Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 156 (2016) 70–77

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

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

Looking for the interactions between omeprazole and amoxicillin in a disordered phase. An experimental and theoretical study

Marcos G. Russo [a,*], Matias I. Sancho [b], Lorena M.A. Silva [c], Hector A. Baldoni [d], Tiago Venancio [c], Javier Ellena [e], Griselda E. Narda [a]

[a] Inorganic Chemistry, INTEQUI, National University of San Luis, Chacabuco and Pedernera-5700, San Luis, Argentina
[b] Physical Chemical Laboratory, National University of San Luis, Chacabuco and Pedernera-5700, San Luis, Argentina
[c] Chemistry Department, Federal University of São Carlos, PO Box 676, 13565-905 São Carlos, Brazil
[d] Institute of Applied Mathematics San Luis (IMASL-CONICET), National University of San Luis, Chacabuco and Pedernera-5700, San Luis, Argentina
[e] Physics Institute of São Carlos, University of São Paulo, CP 369, 13560-970 São Carlos, SP, Brazil

ARTICLE INFO

Article history:
Received 5 February 2015
Received in revised form 8 October 2015
Accepted 20 November 2015
Available online 22 November 2015

Keywords:
Omeprazole
Amoxicillin
Drugs interactions
Disordered phases
DFT
QTAIM
NBO
FTIR
ssNMR

ABSTRACT

In this paper, co-grinding mixtures of omeprazole–amoxicillin trihydrate (CGM samples) and omeprazole–anhydrous amoxicillin (CGMa samples) at 3:7, 1:1 and 7:3 molar ratios, respectively, were studied with the aim of obtaining a co-amorphous system and determining the potential intermolecular interactions. These systems were fully characterized by differential scanning calorimetry (DSC), FT-infrared spectroscopy (FTIR), X-ray powder diffraction (PXRD), scanning electron microscopy (SEM) and solid state Nuclear Magnetic Resonance (ssNMR). The co-grinding process was not useful to get a co-amorphous system but it led to obtaining the 1:1 CGMa disordered phase. Moreover, in this system both FTIR and ssNMR analysis strongly suggest intermolecular interactions between the sulfoxide group of omeprazole and the primary amine of amoxicillin anhydrous. The solubility measurements were performed in simulated gastric fluid (SGF) to prove the effect of the co-grinding process. Complementarily, we carried out density functional theory calculations (DFT) followed by quantum theory of atoms in molecules (QTAIM) and natural bond orbital (NBO) analyses in order to shed some light on the principles that guide the possible formation of heterodimers at the molecular level, which are supported by spectroscopic experimental findings.

1. Introduction

Amoxicillin trihydrate (AMX) belongs to the penicillin class of antibiotics, being a β-lactam antibiotic used to treat bacterial infections caused by susceptible microorganisms. AMX is classified as class III according to the Biopharmaceuticals Classification System (BCS) [1]. Omeprazole (OMZ), a gastric proton-pump inhibitor (H^+/K^+-ATPase), is widely used for the prevention and treatment of stomach and esophagus diseases [2]. Moreover, OMZ is a slightly water-soluble drug and is classified as class II by the BCS. Both, OMZ and AMX are supplied for the treatment of *Helicobacter pylori* eradication [3], which plays an important role in duodenal and gastric ulcer pathologies.

