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Research paper

Understanding the solid-state forms of fenofibrate – A spectroscopic and computational study

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ABSTRACT

The aim of this study was to investigate the structure of different solid-state forms of fenofibrate, a drug that lacks strong intermolecular interactions such as hydrogen bonding. In addition to a structural analysis of crystalline and amorphous fenofibrate using infrared and Raman spectroscopy combined with density functional theory calculations [B3LYP 6-31G(d)], solid-state changes that occur upon recrystallization of amorphous fenofibrate were monitored and described using *in situ* Raman spectroscopy. A comparison of the calculated vibrational spectra of a fenofibrate monomer and two dimer structures with the experimental vibrational spectra of crystalline and amorphous fenofibrate revealed conformational differences in the orientation of the two benzyl rings in the fenofibrate molecule and structural differences between the different solid-state forms in aliphatic parts of the drug molecule. The spectroscopic analysis suggests that non-hydrogen-bonded drug molecules are likely to exhibit more random molecular orientations and conformations in the amorphous phase since the weak intermolecular interactions that occur between such molecules can easily be disrupted. *In situ* Raman spectroscopy and multivariate analysis revealed multiple solid-state forms of fenofibrate, including the metastable crystalline form II, which were structurally analyzed with reference to the quantum chemical calculations. Overall, the study showed that vibrational spectroscopy, multivariate analysis, and quantum chemical modeling are well suited to investigate and characterize the structure of drug substances that exhibit only small structural differences between different solid-state forms.

1. Introduction

Pharmaceutical solids can exist in various solid-state forms which are classified in terms of molecular structure and composition. Crystalline materials are characterized by orientational and positional long-range order in all three dimensions of space. Even though amorphous materials lack long-range order, they may exhibit short-range order which involves interactions between neighboring molecules, such as the formation of dimers [1]. The diversity of solid-state forms of the same compound is based on differences in non-covalent interactions between neighboring molecules and may additionally be associated with changes in molecular conformation. Of the various interactions, hydrogen bonding plays an important role. Other non-covalent attractive forces include van der Waals interactions such as π–π stacking and mainly depend on dipole moments, polarizability, and electronic distribution in the molecules [2,3]. Based on possible molecular interactions and/or conformations, crystalline materials may exist in different polymorphic forms.

Depending on the solid-state form of a pharmaceutical substance, differences in a range of properties, such as morphology, density, stability, melting point, and solubility, may occur. These differences, in turn, may influence the bioavailability, processability, as well as chemical and physical stabilities of the drug. Usually the pharmaceutical solid is manufactured in a stable crystalline form because the amorphous form tends to convert to the crystalline form due to its thermodynamic instability. However, despite this disadvantage, the amorphous state has attracted considerable interest in recent years in the formulation of poorly soluble compounds. The higher molecular mobility of amorphous materials, for instance, may improve the solubility and dissolution rate of the pharmaceutical compound and, thus, facilitate the gastrointestinal absorption of the active drug [4–6]. Whichever solid-state form is chosen, to ensure reproducible quality and efficacy of the pharmaceutical product, it is necessary

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to comprehensively characterize solid-state forms of a drug and closely monitor solid-state changes that may occur during processing, including recrystallization phenomena and polymorphic transitions.

A range of analytical techniques are available for the physical characterization of crystalline and amorphous compounds [4,7]. X-ray powder diffraction (XRPD), optical microscopy, and thermal methods including differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA), for instance, are utilized to characterize the particulate level [8]. Molecular-level characterization can be carried out using solid-state nuclear magnetic resonance spectroscopy and vibrational spectroscopy, namely, Fourier transform mid-infrared (FTIR), near-infrared (NIR), Raman, and terahertz spectroscopies [1,2,4,9]. Vibrational spectroscopy has become popular to characterize and quantify various solid-state forms of drugs including polymorphs [10–15], solvates [16], amorphous materials [17–19], and salts [20]. However, to gain molecular-level information on solid-state structure from vibrational spectral analysis, the spectral bands of interest must be assigned to the correct molecular vibrations. For pharmaceutical compounds, this is often very challenging. Quantum mechanical calculations, which have only recently been adopted for solid-state analysis in the pharmaceutical setting, are an invaluable tool for interpreting vibrational spectra [21–24]. Together with vibrational spectroscopy, quantum chemical calculations have, for example, been used to analyze conformational differences between the carbamazepine polymorphs form I and form III [21,25], to investigate hydrogen bonding in anhydrate and hydrate forms of theophylline, caffeine, and theobromine [26], to study structural differences between crystalline γ-indomethacin and the amorphous form created by quench-melting γ-indomethacin [22], and to gain insight into the structure of amorphous carbamazepine [27]. It could be shown that both indomethacin and carbamazepine exist predominantly as hydrogen-bonded dimers in the amorphous form.

