Synthesis and thermal behavior of new ambazone complexes with some transitional cations

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Abstract Ambazone is a pharmaceutical compound that possesses antiseptic activity and tested as well for anti-tumor properties. Metal complexes of Zn(II), Fe(III), and Cu(II) containing ambazone as ligand were synthesized using a molar ratio salt:ligand of 1:1, heating the mixture up to 50 °C for 6 h. Coordination compounds were characterized by thin-layer chromatography, FT-IR spectroscopy, elemental analysis, and thermal behavior. The non-isothermal experiments were carried out in order to investigate the thermal degradation process of these complexes and were performed in a dynamic air atmosphere at a heating rate $\beta = 10$ °C min⁻¹ from ambient temperature, up to 500 °C. It was revealed that decomposition process is a multistadial one.

Keywords Ambazone · Metal complex · Coordination compound · Thermal analysis · Transitional cations

Introduction

Ambazone ([4-(2-(Diaminomethylidene)hydrazino)phenyl] iminothiourea) (AMB, Fig. 1) is a pharmaceutical compound, well-known for its bacteriostatic properties, being used against Gram-positive germs that populate the oral cavity, such as streptococcus and pneumococcus [1]. Beside its

G. Vlase · T. Vlase Research Centre for Thermal Analysis in Environmental Problems, West University of Timisoara, Pestalozzi Street 16, 300115 Timisoara, Romania antibacterial activity, ambazone was identified as an antineoplasic agent [2–4], on in vivo tumor models in mice and rats, its antineoplasic effect apparently being mediated by the immune system [5]. Other studies revealed that ambazone has a partial absorption after oral administration [6] which leads to poor bioavailability but, on the other hand, it lacks mutagenic activity [5] and a large number of the usual adverse reactions of anticancer drugs [7]. As a consequence, ambazone became the topic of several studies that attempted an exhaustive characterization of its solid form [8] as well as to increase its bioavailability through improving its water solubility; thus, a number of salts were prepared using different acids: lactic [9], niflumic [10], hydrochloric [11], lipoic [12], glutamic [13], all salts showing a better solubility profile and, presumably, a better bioavailability.

Metal ions play an important role in biological systems [14], particularly transitional metals such as copper or zinc, which are associated with the active sites of enzymes or proteins [15], their concentration disturbancy being involved in pathological processes such as cancer. Metal complexes have been involved in the anticancer therapy beginning with the discovery of the platinum complex, cisplatin, and its analogs followed by researches on complexes of copper [16], gold [17], zinc [18], and ruthenium [19].

Thermoanalytical methods (TG/DTG/HF) are very important to investigate the possible interactions between active substances and excipients [20–23], to characterize from physico to chemical view (thermal stability and decomposition, polymorphic transitions and solid-state transformations), the new synthesized compounds which is supposed to have biological activity [24–28]. For these purposes, the evolved gas analysis using hyphenated TG-FTIR technique, which can present a continuous real time analysis, is used for provide more information about the sequences of the reactions and the obtained products [29].

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Fig. 1 Structural formula of ambazone (AMB)

Given the antineoplasic activity of ambazone manifested in leukemia [30] and melanoma [31] and the variety of metal complexes already involved in cancer treatments, this paper aims to prepare and characterize coordinative compounds containing Zn^{2+} , Fe^{3+} , and Cu^{2+} as complex generators and ambazone as ligand.

According to our knowledge, synthesis and thermal behavior of these metal complexes was not reported before, even if the synthesis and thermal characterization of lipoic acid salt of ambazone is already published [12].

The results which characterized these complexes come from elemental to thermal analysis and FT-IR and evolved gases investigations of ambazone complexes with three transitional cations, respectively.

Experimental

All chemicals were of analytical grade. The active substance, ambazone, was obtained as a gift from pharmaceutical company Terapia Ranbaxy S.A. (Cluj-Napoca, Romania). The metal chlorides were obtained from Sigma Aldrich and were used without further purification. Extra pure samples of metal oxides used for comparative FTIR spectroscopy were used as follows: copper(II) oxide \geq 99 % (Acros Organics), zinc oxide \geq 99 % (Carl Roth), and iron(III) oxide 99.98 % (Carl Roth).

The percentage of C, H, N, Cl, and S were obtained by means of elemental analysis using a Vario El Cube apparatus. The metal content was determined by a standard analytical procedure, namely complexometric titration: Zn(II) with EDTA, in buffer solution (NH_3/NH_4Cl) at pH ~ 10, in the presence of Eriochrome Black T as indicator; Fe(III) with EDTA, at pH ~ 2, in the presence of sulfosalicylic acid as indicator; Cu(II) was determined with EDTA at pH 8 in buffer solution (NH_3/NH_4Cl) using Murexide as indicator.

