



## Solvothermal Synthesis, Characterization and Cytotoxicity Study of Cobalt and Copper of Complexes Sulfamethoxazole

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### Authors' contributions

This work was carried out in collaboration among all authors. Authors UAA, BM and ABM designed, supervised and reviewed all the drafts of the manuscript. Author ZIH carried out the research and author MMS wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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### ABSTRACT

The complexes of Co and Cu with sulfamethoxazole (SMZ) were synthesized in the 1:2 molar ratios. The physical characterization of the ligand and the complexes showed different colours with a good % yield and high melting point/decomposition temperature. The solubility test of the metal complexes and the free drug using various solvents of different polarity and proticity showed that the free drug and the metal complexes are soluble in polar and non-polar solvents. The IR results of the synthesized complexes displayed new peaks that are not present in the free drug, the bands are due to M-O and M-N thus, confirming the coordination. The result from the conductivity measurement shows that the complexes are non-electrolyte. More so, all the complexes were found to be toxic in brine shrimp lethality assay.

*Keywords:* Bioassay; brine shrimp; polar and non-polar solvents; sulfamethoxazole.

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## 1. INTRODUCTION

Most metals ions are essential components to maintain homeostasis and play crucial role in many biological processes by involving as co-factors in the biological functions of proteins and operating a many regulations, stabilization, completion courses of cellular function [1]. Metals plays an important role in medicinal chemistry. First series transition-metal represent the d-block element (group IIA-III A) on the periodic table. They have incompletely filled d-shells. This property gives them the ability to form coordination [1]. Transition metal complexes are also important in catalysis, materials synthesis and photochemistry. They display diverse chemical, optical and magnetic properties. Metal complex or coordination compound is a structure consisting of a central metal atom, bonded to a surrounding array of molecules or anions [1].

Interest in coordination chemistry is increasing continuously with the preparation of organic ligands containing a variety of donor groups [2].

The number and diversity of nitrogen and sulfur chelating agents used to prepare new coordination and organometallic compounds have increased rapidly during the past few years [3]. Sulfur compounds and metallic complexes have antimicrobial activity and showed a high dependence on their substituents [3]. Organic compounds containing  $-C_6H_4S$  moiety are well known for their significant biological activities [4]. The activity may be due to the presence of multi-coordination centers having the ability to form stable chelates with the essential metal ions which the organism need in their metabolism [4]. Research has shown significant progress in utilization of transition metals as drugs to treat several human diseases. Transition metals exhibit different oxidation states and can interact with a number of negatively charged molecules or neutral molecules with lone pair of electrons.

Bioinorganic chemistry exploits the unique properties of metal ions for the design of new drugs. This has, for instance, led to the clinical application of chemotherapeutic agents for cancer treatment, such as cisplatin [5]. The use of transition metal complexes as therapeutic compounds has become more and more pronounce, as these complexes offer a great diversity in their action; they do not only have anti-cancer properties but have also been used as anti-inflammatory, anti-infective and anti-diabetic compounds [1]. Development of

transition metal complexes as drugs is not an easy task; considerable effort is required to get a compound of interest. Besides all these limitations and side effects transition metal complexes are still the most widely used chemotherapeutic agents and make a large contribution to medicinal therapeutics in a way that is, unimaginable in few years back [1]. Several techniques such as hydrothermal [6], coprecipitation [7], ball milling [8], micro-emulsion [9], sol-gel [10] and electrospinning [11] are used for the synthesis of Co(II) and Cu(II) complexes. Among the listed techniques, only solvothermal method is designed to achieve targeted results such as virtuous homogeneity, controlled crystallite size, high limpidness and low agglomeration.

The treatment of infectious diseases still remains an important and challenging problem in our society today because of a combination of factors including emerging infectious diseases and the increasing number of multi-drug resistant microbial pathogens. In spite of a large number of antibiotics and chemotherapeutics available for medical use, at the same time the emergence of old and new antibiotic resistance created in the last decades revealed a substantial medical need for new classes of antimicrobial agents [12]. Due to outbreak of infectious diseases caused by different pathogenic bacteria and the development of antibiotic resistance, researchers are searching for new antibacterial agents [12]. In order to overcome the increasing resistance of microbes to existing antibiotics and to treat new emerging diseases, the toxicity profile of the complexes needed to be assessed. Although synthesis of SMZ-metal complexes has been reported by [13]. However, no information obtained on the cytotoxicity of the synthesized SMZ-complexes thus, we synthesized [Co-SMZ] and [Cu-SMZ] through a modified solvothermal method and characterized by using Fourier transform infrared (FT-IR), melting point/decomposition temperature, electrical conductivity and solubility in various solvents of different polarity and proticity. Along with this, we investigated the antimicrobial activity of the synthesized complexes.

