A Solid Form Of Ambazone With Lactic Acid

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Abstract. In recent years, much research has been carried out on the preparation of pharmaceutical solid forms due to their improved physical-chemical parameters such as solubility, dissolution rate of the drug, chemical stability, melting point and hygroscopic parameter. The aim of this study was to obtain and to investigate the structural properties of the ambazone (AMB) with lactic acid (LA) solid form. The solid form was obtained starting from the mixture of ambazone with lactic acid (1:1), by grinding method at constant temperature. The obtained compound was investigated *via* X-ray powder diffraction (PXRD), thermal analysis (DSC, TG-DTA) and infrared (FTIR) spectroscopy. The difference between the patterns of AMB•LA and of the starting compounds evidenced a new compound. Using X-ray powder diffraction method, by indexing procedure the unit cell and the lattice parameters were determined. Thermal and FTIR measurements on the pure compounds and on the (1:1) ground mixture of AMB with LA confirm the new salt form formation.

Keywords: ambazone lactate salt, powder X-ray diffraction, FTIR, thermal analysis
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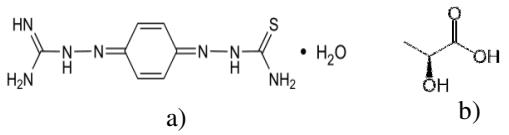
INTRODUCTION

Traditionally, the solid form selection process was limited to the free drug or pharmaceutically accepted salts [1], the form with the best properties for the intended usage being developed. Salts are different as compared to other solid forms: in salts, a proton is transferred from the acidic to the basic functionality, as the pKa difference between the partners is sufficiently large [1,2]. The solid form presents specific physical-chemical parameters: solubility, dissolution rate of the drug, chemical stability, melting point, and hygroscopic parameter. The bioavailability is strongly influenced by the solubility and the dissolution profile and can determine if the compound is further developed. In recent years, much of the research has been carried out on the preparation of pharmaceutical solid forms.

Ambazone monohydrate, $C_8H_{11}N_7S•H_2O$ ([4-(2-(Diaminomethylidene) hydrazinyl)phenyl] iminothio urea), (AMB, Fig. 1.a.) one of the oldest antimicrobial chemicals is a dark brown, odorless, tasteless microcrystalline powder with the melting point around 192 up to 194 °C with decomposition. Ambazone undergoes three protonation reactions with p*K* 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) [3]. With a very slight solubility in water and in the other organic solvents, it presents a bacteriostatic action [2]. The ulterior re-evaluation of AMB properties evidenced an antibacterial activity spectrum similar to that of sulfamides. Recently, the antineoplasm properties of AMB were also demonstrated [4–8] and accelerated the researches on this substance, without mutagenic effects and unpleasant reactions characteristic to other oncostatic drugs.

Lactic acid, $C_3H_6O_3$ (2-hydroxypropanoic acid), see Fig. 1.b, also known as milk acid, is a chemical compound that plays a role in various biochemical processes, is capable of releasing energy to resynthesize ATP without the involvement of oxygen, a process called anaerobic glycolysis. Lactic acid is from a group of weak hygroscopic acids, which contain an alcohol function in the alpha position relative to the carboxyl group [9]. The alpha hydroxy acids (AHAs), are the "star" ingredients in the new anti-aging, anti-wrinkle, and cell renewal products [10].



a) b)

FIGURE 1. Ambazone monohydrate (a) and lactic acid (b) molecules.

There are many methods by which solid forms may be prepared, mostly common being solution-based crystallization and grinding. Mechanical chemical method [11–13], more commonly and usefully described as grinding, has been employed extensively

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in the preparation of solid forms. The range of grinding conditions has been extended by the addition of solvents in the ‘‘solvent-drop’’ method (SDG) [13, 14] and this may represent the introduction of solution conditions on a limited scale to the grinding process. The obtained solid form was characterized by several physical methods such as X-ray powder diffraction, FTIR spectroscopy and thermal analysis. The chosen methods demonstrate the formation of the ambazone lactate AMB•LA compound.

MATERIALS AND METHODS

The Ambazone was obtained from *Microsin* SRL Bucharest, Romania, the lactic acid from *Sigma*, Germany, and both compounds were used without further purification.

The ambazone lactate (AMB•LA) was prepared by grinding a mixture of 255.3 mg AMB and 0.086 ml LA to fill up 1 ml with twice distilled water added in drops in an agate mortar at room temperature, until a dried compound was obtained.

X-ray Powder Diffraction

X-ray powder diffraction pattern was obtained using Bruker D8 Advance diffractometer, sealed Cu tube k = 1.5406 Å equipped with an incident beam Ge 111 monochromator.

FTIR Spectroscopy

FTIR spectra were obtained with a JASCO 6100 FTIR spectrometer in the 4000 to 400 cm^{-1} spectral domain with a resolution of 4 cm^{-1} using KB pellet technique.

