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Synthesis and characterization of Fe(III) complex with thiosemicarbazide-based ligand

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Abstract: Complex iron(III) salicylaldehyde thiosemicarbazone with a formula Na[Fe(STSC)$_2$] have been synthesized. Ligand, salicylaldehyde thiosemicarbazone (SCT) was prepared through the condensation reaction of salycilaldehide with thiosemicarbazide. Na[Fe(STSC)$_2$] has been synthesized by reacting $FeCl_3$ with thiosemicarbazide based ligand bearing ONS donor atoms. Complex has been formulated and characterized by mass spectrometry, infrared and UV/visible spectroscopy. The facts showed the formation of a complex in metal:ligand stoichiometric ratio 1:2. The ligand is coordinated as an ONS tridentate dianion *via* oxygen atom after the deprotonation of the phenolic OH-group, azomethine nitrogen and sulphur in thiol form of the deprotonated thiosemicarbazide residue. Antioxidant activity was determined, where ligand showed significant activity, while complex, at low concentration, exhibited almost no activity.

INTRODUCTION

Thiosemicarbazones have been a focus of a research for a long time due to its biological activities. These compounds and its derivatives showed antiviral, antibacterial and antineoplastic effects with different levels of activity. Moreover, the complexes of thiosemicarbazone with metals showed significant biological activity especially due to the changed lipophilicity that allows better deposition in the cell. This is due to their redox properties and lately discovered mechanism of action that involves the Fenton chemistry in the cases of an iron-thiosemicarbazone complexes. As a result, the production of free radicals, especially reactive oxygen species (ROS) is increased that in turn leads to the antiproliferative effect. In addition, if thiosemicarbazone are used alone as an active substances, they can sequester iron ions from the cell by chelation mechanism that will cause the inhibition of compounds with an iron in the structure such as heme containing enzymes of iron-protein compounds.

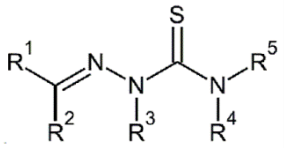

Figure 1.General structure of thiosemicarbazones where X represents sulfur atom while R$_1$, R$_2$ and R$_3$ represents alkyl or aryl groups.

Anti-tumor activity of thiosemicarbazone complexes have been explained by mechanism that involves depletion of iron from the tumor cells first and formation of Fe(III) complex. Due to the number of cellular reductant and oxidant, redox turnover between Fe(III) and Fe(II) complexes occurs. During this process, in the case of iron complexes, Fenton reaction produces ROS that are damaging tumor DNA bases resulting in cytotoxicity and destruction of tumor cells (Alcaraz, Muñiz, Cavia, et al. 2020).