## 2. EXPERIMENTAL

### 2.1 Materials

The chemicals used in this experiment include cobalt (II) chloride hexahydrate, copper (II) chloride pentahydrate, sulfamethoxazole, sodium

chloride. All glass wares were washed and kept in an oven overnight at 110 °C.

## 2.2 Synthesis of Cobalt and Copper Complexes

The cobalt complex was synthesized with slide modification of solvothermal method of Al-khodir [13] using a 5.06 g of sulfamethoxazole (SMZ) in 25 cm<sup>3</sup> of ethanol and mixed with ethanolic solution of cobalt (II) chloride hexahydrate (2.38 g) and in a ratio of 2:1 (Co<sup>2+</sup>: SMZ). The mixture was refluxed for 2 hrs at 60°C, cooled, filtered, washed with ethanol. The precipitates were then dried overnight in an oven at 100 °C. Similarly, we obtained copper complex sample by same procedure with copper (II) chloride pentahydrate (2.5 g).

## 2.3 Instrumental Techniques

The electrical conductivity of the sulfamethoxazole (SMZ) ligand and the complexes was examined by a HI9835 model of Hanna instrument EC/TDS/NaCl meter at Department of Biochemistry, Gombe State University. Vibrational spectra of the sulfamethoxazole and its complexes were recorded the range of 4000 to 400 cm<sup>-1</sup> using PerkinElmer 10.03.09 spectrum with the standard KBr techniques at the Faculty of Pharmaceutical Sciences, Gombe State University. Decomposition temperature of the complexes was determined by using Electro Thermal Melting Point (SMP10).

The solubility of the metal complexes and the standard drug were tested using various solvents with different polarity and proticity namely: methanol, ether, ethanol, dimethyl sulfoxide and n-hexane.

## 2.4 Brine Shrimp Lethality Assay

Artemia salina nauplii (< 48 hrs old) was exposed to the sample solution for 24 hrs and frequencies

of immobility of the 10 nauplii in 5 mL solution were recorded.

## 2.5 Preparation of Artificial Sea Water

Artificial sea water was prepared by dissolving 17.5 g of sea salt in 500 mL of distilled water for hatching the brine shrimp eggs.

## 2.6 Hatching the Brine Shrimp

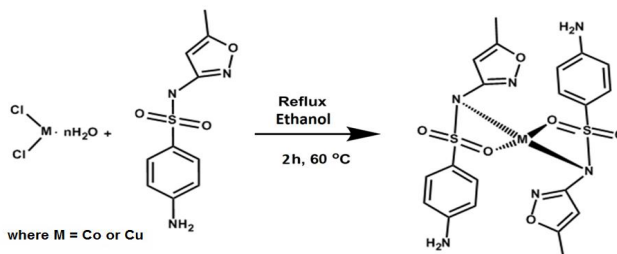
Brine shrimp eggs were hatched in 500 mL beaker filled with artificial sea water 1.0 g of artemia salina was added to the artificial sea water. The air line from the aerator was inserted into the beaker, light was positioned over the culture to synchronize hatching. The eggs were allowed to hatch and mature as nauplii after two days (48 hrs) [14].

The stock solution was prepared by dissolving 0.2 g synthesized complex in 2 mL dimethyl sulfoxide (DMSO) and 1.8 mL of brine solution was added to 0.2 mL of the stock to give 1000 ppm solution subsequent concentration of 100 and 10 ppm were obtained from this by dilution. Ten (10) nauplii were drawn using Pasteur pipette and placed in a test tubes containing 4.0 mL of brine solution and 0.5 mL of the synthesized complex solutions of different concentration and made up to 5 mL with brine solution. The experiments were maintained at room temperature for 24 hrs in the laboratory.

## 2.7 Statistical Analysis

The percentage of death and LC<sub>90</sub> were determined using statistical analysis. Percentage mortality (% m) was calculated by dividing the number of death nauplii by the total number and then multiplied by 100%.

Percentage of death (% m) = (Total nauplii – Alive nauplii) x 100/Total nauplii LC<sub>90</sub> value were obtained from the best fit line from the plot of percentage mortality against log of concentration.



