

Chemical Synthesis, Spectral Characterization and Antimicrobial Studies on complexes of Ni(II), Cu(II) and Zn(II) with N, N-di (o-hydroxybenzenoylmethylene) ethylenediamine

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Abstract: The Schiff's base ligands and their complexes with Ni(II), Cu(II) and Zn(II) were synthesized and characterized by elemental analysis molar conductivity, infrared and ultraviolet-visible spectroscopy and magnetic susceptibility measurements. The ligands, ethylenedianil of o-hydroxyphenylglyoxal, $C_{18}H_{16}N_2O_4$, have been synthesized by condensation of ethylenediamine and orthohydroxyphenylglyoxal. The metal complexes were prepared by mixing of saturated solutions of ligands and metal salts in appropriate molar ratio in acetone and methanol solvents. The study also confirmed the formation of mono-, di- and trinuclear isopolystructures of the complexes in square planar geometry except for Zn(II), which has tetrahedral stereochemistry. The synthesized ligands and their metal complexes were screened for their antimicrobial activities against two bacterial strains (*Staphylococcus aureus* and *Xanthomonas holcicola*), and two fungal strains (*Aspergillus niger* and *Fusarium oxysporum*) using a disc diffusion method. The tests showed that the complexes have higher antimicrobial activity than the free Schiff's base.

Keywords: Antimicrobial Studies, Disc Diffusion Method, Ethylenedianil of O-Hydroxyphenyl Glyoxal, Ortho-Hydroxyphenylglyoxal, Schiff's Base, N,N-di (o-hydroxybenzenoylmethylene) Ethylenediamine

1. Introduction

A metal complex consists of a central metal atom and ion surrounded by a set of ligands, anions or neutral molecules, which have one or more atom(s) bearing lone pair(s) of electrons [18]. The binding of these donor atoms with metal ion is electrostatic and/or covalent. In non-transition metal complexes, the binding is largely electrostatic, while in transition metal complexes there is significant covalent. Generally ligands are Lewis bases that have lone pair on nitrogen, oxygen, sulphur, etc and that bind with transition metals (Lewis acids). Ligands can be divided into unidentate, bidentate and multidentate types, according to the availability of one, two or more donor atoms bonding with metal [9,22]. Multidentate ligands which are bonded to the metal through their two or more atoms form heterocyclic ring(s) (usually five or six member

in which the metal is one of the members. Such a metal complex is termed as metal chelate. But sometimes, the stereochemistry of some ligands does not allow the entire binding site to be simultaneously used [20].

Metal complexes are of great interest for many years. It is well known that N, S and O atoms play a key role in the coordination of metals at the active sites of numerous metal biomolecules. Chelating ligands containing O, N and S donor atoms show broad spectrum of biological activities and are of special interest in the variety of ways in which they are bonded to metal ions [27].

The development of the field of bioinorganic chemistry has increased the interest in Schiff's base complexes, since it has been recognized that these complexes may serve as models for biologically important species [23,33]; coordination compounds derived from arylhydrazones have been reported as anti-tuberculosis, anti-microbial and corrosion inhibitors [18].

Schiff's bases possessing azomethine group (RHC=N-R'), first reported by Hugo Schiff in 1864 (Burger, 1973), are condensation products of carbonyl compounds and primary amines. The presence of a dehydrating agent normally favours the formation of Schiff's bases. Though the Schiff's bases are stable solids, care should be taken in the purification steps as it undergoes degradation. Presence of a lone pair of electrons in 2p orbitals of nitrogen atom of the azomethine group is of considerable chemical importance and imparts excellent chelating ability especially when used in combination with one or more donor atoms close to the azomethine group. This chelating ability of the Schiff's bases combined with the ease of preparation and flexibility in varying the chemical environment about the C=N group makes it an interesting ligand in coordination chemistry [18].

