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Phase transitions in secnidazole: Thermal stability and polymorphism studied by X-ray powder diffraction, thermal analysis and vibrational spectroscopy

Beatriz Pinheiro Bezerra[a], Jéssica Castro Fonseca[a], Yara Santiago de Oliveira[a], Maria Silmara A. de Santana[b], Keilla F. Silva[b], Bruno S. Araújo[b], Alejandro Pedro Ayala[b,*]

[a] Department of Pharmacy, Federal University of Ceará, Fortaleza, Ceará, Brazil
[b] Department of Physics, Federal University of Ceará, Fortaleza, Ceará, Brazil

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ABSTRACT

Secnidazole is an old drug efficient as an antimicrobial agent used against some anaerobic bacteria and parasites, such as giardia, ameba and trichomonas that are causing some neglected diseases. This drug is commercialized as a hemihydrate, being stable at room temperature, but showing relevant phase transitions on heating. The understanding of the dehydration process on solvates provides valuable information for quality control and development of solid formulations. In order to study the thermal behavior of secnidazole, Raman spectroscopy, X-ray powder diffraction, hot-stage microscopy and thermal analysis were employed to verify the structural modifications involved with this drug. Raman spectroscopy was a key technique to monitor the dehydration process *in situ*, providing the spectral fingerprints for identifying the anhydrous form. In addition, the reported results suggest the existence of two anhydrous monotropic polymorphs.

1. Introduction

Secnidazole (SNZ, Fig. 1) is an antimicrobial agent, used against some anaerobic bacteria and parasites, such as giardia, ameba and trichomonas which are responsible for some neglected diseases. According to IUPAC classification, denominated as (hydroxyl-2-propyl)-1-methyl-2-nitro-5-imidazole, it belongs to the 5-nitro-imidazoles chemical class and is structurally related to metronidazole and tinidazole [1–3], employed against a variety of G^+/G^- bacteria, but with significant side effects as it is used in very high concentration [2]. This drug is presented as white crystalline powder, and even though marketed for a few decades, and therefore with a consolidated use, the pharmacopoeias give absolutely no mention of this active substance.

SNZ shows significant degradation under alkaline conditions and in the presence of light, while extenuated in acidic and neutral media. Various analytical procedures have been reported for determining SNZ in pharmaceutical preparations, either alone or in combination with other drugs. The reported methods include potentiometry, polarography, supercritical fluid chromatography, high performance liquid chromatography, spectrophotometry and voltammetry. Most spectrometric methods (UV/VIS) suffer from disadvantages such as narrow determination steps requiring heating or long lasting extraction for reaction completion and the use of non-aqueous systems [4,5].

Secnidazole is formulated as a hemihydrate (SNZh), whose crystalline structure was determined by Novoa et al. and is reported in the Cambridge Structural Database (CSD) with Refcode SIBFUE [6,7]. It has monoclinic symmetry with lattice parameters $a = 12.424$ Å, $b = 12.187$ Å, $c = 6.662$ Å and $\beta = 100.9°$, belonging to the space group $P2_1/c$. Some authors have reported on the X-ray powder pattern of the anhydrous form (SNZa), suggesting that it could be isostructural with the hydrated one, although the crystal structure of SNZa has not been solved. Several physicochemical properties of SZNh have already been reported in the literature as X-ray powder diffraction, solubility, pK_a, dissolution, etc. [3], but the descriptions on the thermal stability of this drug are conflictive.

Water and solids may interact and modify different properties such as flowability, bulk density, providing degradation and consequently the physical-chemistry of the molecule, while dehydration could possibly occur during the manufacturing process, also affecting the compound [8,9]. Raman spectroscopy was successfully applied to the study of hydrate-anhydrous

* Corresponding author.
E-mail address: ayala@fisica.ufc.br (A.P. Ayala).

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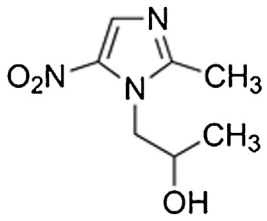

Fig. 1. Chemical structure of secnidazole.

conversion in pharmaceuticals, as well as, in the identification of drug polymorphs [10,11]. This technique can be easily adapted for *in situ* measurements allowing monitoring the water release/absorption under different stress conditions. For example, the water release dynamic of aripiprazole investigated by Raman spectroscopy was correlated with calorimetric studies showing that the dehydration is divided in two processes driven by the heating rate [12]. Polymorphic transitions were also identified in formulated products [13] and/or in raw materials at ambient conditions or under temperature variations [14–17]. The structural specificity of Raman spectroscopy is mainly due to the ability to the lattice and molecular backbone vibrations, which directly reflects polymorphic and/or conformational differences [18]. Considering its key features for the solid-state characterization of pharmaceutical compounds, in this contribution, Raman spectroscopy was applied in combination with hot-stage microscopy, X-ray powder diffraction and thermal analysis to provide a comprehensive description of the structural modifications involving the hydrate of SNZ and its transformation into the anhydrous form.

