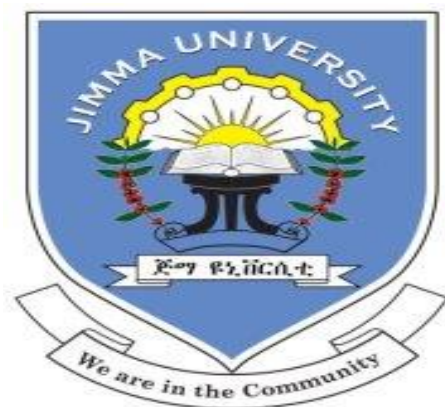


**JIMMA UNIVERSITY  
COLLEGE OF NATURAL SCIENCE  
SCHOOL OF GRADUATE STUDIES  
DEPARTMENT OF CHEMISTRY**



**SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL ACTIVITY OF  
HEXAMETHYLENETETRAMINE COPPER (II) COMPLEX**

**BY**

**BERHANU DEGAGSA DANDENA**

**JUNE, 2013**

**JIMMA, ETHIOPIA**

**SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL ACTIVITY OF  
HEXAMETHYLENETETRAMINE COPPER (II) COMPLEX**

**BY**

**BERHANU DEGAGSA DANDENA**

**Advisor: Prof. Nester Fernandez (PhD)**

**Co-Advisor: Gezahegn Faye (M.Sc)**

**A Thesis Submitted to school of graduate studies, Jimma University, in partial  
fulfillments of the Requirements for the Degree of Masters of Science in  
chemistry**

**JUNE, 2013**

**JIMMA, ETHIOPIA**

**Jimma University**  
**School of Graduate Studies**  
**Department of Chemistry**

**Synthesis, Characterization and Biological Activity of Hexamethylenetetramine Copper  
(II) Complex**

**BY**

**Berhanu Degagsa Dandena**

A Thesis Submitted to school of graduate studies, Jimma University, in partial fulfillments of the Requirements for the Degree of Masters of Science in chemistry

Approved by board of Examiners

External Examiner

Signature

Date

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

Internal Examiner

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

Advisors

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

Department Head

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

## Table of Contents

|  |     |
|--|-----|
| Table of contents.....                                   | i   |
| Acknowledgement .....                                    | v   |
| Abstract.....  | vi  |
| Abbreviations.....                                       | vii |
| 1. Introduction .....                                    | 1   |
| 1.1. Objectives of the study.....                        | 3   |
| 1.1.1. General Objective .....                           | 3   |
| 1.1.2 Specific Objectives .....                          | 3   |
| 1.2. Statement of the problem.....                       | 3   |
| 2. Literature Review.....                                | 4   |
| 2.1. Structural characterization techniques .....        | 5   |
| 2.1.1. Magnetic measurements .....                       | 5   |
| 2.1.2. Electronic spectroscopy.....                      | 6   |
| 2.1.3. Infrared spectroscopy .....                       | 6   |
| 3. Experimental section.....                             | 8   |
| 3.1. Chemicals .....                                     | 8   |
| 3.2. Physical Measurements .....                         | 8   |
| 3.3. Synthesis of the Complex.....                       | 8   |
| 3.4. Gravimetric analysis for sulphate.....              | 8   |
| 3.5. Volumetric analysis for hexamethylenetetramine..... | 9   |
| 3.6. Spectrophotometric analysis for copper.....         | 9   |
| 3.7. Antimicrobial Test.....                             | 9   |
| 4. Results and discussion .....                          | 11  |

|   |    |
|---|----|
| 4.1. Colour, Melting point and Solubility .....                                       | 11 |
| 4.2. Analysis of a hexamethylenetetramine copper (II) complex .....                   | 11 |
| 4.2.1. Gravimetric Analysis for Sulfate .....   | 12 |
| 4.2.2. Volumetric Analysis for hexamethylenetetramine .....                           | 12 |
| 4.2.3. Spectrophotometric Analysis for Copper .....                                   | 13 |
| 4.2.4. Calculation of the Formula of the Coordination Compound .....                  | 14 |
| 4.3. Conductivity of the complex .....  | 15 |
| 4.4. Magnetic measurement .....   | 16 |
| 4.5. Electronic spectra of the salt and complex .....                                 | 18 |
| 4.6. IR spectra of the ligand and complex .....                                       | 20 |
| 4.7. Structure of $[\text{Cu}(\text{HMTA})_3(\text{H}_2\text{O})_3]\text{SO}_4$ ..... | 22 |
| 4.8. Antibacterial Tests .....  | 24 |
| 5. Conclusion and Recommendation .....  | 25 |
| 6. References .....   | 26 |

## List of tables

|  | Page |
|--|------|
| <b>Table 1:</b> Colour and Melting point of the salt, ligand and complex.....  | 11   |
| <b>Table 2:</b> Experimental data for the analysis of sulphate ion.....  | 12   |
| <b>Table 3:</b> Experimental data for the analysis of HMTA.....  | 12   |
| <b>Table 4:</b> Concentration for standard solution.....   | 13   |
| <b>Table 5:</b> Spectrophotometric Data: Absorbance at 645 nm.....   | 13   |
| <b>Table 6:</b> Mass percent of copper in the sample.....  | 14   |
| <b>Table 7:</b> Mass and number of mole of $\text{Cu}^{2+}$ , $\text{SO}_4^{2-}$ and $\text{C}_6\text{H}_{12}\text{N}_4$ .....                                   | 14   |
| <b>Table 8:</b> Comparing the experimental value with the calculated one for $\text{Cu}^{2+}$ , $\text{SO}_4^{2-}$ and $\text{C}_6\text{H}_{12}\text{N}_4$ ..... | 15   |
| <b>Table 9:</b> Calculation for the Percent Yield.....   | 15   |
| <b>Table 10:</b> Electronic spectra of the salt and complex.....   | 18   |
| <b>Table 11:</b> Selected IR Absorption Bands ( $\text{cm}^{-1}$ ) of HMTA and its Complex.....  | 20   |
| <b>Table 12:</b> Vibration characteristic of free sulphate ion.....  | 21   |
| <b>Table 13:</b> Characteristic IR bands for the HMTA and complex.....   | 22   |
| <b>Table 14:</b> Antimicrobial Activities of the salt, ligand and complex.....   | 24   |

## List of figures

|   | <b>Page</b> |
|---|-------------|
| <b>Figure 1.</b> Structure of hexamethylenetetramine.....                   | 2           |
| <b>Figure 2.</b> Calibration plot.....                                      | 14          |
| <b>Figure 3(a).</b> UV-vis spectra of the salt.....                         | 19          |
| <b>Figure 3(b).</b> UV-vis spectra of the complex.....                      | 19          |
| <b>Figure 4(a).</b> IR-spectra of the hexamethylenetetramine.....           | 22          |
| <b>Figure 4(b).</b> IR-spectra of the complex.....                          | 22          |
| <b>Figure 5.</b> Vibration characteristic of free sulphate.....             | 23          |
| <b>Figure 6.</b> Proposed molecular structure for the obtained complex..... | 25          |

## **Acknowledgements**

All praises for the most omnipotent, omnipresent and omniscient AL-MIGHTY GOD who blessed me to accomplish my M.Sc project work.

Any sense of gratitude will be incomplete without giving glory and words of thanks to my research supervisors. So I would like to pay my heartiest gratitude to my research supervisor Prof. Nestor Fernandez (PhD) and my co-advisor Mr. Gezahegn Faye (M.Sc) for their expert guidance and praiseworthy help, keen interest, attention, willingness to help me whenever I was in need of his help, his communicative enthusiasm for chemistry, continuous encouragement during the period of this research work.

Thanks are due to Department of Chemistry, Jimma University for providing laboratory facilities for conducting my research.

It is my pleasure to thankfully acknowledge Mr. Shimalis Addisu for following, helping and strengthening my research work. I would also express sincere thanks to all staff members of spectroscopy laboratory, Department of Chemistry Addis Ababa University.

On the completion of this work, which certainly cannot be stated as a single man's task, I would like to pay my sincere sense of gratitude to Chemistry lecturers laboratory staff members at the Department of Chemistry whose cooperation helped me do my work very well. I also express my deepest gratitude and appreciation to the Department of Biology, Jimma University, for providing me bacterial test strains and lab facilities to carry out antibacterial test.

Finally, I am also thankful to Jimma University for providing financial support.



**Abstract**

[Cu(C<sub>6</sub>H<sub>12</sub>N<sub>4</sub>)<sub>3</sub>(H<sub>2</sub>O)<sub>3</sub>]SO<sub>4</sub> has been synthesized in water and ethanol. The complexes have been characterized by Magnetic measurement, Atomic absorption, infrared and visible spectroscopy as well as conductivity. The results suggest octahedral coordination in which the central metal ion is bonded to aqua ligands and the HMTA. Antibacterial activities of the ligand showed that the ligand is active against *Pseudomonas aeruginosa* and *Salmonella typhi* of *Staphylococcus aureus* (Gram-Positive bacteria), *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi* tested bacteria species but the complex was active against all tested bacteria species. The complex showed greater activity than the reference antibiotic gentamycin against *Salmonella typhi*.

