

Sustainable Health in View: Comparison of Antibacterial Activity of Oxine-Hydrazine Mixed Ligand to the Cobalt (II) Complex of the Mixed Ligand and Standards

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ABSTRACT

Mixed ligand and metal complex of Cobalt(II) of the mixed ligand was prepared and compared. The complex formed is of type ML_2L_2 (where $M=Co(II)$, $L_1=Oxine$ and $L_2=Hydrazine$). The ligand and the complex were characterized using Physical and Spectroscopic methods. The physical analysis carried out are Appearance, Solubility, Melting point and Conductivity; the Spectroscopic analysis are Atomic Absorption Spectra(AAS), Ultraviolet/Visible Spectra(Uv/Vis) and Infrared Spectra(IR). The antibacterial activity of the mixed ligand and Cobalt(Co) complex were carried out. Ligand and metal complex exhibited potent activity against antibacterial compared to standards used. The compound advances the knowledge in pharmacological research. The results of the research have potentials for advancing the standard of human health in a time when humans are more prone to ill health incidences largely cause by bacteria and other pathogens. Further work will be done to prove the potency of the complex.

Keywords: Bioactive ligand; Metal complex; Antibacterial activity; Complexation; Characterization; Synthesis.

INTRODUCTION

Sequel to the outbreak of infectious diseases caused by different pathogenic bacteria and the development of resistance to common antibiotics, researchers are searching for new antibacterial agents. Therefore, new antimicrobial agents and nano-technological materials have to be synthesized for the treatment of resistant bacterial diseases. Many of the natural drugs still originate from wild growing materials. However, plant-based drugs have shortened the life span of these sources of material. Research continues on the possibility of more potent and cheaper raw materials to feed the industry. For this reason, pharmaceutical industries look forward to synthesizing alternative compounds acting as drugs (Saha *et al.*, 2010: 225). Metal ions play a vital role in a vast number of widely different biological processes. The interaction of these ions with biologically active ligands, for example in drugs, is a subject of considerable interest. There has been a tremendous growth of drugs from quinoline family, which began with the discovery of nalidixic acid some 40 years ago. Since then, the exponential growth of this family has produced more than ten thousand analogues.

The complexation of metallic elements with biologically inactive compounds renders them active; and in cases where the compounds are already active, making them more active. There is need to further investigate the mechanism involved in enhancing this biological activity upon complexation (Patel *et al.*, 2013: 55).

2. RELATED LITERATURE

Over the past few decades, scientists have shown interest in the mixed ligand complexes of dibasic acids and amine bases because most of them are potentially biologically active and mixed-ligand complexes of metal(II) containing nitrogen, oxygen and sulphur donor ligands due to their diverse biological activities including being antifungal, antibacterial, anti-inflammatory, antipyretic, herbicidal and anticancer, and antiulcer activities (Arya *et al.*, 2010: 253).

Recently due to the constant emergence of antibiotics resistance to clinically used compounds, there is need to develop novel antibiotics which eventually would target the lipid layer of the organisms and other aspects of pathogens' life cycle. Metal complexes may be subjected for the design and synthesis of such possibilities having such biological activities (Chohan *et al.*, 2005: 463). The synthesized chemical compounds, which are used for the treatment of infectious diseases, are known as chemotherapeutic agents and they also play an important role in the activation of enzymes, and are used for storage as well as for transport of active materials. Every year, researchers synthesise thousands of compounds to find out potential chemotherapeutic agents to combat pathogenic microorganisms. In this regard, heterocyclic bases have great importance in biological and industrial fields. Most of them are used as corrosion inhibitors and their complexes with platinum and copper tested as antitumour and antibacterial properties. Mixed ligand complexes of some metal ions containing different dibasic acids as malonic acid (Reza *et al.*, 2003: 1314), maleic acid (Islam *et al.*, 2003: 289), and phthalic acid (Reza *et al.*, 2003: 1494) with some heterocyclic bases have been reported in some recent communications.

