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Cocrystals of Enoxacin with Heptanedioic Acid: Preparation and Characterization

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Abstract

Enoxacin (EX), a third-generation fluoroquinolone, demonstrates broad-spectrum antibacterial activities and is widely applied for the treatment of various bacterial infections. However, it is challenging to improve its antibacterial activity and drug resistance effects based on solubility changes owing to EX being categorized as a class II drug in the Biopharmaceutics Classification System (BCS). Based on improving the solubility of enoxacin (EX) to enhance the antibacterial activity in vitro, pharmaceutical cocrystals of EX with heptanedioic acid have been designed, synthesized and characterized. Comprehensive analysis structure and Hirshfeld surface reveal that the hydrogen bonds formed by the N atom in the piperazine ring from EX molecule with the carboxylic acid group in the coformer could form a stable crystal structure. In summary, this study provides new insights to solid form of EX.

Subject Areas

Chemical Engineering & Technology

Keywords

Enoxacin, Cocrystal, Heptanedioic Acid

1. Introduction

Enoxacin, as a promising active pharmaceutical ingredient, is a widely used quinolone antibiotic. Because of its broad antibacterial applications, strong bactericidal effect and little cross resistance with other antibiotics, it has attracted the attention of the scientific community [1] [2]. However, the poor water solubility of EX is one of the key elements that limit its clinical optimal antibacterial activity. Therefore, in recent years, our research group has been devoted to solving its low solubility in water, and enhancing its antibacterial activity through

the concept of crystal engineering. Up until now, kinds of EX pharmaceutical salts/cocrystals (namely, pharmaceutical salts of EX with oxalic acid ($C_{15}H_{17}FN_4O_3{\cdot}0.5C_2H_2O_4{\cdot}2H_2O$), malonic acid ($C_{15}H_{18}FN_4O_3{\cdot}C_3H_3O_4$), fumaric acid ($C_{15}H_{18}FN_4O_3{\cdot}C_4H_3O_4$) [3], and with hydroxybenzonic acids [4] by introducing carboxylic acids as conformers have successfully synthesized and reported. Although salts/crystals of EX have been prepared, the optimal salts/crystals for EX clinical drugs have not been obtained and the effect of Charge assisted hydrogen bond on improving physicochemical properties of EX has not been explored. Therefore, the design and synthesis of pharmaceutical salts/crystals with improved EX solubility and avoidance of hygroscopicity by charge assisted hydrogen bond remain a challenge. Based on the above motives, in order to design and synthesis the single crystal of EX pharmaceutical salts/cocrystals avoiding hygroscopicity of enoxacin and improving its solubility, the following factors that promote the formation of charge assisted hydrogen bond in drug salts/cocrystals: 1) Nitrogen atoms with lone pair of electrons on tetrahydro berberine act as acceptors or trap hydrogen proton as hydrogen bond donors. 2) When the structure of coformers contains carboxyl group, the pharmaceutical salts/cocrystals are formed which depend on charge assisted hydrogen bond between the coformers that easy to give hydrogen protons and active pharmaceutical ingredient molecules. [5] Thus, in order to use the action of charge assisted hydrogen bond to prepare EX pharmaceutical salts/cocrystals, heptanedioic acid was selected to prepare EX pharmaceutical salts/cocrystals. Moreover, Hirshfeld surface analysis [6] [7], as an effective technique for the qualitative and quantitative determination of intermolecular interactions, has been successfully adopted to quantitatively analyze the impacts of various interactions on the stability of lattice arrangement.

Therefore, research on novel EX cocrystal will not only provide an upgraded solid form of EX but also offer more choices for treatment of inflammation-associated pain and fever caused by bacterial infection.

