

Dissolution rate enhancement of new co-crystals of ezetimibe with maleic acid and isonicotinamide

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Abstract: Ezetimibe (EZT) is a selective cholesterol absorption inhibitor with poor aqueous solubility (0.012mg/ml 23°C) and low oral bioavailability (about 35-65% for a once 10mg dose). The present study illustrates the preparation and characterization of two new co-crystals of ezetimibe using maleic acid and isonicotinamide as the cofomers by solid grinding method. The co-crystal structures were characterized by X-ray powder diffraction (PXRD), differential scanning calorimetry (DSC), infrared spectroscopy (IR) techniques. Crystallinity and surface morphological characteristics of these prepared co-crystals were observed by scanning electron microscope (SEM). Dissolution rate tests demonstrated that both of the new co-crystals showed significant improvement in sodium lauryl sulfate -sodium acetate buffer solution (PH=4.5) at 15min and 20min. This study enriched the types of EZT co-crystals and identified that pharmaceutical co-crystal engineering technique play an important role in the dissolution rate enhancement of ezetimibe.

Keywords: Ezetimibe, maleic acid, isonicotinamide co-crystal, solubility, dissolution.

INTRODUCTION

Pharmaceutical molecules with limited aqueous solubility can provide a number of challenges in drug development and may lead to slow dissolution rate and poor bioavailability, especially for the oral medicines (Blagden *et al.*, 2007; Aakeroy *et al.*, 2009). Pharmaceutical co-crystal formation is an emerging method to meet the requirements of the improvement of solubility and dissolution behavior of the APIs (Aitipamula *et al.*, 2014; El *et al.*, 2015; Yong *et al.*, 2016). Ezetimibe (EZT, fig. 1a), 1-(4-Fluorophenyl)-(3R)-3-(4-fluorophenyl)-(3S)-hydroxypropyl-(4S)-(4-hydroxyphenyl)-2-azetidinone, is a selective cholesterol absorption inhibitor used for the treatment of elevated plasma cholesterol levels (Garciaalvo *et al.*, 2005; Stitzel *et al.*, 2014). It has a rapid popularization in the clinical application after it approved by the FDA in 2002. Then in 2014, the results of IMPROVE-IT trial which is a large-scale clinical study lasted more than nine years were showed that the combination of ezetimibe and simvastatin could reduce the risk of cardiovascular death by 10% (Anderson, 2014; Spinar *et al.*, 2014; Pirillo *et al.*, 2016). Although EZT has a outstanding clinical value, its aqueous solubility is very poor (0.012mg/ml, 23°C) and for this reason its oral bioavailability is only about 35-65% for a once 10mg dose (Stitzel *et al.*, 2014).

With the aim of improving the pharmaceutical properties of EZT, five co-crystals as EZT- salicylic, EZT- benzoic

acid (Mulye *et al.*, 2012), EZT- methyl paraben (Sugandha *et al.*, 2014), EZT- L-proline and EZT- imidazole (Shimpi *et al.*, 2014; Ludeker and Brunklaus, 2015) have been reported by different research teams in recent years. All of these five co-crystals of EZT have exhibited the improvement physicochemical properties in different degrees. But, the types of EZT co-crystal are still scarce and the co-crystals have been reported were prepared by solvent crystallization methods in most cases. There are diverse rearrangements and hydrolysis reactions of EZT in solvents have attracted our attention (Sánta *et al.*, 2012; Filip *et al.*, 2011; Luo *et al.*, 2015), and we found that it could transfer to its cyclizate ether impurity even in the neutral mediums in the co-crystal screening processes (Hu *et al.*, 2016). Thinking of the stability of EZT in solvent, the solid grinding method is more appropriate than the solution crystallization methods, obviously.

In this paper, new co-crystals of ezetimibe with maleic acid (fig. 1b) and isonicotinamide (fig. 1c) were prepared by solid grinding method and their dissolution rate changes were also determined.

MATERIALS AND METHODS

Experimental section

EZT was obtained from Henan Furen Pharmaceutical Technology Development Co. Ltd (Zhengzhou, China) and was used without further purification (purity \geq 99.5%, 163222-32-0). Maleic acid was got from Shanghai Sanpu Chemical Co., Ltd. (analytical grade). Isonicotinamide

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was bought from Sinopharm Chemical Reagent Co. Ltd. (analytical grade). All other reagents were analytical or chromatographic grade.