2. Experimental

2.1. Materials

SNZh was supplied by EMS Pharma (Brazil). The drug was recrystallized (50 mg) in 1 mL of different solvents, such as ethyl acetate, methanol and acetonitrile. The recrystallized sample was used to perform all the analyses presented in this work. Powder X-ray diffraction patterns of these samples were compared with the calculated diffraction pattern of SNZh listed in CSD in order to confirm the crystalline structure.

2.2. Thermal analysis

Thermogravimetric (TGA) and Differential Scanning Calorimetric (DSC) curves were obtained simultaneously using our thermal analysis equipment (Jupiter STA 449, Netzsch). An infrared spectrometer (model Alpha, Bruker Optics) coupled to this system was used to analyze the released gases (32 scans, resolution 4 cm^{-1}). In addition, DSC curves starting below room temperature were recorded with a Netzsch Maia 200 F3 calorimeter. The sample (~5 mg) was placed insealed aluminum crucibles with pierced lids. Several cooling/heating temperature cycles were performed at different constant rates. The sensors and the crucibles were kept under a constant flow of nitrogen during the experiment.

2.3. Hot-stage microscopy

The crystals were submerged in silicone oil and submitted to several heating/cooling cycles using temperature programs under constant monitoring. A polarized microscope Leica (DM2500P) coupled with a hot-stage Linkam (FTIR600) was used. Images were recorded with a QUICAM (Fast1394) camera and processed with the software *Linkesys32*.

2.4. Vibrational spectroscopy

The Raman spectra were obtained by a LabRAM HR (Horiba) spectrometer equipped with a liquid N_2-cooled CCD detector, using 633 nm laser radiation for excitation (~2 mW at the sample surface); the spectrometer was coupled to a Hot-Stage equipment (Linkam), model FTIR600 for temperature control. Multivariate curve resolution analysis was performed using the MCR-ALS GUI 2.0 software [19].

2.5. X-ray powder diffraction

X-ray powder diffraction (XRPD) was used to investigate the hemihydrate and anhydrous forms of secnidazole. The material was finely ground and mounted on a glass sample holder in a D8 Advanced (Bruker AXS), equipped with a theta/theta goniometer, operating in the Bragg Brentano geometry with a fixed specimen holder, using a Cu Kα (0.15419 nm) radiation source and a LynxEye detector. The voltage and electric current applied were 40 kV and 40 mA, respectively. The slit width used for the beam incident on the sample was 0.6 mm. The sample was scanned within the range of $2\theta = 5–40^\circ$ under continuous scan mode, with a rate of $2^\circ\ min^{-1}$. The patterns as a function of the temperature were recorded between 33 and 80 °C using an Anton Paar TT450 chamber.

3. Results and discussion

In the case of solvates, particularly hydrates, the thermal stability is a limiting factor to determine whether these solid forms are suitable for use in pharmaceutical formulations. The production processes of a new drug may involve the presence of water during crystallization, in the manufacture or formulation of the final product by the addition of excipients. In hydrates the combination of intermolecular forces (hydrogen bonds) and the crystal packing produce strong interactions in the solid state that stabilize new crystalline structures [20]. The dehydration of the crystals may easily occur under the effect of temperature, thus justifying the investigation of the thermal stability of SNZh. The TGA curves for SNZh are shown in Fig. 2. Two heating/cooling cycles were recorded in an inert atmosphere (N_2) at a rate of 1 °C min^{-1}, between 30 °C and 100 °C. Along the first heating, it was possible to observe a mass loss event, whereas no changes were present in the second heating. This event started around 45 °C and is completed by reaching 75 °C, with an associated mass loss of 4.1%, which agrees very well with the theoretical value of 4.6% related to the release of half a water molecule. In Fig. 2, the TGA curve recorded up to 400 °C (5 °C min^{-1}) is also presented showing that SNZ started decomposing around 230 °C. The hypothesis of the SNZh dehydration during the first TGA event can be confirmed by considering the IR-spectra of the evolved gases (Fig. 2), where two well defined processes can be easily identified. The IR-spectra of the gases released at the first event exhibit several weak bands around 1700 cm^{-1} and 3500 cm^{-1}, which clearly fingerprint the rotational-vibrational spectrum of water vapor. At the second event, it was possible to identify, at least, the release of ammonia, roto-vibrational bands around 943 cm^{-1} and 3335 cm^{-1}, water vapor, and carbon dioxide, confirming that SNZ decomposes at this temperature.

In Fig. 3 DSC curves of SNZh recorded under different heating/cooling conditions are presented. During the first heating at 5 °C min^{-1}, a wide endothermic peak is observed, which is well resolved in two peaks at 1 °C min^{-1}. Considering the later curve, the first one (with onset at 70.3 °C) can be associated with the release of water by SNZh, since it is directly correlated with the mass loss event recorded at approximately the same temperature in the TGA curve (Fig. 2). The cooling process at 5 °C min^{-1} is also shown in