**Keywords:** Hexamethylenetetramine; Antimicrobial; Cu (II).

## **Abbreviations**

|      |                                |
|------|--------------------------------|
| HMTA | Hexamethylenetetramine         |
| AAS  | Atomic Absorption Spectroscopy |
| DMSO | Dimethyl Sulphoxide            |
| 1D   | One Dimensional                |
| 2D   | Two Dimensional                |
| 3D   | Three Dimensional              |

## 1. Introduction

The great variety in properties of the bonds around metals is the beauty of coordination chemistry. This is the reason for the nature to use metals on special places in enzymes for highly specialized jobs and for most industrial syntheses of chemicals to employ metal compound catalysis. [1]

### Ligands or coordinating groups

Coordination complexes are formed by the union of a cation with one or more neutral or charged species. The neutral molecules or ions (usually anions) which are attached with the central ion in complex compounds are called ligands or coordinating groups.

The term *ligand*, was first introduced by Alfred Stock in 1896, in relation to silicon chemistry. According to Lewis, in most of the complex compounds, the ligands act as Lewis bases (electron pair donors) and central metal ion acts as a Lewis acid (electron acceptor), i.e., in most of the complexes, the ligands donate one or more electron pairs to the central metal ion.



The ligands are attached to the central metal ion through their donor atoms. The metallic atom with which the ligands are attached through coordinating bonds is called the central metallic atom. The metallic atom may be in zero, positive or negative oxidation state. Ligands are classified according to the number of donor atoms contained and are known as uni, di, tri, or quadridentate ligands [2].

### Coordination number of central metal atom/ion

Coordination number of the central metal atom in a given complex compound is equal to the total number of donor atoms, which are actually attached with the central metallic atom. Thus for coordination number equal to 2, 3, 4, 5 and 6, the geometry of the complex compound formed is linear, trigonal, planar, tetrahedral or square planar, trigonal bipyramidal and octahedral respectively. Coordination number gives us an idea about the way in which the ligands are arranged around the central metallic atom [2].

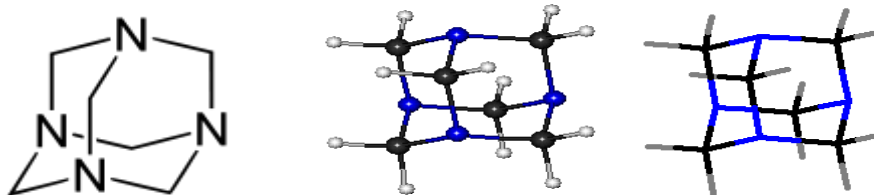
### Coordination sphere and ionization sphere

While writing the structural formula of a given compound, the central metal atom and ligands attached with it are always written in a square bracket, called coordination or inner sphere. The portion outside the coordination sphere is called ionization or outer sphere. Thus in  $[\text{Co}(\text{NH}_3)_5\text{Cl}]\text{Cl}_2$ , the square bracket which contains the central metal ion  $\text{Co}^{3+}$  and the ligands of five ammonia molecules and one chloride ion, is *coordination sphere* and the portion that contains two chloride ions is *ionization sphere* [2].

### Double salts and coordination compounds

Addition compounds are formed when stoichiometric amounts of two or more stable compounds join together. Addition compounds are of two types: those which lose their identity in solution (double salts) and those which retain their identity in solution (complexes). For example if  $[\text{Cu}(\text{H}_2\text{O})_2(\text{NH}_3)_4]^{2+}$  and  $[\text{Fe}(\text{CN})_6]^{4-}$  dissolve, they do not form simple ions-  $\text{Cu}^{2+}$  or  $\text{Fe}^{2+}$  but instead their complex ions remain intact. Thus the ions exist as distinct entities both in solid and solution. Complex ions are shown by the use of square brackets. Compounds containing these ions are called coordination compounds.

Hexamethylenetetramine (HMTA), also known as hexamine or urotropine, can be considered as one such simple heterocyclic compound with a cage like structure, due to its inexpensiveness, commercial availability, high solubility in water and polar organic solvents, has found a broad variety of applications, ranging from the production of phenol resins and solid fuel tablets to uses in organic synthesis, e.g. plastics, pharmaceuticals, rubber additives. It sublimes in a vacuum at  $280\text{ }^\circ\text{C}$



**Figure 1:** Structure of Hexamethylenetetramine

In this paper, we reported the synthesis, characterization, and antimicrobial activities of hexamethylenetetramine complexes of Cu (II).

## 1.1. Objectives of the study

### 1.1.1. General Objective

- To synthesise and characterize Cu (II) complex of HMTA-ligand.

### 1.1.2 Specific Objectives

- To know the composition of Cu (II) complex with HMTA-ligand
- To characterize the obtained Cu (II) complex by using UV-Vis spectrophotometer, molar conductance, Magnetic measurement, Infrared spectral studies and Atomic absorption spectroscopy.
- To study antimicrobial activity of HMTA and its Cu (II) complex on some selected microorganism.

## 1.2. Statement of the problem

A survey of articles in recent issues of journal *Inorganic chemistry* indicates that perhaps 70% could be considered to deal with coordination compounds. Interdisciplinary research is a hallmark of modern inorganic chemistry, and many chemists today are applying the methods and concepts of modern coordination chemistry to problems in areas such as biochemistry, catalysis, energy conversion, and materials chemistry. The rapidly developing field of bioinorganic chemistry is centered on the presence of coordination compounds in living systems.

Therefore, the new complex, that is,  $[\text{Cu}(\text{C}_6\text{H}_{12}\text{N}_4)_3(\text{H}_2\text{O})_3]\text{SO}_4$  was synthesized for testing antibacterial activities on some selected microorganisms.

## 2. Literature Review

Transition elements have an unparalleled tendency to form coordination compounds with Lewis bases that is with groups which are able to donate an electron pair. These groups are called ligands. A ligand may be a neutral molecule such as  $\text{NH}_3$ , or an ion such as  $\text{Cl}^-$  or  $\text{CN}^-$ . Transition metals have the ability to form complexes. The reason transition elements are so good at forming complexes is that they have small, highly charged ions and have vacant low energy orbital's to accept lone pairs of electrons donated by other groups or ligands.

The study of transition metal ion complexes by magnetic and optical techniques has furnished a considerable body of empirical data, much of which can be understood in terms of the phenomenological ligand field theory [3]. Copper ions play a vital role in a number of biological processes. They are recognized to be plant nutrients and serve as catalysts [4] in plant enzyme systems and as urease inhibitors.  $\text{Cu}^{2+}$  complexes with macro cyclic polyamines act as mimics of metalloenzyme active sites [5], redox-active species for electro catalytic processes or photoredox molecular devices for converting the light energy into other forms [5]. Since copper (II) ions are widely distributed in biological systems, a significant amount of research has centered on the search for relatively simple copper (II) complexes which may display some of the properties of the metalloproteins.

Small low-molecular-weight copper (II) coordination complexes with N-donor ligands have frequently been used to model the active site in copper proteins with the aim of obtaining insight into the correlation between structure and the spectroscopic and magnetic behavior [6-9]. The major portion of this data is primarily concerned with complexes containing a single paramagnetic transition metal ion [10].

The emergence of antibiotic resistant pathogens and the continuing emphasis on health care costs has provoked a renewed interest in the design and development of novel and cost-effective antimicrobial agents with increased biological activity against the resistant strains [11-15]. Strategies currently being explored to tackle this problem include the structural modification of

existing antimicrobial drugs to which resistance has developed and the development of entirely new classes of antimicrobial agents that work on different target sites [11, 12]. Broad empirical screening of chemical entities for antimicrobial activity represents an alternative strategy for the development of new antimicrobials [12]. It has been shown that coordination of biologically active organic ligands to metal ions increases their biological activity [16].

Heterocyclic compounds do play important roles in regulating biological activities [17, 18]. Hexamethylenetetramine,  $C_6H_{12}N_4$ , is a heterocyclic tetra- dentate donor ligand, which has been used medicinally as an antiseptic agent for the treatment of urinary tract infections [19].

With regard to coordination chemistry, HMTA is a versatile ligand capable of adopting different coordination modes that span from the terminal monodentate to bridging bi-, tri- and tetra dentate modes [20, 21]. However, due to the sweeping development of crystal engineering the interest in HMTA was regained in recent years, resulting in the synthesis of a good number of one- (1D), two- (2D) and three-dimensional (3D) HMTA-driven coordination polymers bearing different metals.

