmaceutical co-crystal can be explained as a multicomponent crystal in which at least one of the molecular components is an API and along with the other component called co-crystal former [3]. The role of co-crystal former is believed to help the active drug to disintegrate into small particles and to be transported to the blood stream where the drug is intended to play its role and still protect the product's stability so that it will be at greatest benefits and effectiveness [4]. It has been reported that the co-crystallisation process allowed the binding of two or more crystal components in single crystalline lattice via hydrogen bonding and van der Walls intermolecular interactions without breaking the bonds or making a new covalent bonds [5], [6].

Co-crystals are considering as a major class of pharmaceutical materials to promote the solubility and dissolution. Apart from co-crystals, other materials such as polymorphs, salts, and amorphous solids are also widely used to enhance dissolution and bioavailability of less soluble API [7], [8]. The enhancement of drug solubility is required in the pharmaceutical industry [9] and solubility is also known as the key factors in determining the efficacy as well as the activity of a drug.

Nowadays, most of the generic drug manufacturers are competing in the increasingly sophisticated market. The improvement of the drug solubility will dramatically draw their attention as this can make profit towards their business [10]. The improvement of the solubility can maximize the bioavailability because poor solubility correlates with the poor bioavailability.

In recent years, carbamazepine (CBZ) has served as a model element for researchers to engage in the study of crystal engineering. CBZ is practically insoluble in water and facing challenges of dissolution-limited bioavailability [3], [11]-[15]. Previous study has reported that co-crystals have been successfully formed using screening methods such as slow evaporation, reaction co-crystallisation, solid state grinding and slurry [16]-[21]. Some examples of the successful CBZ co-crystal are CBZ-nicotinamide co-crystal and CBZ-saccharin co-crystal [6], [16], [18], [22], [23].

The aim of this study is to investigate the CBZ-FUM co-crystal formation using non-stoichiometric method which including continuous shaking and stirring and stoichiometric method of solvent evaporation in five different solvent systems (acetonitrile, ethyl acetate, propanol, ethanol and formic acid).

2 Materials and methods

2.1 Materials

The CBZ and FUM were purchased from ECA International Corporation and Sigma-Adrich Company respectively. Whereas, the solvents (acetone, propanol, ethyl acetate, formic acid, acetonitrile and absolute ethanol) were supplied by Fisher Scientific with purity exceeding 99%.

2.2 Experimental procedures

2.2.1 Non-stoichiometric crystallisation

Saturated solution of the CCF (FUM) was prepared using propanol, ethyl acetate, formic acid, acetonitrile and absolute ethanol in 100 mL conical flask each at room temperature. The solid CCF was added into a fixed amount of solvent. The mixture was agitated at room temperature until the entire CCF was dissolved. More solid CCF was added to the solution manually until some precipitation occurred. The solutions were left to equilibrate for about 72 hours. After 72 hours, the final solutions were filtered from the conical flask using 0.22 μm syringe filter in order to remove excess solid. After filtration, the saturated solution of the CCF was filled in another clean 100 mL conical flask.

The co-crystal was prepared by adding about 0.1-1.2 g of solid CBZ to the prepared saturated solution. The amount of CBZ added varied depending on the type of solvent systems. The solutions were left to equilibrate for about 72 hours in two conditions i.e. in an automated shaker with 150 rpm at room temperature and stirred using a magnetic stirrer. Finally, filter paper was used to separate the final solution slurry and the solid was dried at room temperature. All experiments were performed in triplicate.

2.2.2 Stoichiometric crystallisation

A combination of 1:1 molar ratios of the CBZ and CCF was mixed and stirred to dissolve in a 25 mL of solvent i.e. propanol, ethyl acetate, formic acid, acetonitrile and absolute ethanol in 100 mL conical flask each, at room temperature. More solvent was added until the solutes were fully dissolved in any cases where the complete dissolution was not obtained. Once the solute had fully dissolved, another 10 mL of solvent was added into the final solution. The solution was removed from the conical flask using 0.22 μm syringe filter to remove any impurities. The solution was filled in the 20 mL vial and covered with parafilm, with a few holes poked in in and left to evaporate at room temperature. The produced crystal was filtered using filter paper and dried in the oven at 30^0C for 24 hours. The dried crystal was kept in a vial. All experiments were performed in triplicate.

2.3 Co-crystal characterisations

2.3.1 Powder X-ray diffraction (PXRD)

PXRD was used to determine the presence of co-crystal in the samples by showing different peak profiles. A RIGAKU (Miniflex II) diffractometer was operated at the operating conditions as following: (Cu Kα radiation, voltage 30kV, current 15mA, step size 0.01, step time 1 s and angular range between 3^0 and 40^0 2θ scales.

2.3.2 Differential scanning calorimetry (DSC)

DSC model G1000 with series no. of Q1000-0567 was used to determine the melting point of the co-crystal. The samples (1-3 mg) were crimped in aluminium pans and lid then heated from 30 to 300^0C, under nitrogen purge with flowrate of 50 ml/min at a heating rate of 10 ^{0}C/min.

