Thermochimica Acta 550 (2012) 13–18

Contents lists available at SciVerse ScienceDirect

Thermochimica Acta

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

Ambazone-lipoic acid salt: Structural and thermal characterization

Irina Kacso[a], Csaba-Pal Racz[b], Szabolcs Santa[b], Lucia Rus[c], Dorin Dadarlat[a], Gheorghe Borodi[a], Ioan Bratu[a,*]

[a] National Institute for Research and Development of Isotopic and Molecular Technologies, 65-103 Donáth street, 400293 Cluj-Napoca, Romania
[b] Babes-Bolyai" University, Faculty of Chemistry, 11 Arany Janos street, Cluj-Napoca, Romania
[c] "Iuliu Haţieganu" University of Medicine and Pharmacy, Faculty of Pharmacy, 6 Louis Pasteur street, 400349 Cluj-Napoca, Romania

ARTICLE INFO

Article history:
Received 13 June 2012
Received in revised form
11 September 2012
Accepted 12 September 2012
Available online 3 October 2012

Keywords:
Ambazone
α-Lipoic acid
Solid form
FTIR spectroscopy
XRPD
Thermal methods

ABSTRACT

A suitable method for increasing the solubility, dissolution rate and consequently the bioavailability of poor soluble acidic or basic drugs is their salt formation. The aim of this study is to investigate the structural and thermal properties of the compound obtained by solvent drop grinding (SDG) method at room temperature, starting from the 1:1 molar ratios of ambazone (AMB) and α-lipoic acid (LA). The structural characterization was performed with X-ray powder diffraction (XRPD) and infrared spectroscopy (FTIR). The thermal behaviour of the obtained compound (AMB·LA) was investigated by differential scanning calorimetry (DSC) and thermogravimetry (TG). The photopyroelectric calorimetry, in front detection configuration (FPPE), was applied to measure and compare the room temperature values of one dynamic thermal parameter (thermal effusivity) for starting and resulting compounds. Both structural and supporting calorimetric techniques pointed out a salt structure for AMB·LA compound as compared to those of the starting materials.

1. Introduction

Pharmaceutical scientists constantly work to improve physical properties of active pharmaceutical ingredients such as solubility, stability, dissolution rate, hygroscopicity, density and taste [1–3] by making different solid form of these. The most active areas of modern solid state chemistry represent the identification and characterization of different solid forms (polymorphs, solvates, salts or co-crystals) of the same molecule [4]. In recent years, much of the research on the preparation of pharmaceutical solid forms has been carried out [2,4]. Usually, there are two methods by which solid forms can be prepared: solution-based crystallization and grinding. Mechanical chemical methods [5–10], more commonly and usefully described as grinding, have been employed extensively in the preparation of solid forms. The use of the so-called "solvent-drop grinding", in which a small quantity of a solvent is added to the solid substance or mixture prior to grinding has been developed [4].

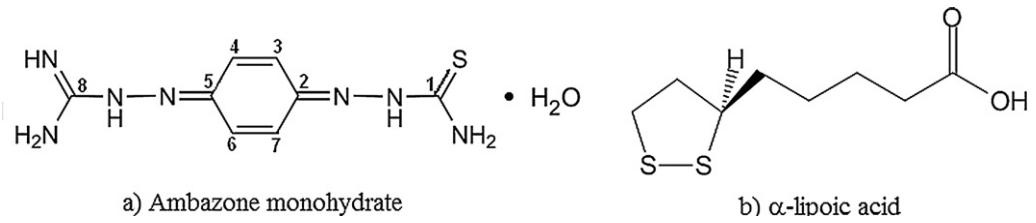
Ambazone monohydrate, $C_8H_{11}N_7S{\cdot}H_2O$ (AMB) ([4-(2(diaminomethylidene) hydrazinyl)phenyl] iminothiourea) (Fig. 1a), is one of the oldest antimicrobial chemicals. The studies performed during the 1950–1960 period have shown the local antibacterial properties of the compound when it is administrated at the buccal pharyngeal cavity level. In such a way it was demonstrated that ambazone monohydrate is an efficient antimicrobial drug [11].

The subsequent re-evaluation of the antibacterial AMB properties evidenced an antibacterial activity spectrum similar to that of sulphamides [12]. The antineoplasm properties of AMB were also demonstrated [13–19], fact that accelerated the researches on this substance without mutagenic effects and unpleasant reactions characteristic to other oncostatic drugs [12]. Ambazone undergoes three protonation reactions with pK_a values at 10.69 (equilibrium between the negatively charged and neutral forms), 7.39 (equilibrium between the neutral and singly positively charged form) and 6.22 (equilibrium between the singly and doubly positively charged form) [20].

α-Lipoic acid, $C_8H_{14}O_2S_2$, LA ((*R*)-5-(1,2-dithiolan-3-yl)pentanoic acid) is a disulphide derivative of octanoic acid (Fig. 1b), that forms an intramolecular disulphide bond in its oxidized form. High electron density resulting from special position of the two sulphur atoms in the 1,2-dithiolane ring confers upon LA a high tendency for reduction of other redox-sensitive molecules according to environmental condition [21]. Lipoic acid is bound to proteins and, consequently, free lipoic acid has not been detected in human beings unless it has been supplemented for its therapeutic effects [22]. LA is a weak acid (with different

* Corresponding author.
E-mail address: ibratu@gmail.com (I. Bratu).

