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Synthesis, Characterization and Studies Antibacterial Activity of Iron and Zinc Metal Complexes derived from Sulfamethoxazole

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Abstract
Sulfa drugs act as good ligand to transition metal ions and they exhibit several coordination modes. Metal complexes of Fe (II) and Zn (II) were synthesized and characterized using Physical Characterization, Solubility Test, Melting Point, Conductivity and FTIR. The complexes have lower melting points compare to the ligand. The result of the Conductivity measurement shows that the complexes are non-electrolyte. The FTIR result shows that the ligand (Sulfamethoxazole) bind to the complex through NH_2 and NH group of the sulfanilamide in Fe complex and the NH of the sulfanilamide and the nitrogen of the isoxazole ring in case of Zn complex. Sulfa drugs concentrate in the urine before being excreted and treat urinary tract infections.The ligand (standard drug) and its complexes were also tested for their antibacterial activity using agar well diffusion method against Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Mycobacterium leprae. The results of the zone of inhibition show that the complexes have good activity on Escherichia coli. The result of the antibacterial study reveals that the complexes showed enhanced activity against the tested organisms than the standard sulfamethoxazole.

Keywords: Sulfamethoxazole, metal complexes of Fe (II) and Zn (II), Antimicrobial activity, Infrared.

INTRODUCTION
The First series Transition metals represent the d block elements which include group's IIA - IIIA on the periodic table. Their d shells are incompletely filled. The properties of transition metals are the foundation of coordination complexes (Rafique *et al.,* 2010). Transition metal complexes are important in catalysis, materials synthesis, photochemistry, and biological systems. They display diverse chemical, optical and magnetic properties. Interest in coordination chemistry is increasing continuously with the preparation of organic ligands containing a variety of donor groups, and it is multiplied manifold when the ligands have biological importance (Latika *et al.,* 2012).

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 (Basu, 2008). Sulfur compounds and their metal complexes have antimicrobial activity and showed a high dependence on their substituents (Basu,

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2008). Organic compounds containing $-C_6H_4S$ moiety are well known for their significant biological activities. 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 organisms need in their metabolism (Singh *et al.,* 2006).

Research has shown significant progress in utilization of transition metal complexes as drugs to treat several human diseases. Transition metals exhibit different oxidation states and can interact with a number of negatively charged molecules. This activity of transition metals has started the development of metal based drugs with promising pharmacological applications and may offer unique therapeutic opportunities. (Rafique *et al*., 2010). The advances in inorganic chemistry provide better opportunities to use metal complexes as therapeutic agents. The mode of action of metal complexes on living organism is differing from non metals. These complexes show a great diversity in action. Warra (2011). Bioinorganic chemistry can exploit 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 (Pieter *et al.,* 2008). The use of transition metal complexes as therapeutic compounds has become more and more pronounced. 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.

Some antibiotics, also called antibacterial, are a type of antimicrobial drug used in the treatment and prevention of bacterial infections. They may either kill or inhibit bacterial activities. A limited number of antibiotics also possess antiprotozoal activity.

Sulfonamides were the first effective chemotherapeutic agents employed systematically for the prevention and cure of bacterial infections in humans. Among the many and so different families of organic–inorganic chemicals being currently investigated today because of their applications, sulfonamides and their N-derivatives are some of the outstanding groups (Alkhoodir, 2015).
It is well documented that toxicological and pharmacological properties are enhanced when sulfonamides are administered in the form of their metal complexes (Dai *et al.,* 2011).

Sulfamethoxazole belongs to the sulfonamide group of antibiotics. This was the first synthetic antibiotic group to be used as a pharmaceutical and these antibiotics have been used in human and veterinary medicine since the 1940s (Sheraby, 2005). Sulfamethoxazole, mainly used in human medicine, was chosen to be the representative of this group due to its widespread use and detection frequency in the aquatic environment. It is effective against both gram-positive and gram-negative bacteria and inhibits growth by a competitive binding to dihydropteroate synthetase which stops the conversion of Para-Aminobenzoic Acid (PABA) to dihydropteroate, a precursor to tetrahydrofolic acid, which is essential for the synthesis of nucleic acids (Rizzotto *et al.,* 2005). An additional mechanism of action is that sulfonamides

block cross-membrane transport of glutamic acids which also is an essential component for synthesizing folic acid (Baran *et al.,* 2011).
The aim of the research is to synthesized, characterize and study the antibacterial activity of complexes of zinc and iron with Sulfamethoxazole.

