Vibrational Spectroscopic Investigation into Novel Ternary Eutectic Formed between Pyrazinamide, Fumaric Acid, and Isoniazid

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ABSTRACT: To improve the efficacy of anti-tuberculosis (anti-TB) therapy, drug–drug co-crystallization stands for an alternative approach to settle the tuberculosis problem. Directly co-crystallizing two typical parent anti-TB drugs (pyrazinamide, PZA and isoniazid, INH) into a single binary co-crystal could not be obtained successfully. Multicomponent eutectic are highly effective and useful for enhancing the dissolution rate, bioavailability, and physical stability of the poorly water-soluble active pharmaceutical ingredient (API) drugs, when the attempts of forming a binary co-crystal have failed. Therefore, the ternary eutectic composition conception was proposed in this study, in which fumaric acid (FA) was chosen as the molecule to connect two first-line anti-tubercular drugs. First of all, three starting materials (including PZA, INH, and FA) were grinded at a 1:1:1 molar ratio, the eutectic composition was investigated through vibrational spectroscopic techniques, including terahertz time-domain spectroscopy (THz-TDS) and Raman spectroscopy. Additionally, the density functional theory (DFT) was utilized to simulate the optimized structures and vibrational modes of two possible theoretical eutectic composition forms. The THz absorption spectrum of the theoretical form I shows much more consistency with the experimental results than that of form II. Raman spectra also help to characterize the differences in vibrational modes between the eutectic composition and the starting parent compounds. The results provide us with both structural information and intermolecular hydrogen bonding interactions within specific multicomponent eutectic composition formulations based on Raman and terahertz vibrational spectroscopic techniques in combination with theoretical calculations.

1. INTRODUCTION

Tuberculosis (TB) is a pandemic and it has become a main health problem in the world today.1,2 According to the World Health Organization (WHO), there were 10.4 million new TB cases and 1.3 million TB-related deaths in 2016.3 In addition, the increased incidence of TB is related to the human immune-deficiency virus (HIV) epidemic, which impairs the human immune system.4 To control the rapid growth of TB and ensure a superior therapeutic effect, combination drugs and fixed dose combinations (FDCs) have become increasingly important. Among these FDCs, pyrazinamide (PZA) and isoniazid (INH) are two typical active pharmaceutical ingredients (APIs) along with rifampicin and ethambutol, which are the most effective drugs in treating TB.5 But the traditional FDCs are only the physical mixtures, which do not affect drug properties and solubility.5,6 The novel multidrug co-crystallization is an alternative method to manage these issues about such traditional FDCs.5,6

As the first-line anti-tuberculosis drugs, INH and PZA exhibit almost similar therapeutic effects and efficacy,9,10 but these two drugs could not form corresponding drug–drug co-crystal directly even with any common preparing methods.11 Meanwhile, there is inability to form a salt between PZA and INH. From the perspective of structure, PZA and INH possess multiple hydrogen bond donor and/or acceptor sites (shown in Figure 1). Typically, both INH and PZA own the N-containing electron-rich aromatic heterocycles as the hydrogen bonding acceptors,12,13 which have been well recognized to form the pyridine–carboxylic acid heterosynthons with carboxylic acid. Moreover, PZA contains a primary amide group, while INH possesses a carbon-hydrazide group, which can form various heterosynthons and homosynthons with other functional groups. Évora et al.14 had reported a drug–drug co-crystal involving dif fusil (DIF) and PZA through the experimental infrared spectroscopy (IR), X-ray diffraction (XRD), and differential scanning calorimetry (DSC) techniques. They found that the co-crystal formed between DIF and PZA was compatible with the respective pharmacological effects of two parent drugs, and the aqueous solubility of DIF was enhanced efficiently by the co-crystallization process.
ternary co-crystal,21 even though it is di

component molecules is required in the synthesis process of Cherukuvada et al.11 failed in their attempt to make a binary consequently led to further therapeutic advantages. In addition, ff

eutectics. Liu et al.16 adopted FA as a bridge molecule to link and thermal analysis (TA), to characterize the above ternary INH and 4-aminosalicylic acid to verify whether a potential structure determines the superior physicochemical properties of this ternary co-crystal was carried out, including aqueousbridge ratio). Besides, they also put forward the concept of INH and PZA, which was able to propagate the growth unit−

