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# Ambazone-lipoic acid salt: Structural and thermal characterization

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### 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  $\alpha$ -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.

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## 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].

 $\alpha$ -Lipoic acid, C<sub>8</sub>H<sub>14</sub>O<sub>2</sub>S<sub>2</sub>, LA ((*R*)-5-(1,2-dithiolan-3yl)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

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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  $\alpha$ -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  $2\theta$  range between 5° and 45°, Cu K $\alpha$ 1 radiation ( $\lambda$  = 1.5406 Å)(40 kV; 40 mA), Ge 111 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,  $4 \, \text{cm}^{-1}$ ; range, 4000–400 cm<sup>-1</sup>). 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  $3 \times 10^6 \, \text{N/m}^2$  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<sub>2</sub> 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 LiTaO3 sensor  $(e_1 = 3.92 \times 10^3 \,\mathrm{Ws^{1/2}}\,\mathrm{m^{-2}}\,\mathrm{K^{-1}};$   $\alpha_1 = 1.56 \times 10^{-6}\,\mathrm{m^2}\,\mathrm{s^{-1}})$ was 215 µm thick and the coupling fluid was ethylene glycol  $(e_2 = 814 \text{ Ws}^{1/2} \text{ m}^{-2} \text{ K}^{-1}; \alpha_2 = 9.36 \times 10^{-8} \text{ m}^2 \text{ s}^{-1})$ . A 220  $\mu$ m thick glass separator ( $e_3 = 1330 \text{ Ws}^{1/2} \text{ m}^{-2} \text{ K}^{-1}$ ;  $\alpha_3 = 8.5 \times 10^{-7} \text{ m}^2 \text{ s}^{-1}$ ) 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  $0-700 \,\mu\text{m}$  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  $3 \times 10^6$  N/m<sup>2</sup>.

## 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  $\alpha = 76.41^{\circ}$ ,  $\beta = 94.38^{\circ}$ ,  $\gamma = 99.32^{\circ}$ .



Fig. 2. X-ray powder diffraction pattern for AMB, LA and AMB-LA obtained by SDG.

#### 3.2. FTIR spectroscopy

In the case of pure AMB, the FTIR spectrum contains two vibrations, the band at  $\sim$ 3400 cm<sup>-1</sup> and 3428 cm<sup>-1</sup> (shoulder) that can be assigned to N–H stretching from primary amine (see Fig. 3) [27,28] and NH stretching band of the secondary amine (3226 cm<sup>-1</sup>) [28,29].

Salt formation has been shown to modify the NH stretching  $(3233 \, \mathrm{cm}^{-1})$  absorption in amines [27]. This band is shifted to  $3218 \, \mathrm{cm}^{-1}$  and reduced in intensity in the spectra of the AMB·LA. The band at  $3145 \, \mathrm{cm}^{-1}$  corresponds to the NH vibration [28] for pure AMB, this band being shifted to  $3128 \, \mathrm{cm}^{-1}$  in the salt spectrum (assigned to N–H stretching of secondary amine). The bands located at 2930 and 2863 cm<sup>-1</sup> are attributed to aliphatic CH<sub>2</sub> stretching vibrations and these bands remain in the same positions in the spectrum of AMB·LA. A new shoulder appeared at ~2767 cm<sup>-1</sup> and is probably due to the protonated secondary amine.

Primary amine has an absorption band of medium intensity at  $\sim 1613 \, \mathrm{cm}^{-1}$  (see Fig. 4), being shifted to  $\sim 1615 \, \mathrm{cm}^{-1}$  by salt formation [29]. Cleaves and Plyler [30] correlated the spectral bands at  $1625 - 1516 \, \mathrm{cm}^{-1}$  with NH deformation vibration.

In the spectrum of the pure LA a strong absorption band appears at 1691 cm<sup>-1</sup> corresponding to the COOH stretching vibration [31]. This band is shifted to 1686 cm<sup>-1</sup> in the salt spectrum, due to the



Fig. 3. FTIR spectra of AMB, LA acid and their salt obtained by SDG method,  $4000\text{-}2500\,\text{cm}^{-1}$  spectral range.