Schiff's bases are important class of ligands in coordination chemistry and find extensive application in different fields [15, 21, and 44]. Schiff's base ligands and their complexes have been extensively studied for their structures and applications. Schiff's bases derived from the carbonyl compounds and ethylenediamine are well known polydentate ligands forming neutral complexes [41,50]. Metal complexes of the Schiff's bases are generally prepared by treating metal salts with Schiff's base ligands under suitable experimental conditions [24]. However, for some catalytic application the Schiff's base metal complexes are prepared in situ in the reaction system [36]. The interactions of these donor ligands with metal ions give complexes of different geometries which are potentially biologically active [18]. Thus, in recent years metal complexes of Schiff's bases have attracted considerable attention due to their remarkable antifungal, antibacterial, antitumor and anticancer activities [7, 17, and 18]. Many biologically important Schiff's bases have been reported in the literature possessing, antibacterial, antifungal, anti-inflammatory, anticonvulsant, antitumor and anti HIV activities [34,44].

Transition metal complexes with Schiff's bases have expanded enormously and embraced wide and diversified subject comprising vast areas of bio-organic compounds. Many studies have been done on transition metal complexes of Schiff's bases due to the fact that Schiff's bases offer opportunities for inducing substrate chirality, tuning metal centred electron factor, enhancing the solubility and stability of either homogenous or heterogeneous catalyst [13]. Schiff's base ligands are able to coordinate many different metals and stabilize them in various oxidation states [4].

Transition metal complexes of N-donor ligands (Schiff's bases) showed anti-*Candida* activities [35]. Transition metal complexes of Schiff's base have become important due to their ability to serve as polymeric ultraviolet stabilizers, as laser dyes and molecular switches in logic or memory circuits. The first row transition metal complexes such as Co(II), Ni(II), Cu(II) have been found to exhibit fungicidal, bactericidal and antiviral activity [8].

Ketoazomethines, a class of Schiff's bases, possessing adjoining azomethine (RHC=N-R') and ketonic (C=O)

groups, well known for their biological properties, dyeing characteristics and analytical applications, have been identified as novel ligands in forming complexes of isomeric structures, unusual stereochemistries and iso- and polynuclear species [44]. The coordination activity of the carbonyl group of the ketoanils derived from primary substituted aromatic amines generally depends upon the nature and position of the substituent(s), which is another interesting feature of these compounds as ligands [48].

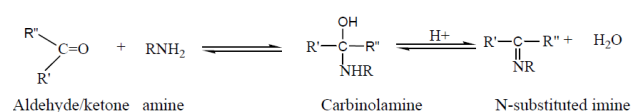
General Objective of the Study was to study spectra, magnetic and antimicrobial properties of complexes of Ni(II), Cu(II) and Zn(II) complexes with N,N-di(o-hydroxybenzenoylmethylene)ethylenediamine, a polydentate ligand

Specific Objectives were:

- To synthesize Schiff's base ligand, ethylenedianil of o-hydroxyphenylglyoxal.
- To determine structure of mentioned new ligand and its Ni(II), Cu(II) and Zn(II) complexes by elemental analysis, conductometric, spectra and magnetic studies.
- To investigate antimicrobial (bactericidal and fungicidal) properties of ethylenedianil of o-hydroxyphenylglyoxal and its complexes.

2. Literature Review

Schiff's base was first reported by Hugo Schiff in 1864 [49]. Schiff's bases can be prepared by condensing carbonyl compounds and amines in different conditions and in different solvents with the elimination of water molecules (scheme 1). The presence of a dehydrating agent normally favours the formation of Schiff's bases [16]. Though the Schiff's bases are stable solids, care should be taken in the purification steps as it undergoes degradation. Chromatographic purification of Schiff's bases on silica gel is not recommended as they undergo hydrolysis [1]. The common structural feature of these compounds is the azomethine group with a general formula RHC=N-R', where R and R' are alkyl, aryl, cyclo alkyl or heterocyclic groups which may be variously substituted. Presence of a lone pair of electrons in a sp² hybridized orbital of nitrogen atom of the azomethine group is of considerable chemical importance and impart excellent chelating ability especially when used in combination with one or more donor atoms close to the azomethine group [45]. Examples of a few compounds are given in scheme 2 and 3. This chelating ability of the Schiff's bases combined with the ease of preparation and flexibility in varying the chemical environment about the C=N group makes it an interesting ligand in coordination chemistry [47].

