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PHYSICAL-CHEMICAL AND STRUCTURAL CHARACTERIZATION OF AMBAZONE AND OF ITS SYNTHESIS SECONDARY PRODUCT

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ABSTRACT. 1,4-benzoquinone guanyl-hydrazone thiosemycarbazone (Ambazone) is an antimicrobial compound, usually employed in Faringosept drug preparation. During the Ambazone synthesis some secondary products appear. One of these secondary products - (1,4-benzoquinon-guanyl-semycarbazone - BGHS) has a chemical structure similar to Ambazone one. In order to investigate the monohydrate and anhydrous forms of Ambazone and of BGHS several techniques were employed such as: powder X-ray diffraction (PXRD), Fourier transform infrared (FTIR) spectroscopy and differential scanning calorimetry (DSC) analysis. It was established that the ambazone monohydrate crystallizes in monoclinic system having $P_{21/c}$ space group. Details of the structures and spectroscopic properties for studied compounds are discussed. The stretching vibrations of primary and secondary amine have been identified by FTIR spectroscopy and showed changes in their characteristic absorption regions during water loss upon heating the ambazone monohydrate sample at 140°C. In the dehydration process, a change in molecular rearrangement was evidenced and the anhydrous ambazone was obtained. DSC data confirm the water molecules loss after 37.5 min. of heating treatment at 140°C and XRPD indicates a new crystalline phase.

Keywords: *ambazone, BGHS, PXRD, DSC, FTIR*

INTRODUCTION

The biologically active compound Ambazone (monohydrate form) (AMB) is an antimicrobial substance with very slightly solubility in water. The studies performed on AMB revealed the fact that this drug has local antibacterial properties if it is administrated at bucofaringeal cavity and it has an antiseptic

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effect against the viruses that produce infections of that type. There is used as active pharmaceutical principle to obtain the Faringosept medicinal product. The investigations regarding AMB in 1990 have described some antineoplastic properties and antitumor effect [1, 2]. Although discovered in 1950's by Domagk, it is less characterized from the point of view of its structural properties. The chemical formula of AMB is $C_8H_{11}N_7SxH_2O$ (M=255.37) (Figure 1a). In the AMB synthesis process some secondary compounds are obtained such as BGHS with molecular formula $C_8H_{12}N_7O$ (M=221) (Fig.1b) [3].

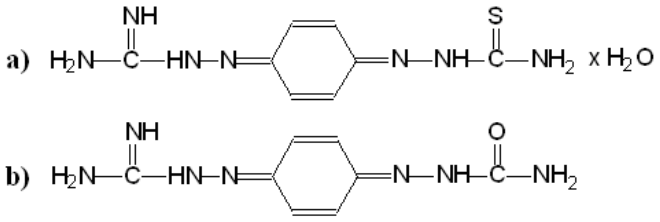

Figure 1. Molecular structures of AMB (a) and BGHS (b) compounds

This investigation shows a comparison between structural characteristics of AMB and of BGHS compounds due to the similarity of their structural formula (sulfur atom being replaced with oxygen one). Another important process that was investigated refers to the transition of AMB from hydrate phase to anhydrous one when heat treatment was applied.

The common techniques applied for characterized these compounds include: powder X-ray diffraction (PXRD), differential scanning calorimetry (DSC) and infrared spectroscopy (FTIR).

RESULTS AND DISCUSSION

Powder X-ray diffraction studies

X-ray powder diffraction patterns for AMB and BGHS are shown in Fig. 2. One can see that the X-ray reflections for BGHS are broader than those for AMB. The crystallinity degree for BGHS is weaker than the corresponding one for AMB. The crystallite size was evaluated using Scherrer relation [6], as being D = 1300Å for AMB and D = 195Å for BGHS.

Using MS Reflex Plus from Accelerys Material Studio suite (Accelerys Software Inc., 2010), the crystallinity degree was evaluated based on the ratio of crystalline peaks to amorphous halos (Neumann, 2003) [4]: 92% for AMB and 44% for BGHS.