and physical stability of the poorly water-soluble API drugs. Nonetheless, the authors succeeded in obtaining the ternary complexes through the formation of carboxylic acid−pyridine synthons, with dicarboxylic acids (succinic acid (SA) and fumaric acid (FA)) being used as the noncovalent linkers between PZA and INH parent drugs (PZA, INH, FA at 1:1:1 molar ratio). In the meantime, they also utilized the traditional analytical technologies, like powder X-ray diffraction (PXRD) and thermal analysis (TA), to characterize the above ternary eutectics. Liu et al.16 adopted FA as a bridge molecule to link INH and PZA, which was able to propagate the growth unit and enable co-crystallization (PZA, INH, FA at 1:1:2 molar ratio). Besides, they also put forward the concept of “drug−bridge−drug” ternary co-crystal for the first time, and the test of this ternary co-crystal was carried out, including aqueous solubility/dissolution, membrane permeability, and in vivo pharmacokinetic properties, so as to predict the enabled clinical efficacy of the co-crystal drugs. Aitipamula et al.17 successfully prepared the ternary co-crystals of INH with nicotinamide (NA) and dicarboxylic acids (like FA and SA), meanwhile, the solubility of INH−NA−SA co-crystal (INH, NA, SA at 1:1:1 molar ratio) was higher than that of INH, with comparable dissolution rate to that of INH.

All of the above-mentioned previous works indicate that the eutectic composition or ternary co-crystals are highly effective and useful for enhancing the dissolution rate, bioavailability, and physical stability of the poorly water-soluble API drugs when the attempts of forming a binary co-crystal have failed.11,18 Ternary co-crystal exists as a new type of extensional form based on the binary one.19,20 Compared with the binary co-crystal, the noncovalent bonding within three different component molecules is required in the synthesis process of ternary co-crystal,22 even though it is difficult to prepare the pretty complex structural form. However, such a complex structure determines the superior physicochemical properties of ternary eutectic composition than the binary one, which also efficiently reduces the drug side effects.22−24 Therefore, it is particularly essential to acquire the related information about the micromolecular structural changes during the formation process of such ternary eutectics and/or co-crystals.

The vibrational spectroscopic techniques have been widely used to understand the vibrational modes of functional groups between various molecules, and they have been regarded as quite useful approach to characterize the solid-state structural structure of specific APIs.25 The vibrational spectroscopy includes Fourier-transformation infrared spectroscopy (FTIR), Raman spectroscopy, and the newly emerging terahertz (THz) spectroscopy. These approaches can provide abundant information on the structure, intramolecular and/or intermolecular interactions with high sensitivity and great selectivity.26−28 Among them, Raman spectroscopy is based on the light scattering effect, and the bands observed in Raman spectra can be ascribed to the energy transfer between the photons of an excitation source and the target molecules,29,30 (namely, the in-elastic scattering of light). In this way, Raman spectroscopy provides an excellent method to investigate and probe into solid-state hydrogen bonding interactions between molecules. Meanwhile, the advances in the THz technique allow to directly yield rich information about the weak collective molecular vibrations and the intermolecular skeleton vibrations for specific molecules within the low-frequency region, particularly below 3.0 THz.31−33 Therefore, the THz vibrational spectroscopy is a promising tool used to distinguish different molecular configurations in pharmaceutical polymorphism and co-crystallization.34 As a supplement to Raman spectroscopy, THz vibrational spectroscopy can fully describe the basic vibrational motions and various crystalline structural changes of APIs.

Compared with other mainstream analytical tools like the single-crystal XRD (SC-XRD) and powder XRD (PXRD), the limitation of the Raman and THz spectroscopic technique lies in the failure about the refined complete three-dimensional (3D) structure and the precise localization of protons between donor and acceptor within specific crystalline compounds. Husák et al.35 successfully determined the crystal structures of two nonsolvated polymorphs of ixazomib citrate by combining the PXRD data with the comparative analysis of nuclear magnetic resonance (NMR) parameters of two nonsolvated polymorphs of ixazomib citrate. They then provided a strategy for high degrees of conformational freedom structure determination from PXRD data36 because the crystal structure determination from PXRD data was largely limited by the degrees of conformational freedom (DOF) analysis to obtain complementary structural data, determine, and refine the complete 3D structure. Other techniques, such as X-ray photoelectron (XPS), and the solid-state NMR have been applied to differentiate eutectic composition, co-crystals, and salts. Of them, only solid-state NMR combined with dynamic nuclear polarization (DNP) is able to quantitatively determine the location and bond lengths in salt, eutectic composition, and co-crystal systems.37

In this work, the eutectic composition strategy was utilized to overcome the failure in directly forming the corresponding binary co-crystal through grinding with the two parent APIs (PZA and INH). The THz and Raman vibrational spectroscopic techniques were employed in combination to investigate PZA, INH, FA, and the eutectic components. To discern the microstructure of such a ternary eutectic (as shown in the two possible forms in Figure 2) and better understand

Figure 1. Molecular structures of PZA (a), INH (b), and FA (c) (black arrows represent hydrogen bond donor positions and purple arrows indicate the possible positions of the hydrogen bond acceptor).
the Raman and THz spectroscopic observations, the quantum chemical density functional theory (DFT) calculation was also adopted to simulate the optimized structure and vibrational frequencies of the PZA–FA–INH ternary eutectic. Abundant information about the structural changes in the ternary eutectic due to various intermolecular hydrogen bonding effects was well obtained by combining experimental vibrational spectroscopic results with DFT simulation.