Preparation of co-crystals

EZT-maleic acid co-crystal (EZT-MA) was prepared by grinding method which was performed with a FRITSCH Pulverisette 7 Premium Line Mill equipped with 70ml stainless steel grinding jar and 5mm zirconium oxide grinding balls. A mixture of a 1:1 molar ratio of EZT and maleic acid at a total of 10g was placed in the grinding jar with 20g grinding balls. The grinding time is 3min, the speed is 800rpm, the interval time is 5min and the cyclic number is 7-9 times.

EZT-isonicotinamide co-crystal (EZT-ISONIC) was prepared by the similar process as EZT-MA.

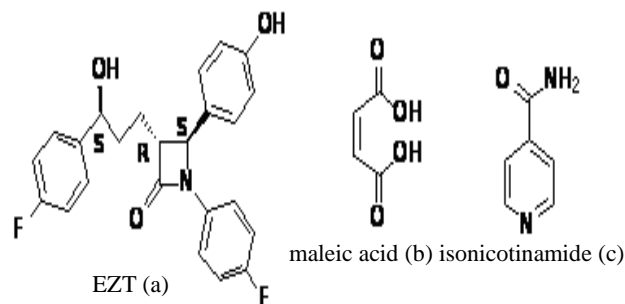


Fig. 1: The structure of ezetimibe and its co-formers

Powder X-ray diffraction (PXRD)

The PXRD patterns of all two co-crystals were measured on a X'Pert PRO X-ray powder diffractometer (PANalytical, Holland) using Cu K α ($\lambda=1.5406$ Å) at 40kV and 40mA. X-ray diffraction data were collected at the 2θ range of 5° to 60° with a step size of 0.02° . The diffraction patterns were processed using JADE 5.0 and calibration line was carried out using a silicon standard.

Thermal characterization (TG-DSC)

The melting point and heat of fusion of both two co-crystals were recorded with a SDT Q600 DSC from TA Instruments. Accurately weighed samples (5-10mg) were placed in an Alumina crucible at the atmosphere of N $_2$ during the experiments. The enthalpy and temperature calibration line was performed using the highly pure zinc. The tested temperature range was 20 to 300°C at the rate of 10K/min. Sample purge flow was 100ml/min.

IR spectroscopy

IR spectra were collected of both two co-crystals with a FT-IR 200 spectrometer (Nicolet Company, USA), the IR spectra range was 4500 to 400cm $^{-1}$ and spectral resolution was 4cm $^{-1}$. Samples mixed with KBr were compressed and IR spectra were recorded. The data were analyzed by Nicolet Omnic 8.2 program.