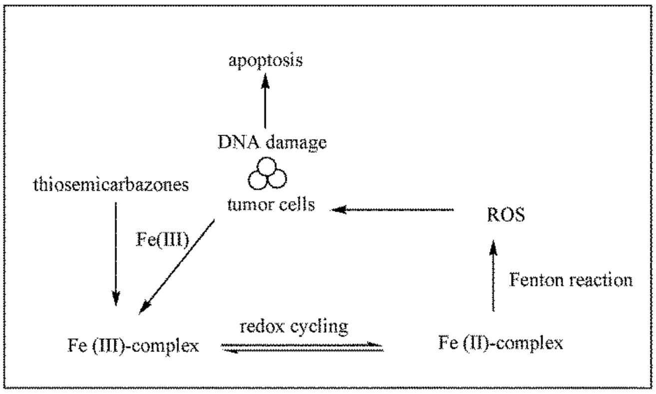

Figure 2. Cytotoxicity mechanism

Besides iron complexes showing antitumor activities, some copper(II) complexes of 3-etoxy-2-oxobutyraledehyde-bis(thiosemicarbazone) showed a strong antitumor activity in animal studies. Further studies proved that variation in a ligand structure of these complexes significantly affected the cytotoxic effect. Wide spectrum of anti-microbial effects was detected for the different thiosemicarbazone with complexes with metals. Generally, complexes showed strong activity against *Staphylococcus aureus*, *Neisseria meningitidis*, while poor activity against the gram-negative bacilli (Dobek, Klayman, Dickson et al. 1989). In these cases, mechanism of the action of the complexes was explained by electron transfer and free radical activity against microbes. Other potent effects that thiosemicarbazone includes are anticonvulsant activity and antiviral activity either as a ligand or in the complex with a various metals. A recent study found that pyridyl thiosemicarbazones are highly potent agents for the treatment of malaria. Generally, standard compounds used for the treatment such as chloroquine showed the interaction with parasitic heme resulting in a pH-dependent decomposition of the heme (Chou, Chevli Fitch, 1980; Slater, Cerami, 1992; Dorn, Vippagunta, Matile et al.,1998). Thiosemicarbazones derived from phenolic aldehydes showed similar anti-malarial activity, based on its ability to chelate iron. Clearly, the synthesis and the investigation of the thiosemicarbazone complexes with metals, especially with iron justify the research in order to detect exact mechanism of it activity and potency in biological systems. In this study, we synthesized and characterized iron(III) salicylaldehyde thiosemicarbazone complex and tested their antioxidant activity.

EXPERIMENTAL

Material

All chemical used in the study were obtained from Sigma Aldrich and Merck. Commercial reagents of analytical reagent grade were used without further purifications. Solvents used in the synthesis of the ligand and their complex were distilled before use.

Methods

ESI ToF MS were obtained on 6210 Time-of-Flight LC/MS instrument (Agilent Technologies, California, USA) connected with Agilent 1200 Series HPLC instrument (Agilent Technologies, Waldbronn, Germany), Instrument consisted of degasser, binary pump, autosampler, column compartment with ZDV (zero dead volume) cell and diode-array detectors (DAD). The mobile phase consists of 0.2% formic acid in water (v/v) (A) and acetonitrile (B) in a 1:1 ratio. Flow mobile phase is 0.20 mL/min, column compartment temperature 25°C, and injection volume of samples c≈1 mg/mL 5 μL. Peak spectral data were stored in the range 190-450 nm. Mass spectra were recorded in the range of 100-2000 m/z in negative ESI ionization mode (capillary voltage 4000 V, fragment voltage 140 V, skimmer voltage 60V, OCT RF voltage 250V); spray gas (nitrogen): pressure 45 psi, temperature 350°C, flow 12 L/min.

FTIR spectra were recorded using KBr pellets on a Perkin Elmer spectrum BX FTIR System in the region 4000-400 cm^{-1}.

The electronic absorption spectra from 200 nm to 7000 nm were recorded with Thermo Scientific Multiscan Go using quartz cuvettes with a 1 cm path length.

Antioxidant capacity of the ligand and a complex was tested by e-BQC lab instrument (bioquochem, CEEI Parque Tecnológico de Asturiasis) based on a redox potential of the tested compounds and its comparison with antioxidant activity of ascorbic acid. Measurements are expressed in charge units- micro-Coulombs (μC). To compare the results (μC) to antioxidant capacity units of Vitamin C Antioxidant Capacity Equivalents (CEAC) are used. Briefly, calibration curve with a standard solution of ascorbic acid diluted in a standard buffer solution was used to measure charge values (μC). Regression line was used to obtain the equation: Charge value (μC)=a Concentration (μM) + b where measured charge values of a tested solutions were used and converted to concentrations of the tested samples that have equivalent antioxidant capacity as a model antioxidant (CEAC) (Rey, Gómez, Muñoz-Cimadevilla et al, 2018). Ligand and complex were dissolved in a dichloromethane and then diluted with the 150 mM phosphate buffer pH 7.4 in order to minimize the effect of the organic solvent on e-BQC measuring strips.

Synthesis of ligand salicylaldehyde thiosemicarbazone

The amount of 0.911 g thiosemicarbazide (0.01 mol) was dissolved in 75 mL of warmed distilled water and volume of 1.044 mL salicylaldehyde (0.01 mol) was added to the solution. The reaction mixture was refluxed for 5 hours at 55-60°C. Afterwards, solution was cooled on ice bath and the crystals of white salicylaldehyde thiosemicarbazone were collected by filtration, dried by vacuum pump and recrystallized from ethanol with 75% yield (Archana, Ezhilarasi, 2012). Ligand is insoluble in water and benzene, soluble in dimethylformamide, dimethyl sulfoxide and dichloromethane.