2. RESULTS AND DISCUSSION

2.1. Powder X-Ray Diffraction Analysis of the Ternary PZA–FA–INH Eutectic. Powder X-ray diffraction is a reliable technique to characterize multicomponent structures and confirm the complex as a co-crystal, salt, or intermediate ionization state. The comparison of PXRD patterns of PZA, INH, FA, and grinding ternary PZA–FA–INH eutectic is shown in Figure 3, meanwhile, the enlarged region in the 2θ range of 15°–25° is shown in Figure S1, where red circles indicate new and distinct diffraction peaks compared with the starting materials. In the ternary PZA–FA–INH eutectic, there are distinct characteristic peaks at 18.95, 22.17, and 29.85° (marked with yellow pentagrams in Figure 3d). However, in the PXRD patterns of the three parent materials, no such characteristic peak is detected at the above three positions. Figures 3 and S1 reveal that the grinding powder does not feature the peaks corresponding to the parent materials and ascertain that it is not the eutectic composition of these three components, which was composed of the co-crystals PZA–(diacid)_{0.5} and INH–(diacid)_{0.5} as it was previously confirmed by Cherukuvada et al. 11 The distinct PXRD patterns in this work are also good agreement with the above mentioned article, but are totally different compared with those of the ternary PZA–FA–INH co-crystal (according to the slow solvent evaporation preparation method from Liu et al. 16), as shown in Figure S2, where gray rectangle shadows indicate new and distinct diffraction patterns between the ternary eutectic and co-crystal. Meanwhile, the ternary PZA–FA–INH eutectic is detected by DSC and the melting point is 151 °C (Figure S3), which is also consistent with the reported melting point of this eutectic composition. 11

2.2. THz Spectral Characterization and Analysis of the Ternary PZA–FA–INH Eutectic. The THz spectra of PZA, FA, INH, the physical mixture, and the grinding ternary PZA–FA–INH eutectic are shown in Figure 4. It is clearly seen that five characteristic peaks of the spectra of the physical mixture are observed at 0.52, 0.73, 1.02, 1.18, and 1.37 THz, respectively, in the recorded range of 0.2–1.4 THz. In addition, the absorption peaks at 0.52 and 0.73 THz are mainly attributed to PZA, while that at 1.02 THz is related to FA. Besides, INH and physical mixture show identical characteristic peaks at 1.18 and 1.37 THz. To sum up, the spectra of the physical mixture is only based on the linear addition of the three parent materials (PZA, FA and INH). The ternary eutectic exhibits five characteristic peaks at 0.58, 0.76, 0.92, 1.25, and 1.35 THz, respectively. Meanwhile, the five characteristic peaks of the physical mixture at 0.52, 0.73, 1.02, 1.18, and 1.37 THz shift to 0.58, 0.76, 0.92, 1.25, and 1.35 THz, respectively, in the spectra of ternary eutectic. The spectral results of the physical mixture are distinctly different from those of the PZA–FA–INH eutectic composition. The THz spectroscopic technique is a useful tool to provide obvious fingerprint information for various solid-state crystalline molecular structures.

Comparisons of THz spectra between experimental results and the two possible theoretical forms (form I and form II) are shown in Figure 5. As displayed in this diagram, the theoretical form I shows five characteristic peaks at 0.51, 0.80, 0.92, 1.20, and 1.35 THz, respectively, which are highly consistent with

![Figure 2](image-url)  
Figure 2. Two possible structures of the ternary PZA–FA–INH eutectic (a) form I and (b) form II.

![Figure 3](image-url)  
Figure 3. PXRD pattern comparison of PZA (a), INH (b), FA (c), and grinding ternary PZA–FA–INH eutectic (d) in the 2θ range of 5°–50°.