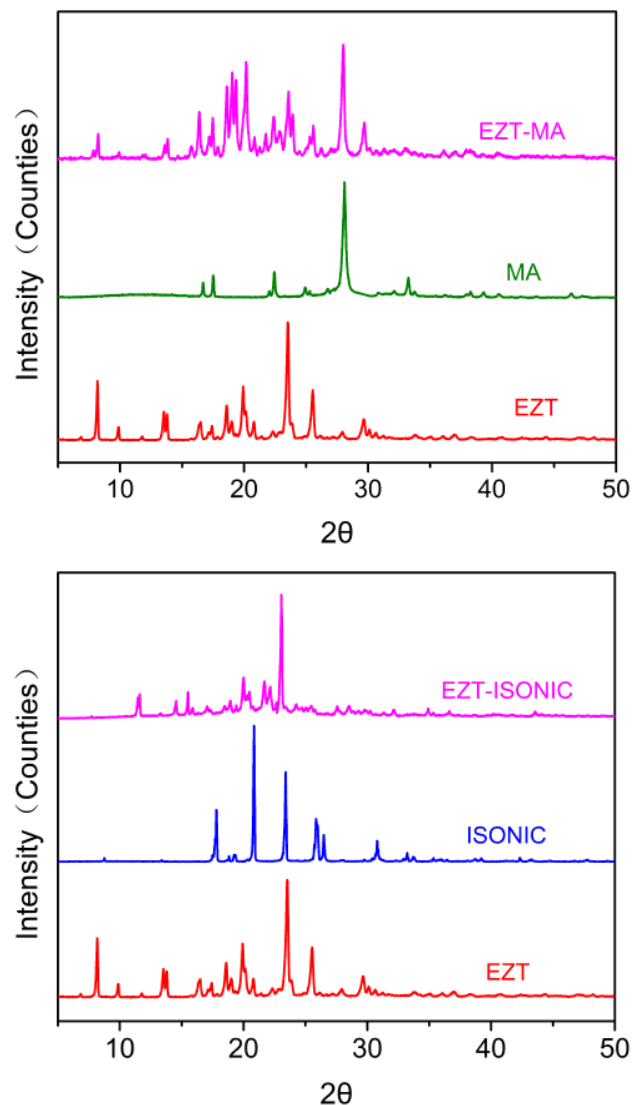


Fig. 2: The PXRD patterns of EZT, co-formers and co-crystals. EZT: ezetimibe; MA: maleic acid; ISONIC: isonicotinamide; EZT-MA: ezetimibe- maleic acid co-crystal; EZT-ISONIC: ezetimibe- isonicotinamide co-crystal.

Scanning electron microscopy (SEM)

The form and surface conditions of specimens of both two co-crystals were observed using a low-vacuum scanning electron microscopy JEOL JSM-7500F at room temperature.

Solubility measurements

Solubility of EZT-MA and EZT-ISONIC co-crystals were measured in distilled water at $25\pm 2^\circ\text{C}$ according to the European Pharmacopoeia method using ezetimibe as the control. Put the accurately weighed fine powders of co-crystal into specified volume water, and then shake 30 seconds every 5 minutes. Observe the visible solute particles at 30 minutes and record the solubility.