The powder-diffraction pattern of AMB was indexed using the XCell (Neumann, 2003) [4] and Dicvol [5] computer programs implemented in the MS Reflex Plus of Accelerys Material Studio suite (Accelerys Software Inc., 2010).

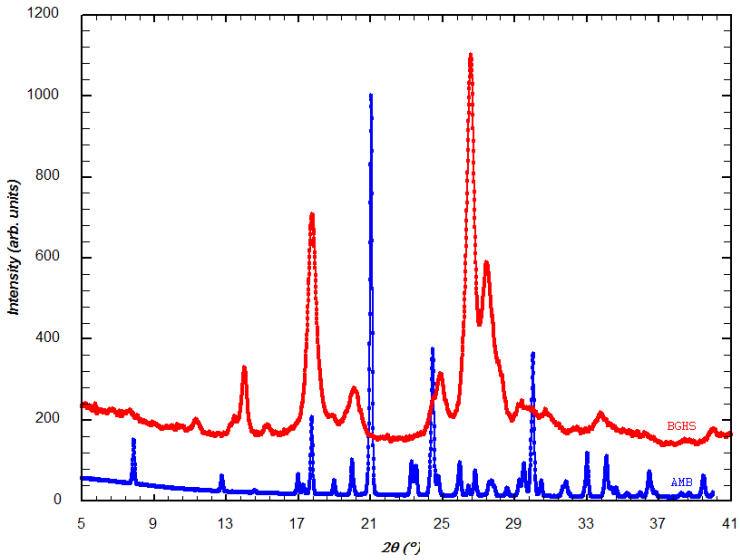

Figure 2. X-ray powder diffraction patterns of AMB and BGHS

From the indexing procedure it was found that AMB crystallizes in monoclinic system, having the following lattice parameters: a = 7.21 Å, b = 7.27 Å, c = 22.43 Å; β = 90.16^0 and unit cell volume is V = 1176Å^3. From forbidden reflection it was found that the space group is $P2_{1/c}$ with Z=4 (number of molecule in the unit cell). From molecular weight, the unit cell volume and Z we obtained the calculated density ρ=1.447g/cm^3, which is a reasonable value for such compounds.

AMB monohydrate was submitted to a thermal treatment at 140ºC for different time intervals. X-Ray powder diffraction patterns for several relevant thermal treatment time intervals are presented in Fig. 3.

After 5 min of thermal treatment the diffraction patterns changes are observed, *i.e.* the diffraction intensities of the starting phase are diminished and diffraction lines, characteristic to a new phase appear. X-ray diffractograms show that the initial phase is diminished steeply until its complete disappearance after a thermal treatment at 140ºC for 37.5 minutes. The crystallinity degree of the new obtained phase is lower that the corresponding one for the starting phase. The crystallite size of the new obtained phase is 590 Å, as compared to that of the initial phase, which is 1300 Å.

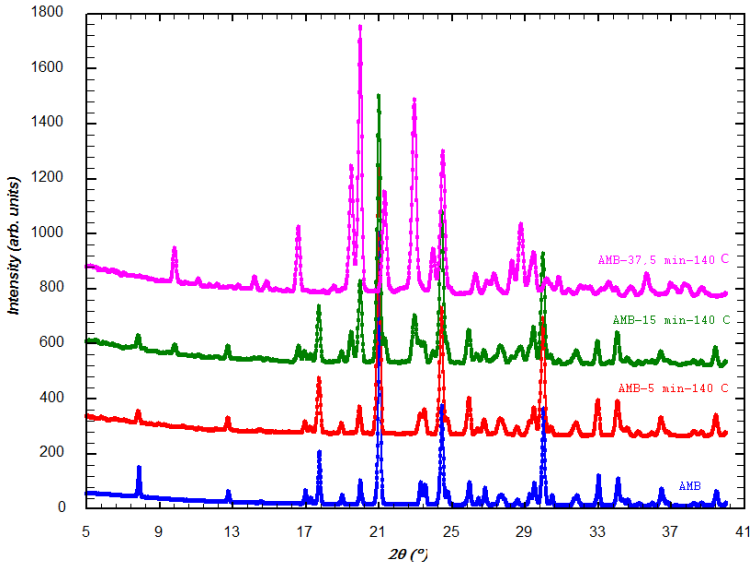

Figure 3. X-ray powder diffraction patterns of AMB compared before and after thermal treatment