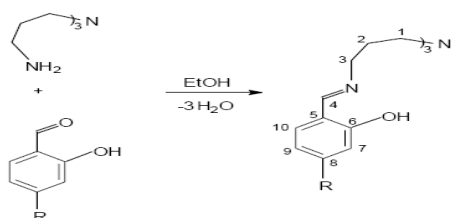


Scheme 1. Formation of Schiff's base from aldehyde or ketone in acid catalyzed dehydration

Polydentate ligands in the field of coordination chemistry and their metal complexes (chelates) are of great interest since many years. It is well known that N, S and O atoms play a key role in the coordination of metals at the active sites of numerous metalloproteins. Chelating ligands containing O, N and S donor atoms show broad spectrum of biological activities and are of special interest because of the variety of ways in which they are bonded to metal ions [8].

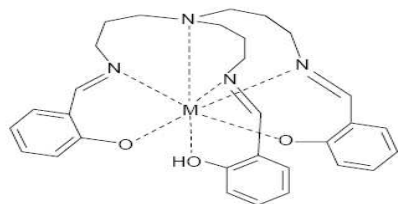
Schiff's bases derived from the salicylaldehydes are well known as polydentate ligands [41, 50]. The interaction of these ligands and metal ions give complexes of different geometries are potentially biologically active [18]. In recent years synthesis and characterization of mixed ligand complexes of 8-hydroxyquinoline and o-hydroxybenzylidene-1-phenyl-2,3-dimethyl-4-amino-3-pyrazolin-5-on with Fe(II), Co(II), Ni(II) and Cu(II) ions have attracted considerable attention due to their remarkable antifungal, antibacterial, antitumor and anticancer activity [5,17,18 and 24]. In particular, Merck company has successfully developed an antibacterial drug cilastatin using chiral copper (II). Several research papers have been synthesized and characterized on transition metal complexes of Schiff's base derived from salicylaldehyde [7].

Two interesting heptadentate Schiff's base ligands, tris[3-(salicylidene-imino)propyl]amine and tris(3-(4'-propyl)amine), derived from condensation reactions of tris(2-aminoethyl) amine (tren) with various ring substituted salicylaldehydes, have been prepared, and the amine phenol ligands, which have been prepared from the reduction of the corresponding Schiff-base ligands, have been reported as shown in scheme 2 and 3 [13].



R = H, H₃L₁, R = OH, H₃L₂

Scheme 2. The procedure of heptadentate ligand synthesis

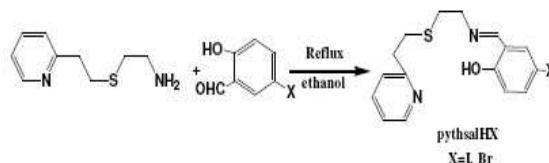


M = Ni(II) or Cu(II)

Scheme 3. The chemical structure for the heptadentate complexes.

Reports on four tetradentate monoanionic ligands pythsalHX (X = I, Br) all having an NSNO donor atoms set synthesized by the 1:1 condensation reaction 1-(2-pyridyl)-

3-thia-5-aminopentane with respective salicylaldehyde derivative in purified ethanol (scheme 4) are known to behave as antitumor agents. Iron complex of these ligands are reported as shown in scheme 3 [10].



Scheme 4. Schematic representation of Schiff's base ligands formation.

In coordination chemistry, Schiff's bases are an important class of ligands and come across widespread applications in different fields [14, 21 and 44]. The interaction of these ligands and metal ions gives complexes of different geometries which are potentially biologically active [18]. The π -system in a Schiff's base often imposes a geometrical constriction and affects the electronic structure as well. Thermochemical properties of Schiff's bases have attracted the attention of many researchers, in view of their ability to bind through NO or N₂O₂ donor atom sets [11]. These Schiff's base metal derivatives involving bidentate or tetradentate bonding of ligands in biological systems, have considerable interest and also contributing to the knowledge of their structure and behavior in various activities [28].

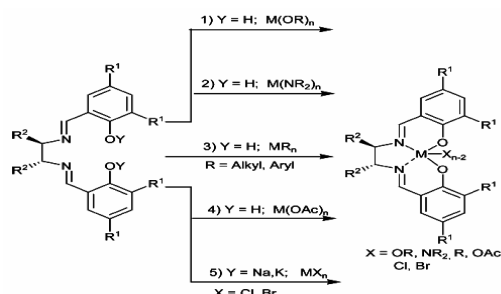
In the different areas Schiff's base complexes do have a number of applications such as; electroluminescent materials, in non-linear optical devices, in electrochemical sensors, in medicinal chemistry etc. Many Schiff's bases are known to be medicinally important and are used to design medicinal compounds [23]. It has been seen that the biological activity of Schiff's bases is generally either increased or decreased upon chelation with metal ions [2]. Cobalt (II), nickel(II), copper(II) and zinc (II) complexes of Schiff's bases show potent antibacterial activity [28,42].