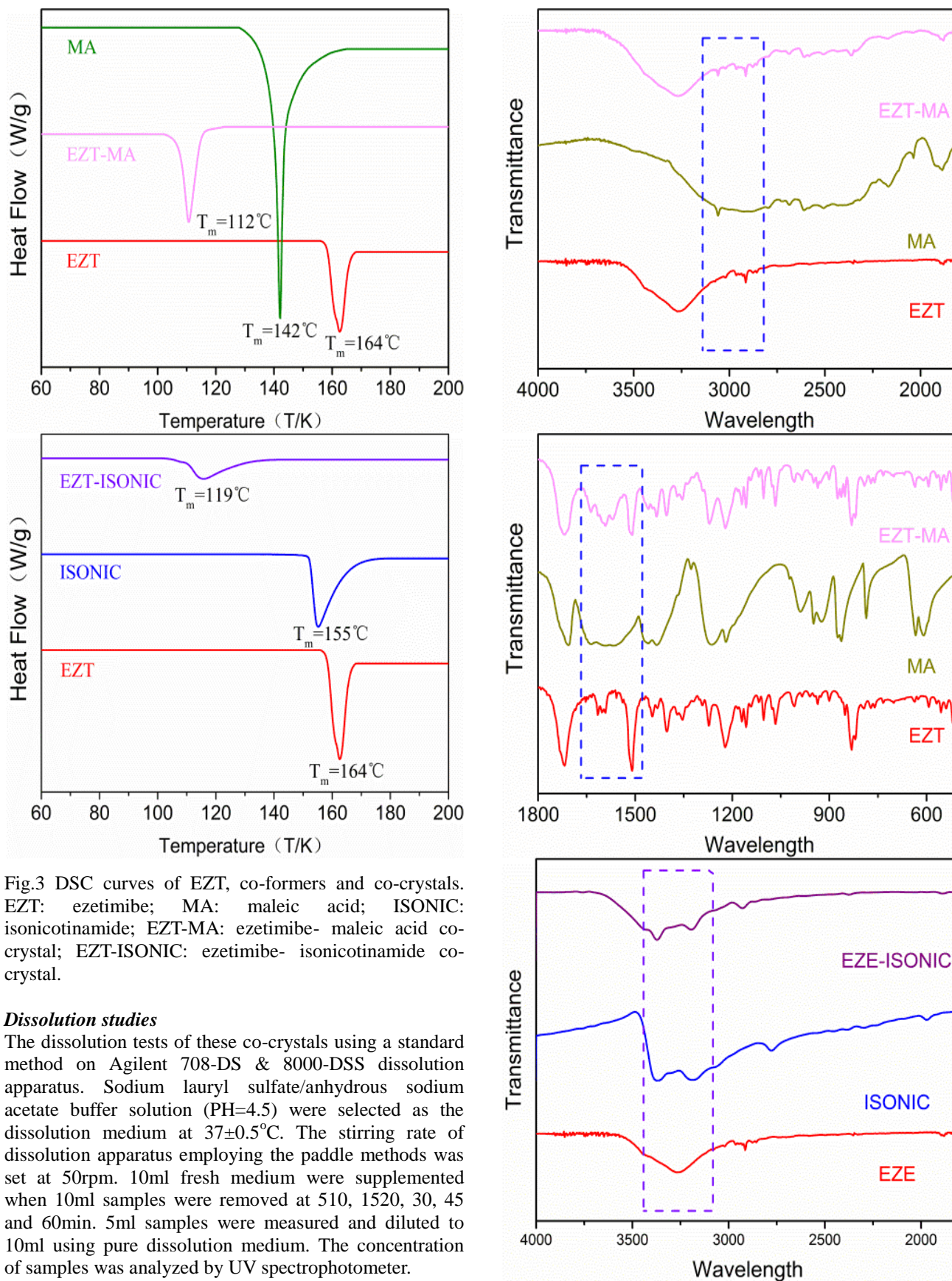


Fig.3 DSC curves of EZT, co-formers and co-crystals. EZT: ezetimibe; MA: maleic acid; ISONIC: isonicotinamide; EZT-MA: ezetimibe- maleic acid co-crystal; EZT-ISONIC: ezetimibe- isonicotinamide co-crystal.

Dissolution studies

The dissolution tests of these co-crystals using a standard method on Agilent 708-DS & 8000-DSS dissolution apparatus. Sodium lauryl sulfate/anhydrous sodium acetate buffer solution (PH=4.5) were selected as the dissolution medium at $37 \pm 0.5^\circ\text{C}$. The stirring rate of dissolution apparatus employing the paddle methods was set at 50rpm. 10ml fresh medium were supplemented when 10ml samples were removed at 5, 10, 15, 30, 45 and 60min. 5ml samples were measured and diluted to 10ml using pure dissolution medium. The concentration of samples was analyzed by UV spectrophotometer.

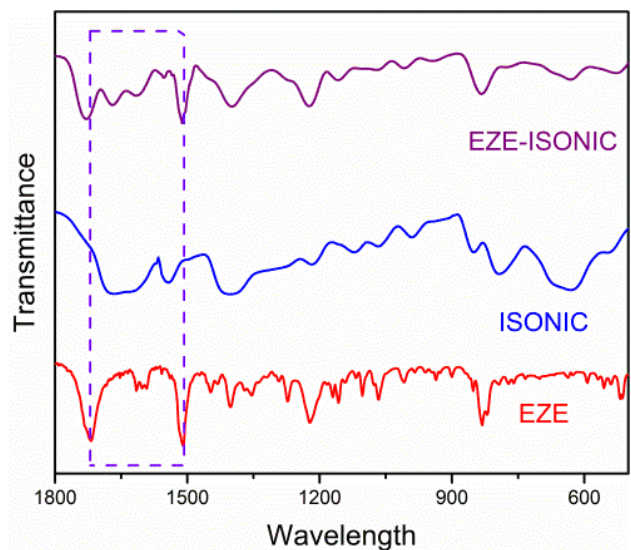
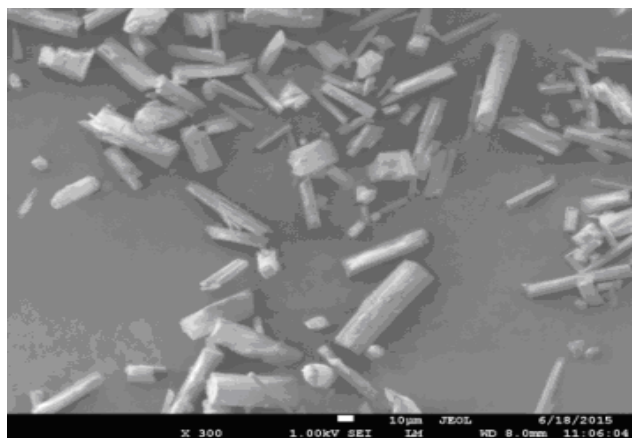
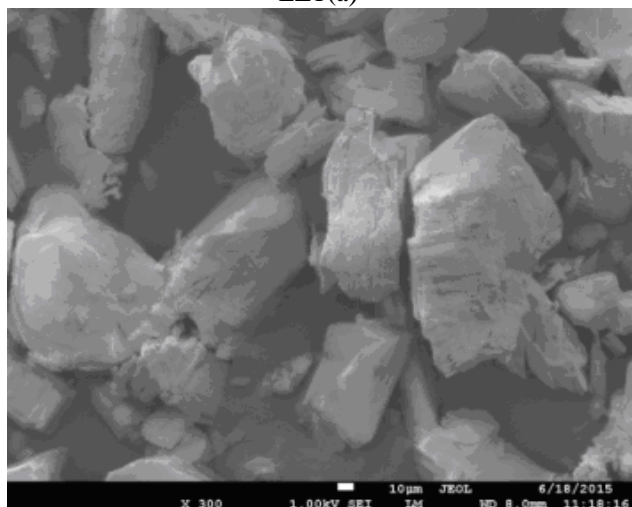