## **2.1. Structural characterization techniques**

Various techniques are used to elucidate the bonding structure and stereochemistry of the ligands and the complexes prepared. While the ligands are characterized by usual methods such as elemental analysis, IR, UV -Visible and NMR spectral techniques, it differs for complexes depending on the nature of the ligands and the metal ions involved. Ligands on complexation with some metal ions having paired or unpaired electrons give diamagnetic or paramagnetic complexes respectively [2]. Some of the common physicochemical methods adopted by inorganic chemists are discussed below.

### **2.1.1. Magnetic measurements**

In paramagnetic complexes, often the magnetic moment gives the spin only value corresponding to the number of unpaired electron. The variation from the spin only value is attributed to the orbital contribution and it varies with the nature of coordination and consequent delocalization. In some cases two magnetic centers may be coupled together and may result in extraordinary increase or decrease in the magnetic moment of the complex. For example, a mononuclear complex of copper

where Cu is in the +2 oxidation state, is expected to have a magnetic moment of 1.73 BM - corresponding to  $d^9$  configuration, but, in the case of  $[\text{Cu}(\text{OAc})_2]$ , the value is lower than 1.73 BM. This is explained on the basis of the assumption that the individual magnetic moments are aligned in opposite directions so that they cancel each other to some extent or can be ascribed as due to anti-ferromagnetic coupling. Thus the value of magnetic moment of a complex would give valuable insights into its constitution and structure. In some cases the variation in the magnetic moment can be explained on the assumption that the compound may be of unpaired electrons differ in either geometries and hence the magnetic moment. The magnetic susceptibility measurements thus help to predict the oxidation state of the metal ion to a limited extent and to establish the possible geometry of the compound.

The most widely adopted method for determining the magnetic moment of a complex is by Gouy's method in which the weight difference experienced by a given amount of a substance in the presence and absence of magnetic field. This is compared with that of a standard substance and magnetic moment is determined with the help of suitable equations. [2]

### **2.1.2. Electronic spectroscopy**

Electronic spectroscopy is an important and valuable tool for most chemists to draw important information about the structural aspects of the complexes. The ligands, which are mainly organic compounds, have absorption in the ultraviolet region -hence do have bands in the region of the 200 to 350 nm of the electromagnetic spectrum- and in some cases these bands extend over to higher wavelength region due to conjugation. But upon complexation with transition metal ions, due to interaction with the metal ion there will be an interesting change in the electronic properties of the system. New features or bands in the visible region due to d-d absorption and charge transfer spectra from metal to ligand (M-L) or ligand to metal (L-M) can be observed and this data can be processed to obtain information regarding the structure and geometry of the compounds. The electronic and structural features of the complexes are widely utilized to investigate the kinetics and mechanisms of the reactions involving transition metal complexes [2].

### **2.1.3. Infrared spectroscopy**

The IR spectroscopy is widely used as a characterization technique for metal complexes. The basic theory involved is that the stretching modes of the ligands changes upon complexation due



to weakening/strengthening of the bonds involved in the bond formation resulting in subsequent change in the position of the bands appearing in the IR spectrum. The changes in the structural features of the ligands are observed as changes in bands observed, mainly in the fingerprint region i.e., in the  $1500 - 750 \text{ cm}^{-1}$  [2]

### **3. Experimental section**

#### **3.1. Chemicals**

Hexamethylenetetramine (HMTA,  $\text{C}_6\text{H}_{12}\text{N}_4$ ),  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , diethyl ether, diethyl sulphoxide, ethanol, acetone, and methanol were obtained from Sigma Aldrich. All the chemicals were of reagent grade and were used without further purification.

#### **3.2. Instruments**

The melting point/ decomposition temperatures of the complexes were obtained using the Mel-Temp II Lab Device. Conductivity measurements were carried out in distilled water using the Vernier LabPro Texas Instruments T189 at room temperature. The infrared spectra of the ligand and complexes were recorded using pressed KBr discs in the range ( $4000\text{-}400 \text{ cm}^{-1}$ ) on Perkin-Elmer 457 Spectrometer. Electronic spectra were recorded on Hitachi U-2000 spectrophotometer.

#### **3.3. Synthesis of the Complex**

The complex was synthesized by adding drop wise a  $1.33\text{mol/L}$  solution of hexamethylenetetramine (2.8 g; 2.0 mmol in 15 mL ethanol) to a  $1\text{mol/L}$  solution of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  (4.99 g; 200 mmol / 2000 mL water) while stirring at ambient temperature. The mixture was further stirred for three hours and the resulting solution allowed to stand for five days, during which formation of light green crystal was observed. These was filtered, and dried *in vacuo*.

#### **3.4. Gravimetric analysis for sulphate**

##### **Experimental Procedure:**

1. Accurately weighed 0.1800g of the complex was dissolved in distilled water in a beaker.
2. 10mL of warm  $\text{BaCl}_2$  solution (0.2M) were added very slowly (drop wise) with stirring
3. The resultant  $\text{BaSO}_4$  precipitate was allowed to settle for 10 minutes.

4. The precipitate was completely transferred into the filter; it was washed with distilled water to remove any chloride ion.
5. The resulting precipitate was dried and ready for the analysis.

### **3.5. Volumetric analysis for hexamethylenetetramine**

#### **Experimental Procedure:**

1. 0.1800g of the complex was placed into 125-mL Erlenmeyer flask
2. Into the sample flask 21ml of HCl (0.01M) were pipetted.
3. 2mL of deionized water and 6 drops of methyl red indicator were added. The solution was gently swirled to dissolve the entire solid.
4. Since the acid was present in excess, the solution has a pink color characteristic of the acid form of methyl red.
5. The pink solution was titrated with the standard NaOH (0.1M) until the color changes sharply from pink to nearly colorless.
6. Over titration was done by adding a drop wise of HCl until the solution turns to pink again (the additional volume of HCl was recorded) then the endpoint were approached again by titrating with NaOH. The total amount of NaOH used was recorded.

### **3.6. Spectrophotometric analysis for copper using Flame Atomic Absorption Spectroscopy**

#### **Experimental Procedure:**

1. A 0.1800 g sample of the complex was weighed in a 250-mL volumetric flask. 30 mL of 6 M HNO<sub>3</sub> were added, mixed thoroughly by swirling, and diluted to the mark with distilled water. Stoppered and mixed by inverting the flask (drained completely in both directions) for 10 times.
2. 0.185 mL of the resulting solution was pipetted into a 100-mL volumetric flask.
3. Mixed thoroughly by swirling and diluted to the mark. Stoppered and mixed by inverting the flask as in step 1
4. Using the blank provided (the same solution above without any complex added) the absorbance of the solution and then concentration were measured.

### 3.7. Antimicrobial Test

The antimicrobial tests were carried out in the Applied Microbiology Laboratory of Jimma University, Jimma. Four species of bacteria namely: *Staphylococcus aureus* (Gram-Positive bacteria), *Escherichia coli*, *Salmonella typhi*, and *Pseudomonas aeruginosa* were used for this study. The microbial isolates were maintained on an agar slant at 4°C in the Laboratory. The strains were sub-cultured on fresh agar plate, 24 hours prior to any antibacterial test.

**Sensitivity Test:** Sample ligand, metal salts and complexes were diluted in sterilized distilled water at 100mg/mL. One milligram of test compound was placed on a sterilized filter paper disc and allowed to dry. Reference antibiotic (RA), Gentamycin, was also prepared in the same manner and 10µg placed on a sterilized filter paper disc and dried, prior to testing.

**Diffusion Tests:** *In vitro* antibacterial activity of the ligand, metal salt and complex were evaluated using disc-diffusion method [21]. Mueller-Hinton Agar was employed as microbial growth medium. The antibacterial diffusion tests were carried out as described by Berghe and Vlietink [21] using a cell suspension of about  $1.5 \times 10^6$  CFU/mL obtained from the McFarland Turbidity Standard N° 0.5. Mueller-Hinton (MH) agar was poured (to a height of 5mm) in to sterile 9 cm diameter Petri dishes and allowed to solidify. The solid Mueller-Hinton agar was inoculated with bacteria strains using a platinum wire loop which had been previously sterilized by heating it red hot in a flame, cooled and then used for the application. The dishes were allowed to dry for 10 minutes at 37°C in an incubator. Sterilized forceps were used for the application of the paper discs containing the test compounds on previously inoculated MH agar dishes, with that of the RA placed at the centre. The plates were kept for 30 minutes at ambient temperature to allow for prediffusion, and then incubated at 37°C for 24 hours. Antimicrobial activity was evaluated [21] by measuring the diameter of growth inhibition zone (IZ) in mm around the discs. Three replicas were performed for each sample and mean values of the growth inhibition zone were calculated. Compounds were considered active when the IZ was greater than 6 mm.