METHODOLOGY

Material

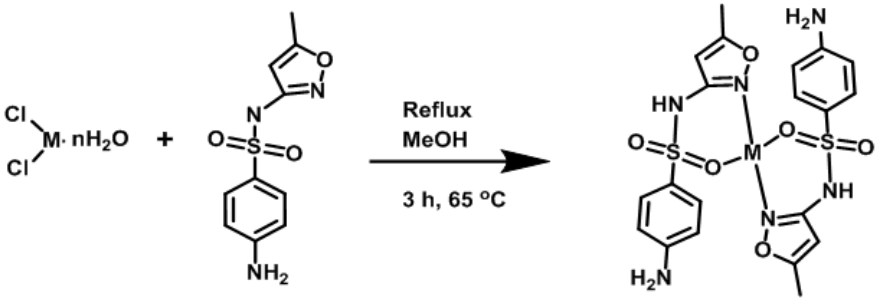
All chemicals and solvents used were of Analar grade and were used as supplied. Metal (II) salts were used in the form of their chlorides.
The complexes were prepared based on the method descibed by Al-khoodir (2015) with slight modification.

SYNTHESIS OF THE ZINC COMPLEXES

5.0 mmol of Sulfamethoxazole (SMZ) ligand was dissolved in 25 cm^3 methanol then mixed with 25 cm^3 of methanolic solution of ($ZnCl_2$). A mixture of 1:2 ratio (metal ions: SMZ) was heated under reflux and continuous stirring at 60–70^oC for about 3hr. The mixtures were then cool to room temperature and left overnight until precipitation occurred. The precipitates obtained were filtered off and washed by methanol then left over anhydrous calcium chloride.

SYNTHESIS OF IRON COMPLEXES

4.0 mmol of SMZ ligand was dissolved in 25 cm^3 methanol then mixed with 25 cm^3 of methanolic solution of ($FeCl_2{\cdot}4H_2O$). A mixture of 1:2 ratio (metal ions: SMZ) was heated under reflux and continuous stirring at 60–70^oC for about 3hr. The mixtures were then cool to room temperature and left overnight until precipitation occurred. The precipitates obtained were filtered off and washed by methanol then left over anhydrous calcium chloride.



Scheme 1: General Synthesis way for the complexes

CHARACTERIZATION

A rising Thin Layer Chromatography was ride on TLC plate, to check the clarity of the synthesized compounds and to monitor the development of the reactions (Morrison & Boyd, 2004). Sport of the compounds, were detected by exposure to iodine vapor. Chromatograms were eluted by Methanol : Petroleum ether (6:4).
Table 1: The melting points were determined on electrothermal melting point apparatus; the solid sample was crushed into a powder using glass rod for the complexes.
The electrical conductances were measured on HI9835 model of Hanna instrument EC/TDS/NaCl meter at Biochemistry Department Gombe State University.

Table 2: The following solvent were used in solubility test: n-Hexane, Dimethylsulphoxide, Petroleum Ether, Ethanol and Distilled water. 6 mg of the ligand and the complexes were dissolves in 2 cm^3 different solvent at room temperature and at elevated temperature separately and checked the solubility.
Table 3: Infrared were recorded as a KBr picture using FTIR model M530 Buck Sci. USA in the range 4000-500 cm^{-1}.