Thin-layer chromatography was carried out on silica gel-coated plates $60F_{254}$ Merck using ethyl acetate:methanol 3:7 as eluant.

The IR spectra were carried out using a Perkin Elmer SPECTRUM 100 device in the range of $4,000-600 \text{ cm}^{-1}$

on an UATR device, with 16 acquisitions for each spectrum.

Thermal analysis was completed using a simultaneous TG/DTA instrument from Perkin to Elmer DIAMOND. The experiments were carried out using aluminum crucibles with approximately 7–8 mg of the sample. For determination of the heat effects, the DTA curves (in μ V mg⁻¹) were changed with the Heat Flow curves (in mW mg⁻¹), so that the peak area corresponds to an energy in J g⁻¹ or kJ mol⁻¹. The experiments were completed in an air atmosphere at a flow rate of 100 mL min⁻¹. These were performed under non-isothermal conditions by increasing temperature from ambient up to 500 °C, at a heating rate $\beta = 7$ °C min⁻¹.

In order to evaluate the accuracy of the measurements, three repetitions have been done with this experimental protocol for the samples, and the obtained results were comparable.

Results and discussion

Synthesis of the metal complexes

The complexes have been obtained through the reaction of aqueous solution of ambazone (0.008 mol/100 mL distilled water) with transitional metal chloride (0.008 mol/30 mL distilled water) in a molar ratio 1:1. The metal salt solution was added dropwise to the aqueous suspension of ambazone at room temperature. After stirring, the mixture was heated up to 50 °C for 6 h. After this, the mixture was allowed to cool down at room temperature, when complexes precipitated as crystalline solids, which were filtered off under vacuum, washed with distilled water $(3 \times 5 \text{ mL})$, and dried for 48 h at 30 °C. Thin-layer chromatography of the synthesized compounds revealed a single spot, confirming their purity.

The complexes of the formulae: $[Cu(AMB)(OH_2)_4]Cl_2$ · H₂O (yield 86 %), $[Zn(AMB)(OH_2)_2]Cl_2 \cdot 0.5H_2O$ (yield 88 %), and $[Fe(AMB)(OH_2)_4]Cl_3 \cdot 2.5H_2O$ (yield 94 %) (where AMB = ambazone C₈H₁₁N₇S) were obtained. The ambazone ligand coordinates as bidentate to the metal ions, in the stoichiometric ratio 1:1. The analytical data of the investigated complexes are shown in Table 1. It can be noticed that a good agreement between calculated and found composition of the metal complexes was obtained, suggesting a high purity of the compounds. The differences obtained regarding the content of metal in complexes can be attributed to the fact that a standard titration protocol presents higher errors than the ones obtained via elemental analysis.

Table 1 Elemental analysis data													
Complex	Molar mass/ g mol ⁻¹	C/%		H/%		N/%		C1/%		S/%		M/%	
		calc.	found										
$[Zn(AMB)(OH_2)_2]Cl_2 \cdot 0.5H_2O$	418.61	22.95	22.11	3.85	3.67	23.42	23.39	16.94	16.88	7.66	7.72	15.62	14.68
$[Cu(AMB)(OH_2)_4]Cl_2 \cdot H_2O$	461.81	20.81	20.14	4.58	4.40	21.23	20.96	15.35	15.11	6.94	7.02	13.76	13.01
$[Fe(AMB)(OH_2)_4]Cl_3 \cdot 2.5H_2O$	516.59	18.60	18.49	4.68	4.54	18.98	19.01	20.59	20.48	6.21	6.35	10.81	11.92



Fig. 2 Thermoanalytical curves TG/DTG/HF obtained in air at $\beta = 10$ °C min⁻¹ for the analyzed complexes and ligand

Ambazone thermal decomposition

The thermoanalytical curves of ambazone obtained during heating in air are shown in Fig. 2a. The first mass loss which can be identified on the thermogravimetric curve and which occurred in the 100–186 °C range corresponds to the loss of one mole of water. The dehydration process is accompanied by a broad endothermic peak (HF_{peak} = 139.6 °C) according to the loss of the hydrate water molecule. Between 188 and 222 °C, the thermal degradation with continuous mass loss is observed. At 204.5 °C, a very sharp exothermic peak is noticed on the HF curve. At the same temperature, on the thermogravimetric curve, a fast loss of mass is recorded that

can be attributed to a superimposition of the degradation and oxidation processes.