## 4. Results and discussion

### 4.1. Colour, Melting point and Solubility

The colour and melting point of the salt, ligand and complex is presented in the table 1. The synthesized complex has a sharp melting point at 310°C, an indication that it is pure, and very different from the starting reagents which are 110°C (decomposition, dehydration) and 280°C for the salt and ligand respectively.

The colour of the synthesized complex is light-green while the colour of the salt and ligand is blue and white respectively. Therefore, the obtained solid is different from the starting materials.

The synthesized complex is insoluble in diethyl ether and acetone but soluble in ethanol, methanol, water and DMSO. The result of solubility shows that the complex is soluble in polar solvents, which indicates that the complex is polar. Because of like dissolves like principle. The same as for the salt and ligand as expected.

Table 1: Colour and Melting point of the salt, ligand and obtained solid

| Compound                                      | Colour      | Melting point (°C) |
|---|-------------|--------------------|
| CuSO <sub>4</sub> .5H <sub>2</sub> O          | Blue        | 110                |
| C <sub>6</sub> H <sub>12</sub> N <sub>4</sub> | White       | 280                |
| Synthesized complex                           | Light-green | 310                |

### 4.2. Analysis of a hexamethylenetetramine copper (II) complex

The objective of this experiment is to determine the molecular formula of a coordination compound of copper. We know only that its general formula is Cu(C<sub>6</sub>H<sub>12</sub>N<sub>4</sub>)<sub>x</sub>(SO<sub>4</sub>)<sub>y</sub>(H<sub>2</sub>O)<sub>z</sub>, and we wish to know the values of *x*, *y* and *z*. To determine this, the mass percentage of each

constituent [Cu, C<sub>6</sub>H<sub>12</sub>N<sub>4</sub> and SO<sub>4</sub><sup>2-</sup>] was determined by an appropriate analytical method and then determine the amount of water by difference [22].

#### 4.2.1. Gravimetric Analysis for Sulfate

To analyze the mass percent of sulfate for the compound, the sulfate ion was precipitated as BaSO<sub>4</sub>. Knowing the molar mass of BaSO<sub>4</sub>, and knowing the actual mass of BaSO<sub>4</sub> isolated from the sulfate in the compound, we can calculate the mass percent of sulfate in the sample of the coordination compound.

Table 2: Experimental data for the analysis of sulphate ion

|   | Sample |
|---|--------|
| Mass of BaSO <sub>4</sub> (g)   | 0.0658 |
| Mass of SO <sub>4</sub> <sup>2-</sup> in BaSO <sub>4</sub> precipitate(g) | 0.0271 |
| Mass % SO <sub>4</sub> <sup>2-</sup> in sample copper compound            | 15.05  |

#### 4.2.2. Volumetric Analysis for hexamethylenetetramine

To analyze the mass percent of C<sub>6</sub>H<sub>12</sub>N<sub>4</sub> for the compound, a weighed sample was titrated with a standard HCl. This gave the number of moles of C<sub>6</sub>H<sub>12</sub>N<sub>4</sub> in the weighed sample, a result that gave in turn the mass of C<sub>6</sub>H<sub>12</sub>N<sub>4</sub> and finally its mass percent.

Table 3: Experimental data for the analysis of HMTA

The concentration of the standard HCl solution is 0.01 M

|  | Sample                  |
|--|-------------------------|
| Moles of HCl used in titration   | 2.0975×10 <sup>-4</sup> |
| Moles of C <sub>6</sub> H <sub>12</sub> N <sub>4</sub> in sample                       | 8.39×10 <sup>-4</sup>   |
| Mass of C <sub>6</sub> H <sub>12</sub> N <sub>4</sub> in sample of copper compound (g) | 0.1191                  |
| Mass % C <sub>6</sub> H <sub>12</sub> N <sub>4</sub> in sample of copper Compound      | 66.16                   |

### 4.2.3. Spectrophotometric Analysis for Copper

The mass percent of copper ion in the compound were determined by spectrophotometer. That is, the greater the amount of  $\text{Cu}^{2+}$  per gram of compound, the more light a given sample will absorb when dissolved in dilute nitric acid. Spectrophotometer were calibrated with solutions containing known amounts of  $\text{Cu}^{2+}$ , the absorbance of unknown solutions were measured and then the amount of  $\text{Cu}^{2+}$  were determined therein.

### Experimental Data

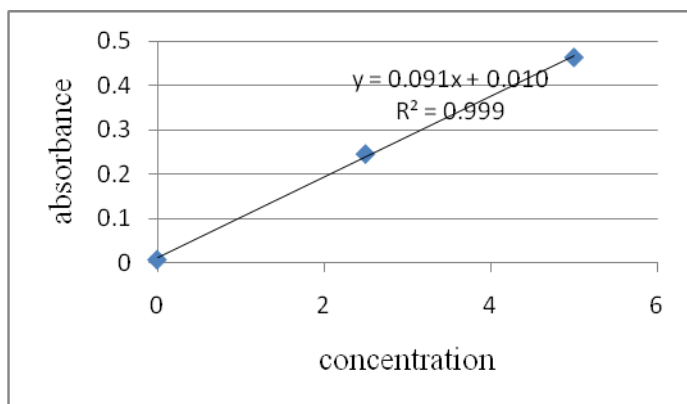
Table 4: Concentration for standard solution

| Standard Number | Sample | mL standard $\text{Cu}^{2+}$ | mL 0.1 M $\text{HNO}_3$ | $[\text{Cu}^{2+}]$ in dilute solution [mg/L] |
|-----------------|--------|------------------------------|-------------------------|--|
| 1               |        | 0.0                          | 10.0                    | 0.000  |
| 2               |        | 4.0                          | 6.0                     | 0.001  |
| 3               |        | 7.0                          | 3.0                     | 2.500  |
| 4               |        | 10.0                         | 0.0                     | 5.00   |

Table 5: Spectrophotometric Data: Absorbance at 645 nm

| Standard Sample Number | Trial 1  | Trial 2  | Trial 3  | Trial 4  | Average    |
|------------------------|----------|----------|----------|----------|------------|
| 1                      | 0.002614 | 0.001918 | 0.00261  | 0.003315 | 0.00261425 |
| 2                      | 0.007612 | 0.006367 | 0.00814  | 0.008329 | 0.007612   |
| 3                      | 0.2454   | 0.245511 | 0.243925 | 0.246807 | 0.24541075 |
| 4                      | 0.4629   | 0.464769 | 0.457415 | 0.466605 | 0.46292225 |

After determining the average absorbance of each reference standard (solutions 1-4) at 645 nm, plot of the average absorbance against the copper concentration were plotted.



**Figure 2:** Calibration plot

### Calculations

Having constructed the calibration plot for the instrument, we can now use the average absorbance of each unknown solution to determine the concentration of the copper ion in those solutions. From that, we can calculate the mass percent of copper in the sample.

**Table 6: Mass percent of copper in the sample**

|                            |        |
|----------------------------|--------|
| [Cu <sup>2+</sup> ], mol/L | 1.516  |
| Mass of copper (g)         | 0.0179 |
| Mass % copper              | 9.94   |

#### 4.2.4. Calculation of the Formula of the Coordination Compound

Based on the mass percentages of Cu<sup>2+</sup>, C<sub>6</sub>H<sub>12</sub>N<sub>4</sub>, and SO<sub>4</sub><sup>2-</sup> that have been found experimentally, the number of moles of each component in a 0.1800 g sample of the unknown compound was calculated.

**Table 7: Mass and number of mole of Cu<sup>2+</sup>, SO<sub>4</sub><sup>2-</sup> and C<sub>6</sub>H<sub>12</sub>N<sub>4</sub>**

|                  | Mass (g)         | Substance amount (mole)  |
|------------------|------------------|--------------------------|
| Cu <sup>2+</sup> | 0.0179 ± 0.00001 | 2.817 × 10 <sup>-4</sup> |

|   |                  |                       |
|---|------------------|-----------------------|
| SO <sub>4</sub> <sup>2-</sup>                 | 0.0271 ± 0.00002 | 2.82×10 <sup>-4</sup> |
| C <sub>6</sub> H <sub>12</sub> N <sub>4</sub> | 0.1191 ± 0.00001 | 8.50×10 <sup>-4</sup> |

**Table 8: Comparing the experimental value with the calculated one for Cu<sup>2+</sup>, SO<sub>4</sub><sup>2-</sup> and C<sub>6</sub>H<sub>12</sub>N<sub>4</sub>**

|   | Experimental (%) | Calculated (%) |
|---|------------------|----------------|
| Cu <sup>2+</sup>                              | 9.94 ± 0.001     | 10.00          |
| SO <sub>4</sub> <sup>2-</sup>                 | 15.05 ± 0.003    | 15.15          |
| C <sub>6</sub> H <sub>12</sub> N <sub>4</sub> | 66.16 ± 0.002    | 66.29          |

Now, the formula of the compound were determined by taking the ratio of the number of moles of each substance (C<sub>6</sub>H<sub>12</sub>N<sub>4</sub>, SO<sub>4</sub><sup>2-</sup>) to the number of moles of Cu<sup>2+</sup>.