Fig. 4. FTIR spectra of AMB, LA and their salt obtained by SDG method,  $1800\text{--}1000\,\mathrm{cm}^{-1}$  spectral range.

appearance of the carboxylate group by deprotonation [32] and can be assigned also to the deformation vibration of the protonated secondary amino group [30]. The pure ambazone spectrum contains the secondary amine vibration at 1508 cm<sup>-1</sup> which is slightly shifted in AMB·LA spectrum to 1511 cm<sup>-1</sup> and is greatly reduced in intensity. Based on these reasons we assumed that ambazone and lipoic acid formed a salt.

## 3.3. Thermal analysis

### 3.3.1. DSC

The DSC thermograms of the pure AMB, LA and of the AMB·LA compound obtained by SDG are presented in Fig. 5. The curve for the pure AMB revealed a broad endothermic signal from 105 to 143 °C, with a maximum at 125 °C and  $\Delta H$ = 36 kJ/mol, that corresponds to the water molecules loss of the AMB monohydrate structure, followed by a sharp exothermic signal with maximum at 204.5 °C,  $\Delta H$ = 75 kJ/mol due to the melting with decomposition of AMB.

The DSC trace of the pure LA revealed a sharp endothermic signal with maximum at 66.6 °C and an associated enthalpy of  $\Delta H$  = 21.8 kJ/mol, that corresponds to the melting of the LA [33].

The DSC curve of AMB-LA presents two broad exothermic signals between 70 and 100  $^\circ\text{C}$ , with a maximum at 89.5  $^\circ\text{C}$  and



Fig. 5. DSC curves of pure AMB, LA and AMB-LA obtained by SDG.





 $\Delta H$  = 29.5 kJ/mol, respectively 115–129 °C with maximum at 122 °C and  $\Delta H$  = 7.7 kJ/mol corresponding to the unbounded and bounded water loss. Between 163 and 186 °C a broad exotherm appears with

peak maximum at 173 °C and  $\Delta H$  = 39 kJ/mol when the melt with decomposition of the sample starts.

#### 3.3.2. TG

The thermal behaviour characteristics of AMB, LA and AMB-LA obtained by SDG procedure, have been investigated by TG and are shown in Fig. 6a–c.

The TG curve for the pure AMB shows the 7.24% mass loss between 97 and 150 °C corresponding to the evaporation of water. In the 184–208 °C temperature range significant mass loss appeared representing 25.41% from mass of AMB due to the starting of decomposition. The next mass losses, 6.91% and 3.17% appear in the temperature ranges 216–288 °C and 294–386 °C, respectively and correspond to the elimination of the volatile components resulted from decomposition. The temperature ranges where mass losses occur are in agreement with DSC results.

The trace of thermogravimetric behaviour of the LA shows a single stage 97.06% mass loss in the 150-300 °C temperature range.

The thermogravimetric curve of the AMB-LA shows the first mass loss, 3.00%, in the range 59–100 °C, followed 8.18% mass loss in the range 135–203 °C. The last mass loss is the most significant, 43.02% and occurs in the 205–400 °C temperature range corresponding to the elimination of volatile components resulting from the decomposition of the sample.

The TG data are in good agreement with the DSC results and support the new obtained compound formation.

## 3.4. Photopyroelectric calorimetry

#### 3.4.1. Theoretical aspects

The theory of the FPPE-TWRC configuration was largely described before [25,34,35]. We present here only the final results.