Thermal decomposition in air atmosphere of the metal complexes

The TG/DTG/HF curves for the three investigated complexes and for the ligand (ambazone) are shown in Fig. 2. All the analyzed complexes are stable up to about 50 °C. The first process which occurs further heating in air atmosphere is the dehydration process which occurs in 50-110 °C temperature range. This process is confirmed by the mass loss on the thermogravimetric curve which is

Complex	First dehyd	lration step/%	Second dehy	dration step/%	Total dehydration mass loss/%		
	Exp.	Calc.	Exp.	Calc.	Exp.	Calc.	
$[Zn(AMB)(OH_2)_2]Cl_2 \cdot 0.5H_2O$	2.50	2.15	8.20	8.80	10.70	10.95	
[Cu(AMB)(OH ₂) ₄]Cl ₂ ·H ₂ O	4.20	3.90	14.8	15.59	19.00	19.49	
[Fe(AMB)(OH ₂) ₄]Cl ₃ ·2.5H ₂ O	8.20	8.71	16.20	15.27	24.40	23.98	

Table 2 The mass loss corresponding to the dehydration processes (experimental and calculated values)

 Table 3
 Thermoanalytical data of the analyzed complexes

Compound	Process	$T_{\rm i}/^{\rm o}{\rm C}$	$T_{\rm f}$ /°C	$T_{\rm max\ DTG}/^{\circ}{\rm C}$	T _{max HF} /°C
[Zn(AMB)(OH2)2]Cl2·0.5H2O	Ι	42	143	_	53
	II	143	193	159	167
	III	216	453	251	251
$[Fe(AMB)(OH_2)_4]Cl_3{\cdot}2.5H_2O$	Ι	45	148	97	102
	II	148	210	198	197
	III	210	460	350; 449	352; 451
$[Cu(AMB)(OH_2)_4]Cl_2 \cdot H_2O$	Ι	50	109	-	77
	II	109	181	132	128
	III	181	442	206; 384	211;388

accompanied by a weak endothermic peak on the HF curve. This fact may point a relatively weak bonding of water molecules in the complex structure. According to literature [32], lattice water is removed at temperatures below 110 °C, whereas coordinated water is eliminated within 110-275 °C temperature range. As a conclusion to this, first mass loss corresponds to the lattice water, not to coordination water. For each complex, the content of lattice water was determined: 0.5 mol for Zn(II), 1.0 mol for Cu(II), and 2.5 mol for Fe(III), respectively. Following the first dehydration step, each complex presents another process with mass loss, in the temperature range between 143 and 193 °C (for Zn(II) complex), 132-181 °C (for Cu(II) complex), and 148-210 °C (for Fe(III) complex), respectively. These processes which occur with mass loss are sustained by the peaks on the HF curve (their endothermic nature suggest the loss of water), and are well defined on the DTG curves. According to the second process, for each metal complex, the composition regarding the coordination water was deduced. The mass loss in this step for Fe(III) complex is 16.2 %, corresponding to 4 mol of water, for Zn(II) complex is 8.2 %, corresponding to 2 mol of water and for Cu(II) complex is 14.8 %, corresponding to 4 mol of water. The analysis of the total mass loss corresponding to lattice water and coordination water is presented in Table 2.

According to Fig. 2, the complexes with Fe(III) and Cu(II) undergo a decomposition process as soon as the dehydration ended, only the complex with Zn(II) is stable up to 216 °C.





Fig. 3 FTIR spectra of AMB-pure ligand and Zn(II), Cu(II), and Fe(III) metal complexes and final decomposition products

Table 4 FTIR characterization of final decomposition products

	Wavenumber/cm ⁻¹	Total mass loss/%		
	Literature	Experimental	Calc.	Exp.
ZnO	3,315; 1,640; 710 [33]	3,317; 1,641; 710	80.55	79.3
CuO	3,450, 1,578, 601 [34]	3,452, 1,578, 602	82.78	82.0
Fe ₂ O ₃	3,440; 1,631; 1,134; 1,035 [35]	3,440; 1,631; 1,135	69.08	70.2

The thermal analysis results of the new metallic complexes with ambazone, determined in non-isothermal conditions at $\beta = 10$ °C min⁻¹, are summarized in Table 3.