Moles of C<sub>6</sub>H<sub>12</sub>N<sub>4</sub> per moles Cu<sup>2+</sup> =  $8.50 \times 10^{-4} / 2.817 \times 10^{-4} = 3.017 \text{ mol} \sim 3 \text{ mol}$

Moles of SO<sub>4</sub><sup>2-</sup> per moles of Cu<sup>2+</sup> =  $2.82 \times 10^{-4} / 2.817 \times 10^{-4} = 1 \text{ mol}$

In order to know the number of moles of H<sub>2</sub>O, first, the mass of H<sub>2</sub>O was calculated:

Mass of H<sub>2</sub>O = 0.1800g – (total mass of Cu<sup>2+</sup>, SO<sub>4</sub><sup>2-</sup> and C<sub>6</sub>H<sub>12</sub>N) = 0.1800 – 0.1641 = 0.0159g

Mole of H<sub>2</sub>O =  $8.83 \times 10^{-4} \text{ mol}$

Moles H<sub>2</sub>O per moles Cu<sup>2+</sup> =  $8.83 \times 10^{-4} / 2.817 \times 10^{-4} \text{ mol} = 3.13 \sim 3 \text{ mol}$

Rounding the ratios above to the nearest whole number, below blanks were filled to give the empirical formula of the copper coordination compound.

**Cu(C<sub>6</sub>H<sub>12</sub>N<sub>4</sub>)<sub>x</sub>(SO<sub>4</sub>)<sub>y</sub>(H<sub>2</sub>O)<sub>z</sub> x = 3, y = 1, z = 3**

**Molecular Mass = 633.5**

**Table 9: Calculation for the Percent Yield**

|                                 |        |
|---------------------------------|--------|
| Mass of product isolated(g)     | 2.9600 |
| Theoretical yield of product(g) | 4.2236 |
| Percentage yield (%)            | 70.08  |



### 4.3. Conductivity of the complex

The measurement of molar conductance is an important tool that gives valuable information about the coordination number, the structure of the complexes and the nature of counter ions present in the complexes. It also helps us to confirm the electrolytic nature and thereby the numbers of ions present outside the coordination sphere [23].

The molar conductance of a solution was obtained from the measured value of specific conductance. The concentration of the solution measured was  $10^{-3}$  mol/L.

Molar conductance is calculated as:

$$\Lambda_M = 1000 \cdot K \cdot S / C$$

$$K = 1 \text{ cm}^{-1}, S = 126.33 \text{ ohm}^{-1}, C = 10^{-3} \text{ mol/L}$$

$$\Lambda_M = 126.33 \text{ } \Omega^{-1} \text{ cm}^2 \text{ mol}^{-1}$$

Where k: cell constant; S: conductivity of the solution in Siemen or the inverse of resistance in ohm ( $1S = 1 \text{ ohm}^{-1}$ ); C: dissolution concentration of complex mol/L. Molar conductivity has the units  $\text{cm}^2 \cdot \text{S} \cdot \text{mol}^{-1}$  or  $\text{cm}^2 \cdot \text{Ohm}^{-1} \cdot \text{mol}^{-1}$ . The molar conductance of the complex were measured at room temperature . The solvent used in the present study for the measurement of conductance was water. The molar conductivities of the metal complex in water was  $126.33 \text{ } \Omega^{-1} \text{ cm}^2 \text{ mol}^{-1}$  which suggest it is a strong 1:1 electrolyte, indicating that the counter ion  $\text{SO}_4^{2-}$  is in the outer coordination sphere.

### 4.4. Magnetic measurement

Magnetic measurements on  $[\text{Cu}(\text{C}_6\text{H}_{12}\text{N}_4)_3(\text{H}_2\text{O})_3]\text{SO}_4$  at  $21^\circ\text{C}$  using a gouy balance gave a mass susceptibility value  $\chi_g = 1.315 \times 10^{-6} \text{ gauss/g}$

Therefore, the molar susceptibility

$$\chi_M = \chi_g \times MW = 1.315 \times 633.54 \times 10^{-6} = 833.10 \times 10^{-6} \text{ (gauss/mol)}$$

The weight change measured with the Gouy balance is the sum of the effects from the paramagnetic metal ion and the diamagnetic ligands and ions present. Thus the value of  $\chi_M$  derived from this weight change is the net magnetism, which is the sum of  $\chi_{\text{paramagnetic}} + \chi_{\text{diamagnetic}}$ . Since we wish to measure the paramagnetic of the metal ion, we must make diamagnetic corrections (sometimes called Pascal's constants) for the atoms and ions in the molecule and contributions from multiple bonds. The molar susceptibility obtained has to be

corrected for the inherent diamagnetic contribution ( $\chi^{\text{dia}}$ ) from the ligands and metal ions using the table of Pascal's constants [25].

The diamagnetic correction is obtained by adding together the contributions from each of the constituent ions and molecules.

$$\text{Cu}^{2+} = 1 \times -11 \times 10^{-6} = -11 \times 10^{-6}$$

$$\text{SO}_4^{2-} = 1 \times -40.1 \times 10^{-6} = -40.1 \times 10^{-6}$$

$$\text{H}_2\text{O} = 3 \times -13 \times 10^{-6} = -39 \times 10^{-6}$$

The value for diamagnetic correction for the above =  $-90 \times 10^{-6}$

Diamagnetic correction  $\text{C}_6\text{H}_{12}\text{N}_4$  is:

$$\text{C} = (-6 \times 6) \times 10^{-6} = -36 \times 10^{-6}$$

$$\text{H} = (-2.93 \times 12) \times 10^{-6} = -35.16 \times 10^{-6}$$

$$\text{N} = (-5.57 \times 4) \times 10^{-6} = -22.28 \times 10^{-6}$$

$$-(36 + 35.16 + 22.28) \times 10^{-6} = -93.44 \times 10^{-6}$$

The value diamagnetic correction for  $\text{C}_6\text{H}_{12}\text{N}_4 = 3(-93.44 \times 10^{-6}) = -280.32 \times 10^{-6}$

Therefore, the value for diamagnetic correction =  $(-90 - 280.32) \times 10^{-6} = -370.32 \times 10^{-6}$

The corrected value for  $\chi_M$  is thus:

$$\chi_M^{\text{corr}} = \chi_M^{\text{exp}} - \chi^{\text{dia}} = (833.10 + 370.32) \times 10^{-6} = 1203.42 \times 10^{-6} = 1203.42 \times 10^{-6}$$

The relationship between molar susceptibility and effective magnetic moment,  $\mu_{\text{eff}}$  is

$$\mu_{\text{eff}} = 2.84 (\chi_M^{\text{corr}} T)^{1/2} \text{ Bohr Magnetron} = 2.84 (1203.42 \times 294 \times 10^{-6})^{1/2} \text{ B.M} = 1.69 \text{ B.M}$$

It can also be shown that the effective magnetic moment,  $\mu_{\text{eff}}$  is given by,

$$\mu_{\text{eff}} = (n(n+2))^{1/2} \text{ B.M}$$

Assuming the spin only formula:

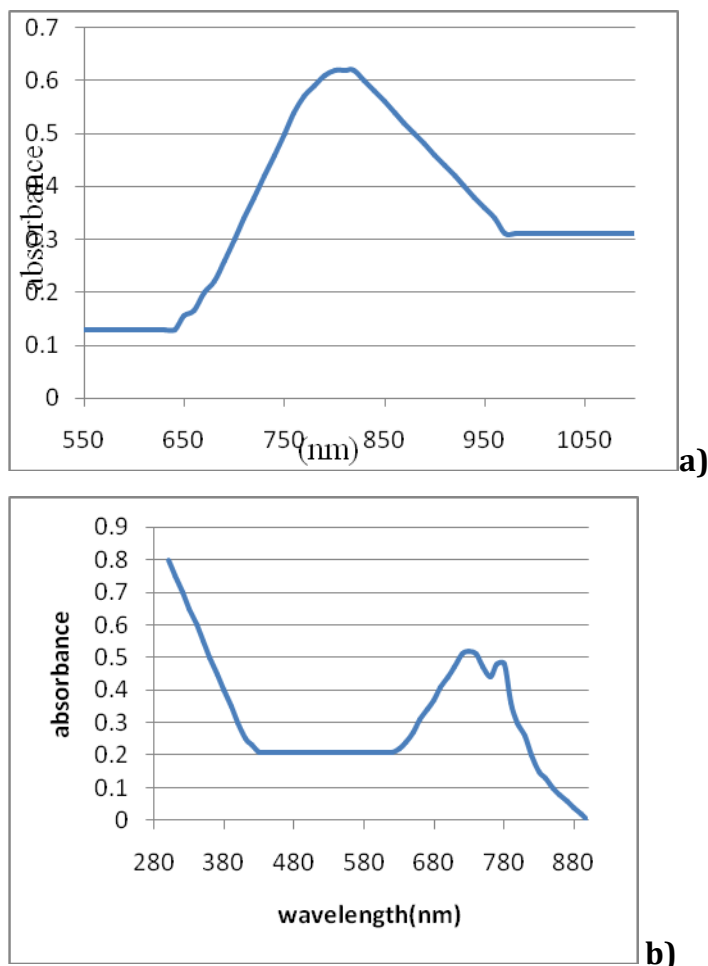
If  $n=1$ ,  $\mu_{\text{eff}} = (n(n+2))^{1/2} \text{ B.M} = 1.73 \text{ BM}$  [26] where  $n$  is the number of unpaired electron