It is well known that mixed ligand ternary complexes of some metals play an important role in the activation of enzymes. It is studied that mixed ligand complexes are biologically active against pathogenic microorganisms (Shivankar and Thakkar, 2003: 45). It becomes clear why the number of publications on the experimental study, theoretical generalization, and practical use of mixed ligand complexes increases at such speed. At the present time, there are large numbers of physicochemical methods for the identification of mixed ligand complexes in solutions.

There is substantial research on mixed ligand- cobalt(II) complex. Taghreed *et al.*, carried out a synthesis, spectral and antimicrobial activity of mixed ligand complexes of Co(II), Ni(II), Cu(II) and Zn(II) with Anthranillic Acid and Tributylphosphine in 2010, Agwara *et al.*, in 2010 work on synthesis, characterisation and antimicrobial activities of cobalt (II), copper(II) and zinc(II) mixed ligand complexes of 1, 10-phenanthroline and 2,2'-Bipyridine. Stanila *et al.* reported about Antibacterial Activity of Copper and Cobalt Amino Acids Complexes in 2011. Chohan, *et al.* reported metal-based antibacterial and antifungal agents, synthesis, characterization, and in vitro biological evaluation of Co(II), Cu(II), Ni(II), and Zn(II) Complexes With Amino Acid-Derived Compounds was reported in 2006. Halli *et al.* reported on Synthesis, characterization, and biological activity studies of (E)-N-((thiophen-2-yl)methylene)benzofuran- 2-carbohydrazide and its metal(II) complexes in 2011.

Furthermore, Patil *et al.* reported the synthesis, characterization, and antibacterial studies of mixed ligand dioxouranium complexes with 8-Hydroxyquinoline and some amino acids in 2011. Again, Patel *et al.* in 2013 reported the metal complexes of 5 [(benzyloxy) methyl], quinolin-8-ol (BeMQ), and 8-quinolinols mixed ligand: a new transition metal complexes with in-vitro antifungal activity.

Despite this rich research, work that involves Co(II) complex of oxine-hydrazine is scanty. This is the gap the instant research attempts to fill. It acknowledges that scientists have found mixed ligand complexes to be acting as an active catalyst in reactions of industrial importance including hydrogenation, hydroformation, and oxidative hydrolysis of olefins and carboxylation of methanol.

Developments in the field of coordination chemistry which is closely bound up with the study of mixed polynuclear complexes have been very extensive in recent years.

The study of mixed ligand complex formation is relevant in the field of analytical chemistry, where the use of mixed ligand complexes allows the development of methods with increased selectivity and sensitivity. They have also great importance in the field of biological and environmental chemistry (Raman *et al.*, 2003: 161). These facts prompted me to synthesize new mixed ligand transition metal complexes, especially biologically important cobalt complex, to study the combined antimicrobial activity effect of mixed ligand in conjugation with the metal ion.

3. RESEARCH DIRECTION AND METHODOLOGY

3.1 Research Direction

The main objective of the present study is to communicate the synthesis, characterization and antimicrobial activity of Cobalt (II) complex with oxine-hydrazine mixed ligand.

3.2 Research Methodology

- ❖ Synthesis: Method used by Singh and Singh, 2012 was used to synthesize the ligand and the complex.
- ❖ Physical characterization of the mixed ligand and the Cobalt(II) complex
- ❖ Solubility Test

The solubility of the metal complex was determined in cold and hot using various polar solvents such as distilled water, dilute HCl, methanol, ethanol, acetone, and non-polar solvents such as benzene, Petroleum ether and di ethyl ether; 10 mg of metal complex was taken and dissolved into 2 ml of corresponding solvent, and then I checked the solubility.

3.3 Colour and Nature determination

Colour and nature of the ligand and the metal complex was determined by the visual observation and the result was noted.

3.4 Melting point determination

The melting point was determined using a capillary tube blocked in one end. I fitted it with thermometer. The ligand and the complex were introduced into the open end of the tube which was attached to a thermometer dipped into the paraffin oil in a beaker. The beaker was heated and the temperature was noted when the ligand and the metal complex became melted to a clear liquid.

Spectrophotometric of the mixed ligand and the Cobalt(II) complex

Ultraviolet/Visible Spectrum

The ultraviolet/visible spectral measurement of the ligand and metal complex between 190-900nm was obtained by using J. P. Selecta Uv/Vis spectrophotometer at the University of Ibadan, Nigeria.

