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Identification of interpolymorph transformations of progesterone by terahertz time-domain spectroscopy

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ABSTRACT

We utilized the terahertz time-domain spectroscopy system to differentiate the two polymorphs of progesterone and to identify the occurrence of interpolymorph transitions. The stable polymorph of progesterone was prepared by ethanol-mediated evaporating/cooling methods, and the metastable state was obtained by using pregnenolone as an additive. These two polymorphs show distinct different absorption spectrum in the terahertz region. As the metastable state of progesterone naturally converted to the stable state over time, the absorption mode of the metastable state disappeared and those of the stable state re-emerged. Interpolymorph transformations of progesterone can be rapidly identified using an apparent absorption mode as an indicator, which represents the existence of the metastable state of progesterone.

1. Introduction

For the development and the production of pharmaceuticals, it is crucial to differentiate the polymorphs of substances. Polymorphs of a given molecule are the crystal systems with various structures characterized by different unit cells or intermolecular packing. The differences in the crystalline structure can result in different physical properties such as stability, dissolution rate, and bioavailability [1]. The stable polymorph is generally preferred in the manufacturing of pharmaceuticals because of its stability and low probability of transformation into other forms which are not identified before [2]. However, for materials such as progesterone, which have separate molecules as the basic unit of interaction with targets (receptors) in the human body, the intermolecular constitution of the solid state is believed to be associated with the efficacy of drug [3,4]. Additional attention needs to be paid to the phenomenon “disappearing polymorph”, which refers to the difficulty of returning to the initial polymorphic form after a more stable polymorphic form is assumed [5]. This phenomenon reveals an inherent danger that all the possible polymorphs and their stabilities are not perfectly predictable. Unintended polymorphs can be generated during the development of a pharmaceutical, in which case controlling and reconstructing the polymorphs would become more difficult.

Therefore, in situ monitoring techniques for determining the polymorphic states during crystallization and manufacture are important. Many types of analytical techniques have been used to determine the physical properties of polymorphs; single-crystal x-ray diffraction, thermal analysis, including differential scanning calorimetry and thermal gravimetric analysis, and optical spectroscopy are all commonly applied. X-ray diffraction has been recognized as the best method to identify the crystal lattice and molecular orientation. However, the x-ray diffraction method is limited by sample type, especially in the case of small crystals or polycrystalline materials [6]. Thermal analytical methods recognize the crystal structures by detecting the transition temperatures of the polymorphs [7]. However, thermal analysis cannot provide direct information about stereo-shape of the molecules. Optical spectroscopy encompasses mid-infrared, near-infrared, and Raman spectroscopy [8–10]. The spectral features of the mid-infrared region represent intramolecular modes. The molecular forms of some polymorphs are not sufficiently distinct from each other for the mid-infrared spectra to reveal differences. Moreover, in the near-infrared region, the interpretation of the spectra becomes difficult. The origin of near-infrared absorptions is the overtones and combinations of the fundamental molecular vibrations located in the mid-infrared region [11,12]. For this reason, classifying the near-infrared peaks is inherently difficult when applied as a probing

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method of polymorphs. Raman spectroscopy should be carefully utilized to probe the polymorphic characteristics because the laser beam used in it could result in a phase transition or other photochemical effects for the final products and during crystallization [13,14]. Solid-state nuclear magnetic resonance (NMR) spectroscopy has recently been demonstrated as a valuable technique that is complementary to the single-crystal x-ray diffraction method [15,16]. The NMR method can determine the structural information about the molecular crystals by analyzing the nuclear-spin energy levels modified by anisotropic interactions [17]. Thus, solid-state NMR is advantageous for the study of noncrystallized materials; it can also be used to study materials in very small amounts. However, the ancillary signal averaging process and experimental techniques are necessary to overcome the intrinsic insensitiveness of the technique [18,19]. Other methods such as optical microscopy and ultrasonic-velocity measurement have been demonstrated as candidates for in situ measurements [20,21]. However, neither method is applicable without supporting techniques. Optical microscopy determines the morphology of a crystal. The morphologies of crystals do not unambiguously correlate with individual polymorphs; therefore, additional techniques such as x-ray spectroscopy should also be used. Ultrasonic velocity measurement, which detects the variation of the viscosity of the solution during the crystallization process, should also be paired with successive samplings and additional analysis for determining the crystal structure of the solid particles. All the above-mentioned methods have some limitation in providing direct information about the polymorphic state during the polymorphic formation or transformation of the crystal.