![Figure 4](image-url)  
Figure 4. THz spectra of PZA (a), FA (b), INH (c), physical mixture (d), and grinding ternary PZA–FA–INH eutectic (e).
the experimental results at 0.58, 0.76, 0.92, 1.25, and 1.35 THz, respectively. Meanwhile, the theoretical form II shows six characteristic peaks at 0.30, 0.41, 0.68, 0.90, 1.23, and 1.33 THz, respectively. However, the absorption peak of the form II at 0.30 THz does not appear in experimental result. At the same time, the other five peaks of the form II show major deviations compared with the experimental THz spectral results. Based on the above-mentioned comparisons of THz spectral results, the ternary co-crystal structure of the theoretical form I is more reasonable than that of the theoretical form II. Most characteristic peaks of the theoretical form I show red shifts relative to the experimental results. The reason for this spectral shift can be attributed to the fact that the theoretical simulation is performed at an absolute temperature of absolute zero degree, while the experimental THz spectrum is obtained at room temperature. 

Different vibrations made contributions to these peaks at different positions. The theoretical THz spectra are plotted against the experimental THz spectra of the ternary PZA−FA−INH eutectic, as shown in Figure 5. Using the dynamic observation function of the Gaussian-view software, the vibrational modes of the ternary eutectic are obtained in Figure 6. The assignment descriptions of these modes are presented in Table 1. The experimental characteristic peak at 0.58 THz corresponds to the vibrational mode calculated at 0.51 THz (Figure 6a), arising from out-of-plane bending of

![Figure 5](image1.png)

**Figure 5.** Comparisons of THz spectra between theoretical (form I (a), form II (b)) and experimental (c) results of the ternary PZA−FA−INH eutectic.

![Figure 6](image2.png)

**Figure 6.** Marked as yellow pentagram: vibrational mode descriptions of the ternary PZA−FA−INH eutectic at (a) 0.51, (b) 0.80, (c) 0.92, (d) 1.20, and (e) 1.35 THz.
Table 1. Vibrational Mode Assignment of the Ternary PZA–FA–INH Eutectic Shown in the THz Spectrum

| experimental result (THz) | theoretical calculation (THz) | mode assignment                        |
|--------------------------|-------------------------------|---------------------------------------|
| 0.58                     | 0.51                          | PZA, FA, and INH molecular out-of-plane bending vibration |
| 0.76                     | 0.80                          | PZA in-plane bending vibration; INH and FA molecular out-of-plane bending vibration |
| 0.92                     | 0.92                          | PZA torsional vibration; INH and FA out-of-plane bending vibration |
| 1.25                     | 1.20                          | PZA, FA, and INH out-of-plane bending vibration |
| 1.35                     | 1.35                          | INH, FA, O24=C28–N26H25H27 out-of-plane bending vibration |

PZA, INH, and FA molecules. The characteristic peak of the ternary eutectic among PZA, INH, and FA at 0.80 THz in theoretical results is mainly due to the PZA molecule’s in-plane bending vibration, and INH and FA molecules’ out-of-plane bending vibration (Figure 6b). The experimental spectra feature at 0.92 THz is entirely agreed well with the theoretical spectra, which is mainly attributed to the PZA’s torsional vibration, and INH and FA out-of-plane vibration (Figure 6c). The characteristic peak at 1.25 THz corresponds to the theoretically calculated modes at 1.20 THz (Figure 6d), which is caused by PZA, INH, and FA out-of-plane bending vibration. The experimental spectrum at 1.35 THz is derived from the out-of-plane bending vibration of INH, out-of-plane bending vibration of FA, and O24=C28–N26H25H27 in the theoretical 1.35 THz (Figure 6e). The above vibrational modes all involve the hydrogen bond formation within PZA, FA, and INH. At the same time, the connecting experimental results and theoretical calculations help to understand the hydrogen bonding interaction effect in the molecular structural changes among PZA, INH, and FA, and also show that these five characteristic absorption peaks of ternary eutectic differ from those of raw materials in experimental THz spectra.

2.3. Raman Spectral Characterization and Analysis of the Ternary PZA–FA–INH Eutectic. Figure 7 exhibits the experimental Raman spectra of PZA, INH, FA, the physical mixture, and the eutectic composition, respectively. Clearly, the Raman spectra of the eutectic composition formed between PZA, INH, and FA is easily differentiated from that of the physical mixture and the individual starting parent materials. The typical spectral changes are shown with gray rectangular shadows for easy observation in Figure 7. Meanwhile, the physical mixture does not involve any hydrogen bond or noncovalent interaction during the mixing process. In the physical mixture, the peaks at 868, 888, and 913 cm⁻¹ are mainly attributed to PZA, INH, and FA, respectively. However, there are only two characteristic peaks at 865 and 902 cm⁻¹ in the same spectral region of the eutectic composition spectra, and the two peaks show red shift compared with those of the physical mixture. Similarly, in the physical mixture, there are two small peaks at 1320 and 1331 cm⁻¹, which are mainly contributed by INH and PZA molecules, but turn into one obvious peak at 1324 cm⁻¹ clearly in the spectra of the ternary PZA–FA–INH eutectic. In addition to the above differences, peaks at the position 1298 and 1002 cm⁻¹ for the physical mixture, arise from the FA molecule but they disappear in that of the eutectic spectra. Another three characteristic peaks at 1602, 1641, and 1685 cm⁻¹ show blue shift, which move to 1621, 1685, and 1724 cm⁻¹, respectively, along with the formation of the ternary PZA–FA–INH eutectic.