Infra/Red Spectrum

The metal complexes were subjected to IR spectral analysis. IR spectrum was recorded on an AVATAR 330 Fourier transform Infrared Spectrophotometer.

3.5 Antibacterial activity

Source of microorganism: The organisms used were Gram-negative *P. aeruginosa*, *E. coli* and Gram-positive *S. aureus*, *K. pneumonia* as bacteria for testing antibacterial activity, were collected from various hospitals in Lagos, Nigeria.

Antibacterial activities of the ligand and the cobalt complex were measured against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* using a well diffusion method according to the National Committee for Clinical Laboratory Standard (NCCLS) in 1993 as reported by Saha *et al.* 2010.

The results obtained were noted in table 5 and 6.

4. RESULTS AND DISCUSSION

The results obtained for percentage yield of the product, the melting point/decomposition temperature, colour appearance of the product and conductivity measurements are presented in table 1 and 2. I present the result of the solubility tests carried out on the product in table 3.

Table 1-Yield/Percentage yield

SAMPLE	YIELD(g)	PERCENTAGE YIELD(%)
Oxine-Hydrazine mixed Ligand	0.5391	54
Co complex of the Ligand	0.5192	52

Table 2-Physico-Chemical Characterization

CHARACTER	MIXED LIGAND	COBALT COMPLEX
Chemical Formular	$C_9H_{10}N_3O$	$(C_{18}H_{16}CoN_6O_2)^{2+}$
Molar weght	175.19	407.29
Exact mass	175.07	407.07
Colour	Creamy White	Pink
Appearance	Crystalline powder	Powdery
Melting point	184-216°C	204-212°C
Conductivity	1789microS/cm	1773microS/cm

Table 3-Solubility

SOLVENT	MIXED LIGAND		COBALT COMPLEX	
	Cold	Hot	Cold	Hot
Distilled Water	Soluble	Soluble	Soluble	Soluble
Dillute Hcl	Soluble	Soluble	Sparingly soluble	Soluble
Petroleum Ether	Insoluble	Insoluble	Insoluble	Sparingly soluble
Acetone	Insoluble	Insoluble	Sparingly soluble	Insoluble
Ethanol	Sparingly soluble	Soluble	Sparingly soluble	Soluble
Methanol	Sparingly soluble	Soluble	Sparingly soluble	Soluble
Benzene	Insoluble	Insoluble	Insoluble	Insoluble
Diethyl Ether	Insoluble	Insoluble	Insoluble	Insoluble

Table 4: Metal Content analysis

Metal Complex	Mg/L
Cobalt Complex	490.01

Physico-Chemical Properties

The mixed ligand by refluxing an appropriate amount of oxine with Hydrazinium sulphate in Methanol. I prepared metal complex was prepared by using the metal salts as chloride (Cobalt chloride hexahydrate) with oxine and Hydrazinium sulphate mixed ligand in the molar ratios of metal: ligand 1: ligand 2 as 1: 1: 1. The mixed ligand and the complex were characterized by some experimental techniques viz: elemental analysis, melting point, conductance and spectroscopic methods. The infrared spectrum of the complex confirms the coordination of metal ion with ligand.

The percentage yield of the Mixed ligand is 54% while for Cobalt complex is 52% (Table 1). The Mixed ligand is white while the complex is pink in colour, non-hygroscopic, thermally stable solids, indicating a strong metal-ligand bond. The molar conductance values of the Mixed ligand and the complex in water were found to be 1789 and 1773 in microS/cm indicating their non-electrolytic nature (Table 2 and 3).

Solubility and Melting point

The mixed ligand and the metal complex was dissolved in all polar solvent such as distilled water, dilute Hydrochloric acid, methanol, ethanol, acetone, petroleum ether and non-polar solvents such as benzene and di ethyl ether. The ligand is insoluble in petroleum ether, acetone, benzene and diethyl ether, sparingly soluble in ethanol and methanol but soluble in distilled water and dillute HCl, and the complex is insoluble in solvents such as petroleum ether, acetone, benzene and diethyl ether, sparingly soluble in ethanol, dilute HCl and methanol, soluble in distilled water. (Table 3).