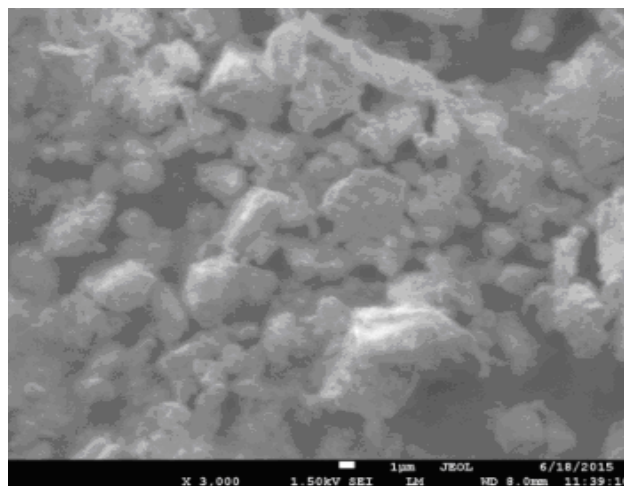
Fig. 4: FTIR spectra for EZT, co-formers and co-crystals. EZT: ezetimibe; MA: maleic acid; ISONIC: isonicotinamide; EZT-MA: ezetimibe- maleic acid co-crystal; EZT-ISONIC: ezetimibe- isonicotinamide co-crystal.



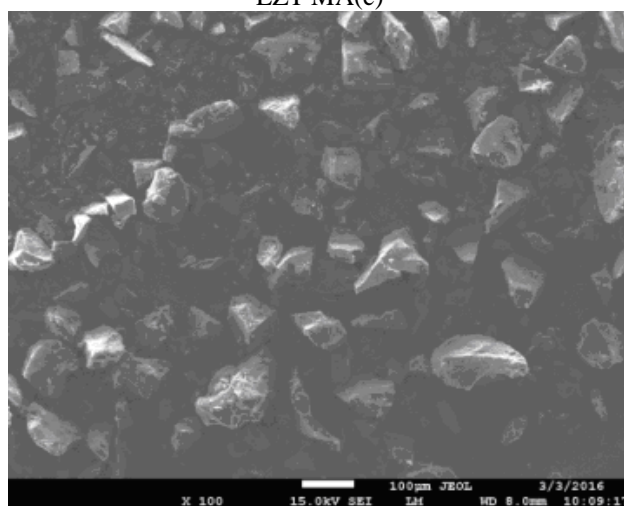
EZT(a)



