Investigation of sulfamethoxazole polymorphism with terahertz time-domain spectroscopical technique

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Abstract: Sulfamethoxazole (SMX), one of the well-known effective and tolerable antibacterial agents, is widely used to treat urinary tract infections. Due to the variations in crystallinity structure, SMX exist in two different polymorphic forms I and II. These polymorphs have the same chemical composition but unique physio-chemical properties which will influence the corresponding different stability, solubility, dissolution rate and also other performance characteristics. Terahertz time-domain spectroscopy(THz-TDS) becomes a promising analytical technique relevant to the pharmaceutical and biological fields. THz-TDS measurement is able to characterize and distinguish the different polymorphs according to the rich information yielded, due to the sensitivity of THz absorption in this low-frequency region to the structures over length scales greater than intramolecular bond distances. In this study, absorption spectra in the terahertz region between 6 and 50 cm^{-1} (0.2-1.5 THz) were measured for SMX pharmaceutical molecule with different polymorphic forms (forms I and II, and also raw material) using THz-TDS technique at room temperature. The temperature-dependent THz-TDS of the raw SMX material was also investigated between 95 K and 296 K. Different absorption features were observed for these two model polymorphs. The observed THz absorption bands are strikingly sensitive to the change of subtle conformational structures existed within such crystal molecules with different polymorphism. The results show that the THz-TDS technique is a promising method in solid-state analytical tools to distinguish or differentiate such compounds with different polymorphs in pharmaceutical and biological fields.

Key words: time-domain terahertz spectroscopy(THz-TDS); absorption features; sulfamethoxazole(SMX); polymorphism, pharamaceutical compound

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基于太赫兹时域光谱技术的磺胺甲噁唑多晶型现象

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摘 要: 磺胺甲噁唑(SMX)作为典型的一类光谱抗生素,经常被用于敏感菌引起的各类感染。由于其

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晶体内部结构的不同,SMX 将以两种不同的多晶型形式存在。不同晶型常常具有不同的物理化学性质,这些差异可能会对药物的流动性、稳定性以及药效等有较大影响。新兴发展起来的太赫兹光谱技术对于分子间弱的相互作用及大分子的骨架振动、偶极子的旋转和振动跃迁以及晶体中晶格的低频振动吸收都非常敏感,在药物以及生物等领域获得了重要的应用。利用太赫兹时域光谱 (THz-TDS) 技术在室温下对两种不同多晶型 SMX 类药物在 0.2~1.5 THz 范围内进行了光谱测量,得到对应的吸收谱,发现其在太赫兹波段都有明显的特征吸收峰,可作为其指纹谱用于该类药物多晶型的识别。不同温度下(95~296 K)获得太赫兹时域波形以及对应的频域吸收谱图,结果发现时域波形以及频域吸收谱均与温度呈现规律变化。该研究结果表明 THz-TDS 技术药物与生物领域研究、区分药物分子多晶型现象等方面具有重要的应用。

关键词: 太赫兹时域光谱; 吸收特性; 磺胺甲噁唑; 多晶型; 药物分子

0 Introduction

Sulfamethoxazole(SMX) is one of the well-known effective and tolerable antibacterial agents, which is widely used to treat urinary tract infections[1-3]. Due to the variations in crystallinity structure, SMX exists in two different polymorphic forms I and II[2-3]. These polymorphs have the same chemical composition but unique physio-chemical properties which will influence the corresponding different stability, solubility, dissolution rate and also other performance characteristics[4]. This makes it important for the pharmaceutical and biological community to investigate and characterize the different polymorphs in drugs to identify these forms and understand the following impact on drug performance for regulatory purposes. There are numerous techniques to characterize the polymorphism in pharmaceutical industry during a number of steps in crystallization, manufacturing and storage, such as powder X-ray diffraction (PXRD)[5-6], near-infrared (NIR) spectroscopy[3], Raman spectroscopy[2], and thermal analysis[7]. But all the above techniques have different limitations. PXRD technique is a time consuming process and complex sample preparation will be required which means that it cannot be used on-line to monitor easily and directly. Also because of its harmful ionizing effect, the safety use of X-ray in PXRD measurement should be taken into consideration. In Raman spectroscopy, the risk of phase transformation and unwanted photochemical reactions in the compounds will occur due to the required high-energy laser irradiation. So it is crucial to develop specific technique to investigate the different polymorphic characteristics in pharmaceutical molecules in order to distinguish them directly and efficiently.