The amorphization of a poorly water-soluble drug can be a useful strategy to enhance its solubility, intrinsic dissolution rate and its bioavailability, which comes as a result of an excess of free energy, compared to that of the crystalline counterpart [4]. However, the amorphous form, being a higher energy state, is physically unstable and leads to the release of excess energy by structural relaxation, going to a lower energy form during storage, which is probably transformed into an unwanted crystalline form. Solid dispersion technology, in which a drug is included into an amorphous polymer, is the most extensive approach to increase the stability of amorphous systems [5], but this method has shown several disadvantages which were reported by Riikka Laitinen et al. [6]. An alternative approach is the synthesis of co-amorphous systems, where a combination of two small molecules, either *Active Pharmaceutical Ingredients* (*API*)-*Generally Recognized As Safe* (GRAS) or API–API, is used instead of drug–polymer mixtures. The improved physical stability and dissolution of these co-amorphous systems is attributed to new intermolecular interactions between the components of the binary system [5,6], which improves physical–chemical properties compared to the pure drugs, particularly solubility and dissolution rate. Several techniques have been used to prepare co-amorphous binary systems: solvent evaporation [7,8], quench cooling [9], grinding [10–12] and so on. The latter could induce defects in the crystal lattice [13,14] altering both the physical and chemical properties of the pharmaceutical ingredients [15,16]. These defects produce disordered phases rather than amorphous compounds and show X-ray patterns similar to both amorphous systems [14,17] and poorly

* Corresponding author
E-mail address: gnarda@unsl.edu.ar (G.E. Narda).

http://dx.doi.org/10.1016/j.saa.2015.11.021
1386-1425/

crystalline [18], while the DSC analysis may or may not detect the glass transition temperature (Tg) [14,17,18].

Within the past few decades, quantum mechanical (QM) chemistry has become increasingly used in the pharmaceutical field, especially due to its applicability in the assignment of vibrational spectra [19]. QM calculations have also been employed for the band assignment of the experimental infrared (IR) and Raman spectra of drugs [20–23]. Especially in the case of amorphous materials, QM calculations can become a powerful tool to get further insight in the near range order of amorphous systems and to help interpret and support experimental spectra. The situation becomes more complex in mixed systems such binary systems of APIs, systems for which the QM calculations could provide a clearer insight. In this paper, the binary system OMZ–AMX, (CGM samples), in addition to OMZ–AMXa, (anhydrous amoxicillin) (CGMa samples), in 3:7, 1:1 and 7:3 molar ratios, respectively, were obtained by co-grinding with the aim to achieve co-amorphous phases and elucidate the potential intermolecular interactions. Although the daily OMZ–AMX molar ratios indicated for the patients are different from the molar ratios used in this study, the latter were selected to account for spectroscopic, thermal or structural changes expected in the molecular environments might be undetectable or lost and as such might be not observable. The physicochemical properties were investigated with an appropriate combination of different techniques such as powder X-ray diffraction (PXRD), FT-infrared spectroscopy (FTIR), differential scanning calorimetry (DSC) and solid state Nuclear Magnetic Resonance (ssNMR). Assays of solubility were determined by HPLC in simulated gastric fluid (SGF). Moreover, density functional theory (DFT) calculations, quantum theory of atoms in molecules (QTAIM) and natural bond orbital (NBO) were employed to shed some light on the possible formation of heterodimers according to the modes of interactions proposed by spectroscopic techniques (FTIR and ssRMN) between both APIs.

2. Experimental

2.1. Materials

OMZ (MW: 345.42 g mol^{-1}) was purchased from Sigma-Aldrich® and used without any further purification. AMX (MW: 419.45 g mol^{-1}) was provided by the Drug Quality Control Laboratory (National University of San Luis, San Luis, Argentina). The purity of the samples was checked by thin layer chromatography. In Fig. 1 the chemical structures and atom numbering corresponding to OMZ and AMX are shown.

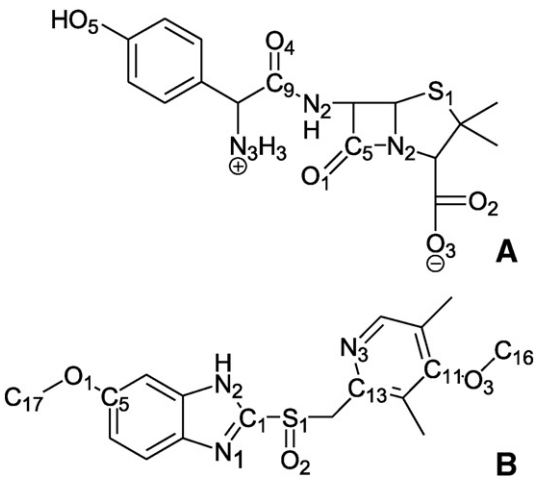

Fig. 1. Chemical structure and numbering of selected atoms of AMX (A) and OMZ (B).

