Journal of Pharmaceutical and Biomedical Analysis 100 (2014) 329–340

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Characterization of polymorphic ampicillin forms

C. Baraldi [a], A. Tinti [b], S. Ottani [c], M.C. Gamberini [a,*]

[a] Department of Life Sciences, University of Modena and Reggio Emilia, via Campi n.183, 41125 Modena, Italy
[b] Department of Biomedical and Neuromotor Sciences, University of Bologna, via Belmeloro 8/2, 40126 Bologna, Italy
[c] Istituto per la Sintesi Organica e la Fotoreattività, ISOF-CNR, via P. Gobetti 101, 40129 Bologna, Italy

ARTICLE INFO

Article history:
Received 19 May 2014
Received in revised form 12 August 2014
Accepted 15 August 2014
Available online 26 August 2014

Keywords:
Ampicillin
FTIR/ATR spectroscopy
Raman microscopy
DFT calculations
X-ray powder diffraction
Thermal analysis

ABSTRACT

In this work polymorphs of α-aminobenzylpenicillin (ampicillin), a β-lactamic antibiotic, were prepared and investigated by several experimental and theoretical methods. Amorphous monohydrate and three crystalline forms, the trihydrate, the crystal form I and the crystal form II, were investigated by FT-IR and micro-Raman. Also data obtained by differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), X-ray powder diffraction (XRPD) and hot-stage Raman spectroscopy are reported. Finally, quantum mechanical calculations were performed by density functional theory (DFT) to assist the assignment of spectroscopic experimental bands. For the first time, the ampicillin molecule in its zwitterionic form was studied at the B3LYP/aug-cc-pVDZ level and the corresponding theoretical vibrational spectra were computed. In fact, ampicillin in the crystal is in zwitterionic form and concentrations of this same form are quite relevant in solutions at physiological pH. Experimental and theoretical results allowed identification of specific features for polymorph characterization. Bands typical of the different polymorphs are identified both in IR and Raman spectra: in particular in the NH stretching region (IR), in the amide I + δNH region (both techniques), in the 1520–1490 cm^{-1} region (IR), in the 1320–1300 cm^{-1} and 1280–1220 cm^{-1} (IR), in the 1200–1170 cm^{-1} (Raman), in the amide V region (IR), and, finally, in the 715–640 cm^{-1} and 220–200 cm^{-1} (Raman). Interconversion among different polymorphs was investigated by hot-stage Raman spectroscopy and thermal analysis, clarifying the complex pattern of transformations undergone as a function of temperature and heating rate. In particular, DSC scans show how the trihydrate crystals transform into anhydrous forms on heating. Finally, stability tests demonstrated, after a two years period, that no transformation or degradation of the polymorphs occurred.

1. Introduction

Several active pharmaceutical ingredients (APIs) crystallize in different forms (polymorphs) or with solvent molecules as integral part of their structures (pseudopolymorphs). Pharmaceutical formulations of these compounds may prove challenging, since differences in the solid state phase of the active ingredient may dramatically alter the final effects of the drug, with marked influence on its bioavailability. Moreover, differences in crystal packing play a major role on drug stability, especially as temperature and humidity effects are concerned. Usually, crystalline forms are strongly preferred for their stability and reproducibility; higher degree of purification can be achieved, as compared to amorphous solids and solid solutions. Thus, the requirement that in pharmaceutical formulations of APIs only a single crystalline form be present, rises a set of problems related to clinical protocols and legal and regulatory issues. The unpredictability (i.e. lack of obviousness) of crystal structures and physical properties may rise legal issues and challenges in terms of obtaining and maintaining patent protection for an API. Among the substances affected by this kind of problems, an important case is provided by ampicillin, a molecule which has obtained new attention in antibiotic therapeutic practices.

Ampicillin or α-aminobenzylpenicillin or 6-[D(-)α-aminophenylacetamido] penicillanic acid (Fig. 1) is a penicillin class antibiotic with betalactamic structure. Literature reports that ampicillin may exist in two anhydrous polymorphic forms (γ form also named B or I; δ form also named II) and hydrate forms (trihydrate; monohydrate or form A) [1–3]. The trihydrate and anhydrous form I have been the most studied forms, while the monohydrate and anhydrous form II did not receive analogous attention. In particular, Shefter et al. [1] refer the existence of form

* Corresponding author. Tel.: +39 59 205 5157; fax: +39 59 205 5131.
E-mail addresses: mariacristina.gamberini@unimore.it, gamber@unimore.it (M.C. Gamberini).

http://dx.doi.org/10.1016/j.jpba.2014.08.021
0731-7085/

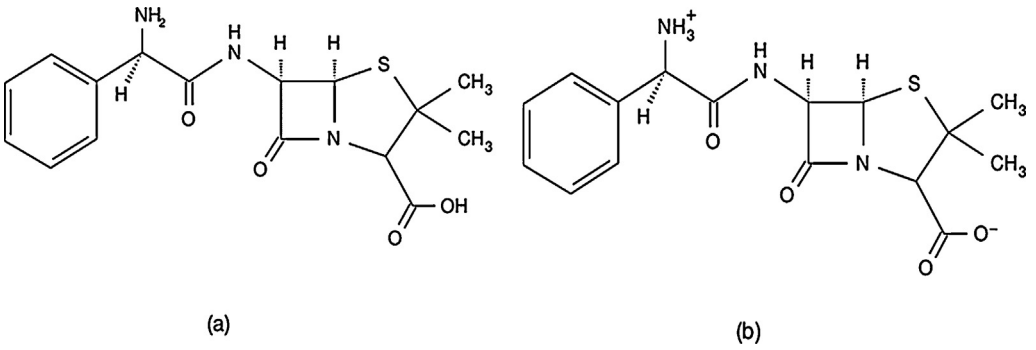

Fig. 1. (a) Anhydrous ampicillin structure and (b) ampicillin zwitterionic form.