**Scheme 1. Synthesis of metal complex**

### 3. RESULTS AND DISCUSSION

The results of the synthesized complexes are shown in the table below, where several analytical techniques, statistical and biological test were carried out in other to fully characterized the synthesized complex. The melting points, colour, % yield and electrical conductivity of the complexes are recorded in Table 1. The temperature at which [Co-SMZ] and [Cu-SMZ] start to decomposed was 150 and 200 °C respectively. While, the melting point of SMZ was 169 °C as presented in Table 1. The cobalt complex was observed to be pink in colour whereas that of copper complex was green in colour. The colors of the complexes could be as a result of either d-d transition, or charge transfer transitions. The cobalt complex has the highest % yield of 94% and the copper complex has 85% yield. The electrical conductivity of the ligand and the complexes was observed in the range of 519-2934  $\mu\text{Scm}^{-1}$ . The high electrical conductivity values indicate that the compounds are good electrolyte [15]. Hence this shows that there is no chloride ion outside the coordination sphere in all the complexes.

The solubility test of the metal complexes and standard drugs using various solvent are shown in Table 2. The ligands were insoluble in water both at room and elevated temperatures but were observed to be soluble in methanol, ethanol, ether, n-hexane and sparingly soluble in ether both at room and elevated temperature which similar to work of [16]. All the metal complexes were soluble in water except [Cu-SMZ] which is insoluble at room temperature. The complexes are all insoluble in ether and n-hexane as reported elsewhere [17].

The IR spectra results of some major peaks are shown in Table 3. The ligand behaves as a bidentate ligand and coordinates to the metal ion with different point of chelation group [4]. Bands due to M-O and M-N were absent in the free ligand as reported elsewhere [17]. However, these bands were observed at 528-529  $\text{cm}^{-1}$  and 631-779  $\text{cm}^{-1}$  for [Co-SMZ] and [Cu-SMZ] respectively confirming the coordination [18].

The result obtained for the mortality rate of brine shrimp *Artemia salina* after 24 hrs exposure to the synthesized metal complexes are shown in Table 4. The results show that the effect of metal complexes on the mortality of *A. salina* depend on the type of the synthesized complex and its concentration. A 100% mortality were observed at 1000 ppm for the two metal complexes, for cobalt complex, 70% and 60% mortality were recorded at 100 and 10 ppm respectively while, for copper complex, 80% and 70% mortality were observed at 100 and 10 ppm respectively. The  $\text{LC}_{50}$  and  $\text{LC}_{90}$  of the two synthesized complexes were determined graphically (Figs. 1 and 2). The  $\text{LC}_{50}$  and  $\text{LC}_{90}$  of the cobalt complex were obtained as 7.07 and 354.85 ppm respectively while, for copper complex, the  $\text{LC}_{90}$  was obtained as 181.96 ppm.

According to the work reported by [19], a crude plant extract as well as synthesized complexes can be considered toxic (active) if it has an  $\text{LC}_{50}$  value of less than 1000 ppm while non-toxic (inactive) if the value is less than 1000 ppm. The results presented in Table 4 indicated that all the synthesized complexes have  $\text{LC}_{50}$  or  $\text{LC}_{90}$  values less than 1000 ppm thus, indicating that all the synthesized complexes are toxic (active) [13,19-20].

**Table 1. Physical properties and electrical conductivity of the ligand and of their metal (ii) complexes**

S/N	Compound	M.P (°C)	E. conductivity ( $\mu\text{Scm}^{-1}$ )	Colour	Yield (%)
1	SMZ	169	519	White	-
2	[Co-SMZ]	150	2934	pink	94.76
3	[Cu-SMZ]	200	987	green	85.85

**Table 2. Result of solubility tests of the ligands and of their metal (ii) complexes in some solvents**

S/N	Compound	Ethanol		Water		Methanol		Ether		n-Hexane	
		RT	ET	RT	ET	RT	ET	RT	ET	RT	ET
1	SMZ	S	S	IS	IS	S	S	SS	SS	S	S
2	[Co-SMZ]	SS	S	S	S	SS	SS	IS	IS	IS	IS
3	[Cu-SMZ]	IS	S	IS	S	SS	S	IS	IS	IS	IS