Thermal Analysis

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

Differential thermal analysis (DTA) and thermogravimetry (TG) were obtained with a Simultaneous Thermogravimetric and Differential Thermal Analyzer of Shimadzu type DTG-60/60H. The measurements were performed by using alumina cells. The sample was heated in the range 30–350°C with a rate of 10°C/min in alumina sample cell under dry nitrogen purge (70 ml/min).

RESULTS AND DISCUSSIONS

X-ray Powder Diffraction

Figure 2 shows the XRD patterns for AMB, and AMB•LA obtained by SDG method.

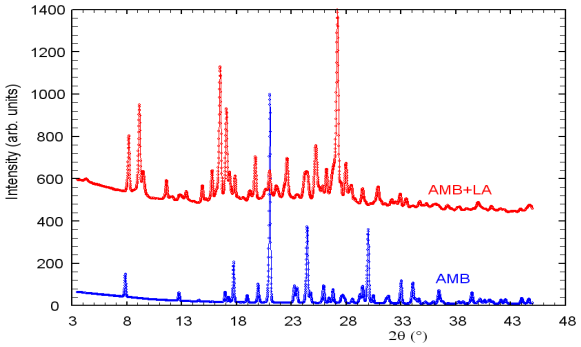

FIGURE 2. XRD patterns of AMB and AMB•LA obtained by SDG method.

Because LA is liquid it is not necessary to put its corresponding pattern, being of amorphous type. These two powder diffraction patterns are different. The obtained compound AMB•LA belongs to triclinic system having the following lattice parameters: a=10.781Å, b=9.352Å, c=7.348Å, α=93.65º, β=92.14º, γ=98.15 º, so we can conclude that a new solid form was obtained.

FTIR Spectroscopy

The band at ~3400 cm^{-1} can be assigned to N–H stretching from primary amine in pure ambazone (see Fig. 3); it can be observed also as a shoulder in the spectrum of AMB•LA.

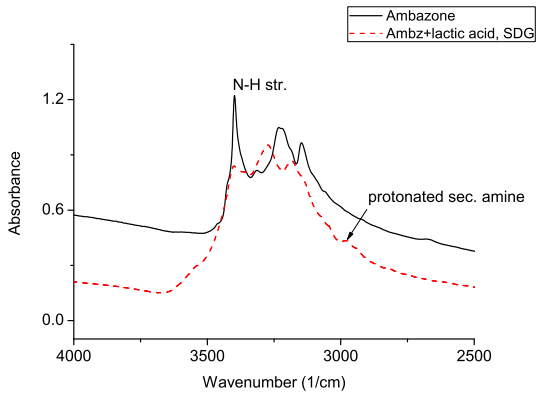

FIGURE 3. FTIR spectra of AMB and AMB•LA, 4000–2500 cm^{-1} spectral region.

In the case of pure AMB, the FTIR spectrum contains two NH_2 vibrations (3300 and 3500 cm^{-1}) [15, 16] and NH (3320–3180 cm^{-1}, i.e., 3226 cm^{-1}) [16, 17]. Salt formation has been shown to modify the NH stretching absorption in amines [16, 18]; it was observed that the free bases have a sharp strong band at ~3226 cm^{-1} due to the NH stretching and that this band is shifted at 3273 cm^{-1} and reduced in intensity in the spectra of the AMB•LA. The band at 3147 cm^{-1} corresponds to the NH vibration [16] for pure AMB, this band shifted at 3182 cm^{-1} in salt spectrum and can be assigned to N–H stretching of secondary amine. A new shoulder appeared at ~2975 cm^{-1} probably due to the protonated secondary amine.

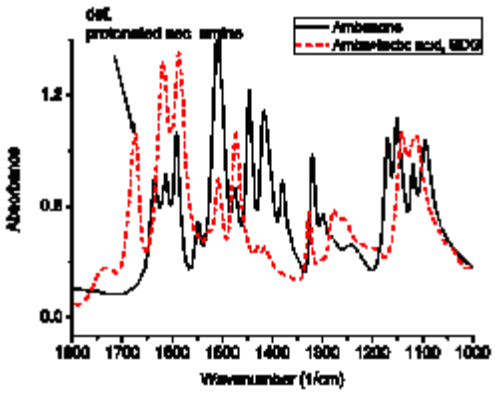

FIGURE 4. FTIR spectra of AMB and AMB•LA, 1800–900 cm^{-1} spectral region.

Primary amine has an absorption band of medium intensity at ~1613 cm^{-1} (see Fig. 4), being located at ~1618 cm^{-1} by salt formation [17]. Cleaves and Phyler [18] correlated the spectral bands at 1625–1516 cm^{-1} with NH deformation vibration. The pure ambazone spectrum contains the secondary amine vibration at 1508 cm^{-1} which is not shifted in AMB•LA spectrum, but greatly reduced in intensity. In the spectrum of the salt a new strong absorption appeared at ~1676 cm^{-1}, which is assigned to deformation vibration of the protonated secondary amino group [19] (Fig. 4). This frequency is not present in the FTIR spectrum of pure AMB, *i.e.,* a salt was formed between ambazone and lactic acid [20].