Terahertz time-domain spectroscopy (THz-TDS) becomes a promising analytical technique relevant to the pharmaceutical and biological fields, which uses the THz electromagnetic waves typically covering 0.1-10 THz(3~333 cm^{-1})[8]. Due to much lower photon energies(~meV) than X-ray photons (one million times weaker), it will not cause any harmful photoionization in the sample and can be used to monitor the process nondestructively. At these low frequencies, collective molecular vibrations, such as lattice vibrational modes (phonons) and intermolecular skeletal modes, predominate in molecular solids, which represents a characteristic "fingerprint" of a molecular substance[8-15]. THz absorption spectroscopy could be used to characterize the different polymorphs according to the rich information yielded. THz-TDS technique has become an effective tool for characterizing and identifying the crystal polymorphs within the whole molecule in the low-frequency range. Clear differences due to the variants in molecular crystals such as different polymorphic forms[9-11], racemic and enantiomeric mixtures[13-15] can be determined precisely with THz-TDS

measurement.

In this work, vibrational properties of SMX pharmaceutical molecule with different polymorphic forms have been investigated using transmission THz-TDS technique at room temperature. The temperature-dependent THz-TDS of the raw SMX material was also investigated between 95 K and 296 K. The experimental results show large difference among absorption spectra of two SMX forms in 0.2~1.5 THz region, which probably originated from the difference of intermolecular interaction forces and lattice vibrational modes due to the variations in crystallinity structure. The study indicates that THz-TDS technology can definitely offer us a potential experimental method to characterize such pharmaceutical compounds with different polymorphs from molecule-level.

1 Methods

1.1 Sample preparation

The raw SMX sample (the chemical structures shown in Figure1) were purchased from Yuancheng Biotech company (Wuhan, China) and used without further purification. The crystals in form I and II were obtained from acetone and ethanol solutions respectively. The samples were weighted into 150 mg aliquots and mixed with 150 mg polyethylene (PE) powder. With the use of a mortar and pestle, the mixture was ground to obtain uniformity powder with several micrometer grain size in order to reduce the scattering effect. Then the mixture was poured into a steel die under ~4 MPa pressure. The resulting 13 mm in diameter, 1.5 mm thick sample discs were extracted and sealed in plastic before analysis.

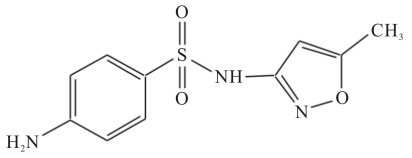

Fig.1 Chemical structure of SMX pharmaceutical molecule

1.2 Apparatus and data analysis procedure

The transmission spectra at room temperature were measured by using a commercial THz-TDS system (Z_2, Zomega Terahertz Corporation, Troy, USA). The THz beam is produced by a Ti:Sapphire oscillator ultrafast laser with a repetition rate of 75 MHz, center wavelength of 780 nm and pulse duration of 100 fs. The power of nearly 960 mW is split into the pump and probe beams after the BS(beam splitter) for THz generation and detection, respectively. After transmitting through the delay line, the pump beam excites a photoconductive semiconductor antenna. The generated THz beam is focused onto the sample by a pair of gold-coated parabolic mirrors, carries sample characteristics, and meets the probe beam at the ZnTe crystal detector, where the probe beam is modulated by the terahertz radiation through the electro-optic effect in which a birefringence is induced in the ZnTe crystal and the polarization of the probe beam is rotated by the terahertz beam. The effective dynamic range is over 70 dB between 0.1 to 2.5 THz. The temperature-dependent THz transmission spectra were taken with a home-made THz-TDS system based on the generation of terahertz radiation by optical recification effect and detection by free space electro-optic sampling. The samples for the measurement were hold in a variable temperature cell (Specac Ltd GS21525 system, Orpington, UK) equipped with 3 mm polyethylene windows for jacket. Data were acquired between room temperature (296 K) and liquid nitrogen (the indicated temperature is 95 K) with sample under vacuum. The path with THz radiation in the above two systems is enclosed and purged with dry nitrogen gas to reduce the absorbance contribution of atmospheric water vapor. A total of three THz spectra representing three complete sets of sample and reference measurements were averaged for each final spectrum reported in this work. The ratio of the power spectra obtained from the Fourier-transformed data sets of the sample and reference yields the corresponding THz absorption spectrum.

2 Results and discussion

Figure 2 shows the THz absorption spectra of

SMX raw material and also the pharmaceutical molecule with different polymorphic forms (form I and II) recorded in the range 0.2–1.5 THz at room temperature. The data below 0.2 THz are not considered due to interference between reflections of the probe pulse inside the sample pellets. A gradual rise of the absorption baseline was observed with increasing frequency which results from scattering. The SMX raw material and polymorphic form I have almost identical spectra and this result means that the raw material which is usually used in medical exists in polymorphic form I. The specific absorption features of raw SMX and polymorphic form I, distinguished at frequencies (0.74, 1.02 and 1.25 THz shown in Figure 2) are clearly observed.