Table 4: Antimicrobial susceptibility tests were carried out using agar diffusion technique. The surface of Muller Hinton's agar in a Petri dish was inoculated uniformly with 0.3 cm^3 of 18hr old test bacteria cultures. 4 mg/cm^3 solution of each complex in DMSO was added to a 9 mm well bore hole into the agar. The plates were allowed to stand on the bench for 30 minutes after inoculation before incubating at 37^oC for 24hr. Inhibitory zones diameter were taken as a measure of antibacterial activities of the complexes (Osowole, wakil, & Okediran, 2015). The experiments were conducted in duplicates.

RESULT

Table 1: Physical properties and electrical conductivity of the ligand and its metal (II) Complexes

S/N	Sample	Melting point (^oC)	Electrical Conductivity (μScm^{-1})	Color	Texture	% yield
1	Sulfamethoxazole	169	7.1	White	Powder	-
2	Zinc complex	135	15.8	White	Powder	69%
3	Iron complex	148	24.2	Brown	Crystal	58%

Table 2: Result of Solubility Tests of the Ligands and of Their Metal (Il) Complexes In Some Solvents

S/N	SAMPLE	ETHANOL		WATER		n-HEXANE		PET. ETHER		DMSO	
		RT	ET	RT	ET	RT	ET	RT	ET	RT	ET
1	Sulfamethoxazole	S	VS	IS	IS	VS	VS	VS	VS	S	VS
2	Zinc Complex	SS	S	IS	IS	VS	VS	S	S	S	VS
3	Iron Complex	IS	SS	IS	IS	VS	VS	VS	VS	S	VS

DMSO = Dimethylsulphoxide; RT = Room Temperature; ET = Elevated Temperature; S = Soluble; IS = Insoluble; SS = Sparingly Soluble; VS = Very Soluble

Table 3: IR Spectra of the ligand and of its complexes in cm^{-1}

Compounds	NH_2	NH	C=C	C=N	Isoxazole Ring	SO_2	M-N	M-Cl
SMZ	3470	3112	1655	1510	1458	1337	-	-
	3317		1633		1423	1127		
Fe-SMZ	3445	-	1657	-	1423	1343	431	396
	3339		1639			1128		367
Zn-SMZ	-	-	1628	1508	1460	1335	441	398
			1620		1421	1125		372

Table 4: Antibacterial Studies Result

S/N	Sample/microorganism	Sulfamethoxazole	Zinc complex	Iron complex
1	*E. Coli*	10	18	17
2	*Staphylococcus aerreus*	00	03	05
3	*Klebsiella pneumoniae*	07	10	12
4	*Mycobacterium leprae*	04	06	09

Where 00: absence of measurable inhibitory action, < 9: Weak, 9-16: Moderate, >16: Significant

DISCUSSION

The melting points of the compounds are recorded in Table 1. The melting point range between 135-169◦C. the melting point of the ligand were higher than that of the metal complexes. The differences in melting points can be as a result of their different structural arrangements and bond strengths within the compounds.

The colours of the synthesized compound were recorded in Table 1. The zinc complex of sulfamethoxazole was found to be white in colour and that of the iron complex of sulfamethoxazole is brown. The colours of these complexes are as a result of either d→d transition, charge transfer transitions, or imperfection in the crystal structure of the compound.

The textures of the compound were presented in Table 1. The ligand and the zinc complex where found to be in powdery formed. The complexes were found to be in crystal formed.

Table 1 present the result of the percentage yield of the synthesis compound. The zinc complex has the highest percentage yields of 69% this may be due to coordination position and the iron complex has 58% yields.

The electrical conductivity of the ligand and the metal complexes were recorded in Table 1. The electrical conductivity was in the range of 7.1-24.2 μScm^{-1}. The low electrical conductivity values indicate that the compounds are non-electrolyte (Cotton *et al*., 1990). Hence this shows that there is no chloride ion outside the coordination sphere in all the complexes.

The results of the tests of solubilities of the compounds in several solvents are recorded in Table 2. The solubilities of the compounds were considered in the following solvents both at room and elevated temperatures; distilled water, methanol, ethanol, petroleum ether, n-Hexane and dimethylsulphuroxide. The ligand and the complexes are all insoluble in water at both room temperature and elevated temperature. the ligand and the metal complexes were both soluble in n-Hexane, DMSO and petroleum ether. The ligand is soluble in both methanol and ethanol at room temperature and very soluble in elevated temperature. The zinc complex is sparingly soluble in methanol and ethanol at room temperature and

soluble at elevated temperature. The iron complexes were insoluble in methanol and ethanol at room temperature and sparingly soluble in elevated temperature.