In comparison with these spectroscopic techniques, the terahertz time-domain spectroscopy (THz-TDS) system has attracted attention as a precise technique for identifying the polymorphic characteristics of pharmaceuticals. Because of the rapid development of the sources and detectors used in THz techniques, THz-TDS can be applied for investigating various materials. For example, semiconductors [22,23] and nanomaterials [24,25], biomedical materials [26–28], pharmaceuticals [29,30], and illicit drugs [31] have been studied with THz-TDS. In particular, THz-TDS is a promising method to probe the long-range order of materials with specific intermolecular arrangements or crystallinities, such as pharmaceuticals because the intermolecular dynamics of motions corresponding to low-frequency bond vibrations, translations and librations of molecules, phonon vibrations, and hydrogen-bonding stretches can be detected in the THz range (0.1–10 THz, 1 THz $= 10^{12}$ Hz). In addition, THz-TDS can be a beneficial technique for detection of polymorphic states compared with the Fourier-transform infrared (FTIR) spectroscopy, which is a common technique used in the far-infrared region. THz-TDS has a high signal-to-noise ratio (~10000:1) with room-temperature sources and detectors and can directly measure complex optical parameters of materials without the need for complicated conversion processes such as the Kramers-Kroning relation, which is required in FTIR. A sensitivity of THz-TDS in change of the dipole moment of the crystals provides complementary results with Raman spectroscopy so that it can descript vibrational structure of molecules including the nearest molecular interactions which is one of the biggest difference between polymorphs. Also, THz-TDS has an advantage to detect thick samples, even they are coated, since THz radiation can penetrate to the dry sample. Low-energy photons used in THz-TDS allow noninvasive inspection of the samples without any hazards; therefore, this method can be utilized for remote and real-time measurements of polymorphic states of pharmaceuticals [32]. Although there are a few minor problems to be surmounted to make commercial THz-TDS systems such as the expensive prices of the current THz-TDS systems based on femtosecond laser, this technique has its distinctive advantages as a good spectroscopic system for polymorph analysis.

Progesterone is a type of steroid hormone and is used for hormone therapy and birth control. Progesterone has emerged as an important material for studying the dependence of medical effects on its interactions with the corresponding receptors in the human body. Because the enantiomers of progesterone are the mirror-image isomers of each other, they can be exploited to induce different physiological effects and to competitively interact with receptors [33]. In this paper, we try to identify two well-known polymorphs of progesterone after and in the middle of inter-polymorph transitions using a THz-TDS system. The fundamental method to find out the existence of the metastable polymorph of progesterone by examining the specific absorption modes in the THz range is also proposed.

2. Materials and methods

The first step is to generate a metastable polymorph that maintains its state for a relatively long time. After the discovery of the progesterone polymorphism, many experimental efforts and computational predictions have been made to develop a reproducible process for obtaining metastable crystals [34–37]. From a careful survey of previous techniques for preparing the metastable state of progesterone, we decided to employ pregnenolone as an additive [38]. The initial mixtures were prepared using different weight ratios of the stable-state progesterone and normal pregnenolone. The stable state of progesterone in these mixtures was changed into the metastable state by recrystallization, which is explained in a later section. The same recrystallization process was repeated for maintaining the stability of the metastable state for every trial. The generation and extinction of the metastable state of progesterone were ascertained in the mixture of the metastable crystals of progesterone with the stable crystals and co-crystals without further separation.

2.1. Materials

To generate a sample of the metastable state of progesterone, commercial progesterone (≥99%, progesterone, 4-pregnene-3,20-dione, CAS number: 57-83-0, empirical formula (Hill notation):$C_{21}H_{30}O_2$, molecular weight: 314.46) and pregnenolone (≥98%, pregnenolone, 3β-hydroxy-5-pregnen-20-one, CAS number: 145-13-1, empirical formula (Hill notation):$C_{21}H_{32}O_2$, molecular weight: 316.48) were purchased from Sigma–Aldrich Co.

2.2. Preparing polymorphs of progesterone

Relatively large crystals of stable-state progesterone were obtained by dissolving progesterone in ethanol at room temperature and evaporating the solution (37). In an alternative method, we dissolved the progesterone in a solution of ethanol and water with the same volume ratio and evaporated the solution by maintaining the temperature at 333 K (60°C) [37].

We induced the transformation from the stable state to the metastable state of progesterone crystals by the following recrystallization process. First, pregnenolone was added to the stable state of progesterone. The mixture was then melted in a heating block whose temperature was maintained at 468 K (195°C). After the mixed powder was melted, we cooled the mixture with 4°C acetone and evaporated the solvent completely [38]. The amounts of the added pregnenolone were varied from 5% to 20% with 5% intervals of weight ratio. The samples for the transmission measurements in the THz-TDS system were prepared as pellets of pure solids with a diameter of 8 mm and a thickness of 8000 μm. All the