2.3.3 Fourier transform infrared (FT-IR)

The samples were analysed by FTIR to determine the presence of certain functional groups in a molecule. The analysis was performed using FT-IR with 50 series model attached with the diamond detector at the wave number from 4000-600 cm^{-1} using 32 scans per spectrum with a resolution 4 cm^{-1} for each sample.

3 Results and discussion

3.1 Powder X-ray diffraction (PXRD)

PXRD had been used to identify the presence of co-crystal since every crystalline solid phase has its unique PXRD pattern [24]. The pattern profile shows in Fig. 1 confirmed that CBZ used in this study was Form III [25]. From the analysis obtained from five solvents using stoichiometric and non-stoichiometric method, it was found that co-crystal had been successfully formed with an exception to formic acid in stoichiometry method where no crystal was precipitated. The results were compared with pure components (CBZ and FUM) in order to confirm the formation of co-crystal.

The findings revealed that CBZ-FUM co-crystal was formed in two forms i.e. Form A and Form B depending on the solvent and method used. This finding was agreed with previous, stated that polymorphic transformation of a crystal may affected with the method used during the crystallisation process [21, 26]. According to Childs and co-workers in 2008, they have reported that CBZ-FUM co-crystal Form A and Form B was formed from saturated aqueous and near saturated or saturated ethanolic solution respectively [27].

In addition, the mole ratio of CBZ to FUM used during the experimental work also gave an effect toward the polymorphic form of the co-crystal [28]. The pattern profile of the co-crystal formed from shaking, stirring and evaporation in acetonitrile and ethyl acetate has confirmed that Form A of co-crystal was obtained from both methods of the screening. On the other hand, for formic acid, Form B of co-crystal was formed from only non-stoichiometric method. Different results were found

for ethanol and propanol solvent. The form A and Form B co-crystal was formed from stoichiometric and non-stoichiometric method respectively for ethanol whereas, in propanol, Form B was formed from shaking condition (non-stoichiometric) and Form A formed from stirring condition (non-stoichiometric) and stoichiometric methods as shown in Fig. 1.

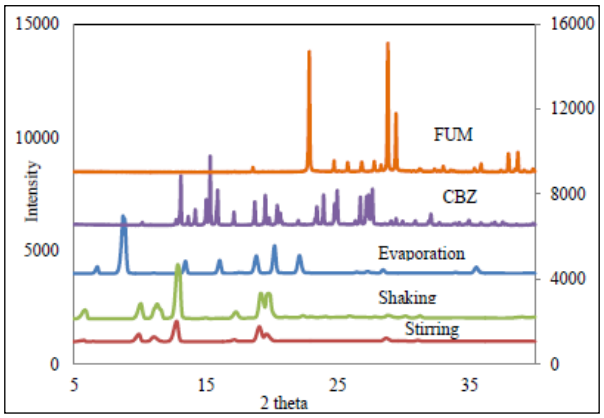

Figure 1. PXRD pattern profile for stoichiometric (form a co-crystal) and non-stoichiometric (form b co-crystal) in ethanol as a solvent.

Table 1. Summary results for stoichiometric and non-stoichiometric method.

Solvent	Screening Method	CBZ-FUM Form A	CBZ-FUM Form B
Acetonitrile	Stirring	√	
	Shaking	√	
	Evaporation	√	
Ethyl acetate	Stirring	√	
	Shaking	√	
	Evaporation	√	
Formic acid	Stirring		√
	Shaking		√
	Evaporation		
Ethanol	Stirring		√
	Shaking		√
	Evaporation	√	
Propanol	Stirring	√	
	Shaking		√
	Evaporation	√	

The overall analysis results including the co-crystals formed from ethyl acetate, formic acid, ethanol and propanol were summarized in Table 1. Table 1 indicated that co-crystals were successfully formed except from solvent evaporation for formic acid which was failed to yield either nor, CBZ, FUM and co-crystal. This is presumably that the solubility of CBZ in the solvent medium directly affected the formation of the CBZ, FUM or co-crystal that required as an optimum condition to induce the precipitation of the crystal in solvent evaporation.

3.2 Differential scanning calorimetry (DSC)

The melting point obtained for FUM was 293^{o}C. On the other hand, there was a weak endothermic peak at 172^{o}C then followed by another endotherm at 191^{o}C for CBZ as shown in Fig. 2. Grzesiak et al. in 2003, has reported that the polymorphic transformation was occurred between 162-175^{o}C and the new phase melted between 189-193^{o}C with a heating rate of 10 ^{o}C/min [25]. This statement has described the polymorphic transformation of Form III to Form I. Therefore the last endothermic peak at 191^{o}C was attributed to the melting point of CBZ Form 1. The finding revealed that DSC analysis is important not only for co-crystal characterization but also for polymorphic characterization, since distinct polymorphs will have different melting temperatures.