Coordination compounds are important due to their role in biological and chemical systems in various ways [31]. It has been observed that metal complexes with appropriate ligands are chemically more significant and specific than the metal ions and original ligand [29, 43]. Currently the significance of complexed metal ions in various biological systems has become important, as they are more powerful biological active compounds [45]. Moreover, the evidences supporting the use of metal complexes in the fight against cancer, tumor, viruses and bacteria have further made this subject a matter of great research interest. There are a large number of metal complexes that are anticancer, antitumor and antibacterial [34]. The complexation of metals with biologically inactive compounds renders them active and in case the compounds are already active, it makes them more active. The mechanism involved in enhancing this biological activity upon complexation is still needed to be further investigated [14, 30, 38 and 50].

Much more attention has been devoted by bioinorganic as well as by medicinal chemists to develop the relationship between the metal ions and their complexes to use as antitumor and antibacterial agents. *In vitro* studies have indicated that some biologically active compounds may become more carcinostatic and bacteriostatic upon chelation [18]. Such interaction of transition metal ions with amino acids, peptides and pyrones, are of immense biological importance. Several reviews show that the metallo-organic chemistry of such compounds greatly influence their biological action highlighting the catalytic function of metals in many biological processes [8].

Evidences supporting the introduction of metallic elements in several biological processes are rapidly accumulating. Kirshener *et al.*, have investigated the antibacterial, antiviral and anticancer activities of more than twenty five inorganic compounds which included the metal atom as potentially significant part of the molecule [25]. They suggested that the transfer of metal ion from the ligand to the cancer-associated viruses was a mechanism for releasing the anticancer drug in the locality of the tumor. Due to significant nature of metallic ions, their metal complexes are now being included in the search for ideal anticancer drugs [7].

Metal complexes of the Schiff's bases are generally prepared by treating metal salts with Schiff's base ligands under suitable experimental conditions. However, for some catalytic applications the Schiff's base metal complexes are prepared in situ in the reaction system [49]. These complexes play a central role in various homogeneous catalytic reactions and the activity of these complexes varies with the type of ligands, coordination sites and metal ions. Literature reports reveal that a large number of Schiff's base metal complexes exhibit catalytic activities. Chiral Schiff's base complexes are more selective in various reactions such as oxidation, hydroxylation, aldol condensation and epoxidation [6]. Cozzi in his review has outlined five synthetic routes that are commonly employed for the preparation of Schiff's base metal complexes and these are depicted in Scheme V [10].



Scheme 5. Preparation of Schiff's base complexes

Route 1 involves the use of metal alkoxides ($M(OR)_n$). Alkoxides of early transition metals ($M = Ti, Zr$), are commercially available and easy to handle. The use of other alkoxide derivatives is not easy, particularly in the case of highly moisture-sensitive derivatives of lanthanides.

Metal amides $M(NMe_2)_4$ ($M = Ti, Zr$) are also employed as the precursors in the preparation of Schiff's base metal complexes (Route 2).

The reaction occurs via the elimination of the acidic phenolic proton of the Schiff's bases through the formation of volatile $NHMe_2$. Other synthetic routes include treatment of metal alkyl complexes with Schiff's bases (Route 3) or treatment of the Schiff's base with the corresponding metal acetate under reflux conditions (Route 4). The synthetic scheme presented in route 5 which is quite effective in obtaining salen-type metal complexes consists of a two-step reaction involving the deprotonation of the Schiff's bases followed by reaction with metal halides. Deprotonation of the acidic phenolic hydrogen can be effectively done by using NaH or KH in coordinating solvents and the excess sodium or potassium hydride can be eliminated by filtration. The deprotonation step is normally rapid at room temperature, but heating the reaction mixture to reflux does not cause decomposition [11].