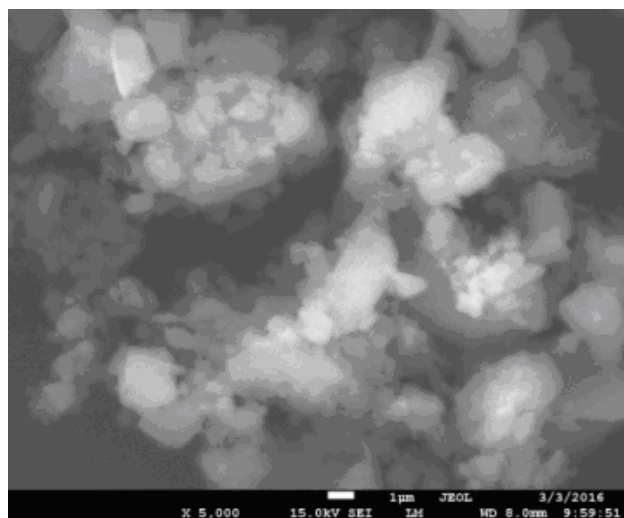
MA(b)



EZT-MA(c)



ISONIC(d)



EZT-ISONIC(e)

Fig. 5: Scanning Electron Microscopy of EZT, co-formers and co-crystals. EZT(a): ezetimibe; MA(b) : maleic acid; EZT-MA(c): ezetimibe- maleic acid co-crystal; ISONIC(d): isonicotinamide; EZT-ISONIC(e): ezetimibe- isonicotinamide co-crystal.

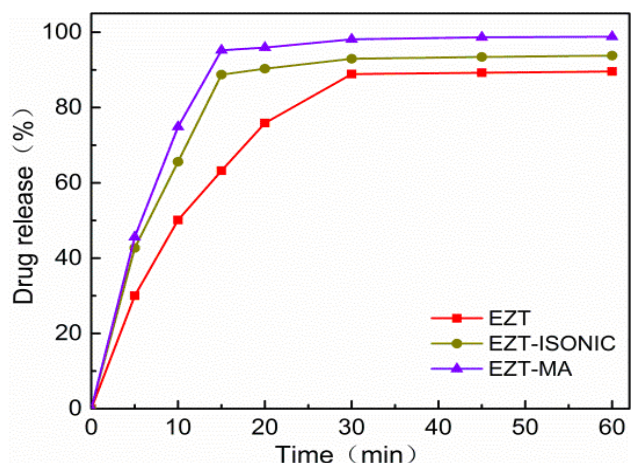


Fig. 6: Dissolution profiles for EZE and its cocrystals EZT-MA and EZT-ISONIC.

RESULTS

Powder X-ray diffraction (PXRD)

PXRD is a useful technique for identified the presence of new crystal form during the co-crystallization processes. Starting materials and co-crystals were characterized by PXRD to identify the solid patterns (fig. 2).

EZT exhibited the characteristic reflections of 2θ at about 8.1 ± 0.1 , 11.8 ± 0.1 , 13.5 ± 0.1 , 16.5 ± 0.1 , 19.0 ± 0.1 , 19.9 ± 0.1 , 25.5 ± 0.1 . Maleic acid exhibited the characteristic reflections of 2θ at about 17.5 ± 0.1 , 22.0 ± 0.1 , 22.4 ± 0.1 , 25.3 ± 0.1 , 33.2 ± 0.1 . The cocrystal EZT-MA produced from EZT and maleic acid exhibited the characteristic reflections of 2θ at about 7.9 ± 0.1 , 15.8 ± 0.1 , 17.2 ± 0.1 , 19.0 ± 0.1 , 22.4 ± 0.1 , 25.6 ± 0.1 .

Isonicotinamide exhibited the characteristic reflections of 2θ at about 8.76 ± 0.1 , 17.80 ± 0.1 , 20.83 ± 0.1 , 23.41 ± 0.1 , and 25.86 ± 0.1 . The cocrystal EZT-ISONIC produced from EZT and isonicotinamide exhibited the characteristic reflections of 2θ at about 7.7 ± 0.1 , 15.5 ± 0.1 , 15.9 ± 0.1 , 20.0 ± 0.1 , 23.0 ± 0.1 .

Thermal characterization (TG-DSC)

The DSC thermograms of EZT, maleic acid, isonicotinamide, co-crystal EZT-MA and co-crystal EZT-ISONIC are shown in fig. 3.

IR spectroscopy

Co-crystal formation often results in conformational and structural variations of the constituent components that can be determined by spectroscopic techniques (Aitipamula *et al.*, 2014). Infrared spectroscopy can be a very powerful tool in detecting co-crystal formation (Schultheiss and Newman, 2009). The IR spectra of EZT, co-formers and co-crystals are showed in fig. 4.