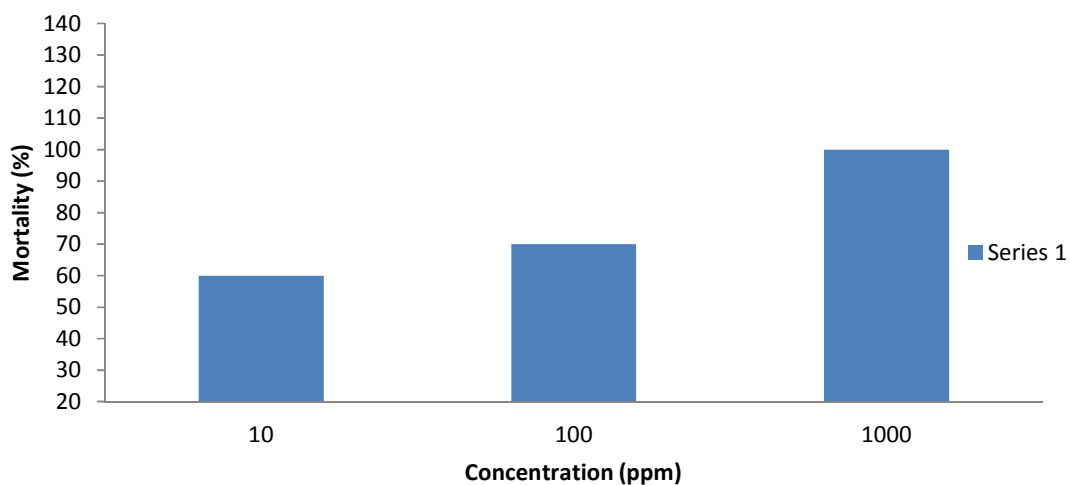
\*RT = Room temperature, ET = Elevated temperature, S = Soluble, SS = Slightly soluble, IS = Insoluble

**Table 3. Major IR spectra of SMZ and of its complexes in cm<sup>-1</sup>**

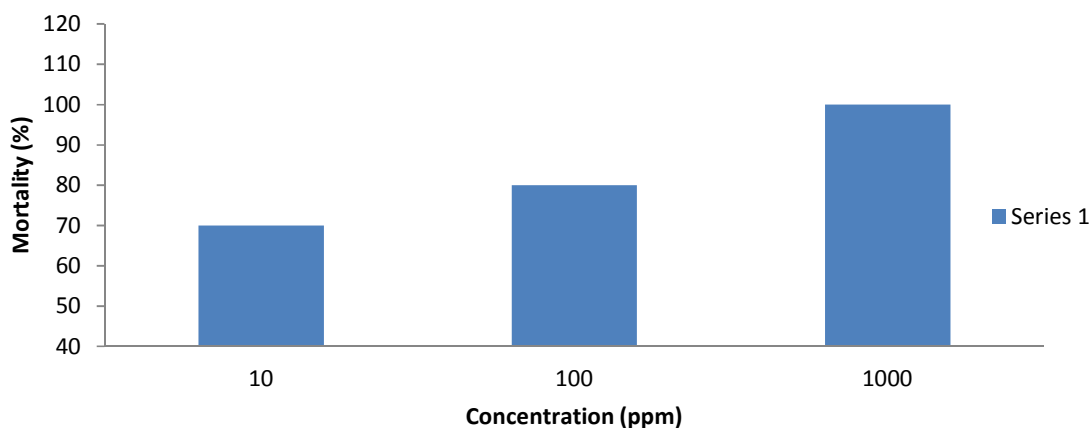
Compound	N-H	C-H	C=C	C=N	S=O	N-O	C-O	M-O	M-N
SMZ	3470	3015	1622	1389	1129	1322	1672	-	-
[Co-SMZ]	3554	3407	1672	1532	1198	1438	1130	529	631
[Cu-SMZ]	3471	3342	1637	1423	1127	1334	1056	528	779

**Table 4. The number of shrimp Nauplii that survived and percentage mortality after treatment with [Co-SMZ] and [Cu-SMZ] complexes**

Metal complexes	Concentration	No. of survivors	% Mortality
[Co-SMZ]	1000 ppm	0	100
	100 ppm	3	70
	10 ppm	4	60
[Cu-SMZ]	1000 ppm	0	100
	100 ppm	2	80
	10 ppm	3	70



**Fig. 1. Lethality rate of [Co-SMZ]**



**Fig. 2. Lethality data for [Cu-SMZ]**

#### 4. CONCLUSION

Complexes of Co and Cu with SMZ were synthesized in the ratio 1:2. They were characterized by high decomposition temperature. The conductivity measurement shows that the complexes are electrolyte in nature. More so, all the complexes were found to be toxic (active) in brine shrimp lethality assay.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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