2. Experimental

2.1. Materials and Methods

Enoxacin (EX, 98%), heptanedioic acid (HDA, 98%) were purchased from Energy Chemical. IR spectra were collected in the 400 - 4000 cm^{-1} region with KBr pellet by Bruker VERTEX 70. X-ray powder diffraction (XRD) were recorded on a Rigaku Ultima IV (Rigaku, Japan) at 40 kV and 200 mA, and Cu Kα radiation in a low background holder. The date was collected at room temperature with a scanning speed of 10˚/min and the 2 range of 5˚ - 40˚. Differential scanning calorimetry (DSC) was carried out by a Netzsch DMA GABO Eplexor. Approximately 5 - 10 mg of samples was placed in Al_2O_3 crucibles and instrument under the nitrogen atmosphere at a heating rate of 10˚C/min and in a range of 20˚C - 500˚C.

2.2. Preparation of EX-HDA

A mixture of Enoxacin (48.0 mg, 0.15 mmol) and heptanedioic acid (24.0 mg, 0.15 mmol) was dissolved into 10 mL solution of methanol. The resulting mixture was sufficiently stirred for 3 h under 50˚C thermostatic water bath. After filtration, the filtrate was evaporated slowly at room temperature. After three days, colorless transparent block crystals of EX-HDA were harvested in 80% yield (based on EX).

2.3. Single Crystal X-Ray Diffraction Analyses

The single crystal X-ray diffraction data of the three EX cocrystals/salts were collected in the CCD system by Mo Kα radiation (λ = 0.71073 Å) at room temperature. Multi-scan absorption corrections were applied, and the structure was solved by direct methods and refined by full matrix least squares on F2 using the SHELXL-2014 and SHELXL-2015 respectively, available within Olex2 or WinGx packages. Selected crystallographic data are provided in Table 1. All non-hydrogen

Table 1. Crystal structure and refinement data for cocrystals of EX.

cell parameter	Crystal data
formula	$C_{39}H_{54}F_2N_8O_{12}$
CCDC	2,256,588
relative molecular mass	864.90
temperature/K	170.00
crystal system	monoclinic
space group	C2/c
a/Å	24.615(4)
b/Å	7.1329(12)
c/Å	24.231(4)
α/˚	90
β/˚	93.963(7)
γ/˚	90
Volume/Å^3	4244.3(13)
Z	4
ρ_{calc}g/cm^3	1.354
μ/mm^{-1}	0.574
F(000)	1832.0
Crystal size/mm^3	0.16 × 0.05 × 0.02
Radiation	GaKα (λ = 1.34139)
2Θ range for data collection/˚	6.262 to 121.492
Index ranges	−32 ≤ h ≤ 32, −9 ≤ k ≤ 9, −31 ≤ l ≤ 31
Reflections collected	21,952
Independent reflections	4818 [R_{int} = 0.0595, R_{sigma} = 0.0598]
Data/restraints/parameters	4818/29/299
Goodness-of-fit on F^2	1.097
Final R indexes [$I \geq 2\sigma(I)$]	R_1 = 0.0860, wR_2 = 0.2581
Final R indexes [all data]	R_1 = 0.1029, wR_2 = 0.2820
Largest diff. peak/hole/Å^{-3}	0.71/−0.65

atoms were refined with anisotropic displacement parameters. Especially, H atoms of N-H or O-H were refined from difference Fourier maps, whereas aromatic and aliphatic H atoms (C-H) were generated by the mixed model in an idealized geometry. Crystallographic data for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Center with CCDC 2256588.

3. Results and Discussion

3.1. Electron Microscope Analysis

The morphology of enoxacin-heptanedioic acid cocrystal is shown in Figure 1, and it can be seen that colorless transparent acicular crystal.

3.2. Infrared Spectral Analysis

Enoxacin has obvious absorption characteristic peaks at 3411 cm^{-1} and 1627 cm^{-1}, corresponding to the stretching vibration of piperazinyl N-H and C=O groups in the structure (Figure 2). In accordance with the norm effect of cocrystal of N-H heptanedioic acid absorption peak at 3435 cm^{-1}, N-H absorption peaks redshift, C=O of cocrystal absorption peak at 1714 cm^{-1}, namely C=O absorption peaks redshift. The characteristic absorption peak shifts confirmed N-H and C=O groups involved in the formation of cocrystal hydrogen bond, consistent with the results of single crystal.