II and a crystallization method is reported [4], but IR characterization is inadequate. In the different commercial pharmaceutical formulations like injectable or oral preparations, capsules or tablets, the API is present as the anhydrous form or as sodium salt or as the trihydrate form. Alburn et al. [5] report that the anhydrous form I has the advantage of high storage stability characteristics, related to its negligible water content. Moreover, anhydrous ampicillin exhibits slower absorption in the gut and prolonged blood levels with more effective action, relatable to its lower solubility in water.

As far as ampicillin is concerned, a major problem is the poor characterization of its polymorphs and the confusion in naming such polymorphs. In fact several notation systems (letters – A, B, C, Greek letters γ, δ or Roman numerals – I, II, III, etc.) are used in parallel, sometimes in inconsistent ways. Thus, it may happen that the form defined as "I" by an author is not necessarily named in the same way by another one. As a result, correlations between crystal forms and experimental data reported in different studies, such as vibrational spectra, may prove difficult and unreliable. The characterization methods reported in the literature include FT-IR [6], X-ray powder diffraction (XRPD) [1], single crystal x-ray diffraction (SCXRD) [2], differential scanning calorimetry/thermogravimetric analysis (DSC/TGA), diffuse reflectance infrared Fourier-transform spectroscopy (DRIFT), THz time-domain spectroscopy (THz-TDS) and NMR spectroscopy [7]. Table 1 summarizes the published studies concerning ampicillin forms and their nomenclature.

The study of ampicillin polymorphs, of their stability and interconversion must face most of the above mentioned difficulties in relating structural features to pharmaceutical formulations and physical properties. SCXRD investigations report that, in the crystals of the anhydrous form I and of the trihydrate, ampicillin molecules are in zwitterionic form (Fig. 1b). In the form I different molecules are held together by intermolecular H-bonds [19]. In the trihydrate the crystallization water molecules participate into a complex network of H-bonds connecting several zwitterions [19]. In contrast, the crystal structure of the anhydrous form II has not yet been reported and the existence of zwitterions in the solid state for this form is not confirmed.

Literature methods report preparation of the monohydrate solvate by heating in vacuo from room temperature up to 95 °C, and preparation of the anhydrous γ form by heating to 80–100 °C [6]. In contrast, the less studied δ form has been crystallized from a boiling xylene solution [4]. However, such methods lead to partial decomposition of samples [1], as indicated also by the light yellow color of the final specimens. Thus, in the present work, different procedures have been used for the preparation, as reported in the subsequent section.

The various polymorphic and solvate forms were crystallized and characterized by different techniques. The molecular vibrations of the different ampicillin forms were investigated by ATR/FT-IR and micro-Raman. Quantum-mechanical calculations allowed assignment of vibrational modes. The hot stage Raman microscopy (HSRM) was able to follow the transition from trihydrate to amorphous monohydrate ampicillin. The same technique allowed, by heating specimens in a sealed capillary, to control solid-solid conversion from trihydrate to anhydrous forms. The results reported in the present work allow a better identification and characterization of the different polymorphs and of their interconversion, with a special focus on transformations undergone by the different forms and their range of stability. From this point of view, they can also be useful in addressing the above reported questions related to clinical protocols and legal and regulatory issues.

2. Materials and methods

2.1. Compounds

The basic ampicillin was a commercial sample from Sigma–Aldrich, Milano, Italy, corresponding to the trihydrate form and was used in sample preparation as reported in the following.

2.1.1. Preparation of polymorphs

Form I (γ). Commercial trihydrate ampicillin (0.5 g) was suspended in water (0.8 ml) and heated to 90 °C. In few minutes crystals of the anhydrous form were precipitated [6]. The mixture was filtered and placed in a dryer. The same anhydrous form was also prepared by placing the trihydrate ampicillin in a sealed capillary subjected to a slow heating (5–10 °C/min) from 25 °C to 150 °C.

Form II (δ). Commercial trihydrate ampicillin (0.6 g) was dissolved in boiling xylene (6 ml, at 136 °C) and then filtered under vacuum. The precipitate was washed with ethyl acetate and diethyl ether, dried in a oven at 50 °C for 15′ and then stored in a vacuum dryer at room temperature [4]. The final product was lightly yellow. Alternatively, the same form was obtained by fast heating (60 °C/min) to 150 °C in a sealed capillary. In this case, white crystals were obtained.

Monohydrate. Commercial trihydrate ampicillin (0.6 g) was heated in a oven from 30 to 95 °C in 12 min and kept at this temperature for 2 h [1,7]. The product obtained was lightly yellow.

Trihydrate. The trihydrate form was purified by recrystallization from saturated aqueous solutions of the commercial powder heated below 50 °C [2]. The resulting precipitate was filtered by sintered glass and the crystals were dried in vacuum at room temperature.