White solid; Yield: (75%). M.p. 250°C. FT-IR(KBr): ν, cm^{-1} 1263 (C-O), 1616 (C=N),777(C=S), UV-Vis (DMF): λ_{max}, nm 234, 309, 334.

Synthesis of iron(III) salicylaldehyde thiosemicarbazone, $Na[Fe(STSC)_2]$

The amount of 0.135 g of iron (III) chloride ($FeCl_3.6H_2O$) (0.0005 mol) was dissolved in 15 mL of slightly warmed methanol and mixed with 0.204 g (0.0015 mol) of sodium acetate three hydrate (NaOAc · $3H_2O$). After cooling to room temperature, the solution was filtered and ligand, salicylaldehyde thiosemicarbazone (0.195 g, 0.001 mol) was added. The mixture was gently heated at 45-50 °C for about 30 minutes with continuous stirring. After the mixture was cooled to a room temperature, separation of the solid was observed. The mixture was left at room temperature over the next 24 hours resulting in complete precipitation of a black solid. Subsequently, the obtained complex was filtered, the crystals washed with methanol and ether and dried in a vacuum desiccators (Semanti, Rama, Falgun et al., 2010; Sulekh, Lokesh, 2005).

Insoluble in water, benzene, soluble in dimethylformamide, dimethyl sulfoxide and dichloromethane.

Black solid; Yield: (71%). M.p. 227°C. FT-IR (KBr): ν, cm^{-1} 11304 (C-O), 1603 (C=N), 737 (C-S), 590 (Fe-N), 457 (Fe-O), 427 (Fe-S); UV-Vis (DMF): λ_{max}, nm 238, 308, 337. ESI ToF m/z =441.99746 $[M+ H]^+$

RESULTS AND DISCUSSION

Ligand salicylaldehyde thiosemicarbazone and a complex iron(III) salicylaldehyde thiosemicarbazone were synthesized from the starting compound thiosemicarbazide by modified synthesis procedures. Complex was synthesizes in the presence of sodium acetate three hydrate with the addition of $FeCl_3{\cdot}6H_2O$ (Figures 3 and 4).

Obtained ligand and a complex were insoluble in the water, benzene or chloroform, but soluble in the dimethylformamide, dimethylsulfoxide and dichloromethane. Synthesized compounds were characterized by UV/Visible spectroscopy and FT-IR. Mass spectroscopy confirming the presence of a newly formed complex.

ESI ToF mass spectrometry confirmed existence of $[C_{16}H_{14}N_6O_2S_2Fe]^-$, ion with m/z values at 441.99746 amu. Spectra showed a series of peaks at 199.8; 194.039; 318.901; 640.8082 that are corresponding to the various fragments (Figure 5).

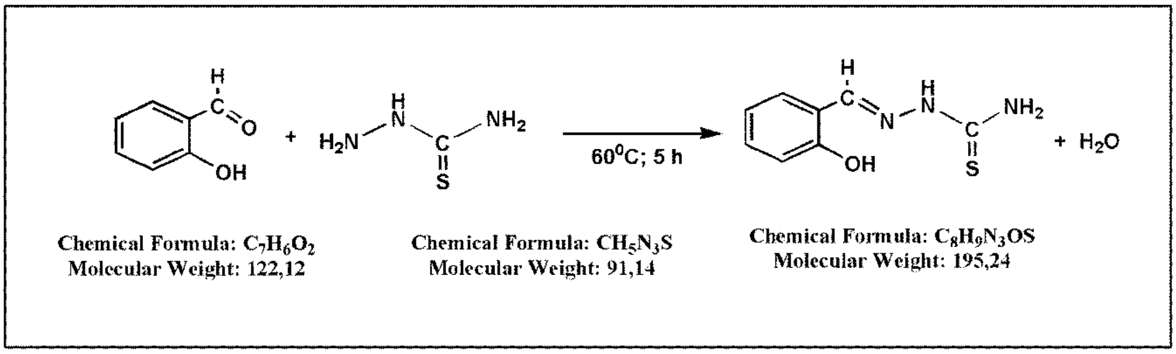

Figure 3. Ligand synthesis, thiosemicarbazone salicylaldehyde (STSC)