3.3. X-Ray Powder Diffraction Analysis

It can be intuitively seen from Figure 3 that the PXRD diffraction peaks of enoxacin-heptanedioic acid are different from those of enoxacin and heptanedioic acid. The specific data are as follows: the position of enoxacin-heptanedioic acid diffraction peak: $2\theta = 7.08^{\circ}$, 9.28°, 16.4°, 20.4° which is not found in enoxacin and heptanedioic acid; the position of enoxacin diffraction peak: $2\theta = 7.92^{\circ}$, 10.28°, 13.04°, 15.96°; the position of the peak of perimelic acid diffraction: $2\theta = 8.32^{\circ}$, 12.12°, 14.04°, 15.8. This shows that the analytical results of the crystal structure are reasonable and reliable, which proves that enoxacin-heptanedioic acid is a new crystal phase.

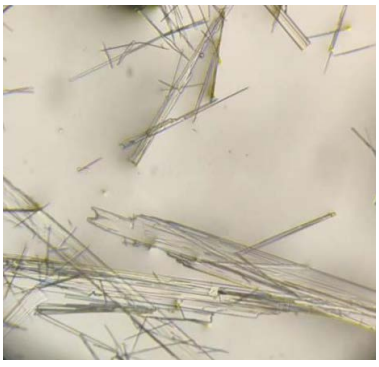

Figure 1. Electron microscope image of enoxacin-heptanedioic acid cocrystal.

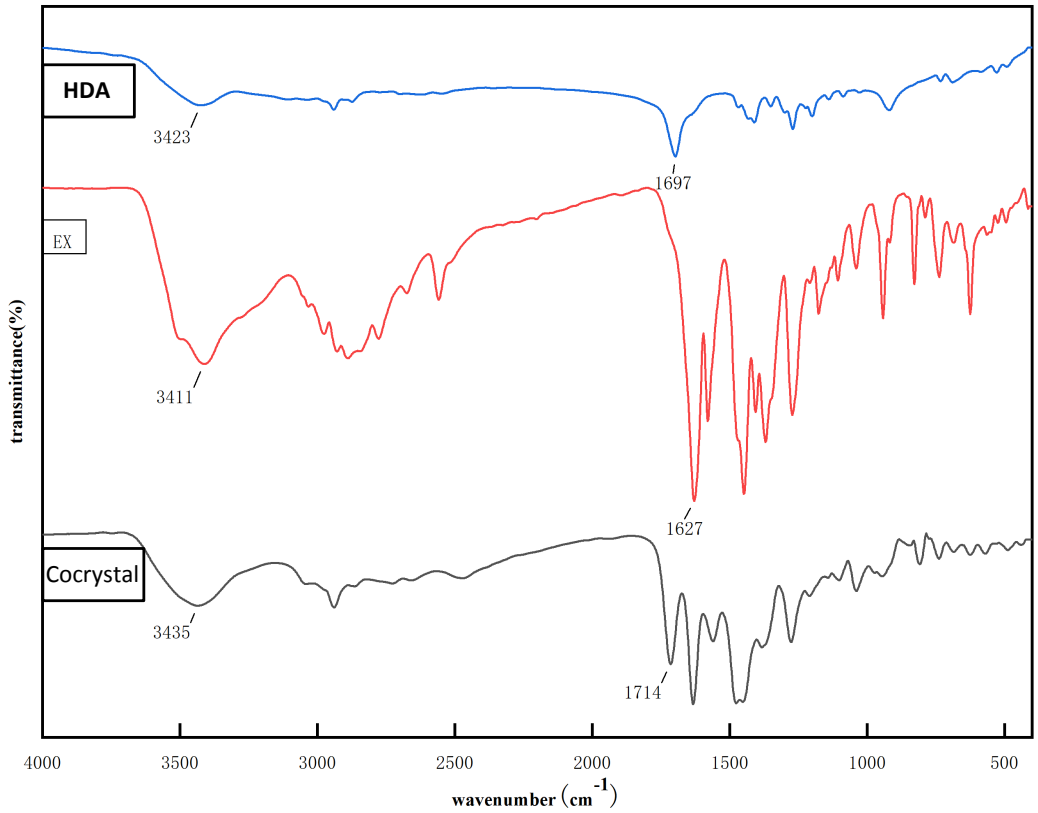

Figure 2. FT-IR of enoxacin-heptanedioic acid cocrystal.