Figure 8 shows the comparison between experimental Raman spectral results and the theoretical form I of the ternary PZA–FA–INH eutectic and it could be seen that the theoretical Raman spectrum is in quite agreement with the experimental result. Table 2 exhibits the vibrational mode assignment of Raman characteristic peaks for the ternary eutectic formed by PZA, INH, and FA. The characteristic peak in the experimental Raman spectra at 1387 cm⁻¹ is derived from in-plane bending vibrations of O22–H23, C19–H39, C18–H38, and O15–H14, together with the scissor vibrations of H11–N10–H12 and H25–N26–H27. The peak at 1485 cm⁻¹ in the experimental spectra is caused by in-plane bending vibrations of FA molecule, N9–H39, N26–H25, and N10–H11. At 1724 cm⁻¹, this characteristic peak is originated from
Table 2. Vibrational Mode Assignment for Characteristic Peaks of the Ternary PZA–FA–INH Eutectic Shown in the Raman Spectrum

| mode | theoretical wavenumber (cm⁻¹) | experimental wavenumber (cm⁻¹) | mode assignment |
|------|-------------------------------|-------------------------------|-----------------|
| υ₁   | 253                           | 251                           | ρ(PZA), ρ(C₃₀−H₄₁, C₃₂−H₴₂) |
| υ₂   | 290                           | 282                           | ρ(H₁₁−N₁₀−H₁₂, R₂) |
| υ₃   | 326                           | 329                           | τ(H₁₁−N₁₀−H₁₂, FA) |
| υ₄   | 366                           | 368                           | τ(R₁), ρ(C₁₇−O₄₈) |
| υ₅   | 408                           | 408                           | Def(R₁), ρ(O₂₄−C₂₈−N₂₆H₂₅H₂₇) |
| υ₆   | 452                           | 438                           | ω(PZA) |
| υ₇   | 509                           | 525                           | τ(H₁₁−N₁₀−H₁₂, H₂₅−N₂₆−H₂₇), ρ(R₁, FA) |
| υ₈   | 553                           | 567                           | τ(H₁₁−N₁₀−H₁₂, H₂₅−N₂₆−H₂₇), ρ(R₁, FA, O₂₄−C₂₈−N₂₆H₂₅H₂₇) |
| υ₉   | 581                           | 581                           | ρ(INH, FA) |
| υ₁₀  | 606                           | 606                           | δ(H₂₃−O₂₂−C₂₀, O₁₇−C₁₆−O₁₅−H₁₄), ρ(INH) |
| υ₁₁  | 667                           | 664                           | Def(R₁), δ(O₂₄−C₂₈−N₂₆) |
| υ₁₂  | 681                           | 695                           | Def(R₂) |
| υ₁₃  | 709                           | 715                           | ω(R₁, R₂) |
| υ₁₄  | 750                           | 748                           | Def(R₂), ρ(H₁₁−N₁₀−H₁₂, N₉−H₁₃) |
| υ₁₅  | 808                           | 809                           | ω(H₂₅−N₂₆−H₂₇, R₁) |
| υ₁₆  | 861                           | 865                           | ω(INH) |
| υ₁₇  | 904                           | 902                           | ω(R₂) |
| υ₁₈  | 943                           | 958                           | ω(H₁₁−N₁₀−H₁₂, O₁₅−H₁₄) |
| υ₁₉  | 982                           | 988                           | ω(R₂, H₁₁−N₁₀−H₁₂) |
| υ₂₀  | 1021                          | 1027                          | ω(O₂₂−H₂₃) |
| υ₂₁  | 1055                          | 1049                          | Def(R₁) |
| υ₂₂  | 1083                          | 1083                          | Def(R₁) |
| υ₂₃  | 1094                          | 1094                          | Def(R₂) |
| υ₂₄  | 1195                          | 1186                          | ρ(C₃₀−H₄₁, C₃₂−H₴₂) |
| υ₂₅  | 1236                          | 1242                          | ρ(C₂−H₃₇, C₃−H₄₃, C₅−H₃₆, C₆−H₳₅) |
| υ₂₆  | 1289                          | 1267                          | τ(O₁₅−C₁₆, C₂₀−H₂₂) |
| υ₂₇  | 1307                          | 1307                          | τ(H₁₁−N₁₀−H₁₂) |
| υ₂₈  | 1320                          | 1320                          | ρ(C₃₀−H₄₁, C₃₃−H₴₀, C₃₂−H₴₂) |
| υ₂₉  | 1348                          | 1362                          | ρ(R₂) |
| υ₃₀  | 1395                          | 1387                          | δ(O₂₂−H₂₃, C₁₉−H₃₉, C₁₈−H₳₈, H₁₄−O₁₅), δ(H₁₁−N₁₀−H₁₂, H₂₅−N₂₆−H₂₇) |
| υ₃₁  | 1434                          | 1451                          | Def(R₂) |
| υ₃₂  | 1473                          | 1485                          | ρ(F₈, N₉−H₳₉, N₂₆−H₂₅, N₁₀−H₁₁) |
| υ₃₃  | 1529                          | 1526                          | ρ(R₂) |
| υ₃₄  | 1563                          | 1563                          | Def(R₁) |
| υ₃₅  | 1590                          | 1580                          | δ(H₂₅−N₂₆−H₂₇) |
| υ₃₆  | 1624                          | 1621                          | Def(R₁) |
| υ₃₇  | 1641                          | 1655                          | Def(R₂) |
| υ₃₈  | 1711                          | 1724                          | δ(O₂₄−C₂₈, O₂₁−C₂₀), δ(H₂₅−N₂₆−H₂₇, H₁₁−N₁₀−H₁₂) |