The mixed ligand melted between 184-216°C and the metal complex melted between 204-212°C respectively (Table 2).

Uv/Vis Absorption Spectroscopy

The electronic spectra of the mixed ligand and the metal complex were recorded in the UV-visible region. The result has supported the presence of cobalt molecule in the ligand metal complex, as cobalt molecule shows the highest absorption in the range between 200 to 300 nm wavelengths. The spectra of the mixed ligand show transition at 240nm ascribed to $n \rightarrow \pi^*$ while spectra of the complex shows transition at 255nm and 290nm ascribed to $\pi \rightarrow \pi^*$. A negative shift in this vibrational mode on complexation indicates the coordination through ternary nitrogen donor of HQ. For the present study, the frequency of first two bands was in range of 255-290 nm. Hence by comparing with the literature values, these transitions observed in the UV spectrum of complex were assigned for $\pi \rightarrow \pi^*$ transition for the aromatic chromophore in the complex while $n \rightarrow \pi^*$ transition was assigned to 240nm in the ligand.

Fourier Transform-IR Spectroscopy

The FT-IR spectra of the mixed ligand and the metal complex were recorded over the range 4000– 400 cm^{-1} . On the basis of the reported infrared spectra of Hydrazine, 8-hydroxyquinoline, and its metal complex, some of the important bands have been assigned. The IR spectrum of the mixed ligand was compared with the spectra of the metal complex. The IR spectra of the complex have been compared with those of the free ligand in order to determine the coordination sites that may get involved in chelation.

The research observed a broad band in 3446 cm^{-1} due to asymmetric and symmetric O-H stretching modes in the complex which is absent in the mixed ligand. The $\nu(\text{CO})$ band is observed at 1120 cm^{-1} in the complex which appear in 1057 cm^{-1} on the spectrum of the mixed ligand. The position of this band undergoes variation depending on metal complex under study. A strong $\nu(\text{CO})$ band observed in the 1074 cm^{-1} indicates the presence of oxine moiety in the complexes coordinated through its nitrogen and oxygen atoms as uninegative bidentate ligand.

The $\nu(\text{C-N})$ mode aobserved at 1018 cm^{-1} in the mixed ligand shifted to 1317 cm^{-1} in the metal complex. Also the $\nu(\text{C=N})$ mode observed at 1558 cm^{-1} in the spectra of the mixed ligand is found to be shifted to lower wave number, in the range of 1500 cm^{-1} in the spectra of the complex, indicating coordination of nitrogen towards the metal ion (M-N). A new band at 422 cm^{-1} due to $\nu(\text{M-N})$ is further confirmed the coordination of metal to nitrogen and band at 484 cm^{-1} due to $\nu(\text{M-O})$ and also 609 cm^{-1} due to $\nu(\text{M-O-C})$. . A negative shift in this vibrational mode on complexation indicates the coordination through ternary nitrogen donor of HQ. The in-plane and out-of-plane ring deformation modes observed at 484 cm^{-1} and 609 cm^{-1} respectively, confirm coordination through nitrogen atom of HQ with the metal.

The other band observed in 2924 cm^{-1} and 2816 cm^{-1} were assigned for -NH asymmetric and -NH symmetric stretching vibrations of -NH² group in mixed ligand are shifted to higher wave numbers, in 3072 cm^{-1} and 2939 cm^{-1} respectively, in the spectra of metal complex, suggesting coordination of the mixed ligand through nitrogen with the metal ion. The other bands in the spectrum of cobalt complex were due to the carbon and hydrogen bonding.

Atomic Absorption Spectrometry

The AAS result reads 490.01mg/L which shows significance presence of cobalt metal in the complex as given in table 4.