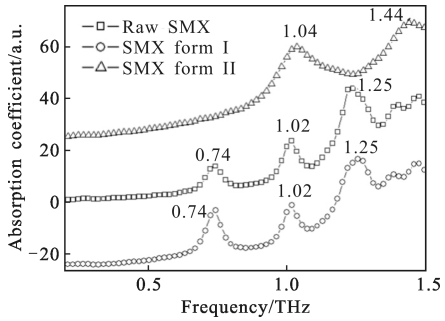

Fig.2 THz absorption spectra of SMX raw material and different polymorphic forms (form I and II) in 0.2–1.5 THz region obtained from THz-TDS measurements

As for the polymorphic form II SMX sample, there are obvious different resonance peaks between 0.2–1.5 THz (1.04 and 1.44 THz seen in Figure 2). It is known that many factors contribute to the low-frequency spectrum and result in obvious absorption. Intermolecules vibrational modes or lattice vibration are considered as the main mechanisms in the THz frequency region. Large difference among absorption spectra of two SMX polymorphic forms in 0.2–1.5 THz region originated from the difference of intermolecular interaction forces and lattice vibrational modes due to the variations in crystallinity structure. This also indicates that the vibrational modes found in these spectra are mainly intermolecular character in nature as any intramolecular modes should be common in both spectra.

Upon cooling the raw SMX material sample from room temperature to low temperature, there are clearly the trends observed in THz time-domain waveforms and Figure 3 shows the waveforms at selected different temperatures (296 K, 193 K and 95 K). The arrows indicate that, with the temperature decreases, the oscillatory peaks resulting from sample in time-domain will shift to less time-delay and the corresponding intensity will increase, while the FWHM of these peaks will become narrower.

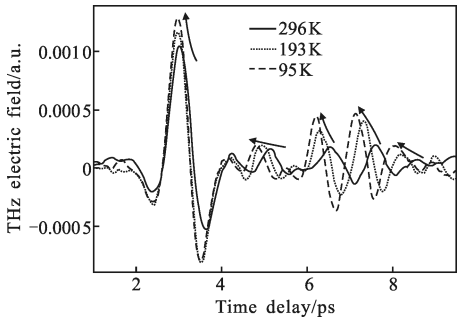

Fig.3 Experimentally obtained THz waveforms of raw SMX material at selected different temperatures

The drawn through arrows indicate how the peaks in time-domain change with the temperature decrease. Figure 4 shows the THz absorption spectra change of the raw SMX material sample over the temperature range of 296–95 K. As the temperature was reduced from room temperature to 95 K, the observed absorption bands become narrower and shift toward higher frequencies, and also different features shift with different amounts. The peaks shift between 0.07 and 0.12 THz with the peak centered at 1.25 THz showing the largest shift to 1.37 THz. The reason for such shift is already well known because of the contraction of the lattice cell dimensions with decreasing the temperature. Narrowing of the absorption features is also achieved by cooling SMX raw material sample and the spectral line-width reduces resulting from the reducing excited vibrational state populations and sequence band transition

intensities. Obtaining THz spectra at cryogenic temperatures is necessary for the proper further assignment of vibrational modes and the evaluation of the quality of THz spectral theoretical simulations.

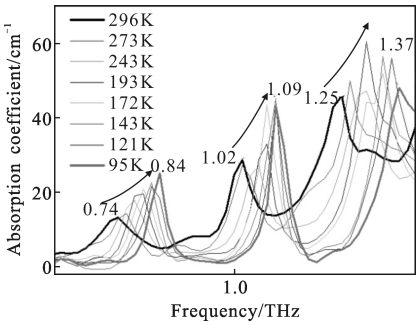

Fig.4 Experimentally obtained THz absorption spectra of raw SMX material at different specific temperatures from THz-TDS measurement

The drawn through arrows indicate how the absorption peak trends change with the temperature decrease. The results from THz-TDS measurements in this study show that THz absorption spectra can give us pretty rich information on both molecular conformation and intermolecular interactions. The low-frequency vibrational features have been demonstrated to be mainly associated with the collective vibrational modes of molecules held together by the weak intermolecular molecular interactions. For different polymorphic forms SMX compounds (form I and II), the intermolecular molecular interactions will be different due to the change of subtle conformational structures within the crystalline polymorphs and the corresponding vibrational modes will be totally different. Their distinct fingerprint absorption features in THz region indicates that THz-TDS technology can absolutely offer a potential experimental tool to identify and monitor such polymorphism effect in pharmaceutical fields.

3 Conclusions

Thecompleted THz spectra of SMX pharmaceutical molecule with different polymorphic forms have been recorded, represented as absorption coecient in frequency region between 0.2 and 1.5 THz using THz -TDS technique. The THz -TDS spectroscopy provides a finger-print spectrum associated with both molecular conformation and intermolecular interactions, which may be useful for the discrimination of polymorphism effect within pharmaceutical molecules. The reported results indicate that THz -TDS technique is an effective and also promising tool in solid-state analysis to distinguish or differentiate such compounds with different polymorphs directly and also in-line in pharmaceutical and biological fields.

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