Plenty of Schiff's base complexes have been worked out including ketoanils [46] but a search through literature reveals that there is no work that has been done on the transition metal complexes of ethylenediamine of o-hydroxyphenylglyoxal. Therefore in the present project synthesis of mentioned ketoanil Schiff's base and its complexes with a few biologically important metal ions, viz. $Ni(II)$, $Cu(II)$ and $Zn(II)$ and their characterization for structures and antimicrobial properties is planned and carried out.

3. Experimental

3.1. Chemicals and Reagents

Metal salts that are used for synthesis of the complexes include $NiCl_2 \cdot 6H_2O$, $ZnCl_2 \cdot 2H_2O$ and $CuCl_2 \cdot 2H_2O$; all were BDH/Uni-Chem products. Ethylenediamine (Fisher scientific make), Selenium dioxide, (from S.Merck, India) and orthohydroxyacetophenone (SISCO, India) were used as supplied. Solvents such as methanol, ethanol, butanol, chloroform ($CHCl_3$), toluene, acetone, DMSO, DMF, benzene, methyldichloride (dichloromethane), propanol, acetic acid, methyl cyanide, ether etc. were BDH reagents.

3.2. Instruments and Apparatus

Melting point of the ligands and their metal complexes determined in open glass capillaries with Stuart SMP10 digital melting point apparatus (Table 1). Chemical analysis for C, H and N elements of the samples conducted at IIT, Roorkee (India) on Vario EL-III Analyser. Infrared (IR) spectrum of samples also recorded using a SHIMADZU Prestige 21 FT-IR spectrometer in the range $4000-200\text{ cm}^{-1}$ in KBr medium in India, Roorkee at IIT. UV-Vis spectrophotometer studies conducted in the range 200-900 nm using SP65 UV-Vis spectrophotometer at research laboratory, Haramaya University. Magnetic

susceptibility measurements performed at room temperature (295 °K) using MSB-AUTO, (Sherwood Scientific Ltd.) magnetic susceptibility balance at Addis Ababa University. The molar conductance measurements carried out using Jenway digital conductivity meter in methanol at research laboratory, Haramaya University.

A number of supplementary common laboratory equipment such as beakers, round bottom flask, test tubes, analytical balance, magnetic stirrer, separatory funnel, oven, condensers, water bath, suction filter pump were also used during the investigation.

3.3. Synthesis of O-Hydroxyphenylglyoxal and Ligand (Ethylenedianil of O-Hydroxyphenyl Glyoxal)

Orthohydroxyphenylglyoxal, which is precursor of ketoanil, was prepared by oxidation of o-hydroxyacetophenone (68 g, 0.5 mol) with selenium dioxide (55.6 g, 0.5 mol) in 95% alcohol followed by refluxing for 6 h. Refluxed reaction mixture was allowed to cool and yellow brown solution was decanted, filtered,

concentrated over water bath, dissolved in diethyl ether and filtered off to remove selenium metal from the product, if any and left for crystallization.

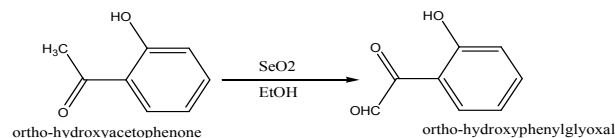


Figure 1. Preparation of Orthohydroxyphenylglyoxal

The ligand ethylenedianil of ortho-hydroxyphenylglyoxal(L) was synthesized by the condensation of ethylenediamine and ortho-hydroxyphenylglyoxal. The reaction mixture containing ethylenediamine (0.2 mol) and ortho-hydroxyphenylglyoxal (0.5 mol) in ether was stirred at room temperature (~20 °C). The yellow brown precipitate obtained was crystallized from acetone and product was finally washed with ether and dried in air.