The normalized FPPE signal obtained with a 4-layers cell: (1) directly irradiated pyroelectric sensor, (2) liquid layer – coupling fluid, (3) thin solid separator, (4) semi-infinite solid backing, is given by [25,34]:

$$V_n = \frac{1 - R_{21} e^{-2\sigma_1 L_1}}{1 - \rho_{21} e^{-2\sigma_1 L_1}} \times \frac{(e^{-\sigma_1 L_1} - 1) - \rho_{21}(e^{-\sigma_1 L_1} - e^{-2\sigma_1 L_1})}{(e^{-\sigma_1 L_1} - 1) - R_{21}(e^{-\sigma_1 L_1} - e^{-2\sigma_1 L_1})}$$
(1)

with 
$$R_{21} = \frac{1 - b_{21}}{1 + b_{21}}; \quad \rho_{21} = \frac{(1 - b_{21}) + \rho_{32}(1 + b_{21})e^{-2\sigma_2 L_2}}{(1 + b_{21}) + \rho_{32}(1 - b_{21})e^{-2\sigma_2 L_2}}$$

$$\rho_{32} = \frac{(1 - b_{32}) + \rho_{43}(1 + b_{32})e^{-2\sigma_3 L_3}}{(1 + b_{32}) + \rho_{43}(1 - b_{32})e^{-2\sigma_3 L_3}}; \quad \rho_{43} = \frac{1 - b_{43}}{1 + b_{43}} \tag{2}$$

In Eqs. (1) and (2)  $\sigma_j = (1+i)a_j$ ,  $\mu = (2\alpha/\omega)^{1/2}$ ,  $b_{ij} = e_i/e_j$ ,  $\alpha$  and e are the thermal diffusivity and effusivity,  $\omega$  is the angular chopping frequency of radiation,  $\sigma$  and a are the complex thermal diffusion coefficient and the reciprocal of the thermal diffusion length  $(a = 1/\mu)$ , respectively. "Normalized signal" in Eq. (1) refers to the signal obtained with a 4 layers cell normalized with respect to the signal obtained with semi-infinite coupling fluid.

Eq. (1) indicates that the normalized PPE signal depends on the thermal diffusivity and effusivity of the pyroelectric sensor and coupling fluid and on the thermal effusivity of the backing material. We will obtain the value of the thermal effusivity of the backing by performing a coupling fluid's thickness scan of the phase of the PPE signal at constant chopping frequency (TWRC method) [34,35].

#### 4. Results

Typical experimental results for pure components and AMB·LA compound in 1:1 molar ratio are displayed in Fig. 7.

The PPE investigations showed that the value of the thermal effusivity of the compounds are different from the values of the starting materials and are concentration dependent, see Fig. 8. In the mean time the shape of the curve indicates deviations from a typical simple mixture law.



**Fig. 7.** Normalized PPE phase as a function of coupling fluid's thickness for pure LA ( $\blacksquare$ ), the compound AMB·LA ( $\bullet$ ) and AMB ( $\blacktriangle$ ). The best fit performed with Eq. (1) is also displayed for each curve.



Fig. 8. The values of the thermal effusivity of the compound as a function of the molar content.

Having in mind that the solid samples were in fact pressed powders, this result can be ascribed to two reasons: (i) a different confinement of the powders or (ii) the formation of the compound. Due to the fact that the pressure used for samples' preparation was the same, the second alternative is more probable. In conclusion, the PPE calorimetry also supported the results obtained by X-ray analysis and FTIR spectroscopy concerning the formation of the compound.

## 5. Conclusions

The XRPD pattern of the AMB with LA is clearly different as compared to those of the starting materials, confirming the presence of a new crystalline phase, crystallized in triclinic system, the lattice parameters being reported.

FTIR spectrum of this compound suggested the deprotonation of the carboxylic group and the protonation of the secondary amine that means a solid form of AMB with LA, *i.e.* the ambazone lipoate salt was obtained.

DSC and TG data, demonstrated different thermal behaviour of AMB-LA compound as compared to the starting ones.

The PPE calorimetry obtained for the room temperature thermal effusivity, values that are composition dependent, and different from the values of the thermal effusivity of the starting materials. In the mean time, the dependence of the thermal effusivity as a function of composition shows deviations from the additive rule (valid for simple mixtures). Consequently, it also supports the idea of the formation of a new compound.

Our results are in agreement with the previous ones referred to the compounds of AMB with several different acids ( $\Delta pK_a > 2$ ) and obtained by using SDG method [36,37].

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