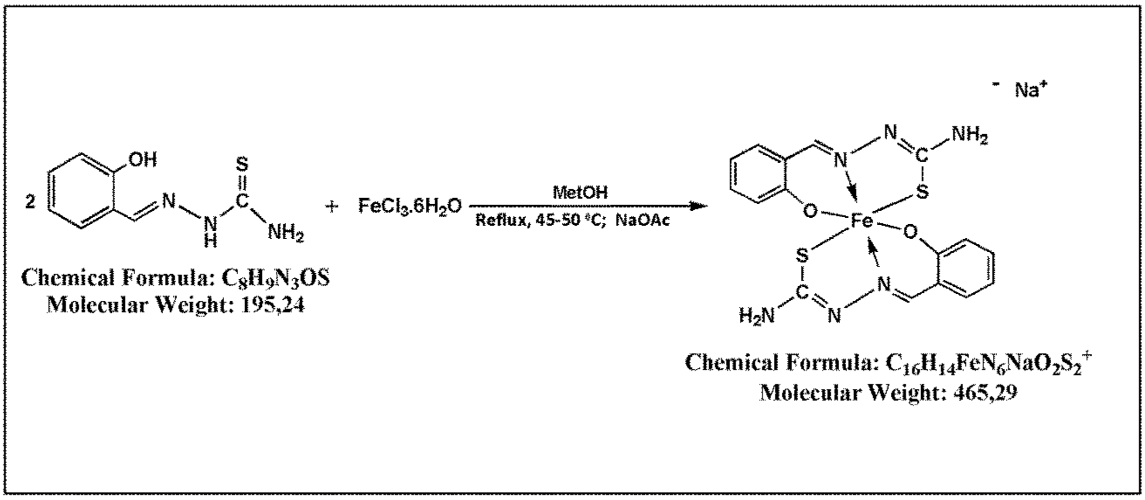

Figure 4. Synthesis of iron(III) complex with salicylaldehyde thiosemicarbazone, $Na[Fe(STSC)_2]$

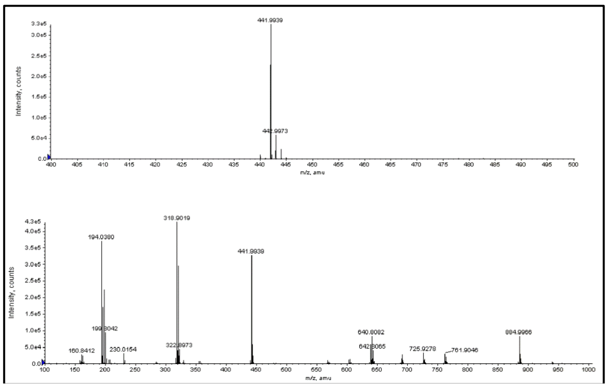

Figure 5. Mass spectrum of Na [Fe (STSC) 2]

Table 1. Calculated and experimental mass spectra data for the molecular ion

Molecular ion	$C_{16}H_{14}N_6O_2S_2Fe^-$
Theoretical mass	441.99746
Experimental mass	441.99775
Error in mass /mDa	0.29458
Error in mass/ppm	0.67

IR spectra were recorded for the ligand and a complex in the region of 2300 to 400 cm^{-1}, analyzed and the assignment of the detected frequencies have been compared with the literature values for the specific group vibrations. The Table 2 shows the assigned values for the ligand and the complex.

Table 2. FT-IR data for the ligand and complex (2300 to 400 cm^{-1})

Peak assignment	ν(C=N) cm^{-1}	ν(C-O) cm^{-1}	ν(C=S) cm^{-1}	ν(C-S) cm^{-1}	*ν*(Fe–N) cm^{-1}	*ν*(Fe–O) cm^{-1}	*ν*(Fe–S) cm^{-1}
STSC	1616	1263	777	-	-	-	-
Na[Fe(STSC)$_2$]	1603	1304	-	737	590	457	427

STSC-salicylaldehyde thiosemicarbazone, Na[Fe(STSC)2]-iron (III) salicylaldehyde thiosemicarbazone

The ligand is coordinated to iron center as a ONS tridentate dianion *via* oxygen atom after the deprotonation of the phenolic OH-group, azomethine nitrogen and sulphur in thiol form of the deprotonated thiosemicarbazide. This was confirmed by IR spectra: shift of azomethine stretching to lower frequency, shift of C-O(H) vibration to higher frequency and disappearance of the vibration of C=S double bond in the spectra of the complex (El-Bahnasawy et al., 2014).