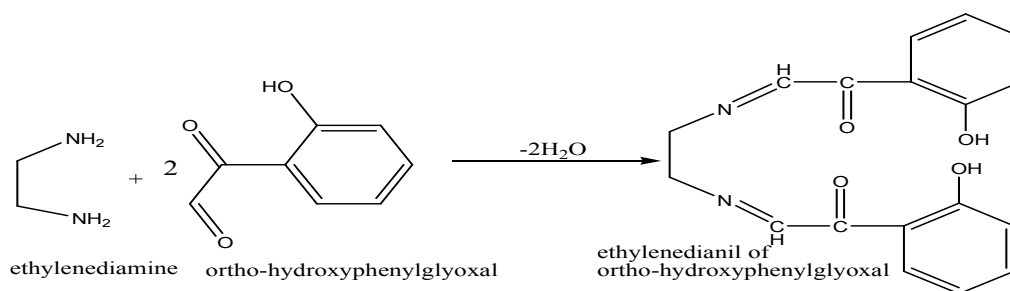


Figure 2. Preparation of N,N-di(o-hydroxybenzenoylmethylene)ethylenediamine

3.4. Preparation of the Metal Complexes

For the preparation of metal complexes saturated solutions of ligand and metal chlorides in acetone/methanol were mixed together in 3:2 molar ratios. The black green copper complex precipitated immediately was filtered out, washed with acetone repeatedly and dried in air; on adding excess of the ligand to the filtrate a green brown precipitate of other copper complex obtained was filtered, washed with acetone and dried. Ni(II) and Zn(II) complexes were obtained as residues on evaporation of their reaction mixtures on water bath at ~50°C; the residues were washed with water, and finally crystallized from methanol.

3.5. Purification

Each of the products including the Schiff's base ligand and its complexes were tested for their purity by thin-layer chromatography (TLC) on silica gel layers using chloroform, acetone and butanol solvents. TLC plates were activated by heating at 60°C to ensure compactness of spots before use. Sample solutions in methanol were spotted on warm plates as small drops using glass capillaries in a series on a line 2 cm from the lower edge of the plate. The oven dried loaded plates were developed in rectangular glass chambers with ground-in-lids by ascending

techniques. To obtain reproducible results, the development chambers were saturated with solvents before use. When the development had proceeded for 8 cm the plates were removed from the chamber. Owing to the dark colors of the analytes they were only discernible in day light. All the products migrated as single spot except Ni(II) complex in all the solvents used. Since Ni(II) complex showed two spots in acetone, it was purified by washing with acetone.

3.6. Chloride Analysis

Every complex (0.05 gm) was decomposed with conc. HNO₃ and diluted. To the resultant solution of the complexes, aqueous solution of AgNO₃ was added. A cruddy white precipitate of AgCl was formed in the case of Cu(II) and Zn(II) complexes, which was soluble in ammonia, whereas in the case of Ni(II) complex no precipitate was observed. To the solution of complexes in methanol, addition of AgNO₃ solution also gives no precipitate of AgCl.

3.7. Determination of Molecular Weight

Molecular weight of the ligand was determined by Rast's method using camphor (melting point, 176 °C) as solvent. Molecular weights of complexes could not be determined

owing to their high melting points (greater than 200 °C). 20 mg of the ligand was mixed with 300 mg camphor and the mixture was ground and melted in crucible to ensure homogeneity. Then the freezing point of molten mixture was determined in open glass capillary with melting point apparatus. Once ΔT_f 's were recorded the molecular weight of the ligand was calculated by Rast's formula as follows.

$$M_w = (1000 k w) / \Delta T_f W \quad (1)$$

where k is cryoscopic constants for camphor (39.7°C); w is weight of camphor; W is weight of ligands; ΔT_f is depression in freezing or melting point, i.e. difference between T_f of camphor and T_f of mixture.

3.8. Atomic Absorption Spectroscopy (AAS)

This analysis was carried out by direct method to get total metal content. A number of reference standard solutions of varying concentrations of each metal were prepared. Absorbance of these solutions was measured at the specific wavelength of each metal using background correction technique [40] and a graph was plotted between absorbance and concentration (calibration curve) of each metal solution, which should show a straight line in each case. Accordingly, concentrations of the unknown solutions of the complexes were calculated from the absorbance of the known solutions by using the standard values. Calibration curves of the metals under study are depicted in Figure 21, 22, 23 and 24. The experimental percentage of metal in the complexes was obtained from the AAS data using the following formula:

$$M (\%) = 0.1 A V / m \quad (2)$$

Where A is absorbance (ppm); V is volume of metal complex solution (ml); m is mass of the sample (g).