0040-6031/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.tca.2012.09.026



a) Ambazone monohydrate b) α-lipoic acid

Fig. 1. Chemical structures of the starting compounds.

reported values for pK_a = 5.3–4.76 [23]) with both low aqueous solubility and low bioavailability.

Generally, pharmaceutical salts present higher solubility in gastric and intestinal fluids than non-ionic species and, consequently, they are useful in solid dosage forms. Furthermore, due to the fact that their solubility is often a function of pH, selective dissolution in one or another part of the digestive tract is possible and this capability can be manipulated as one aspect of delayed and sustained release behaviours. Even more, the salt-forming molecule can be in equilibrium with a neutral form and consequently, the passage through biological membranes can be adjusted [24].

The aim of this study is to obtain a salt of ambazone with α-lipoic acid (AMB·LA), based on the sufficiently large pK_a difference between these compounds [4] and to characterize its physical–chemical and structural properties by using several investigation methods.

2. Experimental procedure

2.1. Materials and preparation

AMB was obtained from Microsin SRL Bucharest Romania, LA commercially available was obtained from Alfa Aesar, Germany and ethanol of PA grade from Merck, Germany. These compounds were used without further purification. Solvent-drop grinding (SDG) experiments were performed by placing 255.3 mg AMB with 206.32 mg LA (1:1 molar ratio) and grinding this mixture in an agate mortar by adding in drops of 2 ml ethanol at room temperature, until a dried compound was obtained. For PPE experiments various mixtures having different molar ratios of AMB and LA were also prepared: 4:1; 2:1; 1:2. The resulting samples were analysed using powder X-ray diffraction (PXRD), thermal methods (DSC, TG, FPPE) and FTIR spectroscopic technique.

2.2. Powder X-ray diffraction

PXRD patterns were obtained with a Bruker D8 Advance diffractometer, at 20 °C and the whole experiment was computer controlled. The experimental conditions were: the 2θ range between 5° and 45°, Cu Kα1 radiation (λ = 1.5406 Å) (40 kV; 40 mA), Ge 1 1 1 monochromator on the diffracted beam. The step scan mode was performed with a step width of 0.01 at a rate of 1 step/s. The samples were mildly pre-ground in an agate mortar to make them homogeneous, to control crystals size and to minimize the preferred orientation effects.

2.3. FTIR spectroscopy

The FTIR spectra were recorded with a JASCO 6100 FTIR spectrometer (number of scans, 256; resolution, 4 cm^{-1}; range, 4000–400 cm^{-1}). The KBr pellets were prepared by mixing 0.8 mg of sample and 150 mg KBr and pressing the mixture into a 13 mm disks at 3×10^6 N/m^2 pressure. The spectra were analysed using Spectra Analysis and ORIGIN software.

2.4. DSC – TG thermal analysis

Differential scanning calorimetry (DSC) was carried out by means of a Shimadzu DSC-60 calorimeter, the 1.5–2 mg amounts of sample were heated in the range of 30–350 °C with a heating rate of 10 °C/min in crimped aluminium sample cell. The purge gas was nitrogen purged of 60 ml/min. For data collection and analysis Shimadzu TA-WS60 and TA60 2.1 software were employed.

TG curves were obtained with a TGA/SDTA 851e thermobalance in the temperature range of 25–400 °C, using alumina crucibles with approximately 5 mg of sample, under dynamic N_2 atmosphere (50 ml/min) and at a heating rate of 10 °C/min.

2.5. Photopyroelectric method

The experimental set-up for front photopyroelectric (FPPE) calorimetry contains as radiation source a 30 mW HeNe laser, chopped by an acousto-optical modulator [25]. The detection cell contains 4 layers, the investigated samples being inserted in the backing position. The $LiTaO_3$ sensor ($e_1 = 3.92 \times 10^3$ $Ws^{1/2}$ m^{-2} K^{-1}; $\alpha_1 = 1.56 \times 10^{-6}$ m^2 s^{-1}) was 215 μm thick and the coupling fluid was ethylene glycol ($e_2 = 814$ $Ws^{1/2}$ m^{-2} K^{-1}; $\alpha_2 = 9.36 \times 10^{-8}$ m^2 s^{-1}). A 220 μm thick glass separator ($e_3 = 1330$ $Ws^{1/2}$ m^{-2} K^{-1}; $\alpha_3 = 8.5 \times 10^{-7}$ m^2 s^{-1}) was inserted between the sample and the coupling fluid to prevent sample's contamination. The thermal contact between the sample and the glass separator was performed with a very thin layer of silicon oil. This layer was considered very thin from thermally point of view and, in the mean time, it will not contaminate the sample's volume. The chopping frequency was 1 Hz. The PPE signal was processed with a SR 830 lock-in amplifier. The data acquisition and processing was performed with adequate software. The coupling fluid's thickness scan was performed in the 0–700 μm range with a step of 30 nm. Typical signal/noise ratio was better than 1000.

For PPE investigations, the samples, in powder form, were compressed as disks with 8 mm radius, and 5 mm thickness (from thermal point of view they are thermally thick) at a pressure of 3×10^6 N/m^2.

3. Results and discussion

3.1. X-ray powder diffraction

X-ray powder diffraction patterns for AMB, LA and AMB·LA are shown in Fig. 2. One can see that the powder diffraction pattern of AMB·LA solid form presents different features comparing with both AMB and LA ones.

From powder pattern indexing by using Dicvol method [26] it was established that AMB·LA crystallizes in triclinic system having the following lattice parameters: a = 9.36 Å, b = 7.73 Å, c = 7.35 Å and α = 76.41°, β = 94.38°, γ = 99.32°.