The effective magnetic moment value of  $\text{Cu}^{2+}$  in  $[\text{Cu}(\text{C}_6\text{H}_{12}\text{N}_4)_3(\text{H}_2\text{O})_3]\text{SO}_4$  (i.e.  $\mu_{\text{eff}} = 1.69 \text{ BM}$ ) is very close to the spin only value of  $d^9$  ( i.e  $\mu_{\text{eff}} = 1.73 \text{ BM}$ ), which indicates that  $\text{Cu}^{2+}$  in  $[\text{Cu}(\text{C}_6\text{H}_{12}\text{N}_4)_3(\text{H}_2\text{O})_3]\text{SO}_4$  has one unpaired electron. Therefore, the complex is paramagnetic.

## 4.5. Electronic spectra of the salt and complex

Table 11: Electronic spectra of the salt and complex

| Compound  | Maximum absorption (nm) |
|---|-------------------------|
| $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$   | 817                     |
| $[\text{Cu}(\text{C}_6\text{H}_{12}\text{N}_4)_3(\text{H}_2\text{O})_3]\text{SO}_4$ | 731                     |



**Figure 3:** UV-vis spectra of a) salt and b) UV-vis spectra of the complex

The electronic spectra of  $[\text{Cu}(\text{C}_6\text{H}_{12}\text{N}_4)_3(\text{H}_2\text{O})_3]\text{SO}_4$  showed a maximum absorption band at 731nm with a molar absorptivity of 5.2. According with the intensity and the position of the observed band, it can be assigned to a d-d transition in octahedral geometry. In a  $\text{Cu}^{2+}$  complex with electronic configuration  $t_{2g}^6 e_g^3$  only one spin allowed electronic transition is observed corresponding to  $t_{2g}^6 e_g^3 \rightarrow t_{2g}^5 e_g^4$ , which corresponds to the only observed band in the visible region spectrum of our analyzed complex.

The band in the visible region for the obtained complex is shifted to lower wavelength (higher energy) in comparison to the corresponding band in the copper salt solution where the existing species is the slightly distorted octahedral complex  $[\text{Cu}(\text{H}_2\text{O})_6]^{2+}$ . The observed shifting of the band is explained by the substitution of water molecules in the inner sphere for the stronger ammine ligand, as according with the spectrochemical series, which causes a larger splitting parameter  $\Delta_O$  and increases the energy of the electronic transition.

The electronic spectra of Cu (II) compounds are not easily interpreted because of its low symmetry. Virtually all the complexes of copper (II) are either blue or green in colour, but there are exceptions caused by strong ultraviolet band charge-transfer band tailing off into the blue end of the visible spectrum which cause the substance to appear red or brown [27]. The complexes have the characteristic blue and green colour associated to with copper salts and is assigned a distorted octahedral shape. In hexa- coordinated copper (II) complexes, tetragonal distortion from the octahedral symmetry due to the Jahn–Teller distortion is very common. Absorption spectra for Cu (II) complexes in the region 625-909nm, for which octahedral geometry was proposed. However, octahedral complexes of Cu (II) are appreciably distorted (Jahn-Teller effect).

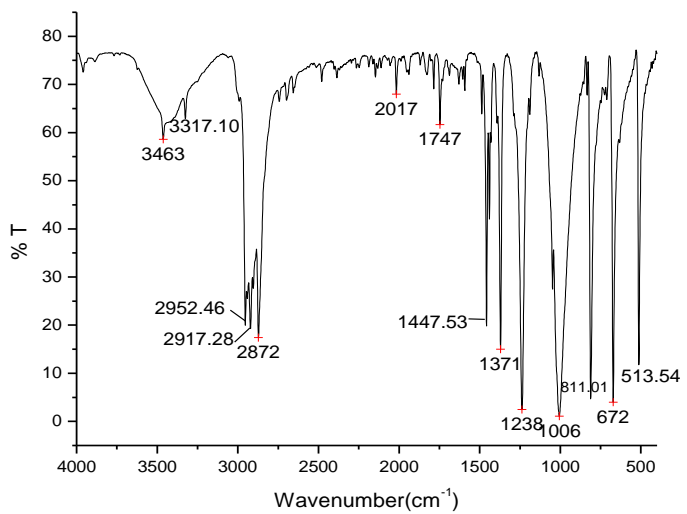
The complex species  $[\text{Cu}(\text{HMTA})_3(\text{H}_2\text{O})_3]^{2+}$ , either with mer- or fac- geometrical configuration will be far apart from regular octahedral symmetry. The reasons for the asymmetry are the distribution of the ligands in the coordination sphere, the presence of different ligands and consequently different coordination bond strengths and distances, and the Jahn-Teller effect. These facts explain why the absorption curve has an irregular shape showing splitting into two maxima.

In the ultraviolet region of the spectrum of the complex it can be observed the low energy branch of a very intense band which maximum occurs in a region out of the range of the equipment. This is a charge transfer band that is frequently found in Cu (II) complexes.

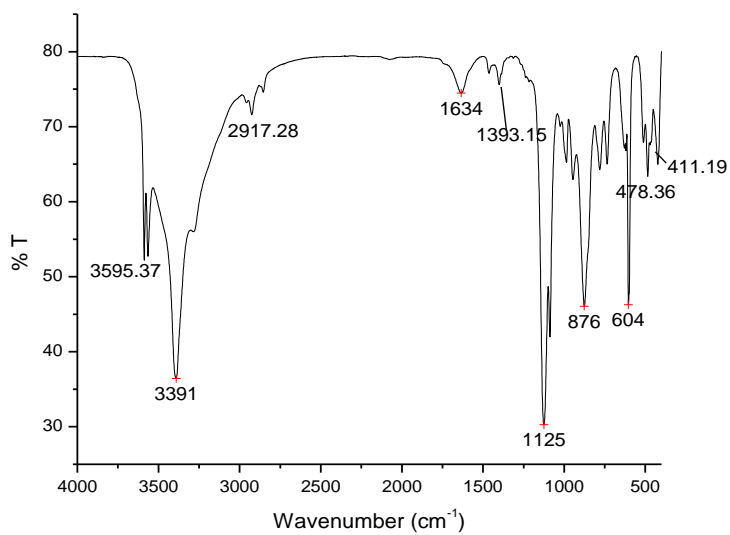
#### 4.6. IR spectra of the ligand and complex

Table 12: Selected IR Absorption Bands ( $\text{cm}^{-1}$ ) of HMTA and its Complex

|   | $\nu(\text{O-H})$ | $\nu(\text{H}_2\text{O})$ | $\nu(\text{C-N})$ | $\nu(\text{SO}_4^{2-})$ | $\rho(\text{CH}_2)$ | $\nu(\text{M-O})$ | $\nu(\text{Cu-N})$ |
|---|-------------------|---------------------------|-------------------|-------------------------|---------------------|-------------------|--------------------|
| $\text{C}_6\text{H}_{12}\text{N}_4$   |                   |                           | 1238s             |                         | 811s                |                   |                    |
| $[\text{Cu}(\text{C}_6\text{H}_{12}\text{N}_4)_3(\text{H}_2\text{O})_3]\text{SO}_4$ | 3391br            | 1634m                     | 1050w             | 1125s                   | 773w                | 731w              | 876s               |