Spectroscopic techniques have become popular to investigate solid-state transformations since they are rapid, nondestructive and provide molecular-level information. Studies have investigated, for instance, the solid-state forms that appear during isothermal dehydration of piroxicam and carbamazepine hydrate forms using *in situ* NIR and Raman spectroscopies [28] or the solvent-mediated phase transformations of theophylline and nitrofurantoin using *in situ* Raman spectroscopy [29]. Multivariate methods, such as principal component analysis (PCA), have shown to be useful tools to interpret the spectral changes in these cases as they visualize spectral variation in a way that makes it possible to better detect and understand solid-state conversions [28,30–32].

To our knowledge there has never been a study that combines spectroscopy, quantum mechanical calculations, and multivariate analysis for physicochemical analysis of a pharmaceutical compound. In this study, the combination of these techniques was used to investigate the solid-state structure and recrystallization behavior of the model drug fenofibrate. Fenofibrate exhibits polymorphism and is known to exist in two polymorphs, the stable crystalline form I [33] and the metastable form II which has only recently been reported [34]. Fenofibrate is an interesting model drug that is challenging for solid-state analysis by vibrational spectroscopy because it lacks hydrogen-bond donating groups and, hence, prohibits hydrogen bonding to other fenofibrate molecules. The aim of this work was to obtain a deeper understanding of the structure of different solid-state forms of fenofibrate and the solid-state conversions between these forms during recrystallization of amorphous fenofibrate by combining vibrational spectroscopy with quantum mechanical calculations and multivariate analysis.

2. Materials and methods

2.1. Materials

Fenofibrate is known to exist in two polymorphs, the stable form I and the metastable form II [34]. Form I crystallizes in the centrosymmetric triclinic space group $P\bar{1}$ [33]. The crystalline form I of fenofibrate (Fig. 1) was obtained from Sigma–Aldrich, Inc. (St. Louis, MO, USA) and was analyzed as received. Amorphous fenofibrate was prepared by melting the crystalline form I on an aluminum pan in a moisture analyzer (MA 100, Sartorius AG, Göttingen, Germany) at 80 °C [35]. The melt was adjusted to ambient temperature in a desiccator over phosphorus pentoxide to prevent atmospheric moisture condensation on the sample. The amorphous samples were analyzed directly after preparation.

2.2. Analytical techniques

2.2.1. X-ray powder diffraction

XRPD was used to confirm the solid state of crystalline and amorphous fenofibrate. The diffraction patterns were recorded using a theta–theta X-ray powder diffractometer (D8 Advance, Bruker AXS GmbH, Karlsruhe, Germany) equipped with Göbel mirror bent multilayer optics. Measurements were performed in symmetrical reflection mode with CuKα radiation (λ = 0.15418 nm) at 40 kV and 40 mA. The powder samples were scanned in the angular range of 5° (2θ) to 40° (2θ) with a step size of 0.1° (2θ) and a count time of 5 s per step.

The crystal structure of fenofibrate was verified by comparing the experimental XRPD pattern with the theoretical diffractogram obtained from the Cambridge Crystallographic Data Centre (CCDC) using Mercury software (v. 1.4.2, CCDC, Cambridge, UK). A structure with the reference code TADLIU was used to generate the theoretical diffraction patterns of crystalline fenofibrate [33].

2.2.2. Differential scanning calorimetry

DSC was carried out on a Q100 (TA instruments, New Castle, DE, USA). The samples (3–5 mg) were analyzed in crimped aluminium pans at temperatures between −60 and 100 °C. The heating rate was 10 °C min^{-1} and the nitrogen gas flow 50 ml min^{-1}. The calibration of the instrument was performed using indium as metal standard. The thermograms were analyzed using TA Universal Analysis 2000 software (v. 4.0, TA Instruments).

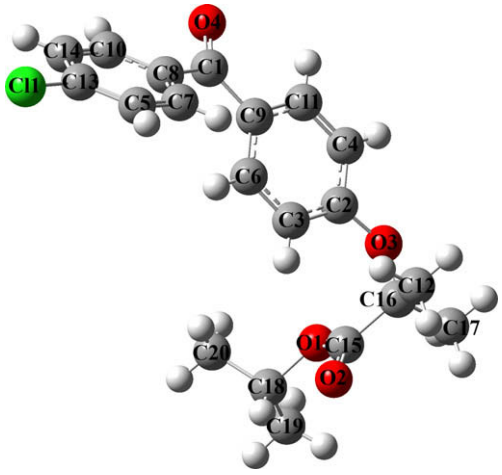

Fig. 1. Molecular structure of fenofibrate with atomic numbering introduced by Henry et al. [33].