*υ*—stretching vibration, ρ—in-plane bending vibration, ω—out-of-plane bending vibration, τ—torsion, δ—scissor, Def—Deformation.

the stretching vibration of O₂₄−C₂₈ and O₂₁−C₂₀, the scissor vibration of H₁₁−N₁₀−H₁₂ and H₂₅−N₂₆−H₂₇, and the in-plane bending vibration of O₂₂−H₂₃ and N₁₉−H₁₃. It could be inferred from the vibrational mode assignment of the above three characteristic peaks that the hydrogen bond formation in N₁₀−H₁₄, N₉−H₁₃···O₁₇=C₁₆, C₂₈=O₂₄···H₂₃−O₂₂, and N₂₆−H₂₅··O₂₁−C₂₀ among PZA, INH, and FA exerted vital parts during their corresponding eutectic composition processes. Such findings also suggest that the ternary eutectic is not simply the combination of the individual starting materials (PZA, INH, and FA) but the structural transformation during the formation of the corresponding ternary PZA–FA–INH eutectic.

As known to those skilled in the art, changes in the bond length between different atoms account for the most direct cause of the characteristic vibration mode information shown in the Raman spectra of various compounds. There are significant changes in the bond length of the ternary eutectic molecules under the action of the hydrogen bonding effect. To further understand the structure of the ternary eutectic, the typical bond length changes during the ternary PZA–FA–INH eutectic have been investigated in this work. Figure 9 presents the changes in the bond length of the PZA–FA–INH eutectic composition based on their DFT-optimized structure. Table 3

![Figure 9](https://dx.doi.org/10.1021/acsomega.0c01486)

**Figure 9.** Typical bond lengths of the ternary PZA–FA–INH eutectic.
Table 3. Change of Typical Chemical Bond Lengths of PZA, INH, FA, and the Ternary Eutectic

| chemical bond | PZA (Å) | INH (Å) | FA (Å) | eutectic composition |
|---------------|---------|---------|--------|----------------------|
| O24=C28       | 1.258   |         |        | 1.242                |
| N26=C28       | 1.468   |        | 1.331  |                      |
| N26-H25       | 0.998   |        | 1.018  |                      |
| N9-H13        | 0.984   |        | 1.022  |                      |
| N9-N10        | 1.408   |        | 1.420  |                      |
| O22-C20       | 1.432   |        | 1.320  |                      |
| O22-H23       | 0.962   |        | 1.006  |                      |
| C20=O21       | 1.259   |        | 1.223  |                      |
| O17=C16       | 1.234   |        | 1.213  |                      |
| C16=O15       | 1.429   |        | 1.330  |                      |
| H14=O15       | 0.984   |        | 0.994  |                      |