2.2. Methods

2.2.1. OMZ–AMX binary system preparation (PM and CGM samples)

A total of 1 g of fresh binary mixtures of OMZ and AMX in 7:3, 1:1 and 3:7 molar ratios were mixed with a similar tumbling mixer during 10 min and named 7:3, 1:1 and 3:7 physical mixtures (PM), respectively. The PM samples were prepared in triplicate. The samples named co-ground mixture (CGM) in 7:3, 1:1 and 3:7 molar ratios were obtained by manually grinding 500 mg of the PM samples for 60 min, in an agate mortar at 25 °C. Moreover, 250 mg of pure OMZ and AMX was ground during 60 min and then it was confirmed by FTIR and PXRD that the mechanical treatment did not alter their spectroscopic and structural properties (data not shown). All samples were kept in the dark at 4 ± 2 °C in a desiccator with silica for further examination.

2.2.2. OMZ–AMXa binary system preparation (PMa and CGMa samples)

A total of 100 mg of physical mixture and co-ground mixtures with amoxicillin anhydrous (PMa and CGMa samples, respectively), in 7:3, 1:1 and 3:7 molar ratios, was prepared as described in Section 2.2.1 but using AMXa. In order to prevent the rehydration of AMXa during grinding, this process was carried out in a PVC bag at 25 °C with silica gel. Amoxicillin anhydrous was obtained placing AMX in a DSC equipment (see Section 2.3.1.) and heating to the dehydration temperature; HPLC analysis shows that the chemical integrity of AMXa is not altered by the dehydration process (Fig. S1). The samples were maintained in the dark at 4 ± 2 °C in a desiccator with silica for further examination.

2.2.3. Stability studies

2.2.3.1. Physical stability. The physical stability of the X-ray amorphous samples, AMXa and 1:1 CGMa, kept under dry conditions in a desiccator at 4, 25 and 40 °C for 45 days, was determined by PXRD and DSC analysis.

2.2.3.2. Chemical stability. The chemical stability of the X-ray amorphous samples, AMXa and 1:1 CGMa, stored for 45 days to 4, 25 and 40 °C was evaluated by HPLC. The analysis was performed by dissolving a known amount of the sample in 50% (*v*/*v*) acetonitrile–DMSO solution and analyzing the sample by HPLC (see Section 2.3.5). No additional peaks corresponding to degradation products were observed in the chromatograms (Fig. S2), suggesting that the storage condition does not alter the chemical integrity of the samples.

2.2.4. Solubility assays

The solubility studies of OMZ, AMX, AMXa and 1:1 CGMa were carried out in simulated gastric fluid (SGF) free of enzymes [24]. An excess of the samples was added to 10 mL of dissolution medium. These suspensions were shaken in a JEIO TECH SI-300R shaker at 100 rpm for 40 min at 37 °C. The resulting suspensions appropriately diluted were filtered with a 0.22 μm Millipore® membrane filter. The concentration of the drugs was chromatographically determined by HPLC (see Section 2.3.5.). All data are presented as the mean of three individual observations with the corresponding standard deviation. The method used to quantify both drugs was linear in the range of 0.345–2.487 g L^{-1} for OMZ (R^2: 0.9990) and 0.167–4.194 g L^{-1} for AMX (R^2: 0.9950).

2.3. Analytical techniques

2.3.1. Thermal analysis

DSC curves were obtained with a Shimadzu TA-60WS Thermal Analysis System using 3–4 mg of the powder in open aluminum pans, in flowing air, at 50 mL min^{-1} and with a heating rate of 10 °C min^{-1} from room temperature to 250 °C.

Thermogravimetrical Analyses (TGA) were performed using a Shimadzu TGA-51 Thermal Analyzer using platinum pans, flowing air at 50 mL min^{-1} and a heating rate of 10 °C min^{-1} from RT to 800 °C.