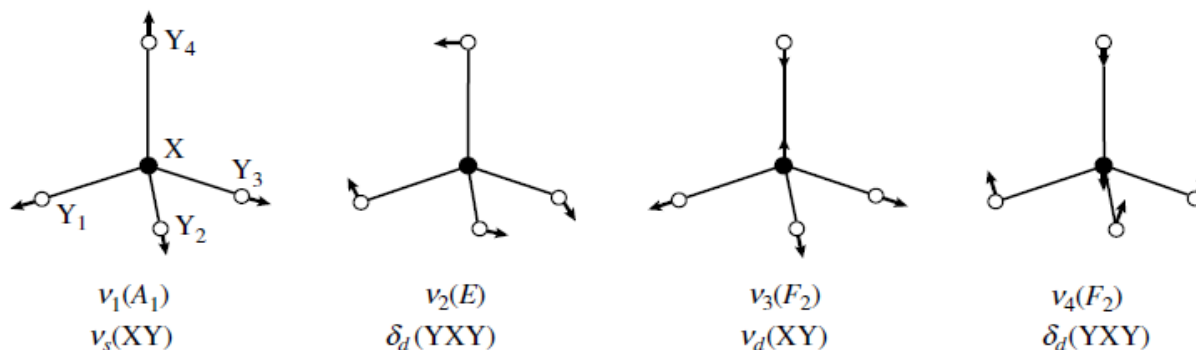


a)



b)

**Figure 4:** IR spectra of a) ligand b) complex



**Figure 5:** Vibration characteristic of free sulphate ion

**Table 13:** Vibration characteristic of free sulphate ion:

|                      | $\nu_s$ | $\delta_d$ | $\nu_d$ | $\delta_d$ |
|----------------------|---------|------------|---------|------------|
| $[\text{SO}_4]^{2-}$ | 983     | 450        | 1125    | 604        |

In the IR spectrum of the ligand are observed many signals which are present also in the spectrum of the complex. This fact makes uncertain many assignment presumptions in several regions of the spectrum of complex. Nevertheless, some facts are clearly appreciated in it.

The free sulphate ion belongs to the high symmetry point  $T_d$ . It is a symmetric tetrahedron. From the four fundamentals, only  $\nu_d$  and  $\delta_d(611 \text{ cm}^{-1})$  are infrared-active. For free sulphate in the outer coordination sphere  $\nu_d$  and  $\delta_d(611 \text{ cm}^{-1})$  do not split,  $\delta_d(450 \text{ cm}^{-1})$  does not appear,  $\nu_s$  might be observed only as a very weak signal around  $983 \text{ cm}^{-1}$ . In the spectrum of our copper complex  $\nu_d$  ( $1125 \text{ cm}^{-1}$ ) and  $\delta_d$  ( $604 \text{ cm}^{-1}$ ) are clearly observed and they do not split, which indicates that the sulphate ion is found freely outside the coordination sphere.

The very broad band at  $3391\text{-}3600 \text{ cm}^{-1}$  observed in the complex can be assigned to  $\nu_s$  and  $\nu_{as}(\text{O-H})$  which usually overlap as a single broad signal in  $3500\text{-}3700 \text{ cm}^{-1}$ . In our complex the signals of  $\nu(\text{O-H})$  are shifted to lower frequency, become sharper and can be differentiated, as corresponds with the effect of oxygen donation to the metal cation which weakens the O-H bonds. These bands additionally overlap with the near bands from HMTA Methylene groups showing  $\nu_{as}$  about  $2930 \text{ cm}^{-1}$  and  $\nu_s$  at  $2850 \text{ cm}^{-1}$  [28]

A single band for complex at  $1634\text{ cm}^{-1}$  which can be assigned to  $\delta(\text{HOH})$  indicates that all the water molecules are equivalent [29] and that is no strong hydrogen bond neither with sulfate anion nor with other HMTA molecule. The coordination of water molecules to the cations results in the appearance of a weak vibration band at  $680\text{-}732\text{ cm}^{-1}$  and tentatively assigned to  $\nu\text{M-OH}_2$  [21], although it is in a region obscured by other stronger vibrations that makes the assignment uncertain. The expected rocking and wagging bands reported around  $560\text{ cm}^{-1}$  and  $470\text{ cm}^{-1}$  respectively. [30], cannot be identified in a reliable way for the same reason.

**Table 14: Characteristic IR bands for the HMTA and complex**

| HMTA ( $\text{cm}^{-1}$ ) | Complex ( $\text{cm}^{-1}$ ) | Assignment  |
|---------------------------|------------------------------|-------------|
| 1238                      | 1393<br>1440                 | $\nu$ (C-N) |
| 1006                      | 1050<br>1083<br>1097         | $\nu$ (C-N) |

The C-N ring vibrations of the HMTA are reported in  $511, 672, 1009, 1238\text{ cm}^{-1}$  [31]. All they are observed in the spectrum of the pure ligand. Formation of Cu-N(HMTA) bonds lowers the HMTA molecule symmetry from  $T_d$  to  $C_{3v}$ . The two higher frequency bands in complex split into five weak signals each [30] observed between  $1050$  and  $1440\text{ cm}^{-1}$ . In this region of spectrum of the complex it can be appreciated a number of weak signals attributable to the expected splitting of C-N vibrations. However, in this region  $\nu_{\text{as}}$  of sulfate ion is present as a very intense peak and superimposes to any other weak signal from the C-N bonds. Despite of the uncertainty of any assignment purpose, these facts could be taken as indication of HMTA coordination to the metal ion.

#### 4.7. Structure of $[\text{Cu}(\text{HMTA})_3(\text{H}_2\text{O})_3]\text{SO}_4$ .

Sulfate anion location in the outer sphere of the complex is proved by the molar conductivity value which shows a 1:1 electrolyte behavior in solution. The presence in the IR spectrum of single intense bands corresponding to  $\nu_d$  ( $1125\text{ cm}^{-1}$ ) and  $\delta_d$  ( $604\text{ cm}^{-1}$ ) indicates tetrahedral symmetry and supports the conductivity result.



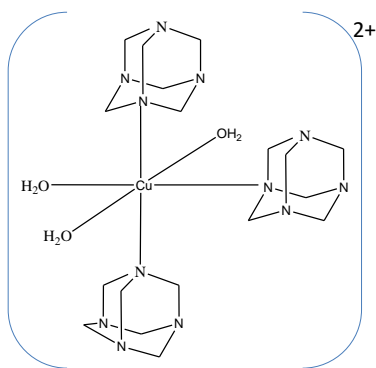
The value obtained for the magnetic moment is consistent with the presence of one unpaired electron as expected for a  $d^9$   $\text{Cu}^{2+}$  central ion in a mononuclear complex.

The presence of water molecules is apparent from the good empirical formula consistency with experimental data obtained from chemical analysis and is supported by the IR spectrum. In this spectrum the signals of  $\nu_s$  and  $\nu_{as}(\text{O-H})$  are observed at lower frequency values than in pure water indicating coordination. The presence of a single signal for  $\delta(\text{HOH})$  ( $1634 \text{ cm}^{-1}$ ) point out that all the water molecules are equivalent.

The position and the intensity of the band in the visible region characterize octahedral metal complexes of  $\text{Cu(II)}$ . The shifting to higher energies (lower wavelength) of the maximum in the HMTA complex in comparison with the aquo-complex is a sign of the addition of the ammine to the coordination sphere. This fact point out to support the uncertain indications of HMTA molecule coordination obtained from IR spectrum. The irregular shape of the absorption curve which presents two maxima is an indication of the distorted geometry of the complex attributable to the presence of different ligands in the coordination sphere and to the inherent Jahn-Teller effect in all  $\text{Cu (II)}$  complexes.

For the proposed structural formula  $[\text{Cu}(\text{HMTA})_3(\text{H}_2\text{O})_3]^{2+}$  two possible geometric isomers can exist, said the *mer*- and the *fac*-isomers. From the data obtained in this study is not possible to decide for one of the two geometries. Nevertheless, steric hindrance in the coordination sphere should be smaller if the geometry were *fac*- and two HMTA molecules were placed in trans position.

According with the results here obtained, the most likely molecular structure of  $[\text{Cu}(\text{HMTA})_3(\text{H}_2\text{O})_3]^{2+}$  could be schematically represented in figure 6:



**Figure 6:** Proposed molecular structure for the obtained complex.

Computational Chemistry Molecular Modeling process and X-Ray Diffraction Analysis could bring a better and complete picture for the structure of the complex. Unfortunately, these techniques are beyond the scope of present work and out of our available possibilities.

#### 4.8. Antibacterial Tests

The ligand, metal salt and the metal complex were tested for antibacterial activity in vitro against four bacteria strains. The susceptibility of the bacterial strains towards the compounds, judged by measuring the diameter of the growth inhibition zone.