FTIR spectroscopy analysis

FTIR spectra of AMB and BGHS are presented in Figs.4 a, b. In the 3400 – 3200 cm^{-1} spectral range (Figure 4a), strong IR absorption bands at ~3398 and 3232 cm^{-1} were observed for AMB, respectively at ~3463 and 3340 cm^{-1} for BGHS, being assigned to the stretching vibration of primary amino group. The stretching vibrations of secondary amine have been identified at ~3147 cm^{-1} for AMB, respectively ~3151 cm^{-1} for BGHS [7, 8].

In the 1800-1650 cm^{-1} carbonyl region of BGHS spectrum two peaks at ~1750 and 1699 cm^{-1} have been assigned to C=O vibration group (Figure 4.b) [8]. In the 1700–1600 cm^{-1} fingerprint region the medium intensity bands at ~1636 and 1613 cm^{-1} for AMB, are attributable to C=N stretching vibration and primary amine bending vibration, respectively.

In the 1600–1500 cm^{-1} spectral range for AMB the primary amino bending at ~1592 cm^{-1} for AMB and at ~1607 cm^{-1} for BGHS is identified. The secondary amine deformation vibration is located at ~1509 cm^{-1} in the spectrum of AMB and at ~1525 cm^{-1} for BGHS, respectively.

The medium intensity bands at ~1548 and 1562 cm^{-1} for AMB and BGHS is assigned to N-C=S and to C=O vibrations, respectively [8].

In the 1230–1030 cm^{-1} spectral range of AMB spectrum, the vibrations at 1170 cm^{-1} (medium intensity) can be associated with C–N bending vibration. The C=S stretching vibration at 1151 cm^{-1} in the spectrum of AMB [10] and in

the spectrum of BGHS the C=O vibration is identified at 1157 cm^{-1}. The differences in peak positions indicate different environments of the carbonyl and amino-groups in these compounds and probably arise from differences in conformation and crystal packing.

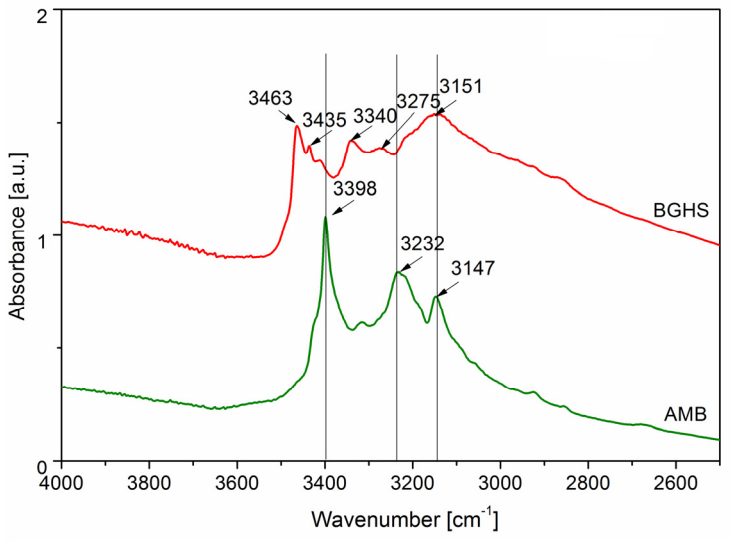

Figure 4. FTIR spectra of AMB monohydrate and of BGHS in the 4000-2500 cm^{-1} (a); 1850-1100 cm^{-1} (b) spectral ranges

Upon heating the AMB at 140°C, (Figs. 5a, b), the shift of some absorption bands in the FTIR spectrum were observed, showing a change in force constant, due to a change in molecular rearrangement promptly after hydrogen bond cleavage and were ascribed to crystal collapse due to dehydration. The differences in peak positions indicate different environments of the carbonyl and amino-groups in these compounds and probably arise from differences in conformation and crystal packing.