In order to analyze the way of ligand coordination to the metal, the most significant infrared spectral frequencies for the metal complex are compared with the frequencies of the free ligand. A band observed at 1616 cm^{-1} attributed to the azomethine C=N stretching frequency of the free ligand where shifted to lower frequency in the spectra of the complex at 1603 cm^{-1} indicating the coordination through N atom. Deprotonated phenolic oxygen – strong absorption band in spectra of ligand positioned at 1263 cm^{-1} after coordination is shifted to 1304 cm^{-1} (Baiu et al., 2009), which corresponds to forming of weaker C-O(Fe) bond comparing to C-O(H) and confirms coordination of ligand to Fe(III) through deprotonated phenolic oxygen. Also, in the IR spectra of ligand, the characteristic vibration of the (OH) band is observed at 3444 cm^{-1}. The absence of this band in the IR spectra of the complex indicates the coordination *via* the phenolic oxygen (Vojinović- Ješić et al., 2011). The ligand showed band at 777 cm^{-1} for ligand for the vibration of the C=S bond (Wiles et al., 1967; Thangadurai et al., 2001). The C=S band was disappeared in the complex (Al-Amiery et al., 2011) and a new band, C–S appeared at 737 cm^{-1}. This confirms that the other coordination site to iron is through thiolate sulphur (Sampath et al., 2016). The new bands at 590, 457 and 427 cm^{-1} are attributed to Fe–N, Fe–O and Fe–S bonds, respectively (El-Bahnasawy et al., 2014) (Figure 6).

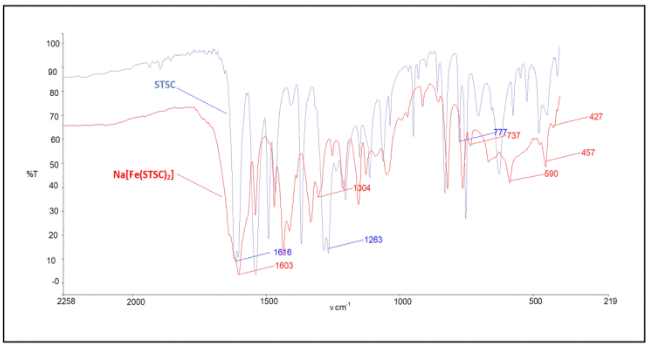

Figure 6. FT-IR spectra of ligand (STSC, blue line) and complex (Na[Fe(STSC)$_2$, red line)

UV/Visible spectra were recorded for the ligand and the complex in the range from 220 to 700 nm. Ligand showed maximum peak with higher intensity at 241 nm, peak at 291nm and one peak at 345 nm with a lower intensity. The spectra of the ligand showed characteristic π→π* and n→π bands due to the substituted benzene group. UV/Vis spectra of the complex showed peaks at 241 nm and 291 nm, and a shoulder at 345 nm due to the complexation.

The d-d transition in this type of complexes usually showed above 500 nm but in this case, probably to the weak transition was not clearly detected (Figure 7).

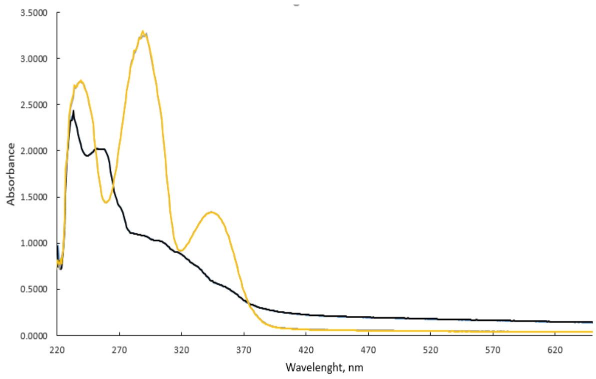

Figure 7. UV/Visible spectra of ligand (yellow line) and a complex (black line)