Scanning electron microscope (SE)

The SEM of all samples was shown in fig. 5.

Dissolution studies

Dissolution experiment were performed on EZT and co-crystals in sodium dodecyl -sulfate-sodium acetate (PH=4.5) at $37\pm 0.5^\circ\text{C}$. Fig. 6 compares the dissolution curves for EZT and its co-crystals.

DISCUSSION

Powder X-ray diffraction (PXRD)

As shown in fig. 2, unique PXRD patterns of both co-crystals distinguishable from EZT and the cofomers were obvious.

Thermal characterization (TG-DSC)

The DSC thermograms of EZT, maleic acid, isonicotinamide, co-crystal EZT-MA and co-crystal EZT-ISONIC showed single melting peak. In both co-crystals, melting endotherm was lower than that of either EZT or its co-former. EZT-MA showed a sharp melting endotherm at 112°C while EZT-ISONIC showed a broad melting endotherm at 119°C .

The TGA profiles of both co-crystals showed no mass loss which suggesting that there was no degradation during the tests.

The changes of co-crystal melting endotherm might be owing to the formation of hydrogen bonding between EZT and its cofomers which resulted in a molecular arrangement.

IR spectroscopy

It observed from fig. 4 that some bands for the starting materials had shifted. The IR spectra of EZT shows strong peaks at 3271.46cm^{-1} and 1718.49cm^{-1} , corresponding to ν (O-H) and ν (C=O), respectively. Strong peaks for pure MA appears at 1706cm^{-1} . It was obvious that original strong O-H stretching band for EZT at 3271cm^{-1} move to 3266cm^{-1} . Unexpected, the band of MA correspondPing to ν (O-H) original at 1706cm^{-1} almost disappear. These results show that O-H group of EZT form hydrogen bonds with C=O group of MA.

The characteristic strong N-H stretching band for ISONIC is at 3368.52cm^{-1} and 3187.19cm^{-1} and the band at 1665.56cm^{-1} corresponds to C=O stretching band. However, these bands had been seen at 3372.16cm^{-1} , 3194.06cm^{-1} and 1398.85cm^{-1} in the co-crystal, respectively.

Scanning electron microscope (SE)

It is obvious from the SEM that EZT appeared as mainly rod-shaped and tetragonal with the average partical size of about $10\ \mu\text{m}$, MA appeared as in regular bulks crystals and ISONIC mainly exhibited bulk crystals. However, the surface morphological properties for EZT-MA co-crystal aggregated fine particles into irregular shape, and the

average partical size of the EZT- ISONIC about 1 μ m are much smaller than others. This is due to the starting material, EZT, MA and ISONIC, were rushed by high-speed rotation of the ball mill, which is a very high entropy synthesis route (Spinar *et al.*, 2014).

Dissolution studies

From fig. 6, it indicated that the EZT complexes show superior dissolution rate by contrast with the original API. The dissolution of MA co-crystals in 15min was more than reaching 90% and ISONIC co-crystal released 88% drug within 15min. However, the release for pure EZT was only 63% in 15min. The dissolution plots of co-crystals reach a maximum value within 20min, and then slowly rise over time. MA co-crystal dissolution is EZT 1.51 times in 15min and it was 1.4 fold from ISONIC co-crystal in 15min.

CONCLUSIONS

Ezetimibe-maleic acid (EZT-MA) and Ezetimibe-isonicotinamide (EZT-ISONIC) co-crystal powders were prepared for the first time by solid grinding method. The co-crystal structures were characterized by X-ray powder diffraction (PXRD), differential scanning calorimetry (DSC), infrared spectroscopy (IR) techniques. Crystallinity and surface morphological characteristics of these prepared co-crystals were observed by scanning electron microscope (SEM). Dissolution rate tests demonstrated that both of the new co-crystals showed significant improvement in sodium lauryl sulfate -sodium acetate buffer solution (PH=4.5) at 15min and 20min. This study enriched the types of EZT co-crystals and identified that pharmaceutical co-crystal engineering technique play an important role in the dissolution rate enhancement of ezetimibe.

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