5. RESULTS AND DISCUSSION OF ANTIBACTERIAL STUDIES

The result of the antibacterial activity of the mixed ligand, complex and standards are presented below;

TABLE 5: Antibacterial activity

Zone of Inhibition(mm)

Name of Bacterial Pathogen	Mixed Ligand			Co Complex			Nft	Ofl
	5mg/L	10mg/L	15mg/L	5mg/L	10mg/L	15mg/L	200µg	5µg
<i>Pseudomonas aeruginosa</i>	30	30	30	30	30	30	25	20
<i>Klebsiella pneumonia</i>	30	30	30	30	30	30	20	30
<i>Escherichia coli</i>	20	20	30	18	18	20	20	20
<i>Staphylococcus aureus</i>	30	30	30	30	30	30	20	25

Key note: 0-9 mm = no inhibition; 10-15 mm = moderate inhibition and ≥ 15 mm = high inhibition
Nft=Nitrofurantoin, Ofl= Ofloxacin

TABLE 6: ZONES OF STANDARDS INHIBITION SHOWING THE ANTIBACTERIAL PROPERTIES OF STANDARDS

Organism	E. Coli	K.pneumoniae	P. aeruginosa	S. aureus
Concentration (µgml-1)	1000 MIC	1000 MIC	1000 MIC	1000 MIC
Augmentin	-	7	10	-
Ofloxacin	20	20	25	30
Gentamycin	17	15	25	30
Nalidixic acid	17	16	20	25
Nitrofurantoin	-	20	20	20
Amoxicillin	-	10	15	-
Tetracycline	16	15	30	30

I studied the antibacterial activity of the mixed ligand and the complex. Generally, the ligands and the metal complex showed antibacterial effect against the tested organism species as presented in the figures above. The tested chemical compounds possessed variable antibacterial activity against gram positive *Staphylococcus aureus*, *Klebsiella pneumonia* and gram negative *Pseudomonas aeruginosa*, and do not show distinct antibacterial activity against gram negative *Escherichia coli*. On the basis of zone of inhibition produced against the test bacterium, I find both the mixed ligand $C_9H_9N_3O$ and complex $C_{18}H_{16}CoN_6O_2^{2+}$ to be most effective against the bacteria with zone of inhibition ranging between 18 mm and 30 mm. This could be due to the different conditions under which the studies were carried out. Both the Mixed ligand and the complex shows more effective antibacterial activity compared to standards.

6. IMPLICATION TO RESEARCH

Cobalt metal complex was synthesized and physico chemically characterized by solubility testing, melting point, UV-spectra, and FTIR. I evaluated the antimicrobial analysis of cobalt metal complex among the different bacterial strains. I obtained the complex as coloured powdered material. Based on these results, one can draw the conclusions that follows.

Electrical conductance studies show non-electrolytic nature of the complex showing the anions is coordinated to the central metal ion. Electronic absorption spectra of the complexes show intraligand and charge transfer transitions, respectively. IR spectra show bonding of the metal ion through N- and O- donor atoms of the mixed ligands. On the basis of elemental analyses, IR, Uv-visible reflectance spectra, it is possible to assign tetrahedral geometry to the Co(II) because coordination number eight is proposed for cobalt complex.

The results of biocidal activities of the mixed ligand and its metal complex clearly show that the compounds have antibacterial potency against the tested organisms. The antibacterial studies suggested that the mixed ligand were found to be biologically active as well as the metal complex against microbial strains as compared to standards. Thus, exhibiting their broad spectrum nature can be further used in pharmaceutical industry for mankind, as an antimicrobial agent, after testing its toxicity to human beings.

7. CONCLUSION

Co(II) complexes have shown promising antibacterial activity against *P. aeruginosa*, *K. pneumonia*, *E. coli* and *S. aureus*. The antimicrobial activity is explored on the basis of overtone concept of cell permeability. The present study reveals that cobalt complex presents a better result for managing bacterial diseases. This result will advance human health, and make the standard of living of humanity better than it currently is, after evaluating the in vitro effect of metal complex on experimental animal and clinical trials. Future research should fill some gaps this study has exposed. I recommend that further analysis on NMR, X-ray should be carried out in furtherance of my findings in order to confirm the proposed structures of . Researchers should also address issues of toxicity to determine the margin of safety that the drug that my research may produce has. I am optimistic that future studies on biological properties complex of 8-hydroxyquinoline, hydrazine, cobalt (II) and their derivatives may lead to the development of a new class of specific and effective pharmaceutical agents

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