shows the bond length changes for both intramolecular typical bonds and intermolecular hydrogen bonds upon the PZA–FA–INH eutectic composition formation. Several bond lengths of the PZA molecule have been changed in such ternary eutectic and the formation of the hydrogen bond length mainly occurred at −COOH and −NH₂. The bonds O24=C28, N26=C28, and N26−H25 in the PZA molecule change their bond lengths to 1.242, 1.331, and 1.018 Å, respectively. The bond lengths of N9−H13 and N9−N10 in the INH molecule reduce from the original 0.984 and 1.408 Å to 1.022 and 1.420 Å, respectively. There are two carboxylic groups in the FA molecule, and the structure changes in the eutectic composition. To be specific, the bond lengths of C−OH are shortened to 1.320 and 1.330 Å from 1.432 and 1.429 Å, respectively; while those of O22−H23 and H14−O15 are extended from 0.962 and 0.984 Å to 1.006 and 0.994 Å, respectively. The carboxyl groups in the FA molecule have been elongated to 1.223 and 1.213 Å from 1.259 and 1.234 Å. The bond length of hydrogen bonds are 1.648, 1.912, 1.994, and 1.856 Å within C28=O24···H23−O22, N26−H25···O21=C20, N9−H13···O17=C16, and N10···H14−O15, respectively. These chemical bonds of C28=O24, N26−H25, H23−O22, O21=C20, N9−H13, N10, H14−O15, and O17=C16 are related via the hydrogen bonds, which make important contributions to the vibrations of Raman peaks at 1395, 1473, and 1711 cm⁻¹ due to the formation of intermolecular hydrogen bonds between them. Investigating the vibrational mode assignment and the changes in the bond length makes it easier to understand the specific causes of Raman peaks’ shift, appearance, and/or disappearance during the eutectic composition formation process. Moreover, this also explains that the changes in intra and/or intermolecular structure caused by the intermolecular hydrogen bond interaction in the ternary PZA–FA–INH eutectic represent the important cause, leading to changes in the characteristic peak positions and intensities of such eutectic composition shown in both THz and Raman spectra.

3. CONCLUSIONS

The two common anti-TB parent drugs (PZA and INH) could not directly form the corresponding binary drug–drug co-crystal. The ternary PZA–FA–INH eutectic was successfully obtained from neat grinding crystallization experiments. Whether the ternary product is a eutectic composition of these three parent compounds or the binary co-crystals was differentiated by PXRD, a careful comparison of the PXRD patterns reveals that the grinding powder does not feature the peaks corresponding to the parent compounds (INH, PZA, and FA) and confirm that it is composed of the binary co-crystals PZA−(diacid)₀.₅ and INH−(diacid)₀.₅. The vibrational spectra of the solid-state PZA, INH, FA, and the eutectic composition were exhibited through the THz and Raman vibrational spectroscopy. Both the Raman and THz spectra are complementary to show the vibrational modes and the structures of the ternary PZA–FA–INH eutectic. Comparing experimental spectra results with DFT-simulated ones, it could help us discern the structural information of the ternary eutectic easily. As for the issues that the THz and Raman spectroscopy are difficult to investigate, such as determining the precise location of protons between the donor and acceptor to differentiate formation of the eutectic composition, co-crystal, and/or salts, it is necessary to use the other alternative analytical techniques to solve such problems. The results could offer us a unique and useful means to obtain an insight into the molecular structures and intermolecular hydrogen bonding interactions within specific multicomponent eutectic compounds in pharmaceutical fields.

4. MATERIALS AND METHODS

4.1. Chemicals and Sample Preparation. FA (purity 98%) was purchased from J&K Chemical Company. The samples of PZA and INH (purity 99%) were purchased from Sigma-Aldrich Company (Shanghai, China). All compounds were used as received without further purification. The physical mixture of raw materials was obtained by placing the three compounds in a vial and gently stirring with a spatula. The ternary eutectic was obtained by neat grinding, which was a commonly used method in the eutectic composition preparation. Grinding the PZA–FA–INH ternary eutectic was performed by co-grinding PZA, INH, and FA at a 1:1:1 molar ratio in 25 mL stainless steel milling jars using a planetary ball mill (QM-3SP, gear type, Nanjing University Instrument Plant) with a frequency of 25 Hz for around 60 min at room temperature.

4.2. Powder X-Ray Diffraction (PXRD) Measurements. The PXRD patterns of PZA, INH, FA, grinding ternary PZA–FA–INH eutectic, and ternary PZA–FA−INH co-crystal samples were obtained using a DX-2700 diffractometer (Dandong Haoyuan Instrument Co. Ltd., China with Cu Kα radiation) at 40 kV and 40 mA. Samples were measured in the 2θ range of 5°−50° with a scan speed of 1.5°/min.