Thermal Analysis

DSC

The DSC curves of the pure AMB and of the compound obtained by SDG between AMB and LA are presented in Fig. 5. The curve for the pure AMB revealed a broad endothermic signal from 109 to 140°C, with a maximum at 125°C that corresponds to the water loss, followed by a sharp exothermic signal at 204°C, due to the melting with decomposition of AMB.

The thermal behavior of aqueous solution of lactic acid presents two endothermic events with maximums at 119 and 126°C, probably due to water elimination, respectively to the subliming of the compound.

The DSC curve of AMB•LA presents two signals: a small broad endothermic peak between 100 and 140°C, with a maximum at 128°C corresponding to the loss of water molecules and an exothermic peak between 170 and 200°C, with peak maximum at 182°C due to the melting with decomposition of the sample.

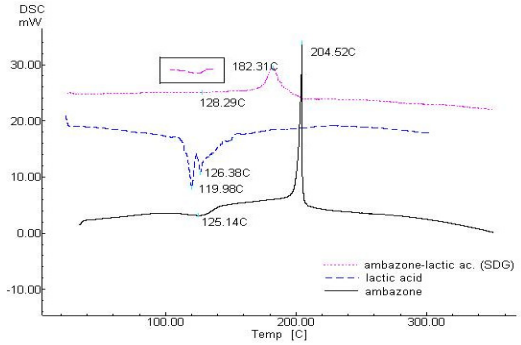

FIGURE 5. DSC of AMB and AMB•LA obtained by SDG.

The difference between the characteristic temperatures of the pure samples and of the AMB•LA shows the formation of a new compound.

DTA-TG

The simultaneously DTA–TG measurements of the AMB revealed the thermal behavior of this compound (Fig. 6). TG–DTA traces of AMB show thermal stability until 85°C. Between 86 and 149°C the first mass loss occurs (6.85%) corresponding to a broad endothermic peak between 100 and 140°C with maximum at 122°C due to water loss. Next mass loss, 26.05%, occurs in the range 185–220°C and corresponds to an exotherm on the DTA curve between 180 and 225°C with peak maximum around 209°C. This signal corresponds to the mass loss of the volatile components resulted from the AMB decomposition.

The obtained data present a very good similarity with DSC measurements.

The DTA–TG measurements of the AMB•LA reveal the thermal behavior of the compound obtained by SDG method (Fig. 7).

TG–DTA traces of AMB•LA indicate in the 105–160°C temperature range the first mass loss of

4.91%,corresponding to a small broad endothermic peak between 105 and 150°C with maximum at 131°C.

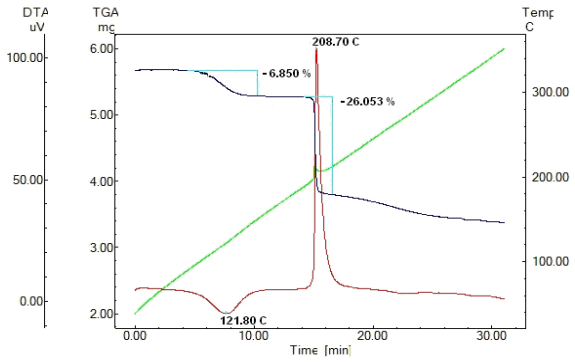

FIGURE 6. DTA–TG of AMB.

The second mass loss occurs between 170 and 210°C with a loss of 18.97%, probably due to lactic acid subliming and corresponds to a sharp exotherm with a maximum around 186°C.

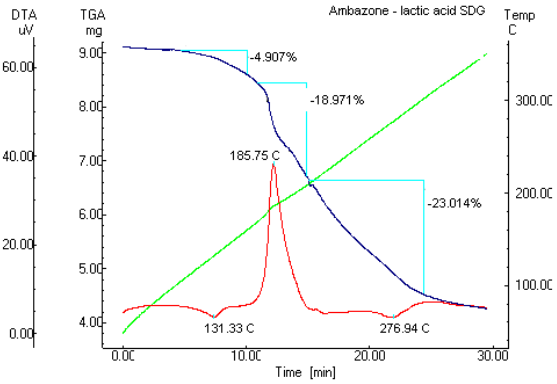

FIGURE 7. DTA–TG of AMB•LA obtained by SDG.

The final mass loss of 23.01% occurs in the range of 210–300°C and corresponding to the elimination of volatile components which results by decomposition of ambazone and evaporation of the lactic acid. This step of mass loss corresponds to a broad endotherm with peak maximum at 277°C.

CONCLUSIONS

The X-ray powder diffraction patterns and DTA–TG–DSC curves of the pure compounds differs from those obtained for AMB•LA by SDG. The final compound shows a good crystallinity.

Based on the FTIR spectrum of AMB•LA the occurrence of the characteristic frequencies of the NH_2^+ group indicates a salt formation between AMB and LA by proton transfer.

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