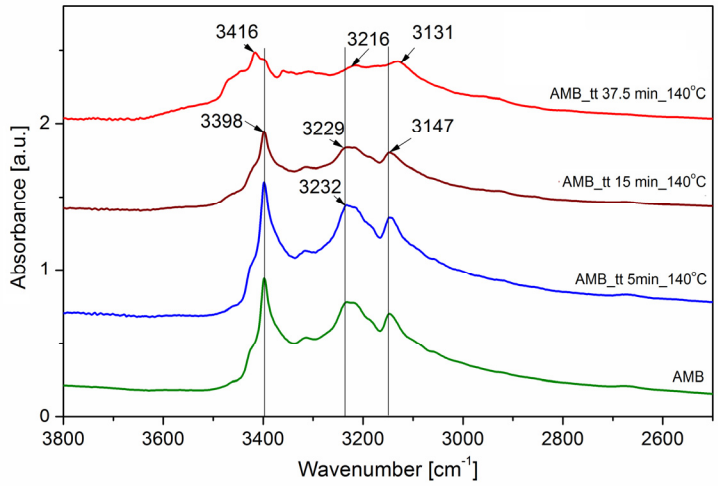

Figure 5. FTIR spectra of AMB before and after thermal treatment in the 3800-2500 cm^{-1} (a); 1700-1400 cm^{-1} (b) spectral ranges

The band at 3398 cm^{-1} corresponding to primary amine is shifted to higher wavenumbers upon thermal treatments (heating up to 140°C for 37.5 min) whereas the band at 3232 cm^{-1} is shifted to 3216 cm^{-1} (Fig. 5a).

The secondary amine band located at 3147 is shifted to 3131 cm^{-1} for AMB_37.5min_140°C [9-12] that corresponds to broadening by intermolecular association of the C-H group [11].

The pure AMB spectrum contains the secondary amine bending vibration at 1509 cm^{-1}, which is shifted to 1519 cm^{-1} in the case of anhydrous form.

In the carbonyl region (Fig. 5b.) there are differences in peak positions at ~1636 and at 1644 cm^{-1} (at 140°C for 37.5 min) showing different environments of the carbonyl groups in AMB, the sample being maintained under thermal treatment for 5, 15 or 37.5 minutes (anhydrate forms) that probably arise from differences in conformation and crystal packing [11, 12]. At the same wavenumber position H-O-H bending vibration of water molecules contributes, also. Its diminishing can support the dehydration of AMB. The C=C stretch at 1613 cm^{-1} shifts to lower wavelength, emphasizing a slight change in orientation of this bond as the water of crystallization bending observed at 1635 cm^{-1} during dehydration is indicative of an alteration in the environment of one of the carbonyl groups. The relatively large intensity increase reflects the loss of hydrogen donation by the water molecule and thus justifies the alterations within the appropriate spectral regions.

Thermal analysis

The DSC thermograms of the AMB, the thermal treated AMB samples and BGHS are presented in Figs. 6, 7. The curve for the pure AMB revealed a broad endothermic signal from 105 to 143°C, with a maximum at 124°C that corresponds to the water molecules loss of the AMB monohydrate structure, followed by a sharp exothermic signal with maximum at 204.5°C, due to the melting with decomposition of AMB.

The BGHS calorimetric curve presents a sharp exothermic signal with maximum at 209°C, attributed to the melting with decomposition of the sample. On this curve any thermal event around 100°C is not observed, so no bounded or unbounded water molecules in the BGHS sample are present.

Comparing the thermally treated samples with starting AMB a decreasing of the dehydration peak intensity was observed with increasing time of the applied heat treatment until the disappearance of this peak for sample heated for 37.5 min (see Fig.7 and Table 1).