4.3. Raman and THz Spectral Measurements. As for Raman spectral measurements, there was no need for further sample preparation. In this study, Raman spectra were obtained using a Fourier-transform Raman spectrometer (Spectra-Physics, Owen, CA) with a diode-pumped solid-state laser (wavelength 1064 nm) as the near-infrared source. Spectra were acquired over 500 scans at the 2 cm⁻¹ resolution over the wavenumber range of 150−3500 cm⁻¹, with the laser operating power of about 150 mW. Total analysis time per sample was of the order of 5 min intervals.

Besides, the Z2 measurement system (Zomega Co. Ltd., New York, NY) was adopted in THz-TDS. A Ti:Sapphire oscillator ultrafast laser pulse system (Spectra-Physics, Owen, CA) was used as the excitation light source at a frequency of 75 MHz, with a pulse width of ~100 fs and a center wavelength of 780 nm. All samples were ground gently to obtain particles with the mean size of several micrometers to minimize the scattering effects of sample particles during THz spectral
measurements. These samples were prepared, respectively, using a hydraulic compression machine (HANDTAB-100, Ichihashi Seiki, Kyoto, Japan), with a diameter of about 13 mm and a thickness of 1.6 mm. A constant pressure of 4 MPa was continuously applied for 30 s to acquire the final pellets, which were then sealed in plastic bags prior to THz spectroscopic measurement and analysis. A total of three spectra representing three complete sets of sample and reference (without sample) measurements were averaged to obtain the THz spectra of each sample. The time-domain of the THz electric field was recorded for the reference (without the sample holder) and each sample. After the fast Fourier-transform operation, the THz spectral absorption was obtained by dividing the frequency response of the sample by that of the reference.

4.4. DFT Theoretical Calculations. Quantum chemistry DFT calculations were performed to simulate the structures of PZA, FA, INH, and their PZA-FA-INH ternary eutectic using the Gaussian 09 program. Meanwhile, the geometry was optimized according to the B3LYP method.39−41 The reliability of B3LYP functional in calculations of the ground-state geometries has been widely assessed previously.42 The vibrational frequencies of all molecular systems were calculated based on 6-311G(d,p) basis set, and the calculated wavenumbers were scaled with a factor of 0.96 by the B3LYP method.43 There were two protons arranged in transoid and numbers were scaled with a factor of 0.96 by the B3LYP based on 6-311G(d,p) basis set, and the calculated wavefunctions were scaled with a factor of 0.96 by the B3LYP method.39−41 The reliability of B3LYP functional in calculations of the ground-state geometries has been widely assessed previously.42 The vibrational frequencies of all molecular systems were calculated based on 6-311G(d,p) basis set, and the calculated wavenumbers were scaled with a factor of 0.96 by the B3LYP method.43 There were two protons arranged in transoid and numbers were scaled with a factor of 0.96 by the B3LYP method.39−41 The reliability of B3LYP functional in calculations of the ground-state geometries has been widely assessed previously.42 The vibrational frequencies of all molecular systems were calculated based on 6-311G(d,p) basis set, and the calculated wavenumbers were scaled with a factor of 0.96 by the B3LYP method.43 There were two protons arranged in transoid and numbers were scaled with a factor of 0.96 by the B3LYP method.39−41 The reliability of B3LYP functional in calculations of the ground-state geometries has been widely assessed previously.42 The vibrational frequencies of all molecular systems were calculated based on 6-311G(d,p) basis set, and the calculated wavenumbers were scaled with a factor of 0.96 by the B3LYP method.43 There were two protons arranged in transoid and numbers were scaled with a factor of 0.96 by the B3LYP method.39−41 The reliability of B3LYP functional in calculations of the ground-state geometries has been widely assessed previously.42 The vibrational frequencies of all molecular systems were calculated based on 6-311G(d,p) basis set, and the calculated wavenumbers were scaled with a factor of 0.96 by the B3LYP method.43 There were two protons arranged in transoid and numbers were scaled with a factor of 0.96 by the B3LYP method.39−41

■ ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c01486.

A comparison of the PXRD pattern of the PZA, INH, FA, and grinding ternary PZA-FA-INH eutectic in the 2θ range of 15−25° (Figure S1); the PXRD pattern comparison of the grinding ternary PZA-FA-INH eutectic and ternary PZA-FA-INH co-crystal in the 2θ range of 5−50° (Figure S2); the DSC profile of the grinding ternary PZA-FA-INH eutectic (Figure S3) (PDF)

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Notes
The authors declare no competing financial interest.

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