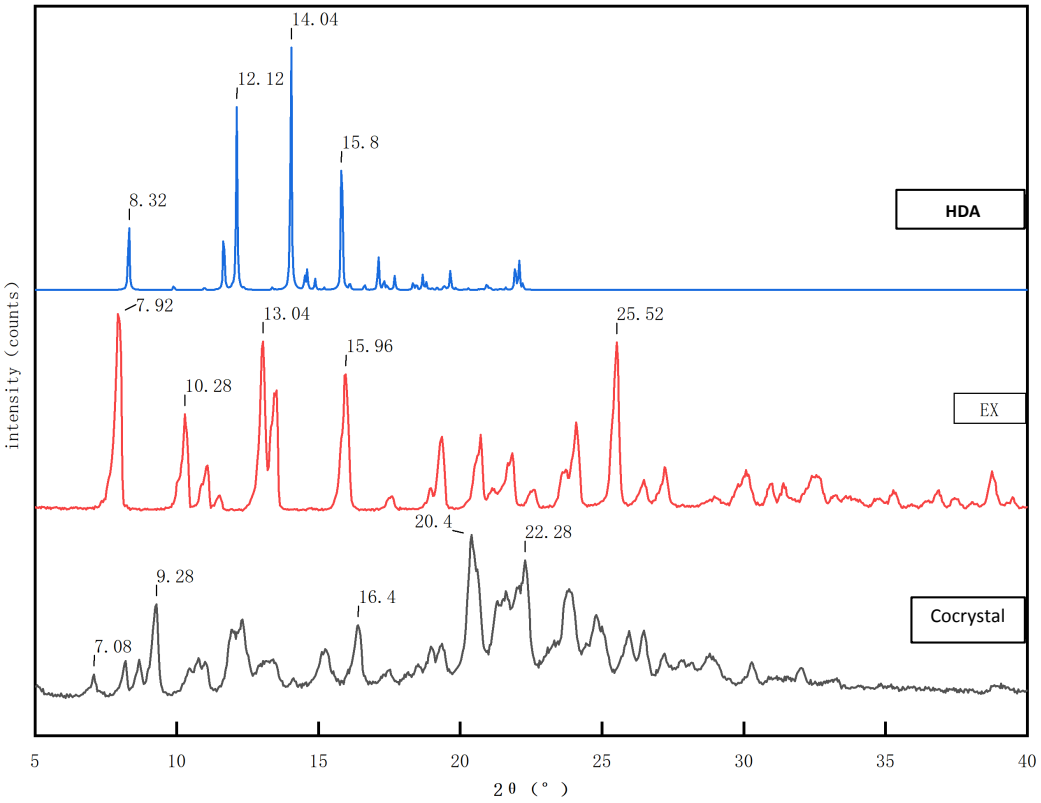

Figure 3. PXRD of enoxacin-heptanedioic acid cocrystal.

3.4. Differential Scanning Calorimetry (DSC)

The melting point of heptanedioic acid is 103˚C - 105˚C, and the melting point of enoxacin is 220˚C - 224˚C. Figure 4 show that the sample has an endothermic peak, the peak value of which is 210.9˚C, which is presumed to be the melting point of enoxacin-heptanedioic acid cocrystal.

3.5. Single Crystal Structure Analysis

Selected crystallographic data are provided in Table 1. All non-hydrogen atoms were refined with anisotropic displacement parameters. Especially, H atoms of N-H or O-H were refined from difference Fourier maps, whereas aromatic and aliphatic H atoms (C-H) were generated by the EX-HDA model in an idealized geometry. Crystallographic data for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Center.