A number of thiosemicarbazide derivatives have been tested for antioxidant activity and attempts have been made to correlate specific structural features with antioxidant ability of the synthesized compounds based on the thiosemicarbazide structure (Ghosh, Misra, Bhatia et al, 2009). The amine group in thiosemicarbazone moiety significantly contributes to the antioxidant activity of the compound. In addition, phenolic hydroxyl group with its electron donation ability is involved in the increased antioxidant activity. However, complexation with the metal decreases its antioxidant ability due to its involvement into metal coordination process.

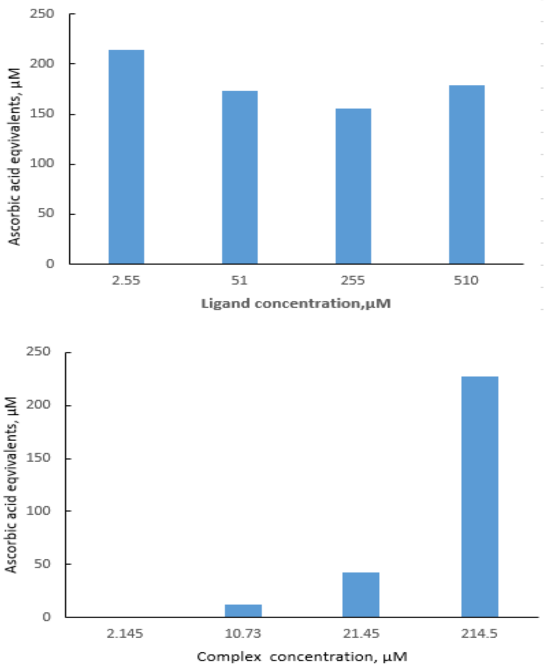

Figure 8. Total antioxidant capacity of a salicylaldehyde thiosemicarbazone (A) and iron(III) salicylaldehyde thiosemicarbazone (B) expressed in ascorbic acid equivalents, μM

Salicylaldehyde thiosemicarbazone showed substantial antioxidant capacity that was not dependent on the concentration of the ligand (Figure 8). Iron(III) salicylaldehyde thiosemicarbazone antioxidant activity was almost negligent at the lower micromolar concentration. Only at a high concentration of the complex (214.5 μM) a high antioxidant activity was observed that can be attributed only to free amine groups in the structure.

CONCLUSIONS

We synthesized and characterized salicylaldehyde thiosemicarbazone ligand and its complex, iron (III) salicylaldehyde thiosemicarbazone. Structures were confirmed by mass spectra, and FT-IR methods and UV/Visible spectra were recorded with characteristic maximum wavelengths. Antioxidant activities that are expected for this type of compounds were measured, where ligand showed a high activity regardless of the tested concentration, while a complex showed significant activity only at the higher concentrations. Further studies should be done in order to correlate specific structural features with antioxidant activity and modulation of it that would allow formation of the stable complex, and at the same time, formation of the stable complex.

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Summary/Sažetak

Sintetiziran je kompleks željezo(III) salicilaldehid tiosemikarbazon formule Na[Fe(STSC)$_2$]. Ligand (SCT) pripremljen je u reakciji kondenzacije salicilaldehida sa tiosemikarbazidom. Na[Fe(STSC)$_2$] sintetiziran je u reakciji $FeCl_3$ sa ligandom na bazi tiosemikarbazida sa ONS donorskim atomima. Kompleks je formulisan i okarakterisan masenom spektrometrijom, infracrvenom i UV/Vis spektroskopijom. Podaci su pokazali formiranje metal:ligand kompleksa u stehiometrijskom odnosu 1:2. Ligand je koordiniran kao ONS tridentatni dianion preko atoma kisika nakon deprotoniranja fenolne OH-grupe, azometinskog atoma azota i atoma sumpora u tiolnom obliku poslije deprotoniranja tiosemikarbazidnog ostatka. Određena je antioksidativna aktivnost, gde ligand pokazuje značajnu aktivnost, dok kompleks u maloj koncentraciji, nije pokazao gotovo nikakvu aktivnost.