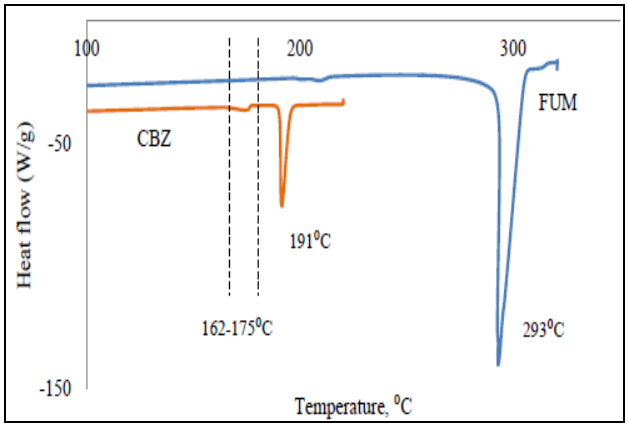

Figure 2. DSC analysis for pure component of CBZ and FUM.

The data obtained from DSC analysis shown that the form A of CBZ-FUM co-crystal has a melting point between 172-176^{o}C [19]. On the other hand, the form B of CBZ-FUM co-crystal has a melting in the range of 186-190^{o}C [21, 27, 28]. The increased of melting point for different of co-crystal polymorphic form was possibly due to the crystal packing nature in the CBZ-FUM co-crystal [1]. The Form A of CBZ-FUM co-crystal has an additional peak appeared in the range of 116-126^{o}C which was due to the existing of water molecule in the co-crystal [27] as shown in Fig. 3.

3.3 Fourier transform infrared (FT-IR)

The peak position in the co-crystal compare with the pure component for acetonitrile was summarized in Table 2. In the FT-IR spectrum of CBZ-FUM co-crystal it shows that the peak profiles was a bit higher or lower frequency

compared to the pure component. The significant shift of wavelength had confirmed the formation of the co-crystal [1]. There were some significant peaks presented in the range of 3500 cm^{-1} to 1550 cm^{-1} and 3400 cm^{-1} to 1650 cm^{-1} for the co-crystal in FT-IR analysis results. The occurred peaks were attributed to amides and carboxylic acids functional group of CBZ and FUM.

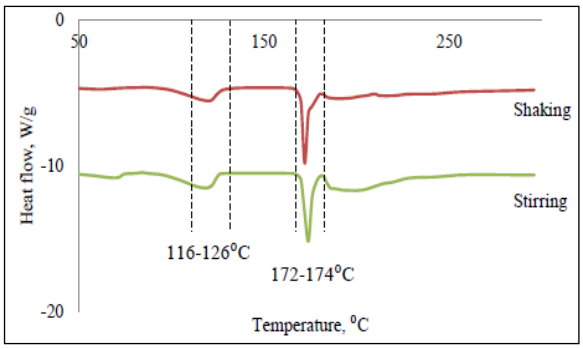

Figure 3. DSC analysis for CBZ-FUM screening obtained from acetonitrile with shaking and stirring (non-stoichiometric method)

Table 2. FTIR data for CBZ, FUM and CBZ-FUM co-crystal based on different screening method in acetonitrile as a solvent.

Type	CBZ	FUM	CBZ-FUM co-crystal		
			Shaking	Stirring	Evaporation
N-H Stretch	3464	-	3455	3455	3455
C=O Stretch	1604	-	1604	1602	1602
N-H Bend	1593	-	1590	1590	1590
O-H Stretch	-	3082	3059	3059	3058
C=O Stretch	-	1659	1660	1659	1656

The FT-IR analysis for ethyl acetate, formic acid, propanol and ethanol had shown the presented of amides and carboxylic acids in the co-crystal but there was some differences between wavelength frequency for both of the functional group in Form A and Form B co-crystal. From the result it shows that Form A with amides and carboxylic acid in range between 3457 cm^{-1} to 1563 cm^{-1} and 3059 cm^{-1} and 1656 cm^{-1} respectively. Whereas for Form B the amides wavelength frequency between 3461 cm^{-1} and 1520 cm^{-1}. The wavelength frequency of 3027 cm^{-1} to1656 cm^{-1} was represented for carboxylic acids.

4 Conclusions

The formation of CBZ-FUM co-crystals were investigated using non-stoichiometric (continuous shaking and stirring conditions) and stoichiometric (solvent evaporation) methods. The PXRD analysis had confirmed that the co-crystal was successfully formed in both methods for all of the solvents systems studied with an exception to formic acid in the stoichiometric method where no crystal was precipitated. From this analysis it was also shows that Form A and Form B co-crystal had been produced from different solvents. This study has revealed that detail screening study is needed for co-crystal formation assessment since the previous and current findings have revealed that different methods, solvent and mole ratio resulted the polymorphic transformation of the co-crystal.

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