In oxidative air atmosphere, the thermal degradation of the organic ligand AMB is accompanied by a strong exothermic effect which can be connected with the oxidative degradation of the ambazone molecule. The decomposition process is continuous, and it is not possible to distinguish the products of the reaction which are formed intermediary. Final decomposition products obtained at 500 °C were subjected to FTIR analysis in order to determine their composition. Due to the fact that the thermal degradation of the metal complexes took place in oxidative air atmosphere, oxides are expected to be formed (see Fig. 3). The literature data and experimental values which characterized the metal oxides are presented in Table 4.

The solid residues obtained during thermal decomposition of metal coordination compounds are in agreement with formation of metal(II) oxides, namely ZnO and CuO, respectively Fe_2O_3 (see Table 4).

Spectroscopic description

The proof for the complex formation was evidenced by comparing the infrared spectra of the ambazone monohydrate (AMB), used as ligand, and complexes in the region of 4,000-600 cm^{-1} (Fig. 3). By the analysis of the FTIR spectra, it can be noticed the stretching vibration of primary amines ($-NH_2$ groups) around 3,394 and 3,230 cm⁻¹, respectively, the stretching vibrations of secondary amine (-NH-) around 3,146 cm⁻¹ [36]. For AMB, the bands at 1,633 and 1,616 cm⁻¹ can be associated with deformation vibration of C=N bond. The literature [36, 37] indicates that functional group -NH-CH(=S)- is characterized by the following bands: $v_{\rm NH} \sim 3,400 \ {\rm cm}^{-1}$; δ_{NH} \sim 1,500 cm $^{-1},$ and $\nu_{C=S}$ \sim 1,100 cm $^{-1}.$ According to this, the vibration bands from $1,502 \text{ cm}^{-1}$ and $1,112 \text{ cm}^{-1}$ can be associated with the deformation vibration of N-H and C=S, respectively.



Fig. 4 Tentatively proposed coordination ways of ligand to central ions for Zn(II), Cu(II), and Fe(III) metal complexes

The FTIR spectra of metal complexes present several differences compared to the one of pure ambazone. The FTIR analysis of only certain group of atoms from the ambazone molecules (namely -NH₂, -NH-, and (C)=S) can be explained by the fact that they can act as electron donors in the dative bond for the formation of the coordination compound, so a modification of their relative intensity or/and position in spectrum can be expected [26]. According to this, it can be noticed that in the case of coordination compounds, a broad signal between 3,600 and $2,900 \text{ cm}^{-1}$ suggests the presence of water molecules in the complexes' structures. This fact is sustained both by elemental analysis and thermal behavior of complexes. Significant shifting to different wavenumbers can be identified for the stretching vibrations of secondary amine (-NH-) from 3,146 cm⁻¹ in AMB to 3,122 cm⁻¹ in the case of Zn(II) complex, 3.096 cm^{-1} in the case of Cu(II) complex and $3,091 \text{ cm}^{-1}$ in the case of Fe(III) complex, respectively. Other significant shifting can be observed for wavenumber corresponding to the deformation vibration of C=S bond, from 1,112 cm⁻¹ in the case of pure AMB to $1,089 \text{ cm}^{-1}$ in the case of Zn(II) complex, $1,099 \text{ cm}^{-1}$ in the case of Cu(II) complex, and 1.090 cm^{-1} in the case of Fe(III) complex, respectively. The shifting of values corresponding to the wavenumbers from the FTIR spectrum of complexes indicates that AMB acts as a bidentate ligand, through lone pairs of sulfur and the ones from the NH group, so the tentatively proposed coordination ways of ligand to central ions are presented in Fig. 4. A comparative analysis of the corresponding wavenumbers of other potential electron-donors groups from AMB molecule and the ones from the metal complexes reveal only insignificant shifting to lower/higher values ($\pm 3 \text{ cm}^{-1}$), so it confirms that these groups are not involved in the formation of coordinative compound.

According to spectroscopic data, and, by corroboration to thermal behavior of coordination compounds and the results from elemental analysis, a tentatively proposed coordination way of ligand to central ion for each metal complex is presented in Fig. 4.

Conclusions

In our study, three coordinative compounds containing Zn(II), Cu(II), and Fe(III) as complex generators and ambazone as ligand were synthesized and characterized by elemental analysis, FTIR spectroscopy, and TG-DTG-HF techniques. It was proven that the formation of complexes occurs by an –NH and –C=S groups from ambazone. From thermal data obtained in oxidative air atmosphere, three main stages of decomposition can be observed for each metal complex. In the first step, lattice water molecules are released, followed the releasing of coordination water molecules, and finally by the decomposition of anhydrous complexes by the degradation of ambazone ligand. By TG technique and FTIR, it was proven that pure oxides ZnO, CuO, and Fe₂O₃ are the final decomposition products.

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