Crystal structure analysis revealed that the stoichiometric ratio of two components in EX-HDA is 1:1, the asymmetric unit contain one EX cation and one HDA anion (Figure 5). In EX-HDA, one proton is transferred to the piperazidine N atom of EX from the HDA. However, in the structure of EX-HDA, since oxalic acid has a shorter carbon chain structure and enters the methanol molecule.

3.6. Hirshfeld Surface Analysis

In order to comprehend the role of hydrogen bonds aim the crystal packing of pharmaceutical salts/cocrystals of EX, Hirshfeld surfaces and 2D fingerprint plots were prepared for EX-HDA using CrystalExplorer software [7]. Hirshfeld surfaces of the three pharmaceutical cocrystal of EX are illustrated in Figure 6 and Figure 7, and showing surfaces that have been mapped over d_{norm}. Hydrogen bonds formed by the N atom of the piperazine ring from EX and carboxyl acid group (O-H···N and N+-H···O-) are all seen in the Hirshfeld surfaces as bright red areas, which mean hydrogen bond OH···N interactions correspond to closer

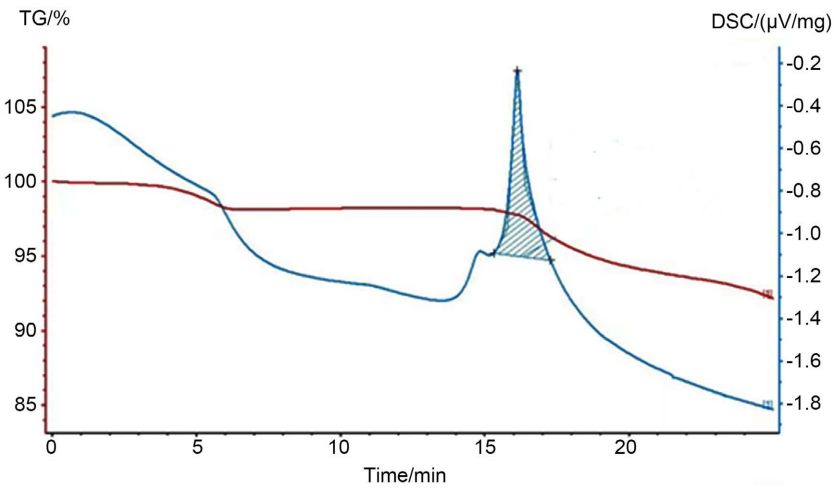

Figure 4. DSC of enoxacin-heptanedioic acid cocrystal.