**Table 15: Antimicrobial Activities of the salt, ligand and complex**

| Compound  | <i>Pseudomonas aeruginosa</i><br>(mm) | <i>Staphylococcus aureus</i> (mm) | <i>Salmonella typhi</i> (mm) | <i>Escherichia coli</i> (mm) |
|---|---------------------------------------|-----------------------------------|------------------------------|------------------------------|
| CuSO <sub>4</sub> .5H <sub>2</sub> O  | 15                                    | 26                                | 17                           | 21                           |
| C <sub>6</sub> H <sub>12</sub> N <sub>4</sub>   | 15                                    | –                                 | 10                           | –                            |
| [Cu(C <sub>6</sub> H <sub>12</sub> N <sub>4</sub> ) <sub>3</sub> (H <sub>2</sub> O) <sub>3</sub> ]SO <sub>4</sub> | 15                                    | 20                                | 23                           | 18                           |
| Gentamycin(reference antibiotic)  | 18                                    | 16                                | 18                           | 19                           |
| DMSO  | –                                     | –                                 | –                            | –                            |

The metal complex tested, showed some activity against the different types of Gram-positive and Gram-negative bacteria while the hexamethylenetetramine ligand (HMTA), was found to be active against only on two ( *Pseudomonas aeruginosa* and *Salmonella typhi*) of the 4 pathogens. All pathogens were inhibited by the complex tested. On coordination, the polarity of the metal ion is reduced due to overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with the ligand's donor atoms. This may increase the lipophilic character of the metal complex, enabling it to permeate the lipid membrane. The complex was found to be more active than the reference antibiotic against *Staphylococcus aureus* (Gram-Positive bacteria), *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*. And also the complex showed greater activity than salt and reference antibiotic against *Salmonella typhi*. Thus, it can be further explored *in vitro* for the treatment of infectious diseases caused by this microorganism.

## 5. Conclusions and recommendation

[Cu(C<sub>6</sub>H<sub>12</sub>N<sub>4</sub>)<sub>3</sub>(H<sub>2</sub>O)<sub>3</sub>]SO<sub>4</sub> have been synthesized and characterized by Magnetic Susceptibility, Atomic absorption spectrophotometer, IR and UV-visible spectroscopy analyses as well as conductivity measurement. In the complex, the copper atom is bonded to three water molecules and three HMTA molecules giving a distorted octahedral geometry about the copper atom. Antibacterial studies of the complex against four bacteria species showed that there is increased activity of the metal ions upon coordination to the ligand. The complex showed greater activity than salt and reference antibiotic, gentamycin, towards *Salmonella typhi* of the tested bacteria, could be further studied and exploited for the treatment of infections caused by this bacterium. Thus, further test is recommended on large number of bacterial strains to decide its potential as candidates in development of antibacterial drugs.

## 6. References

1. Cotton, F.A.; Wilkinson, G. *Advanced Inorganic Chemistry*, 5th edi., John Wiley and Sons: Canada; 1989; p 625.
2. Usman, A.; Nayar, C. R.; Unnikrishnan, P. A.; Sreeja, P. B.; Kurup, M. R. P.; Fun, HK. 2-Hydroxy-3-(4,5,6,7-tetrahydro-1H-imidazo[4,5-c]pyridin-5-ium-4-yl) benzoate monohydrate. *Acta.Cryst, C Cryst. Strnc. Commun.* **2002**, 58, 0724.
3. Kokoszka, G.F.; Duerst R.W. studies of exchange coupled metal ions. *Coordination Chemistry Reviews.* **1970**, 5, 209-244.
4. Miller E.V. *Chemistry of the Plants*; Reinheld: New York, 1957.
5. Ciurla, H.; Hanuza, J.; Talik, Z.; Korabik, M.; Rozinski, J. Vibrational spectra, electronic excited states and magnetic properties of the copper(II) ions in alkylaminoacetylurea complexes *Materials Science-Poland*, **2009**, 27, 5.
6. Collinson S.R.; and Fenton D.E. Metal complexes of Schiff base macrocycles. *Coordination Chemistry Reviews.* **1996**, 148, 19-40.
7. Mandal, S.; Das, G.; Singh, R.; Shukla, R.; Bharadwaj, P.K. Synthesis and studies of Cu(II)-thiolato complexes: bioinorganic perspectives. *Coordination Chemistry Reviews.* **1997**, 160, 191-235.
8. Kaim, W.; Rall, J.; Rall, J. "Copper a "modern" bioelement". *Angewandte Chemie.* **1996**, 35, 43-60.
9. Singh, P.; Jeter, D.Y.; Hatfield, W.E.; Hodgson, D.J. "Out-of-plane interactions in parallel-planar copper (II) dimers. The structure and magnetic properties of dibromobis (2-methylpyridine) copper (II)". *Inorganic Chemistry.* **1972**, 11, 1657-1661.
10. Hay, P. J.; Thibeault, J. C.; Hoffmann, R. Orbital interactions in metal dimer complexes. *Journal of the American Chemical Society.* **1975**, 97, 4884-4899.
11. M. Rai, M.; Yadav, A.; Gade, A. Silver nanoparticles as a new generation of antimicrobials. *Biotechnology Advances.* **2009**, 27(1), 76-83.
12. Tim Cushnie, T. P.; Lamb, A. J. Antimicrobial activity of flavonoids. *International Journal of Antimicrobial Agents.* **2005**, 26, 343- 356.
13. Kim, J.S.; Kuk, E.; Yu, k. N, Kim, J. H.; Park, S. J.; Lee, H. J.; Kim, S. H.; Park, Y. K.; Y.H. Park, Y. H.; Hwang, C. Y.; Kim, Y. K.; Lee, S.; Jeong, D.H.; Cho, M.H. A practical

procedure for producing silver nanocoated fabric and its antibacterial evaluation for biomedical applications. *Nanomedicin. Nanotechnology, Biology, and Medicine*. **2007**, 3, 95– 101.

14. Cloete, T.E. Resistance mechanisms of bacteria to antimicrobial compounds. *International Biodeterioration and Biodegradation*. **2003**, 51, 277-282.

15. Golcu, A.; Tumer, M.; Demirelli, H.; Wheatly, R.A. Cd(II) and] Cu(II) complexes of polydentate Schiff base ligands: synthesis, characterization, properties and biological activity. *Inorg. Chim. Acta*. **2005**, 358, 1785-1797.

16. Shakru, R.N.; Subhashini, J.P.; Sathish Kumar, K.; Shivaraj. Synthesis, characterization and antimicrobial studies on Cobalt (II), Nickel (II), Copper (II) and Zinc (II) complexes of N, O, S donor Schiff bases. *J. Chem. Pharm. Res.*, **2010**, 2(1), 38-46

17. Abou-Melha, K.S.; H. Faruk, H. Synthesis and Characterization of a New N"-[(1Z, 2E)-2-(Hydroxyimino)-1-phenylpropylidene]-N"-[(1E)-phenylmethylene] thiocarbonohydrazide and its Complexes. *Iran. Chem. Soc.* **2008**, 5(1), 122-134

18. Miall, S.; Mackenzie, L. *A Dictionary of Chemistry*; Longmans: London, 1956, pp252.

19. Zheng, S.L.; Tong, M.L.; Chen, X.M. Frontiers in Transition Metal-Containing Polymers. *Coord. Chem. Rev.* **2003**, 246, 185.

20. Allen, F.H. The Cambridge Structural Database (CSD). *Acta Crystallogr. B*. **2002**, 58, 380.

21. Agwara, M. O.; Yufanyi, M. D.; Foba-Tendo, J. N.; Atamba, M. A. Synthesis, characterization and biological activities of Mn (II), Co (II) and Ni (II) complexes of hexamethylenetetramine. *Chem. Pharm. Res.* **2011**, 3, 196-204

22. Synthesis of a Cu Complex, *Spring Rev NF/AEM Sp07*, 2007

23. Levine, I.N. *Physical Chemistry*, 5th edn., McGraw-Hill, 2002.

24. Selwood, P. W. *Magneto chemistry, Interscience*; John Wiley & Sons: New York, 1956.

25. *Journal of Chemical Education*, **2008**, 85, pp532

26. Figgis, B. N.; Lewis, J. In Lewis, J., Wilkins, R. G., Eds. *Modern Coordination Chemistry*; Interscience Publishers Inc.: New York, 1960; pp. 400-454

27. Cotton, F.A.; Wilkinson, G. *Advanced inorganic chemistry fourth edition*; John Wiley and sons: 1980, New York, pp 61-71, 786-791

28. Barbara, H. *Infrared spectroscopy: fundamentals and applications*; Stuart. John Wiley & Sons Ltd, 2004.

29. Balicheva, T. G.; Pologikh, I. V. Thermo gravimetric and Antimicrobial Properties of Some Divalent Metal Complexes of Hexamethylenetetramine. *Russ. J. Inorg. Chem.*, **1975**, 20(12), 1769-1773.
30. Grodzicki, A.; Chrzaszcz, M.; Krajewski, K.; Szlyk, E.; Kontek, J. Thermal properties of complex salts of sulfate and perchlorate Cu(II) with some anti-inflammatory drugs, derivatives of pyrazolin-5-one *Transition Met. Chem.*, **1991**, 16, 413-415.
31. Mecke, R.; Spiesecke, H. Heterocyclic compounds. *Chema. Ber.* **1997**, 88, 1955