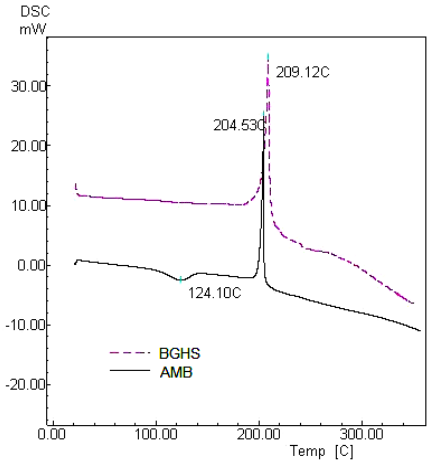

Figure 6. DSC curves of the AMB and BGHS

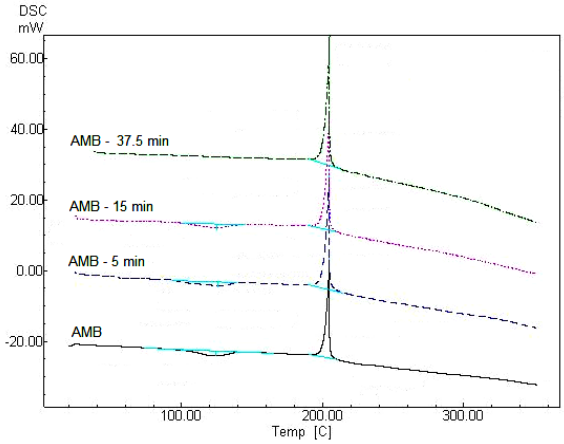

Figure 7. DSC-curves of the AMB monohydrate before and after thermal treatment at 140°C

Table 1. The peaks maxima and heat values for ambazone monohydrate and thermal treated samples at 140°C for different time intervals.

Sample	T peak (°C)	Heat (J/g)	T peak (°C)	Heat (J/g)
AMB	124.1	-215.19	204.5	491.55
Amb (5 min)	126	-182.99	204.5	637.47
Amb (15 min)	124.8	-161.87	204.4	608.00
Amb (37.5 min)	-	-	204.8	732.25

The disappearance of the dehydration peak reveals that water molecule from AMB structure was removed after 37.5 min of heat treatment at 140°C, thus the anhydrous form of the ambazone was obtained.

CONCLUSIONS

From AMB powder diffraction patterns indexing it was established that Ambazone monohydrate crystallizes in the monoclinic system, $P_{21/c}$ space group and the unit cell parameters were determined. The crystallinity and the crystallite size for BGHS are lower than those for AMB. As a result of AMB thermal treatment, its crystalline structure is changed and a new crystalline phase - anhydrous AMB was obtained, its crystallite size (590 Å) being lower than that of the starting compound (1300 Å).

The FTIR analysis showed changes in the characteristic absorption bands of the primary and secondary amines for ambazone monohydrate, BGHS and anhydrous ambazone, respectively.

The BGHS calorimetric curve showed that this compound is an anhydrous form and presents a different melting signal as compared to AMB one. After 37.5 min of the AMB heating treatment at 140°C the water molecule is expelled and the anhydrous form of the ambazone was obtained.

EXPERIMENTAL SECTION

AMB and BGHS investigated in this study were supplied by the Microsin S.A. Bucharest, respectively Research Center for Synthetic Drugs Cluj-Napoca and were used as received.

The thermal treatment of AMB was done maintaining the starting AMB samples at constant temperature (140ºC) for different time intervals, starting with 5 minute up to 37.5 minutes.

Powder X-ray diffraction (PXRD) patterns were obtained at room temperature using SHIMADZU XRD-6000 X-ray diffractometer with CuK_α radiation and Ni – filter, (λ = 1.5418Å) and (30 mA 40 kV). As standard calibration, a quartz powder had been used. The samples were scanned from 3° to 53°, 2θ at a scan speed of 1°/min.

Fourier transformed infrared spectroscopy (FTIR) measurements were recorded with a JASCO 6100 FTIR spectrometer (number of scans 255; 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 12 tones pressure. The spectra were analyzed using Spectra Analysis software.

Thermal analysis (DSC) Calorimetric measurements were performed with DSC-60 Shimadzu differential scanning calorimeter. The 1-2 mg of the accurately weighed samples was heated in crimped aluminium pan from

room temperature up to 350°C under nitrogen flow, the heating rate being 10°C/min. For acquisition and analysis Shimadzu TA-WS-60 and TA60 2.1 system software were used.

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