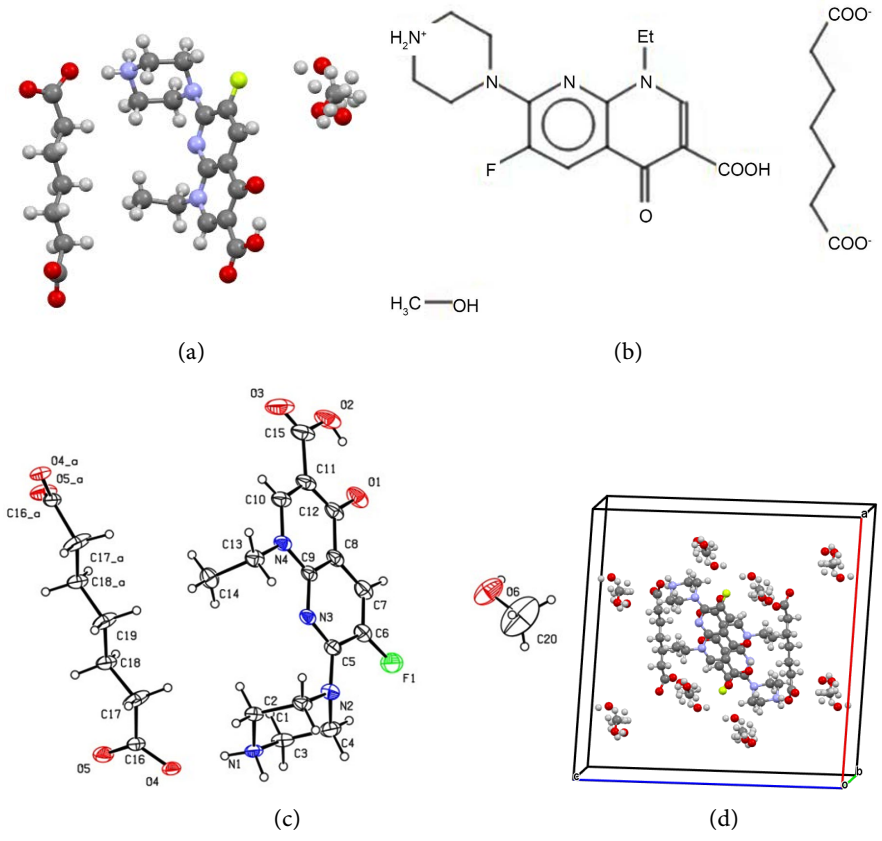

Figure 5. Structures of EX-HDA (a) the building unit with hydrogen-bonded atom label.

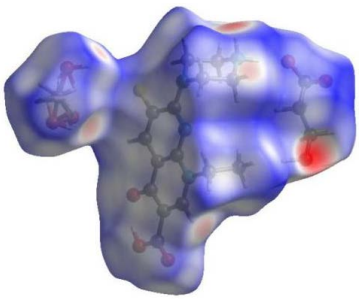

Figure 6. Views of dnorm, mapped on the Hirshfeld surface of EX-HAD.

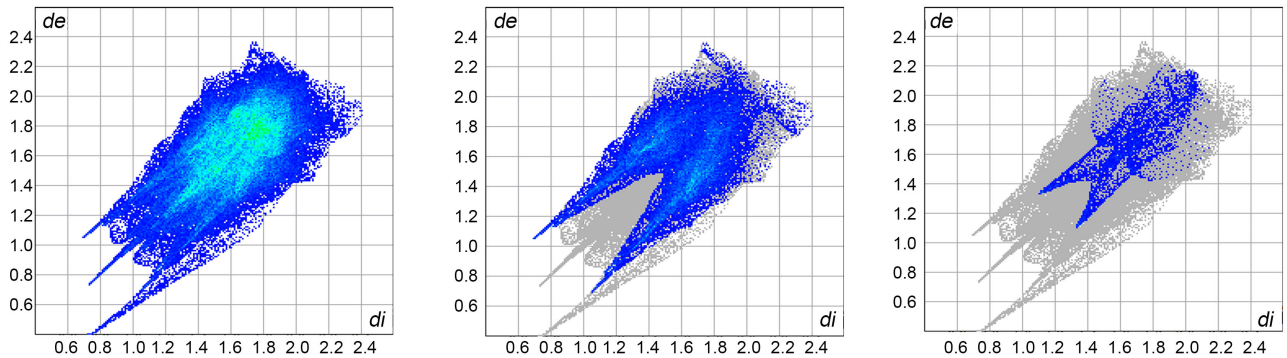

Figure 7. The 2D fingerprint plot of EX-HAD.

contacts relative to other interactions (such as: C-H···O, C-H···F, C-H···N and so on). In the 2D fingerprint plots the interaction of H···O, O···H and F···H from EX-HDA and H···O, O···H has sharp spikes pointing, which these interactions correspond to closer contacts.

4. Conclusion

In this paper, we have designed and synthesized cocrystal of EX with heptanedioic acid, which maybe could effectively enhance the antibacterial activity of EX by improving the solubility and permeability of EX, and improving hygroscopic stability of EX. Comprehensive analysis of structure and Hirshfeld surface showed that the hydrogen bonds formed by the N atom of the piperazine ring from enoxacin and the carboxyl acid group in the coformer could facilitate crystal packing to construct stable crystal structure in the pharmaceutical cocrystals of EX. This suggests that pharmaceutical cocrystals of EX with heptanedioic acid prepared by the pharmaceutical cocrystal technology in the form of methanol solvate.

Conflicts of Interest

The authors declare no conflicts of interest.

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