The sulfamethoxazole ligand behaves as a bidentate ligand and coordinates to the metal ion with different point of chelation group. The IR spectral of the ligand shows two strong's absorption at 3470 cm^{-1} and 3317 cm^{-1} were assigned to asymmetric and symmetric NH_2 group (Mistry, 2009). The absence of this band in the Zn complex shows that NH_2 group might have been deprotonated on metal coordination. The medium strong bands that appear in 3112 cm^{-1} were assigned to NH group of the sulfanilamide. The absence of vibrational band at 3112 cm^{-1} (due to sulfonamide NH stretching) in the infrared spectra of all the metal complexes indicates that sulfonamide NH group might have been deprotonated on metal coordination. The absence of infrared peak at 1510 cm^{-1} and 1458 cm^{-1} in the case of Iron (II) complex is a favourable indication of metal binding possibly through isoxazole ring nitrogen (Mistry, 2009).

Two strong peaks corresponding to asymmetric and symmetric stretching vibrations of the sulfonyl group were observed at 1337 cm^{-1} and 1127 cm^{-1}, respectively in the spectrum of sulfamethoxazole. The infrared peaks at 1655 cm^{-1} and 1633 cm^{-1} in the spectrum of sulfamethoxazole could possibly be due to phenyl ring C=C stretchings. In addition, there is a strong absorption at 1090 cm^{-1} probably due to aromatic C-H in-plane bending vibration. These vibrational bands virtually remain un-shifted in the case of all metal complexes (Mistry, 2009). Furthermore, bands due to M-N, and M-Cl were absent in the sulfamethoxazole. However, these bands were observed at 441-431 cm^{-1} and 398-367 cm^{-1} respectively in the metal complexes confirming coordination (Nakamoto, 1978).

The inhibitory zones of the activities of various compounds at 4mg/cm^3 concentration where measured against the bacteria as presented in Table 4. Sulfamethoxazole ligands were moderately active against the tested bacteria with inhibitory zone of 4-10 mm, except *Staphylococcus aerreus* which shows no activity with the ligand. The Fe(II) and Zn(II) shows great activity with *E. coli* with inhibitory zone greater than greater than 16 mm, both of them shows weak activity with *Staphylococcus aerreus* with inhibitory zone of less than 6 mm and moderate activity with *Klebsiella pneumoniae* with inhibition zone between 10-12 mm. the zinc complex shows weak activity with *M. leprae* with inhibitory zone of 6mm. the iron complex shows moderate activity with *M. leprae* with inhibitory zone of 9 mm. The metal complexes were generally more active than the ligands due to chelation, which reduced the polarity of the metal atom and subsequently increased lipophilic character, favoring its permeation through lipid layers of the bacterial membrane (Osowole, wakil, & Okediran, 2015).

CONCLUSION

Sulfamethoxazole complexes were synthesis by reacting it with a metal salt in the ratio of 1:2 (metal-ligand ratio) progresses and purity of the reaction were determined using thin layer chromatographic technique, the melting point, solubility

and conductivity as well as characterization using FTIR spectroscopic of the synthesis complexes were done. It is found that the ligand is bound to the metal through either NH_2, NH or isoxazole ring. The antibacterial activity studies were done using agar well diffusion technique and the result shows that the complexes have higher antibacterial activity than the free ligand.

The reactions of sulfamethoxazole with metal salts under reflux yielded metal complexes of 1:2 types. The structural investigation on metal-sulfamethoxazole complexes by means of various physico-chemical methods of analysis has thrown light on the nature of interaction between sulfamethoxazole and the metal ions under in vitro experimental conditions. The complexes where found to be biologically active and they exhibit enhanced antimicrobial activity than the free ligand.

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