0.01 g of each metal complex was dissolved in 20 mL of conc. HNO_3 and digested. Then 100 mL standard solutions of deionized water was prepared for each metal complex and analyzed by AAS. The results are tabulated in Table 4.

3.9. Magnetic Measurements

For calculation of magnetic moment of complexes, the gram susceptibility (χ_g) data obtained experimentally for the respective metals was used for calculating their effective magnetic moments (μ_{eff}).

$$\text{Molar magnetic susceptibility } (\chi_M) = \chi_g M_w.$$

Effective susceptibility (χ'_M) = χ_M -diamagnetic correction

$$\text{Magnetic moment } (\mu_{eff}) = 2.84(\chi'_M T)^{1/2}$$

3.10. Antimicrobial Antifungal Tests

Antimicrobial and antifungal activities of the ligands and their complexes were tested *in vitro* against two bacterial and two fungal species using disc diffusion method. Both the bacteria and fungi were cultivated on Mueller Hinton agar (MHA) and potato dextrose agar medium respectively.

The degree of bactericidal and fungicidal activities was determined by measuring diameter of the inhibition zone and compared with the standard drug *chloramphenicol* and *bavistin* respectively (Mapari and Mangaonkar, 2011). The bacterial strains, *Staphylococcus aureus* and *xanthomonas holcicola*, were transferred from the stock cultures and streaked on Mueller Hinton agar (MHA) plates and incubated for about 24 h. Bacteria were transferred using bacteriological loop to autoclaved MHA that was cooled to about 45 °C in water bath and mixed by gently swirling the flasks. The medium was then poured to sterile Petri dishes, allowed to solidify and used for the biotest. For the test fungi, mycelia plugs from the stock cultures were transferred to PDA plates and incubated for 6 days. Then spores of the test fungi were harvested by washing the surface of the colony using 10 mL sterile distilled water and transferred to 50 mL autoclaved PDA cooled to about 45 °C in a water bath. The medium containing spore suspension was poured to sterile plates, allowed to solidify and was used for the paper disc diffusion bioassay. The synthesized ligand and its complexes were dissolved in DMSO at a concentration of 5 mg/ml.

Paper discs about 6 mm in diameter were cut from Watman-filter paper and punched with an office paper punch and placed in a beaker covered with aluminum foil and sterilized in an oven at 180 °C for 1 h. Aliquots of 10 μ L and 20 μ L of the sample solutions of the ligand and its complexes were pipetted to the discs in three replications each. The paper discs impregnated with the sample solutions were then transferred using sterile forceps to PDA seeded with spore suspension of test fungi as described under inoculum preparation above. The petri dishes were incubated at 26 °C for 6 days. All the tests were performed in triplicate. The effectiveness of each sample was evaluated by measuring the inhibition zone against the tested organisms.

Similar procedures were followed for testing antibacterial activities. Paper discs were transferred to Mueller Hinton agar (MHA) plate seeded with bacteria and incubated at 37 °C for 24 h. All the tests were performed in triplicate. Antibacterial activity was evaluated by measuring the zone of inhibition against the tested organisms.

4. Results and Discussion

4.1. Composition of Ligands and Their Complexes

Stoichiometries of the ligand and its complexes have been deduced from their elemental analyses data (Tables 1 and 2).

Freezing point of mixture of ligand with camphor was 168°C and ΔT_f was 8°C; these data were used for determination of molecular weight (M_w) by Rast's method (Table 1). The experimental M_w of the ligands was consistent with its theoretical value.

Table 1. Characteristics of ligands and their complexes

Empirical formula	M _w	Yield (%)	Color	T _m (°C)	Elemental analyses (%)		
					Calc. (found)	H	N
C ₁₈ H ₁₆ N ₂ O ₄ (L)	324 ^t (330.8) ^e	41	Yellow	152±2	66.67 (67.01)	4.94 (4.62)	8.64 (8.43)
Ni(C ₁₈ H ₁₄ N ₂ O ₄).H ₂ O	398.7 ^t	64	Brown yellow	245±2	54.18 (54.42)	4.01 (4.86)	7.59 (8.06)
[Cu ₃ (C ₁₈ H ₁₄ N ₂ O ₄) ₂] ⁺² .2Cl ⁻ .4H ₂ O	977.5 ^t	58	Black green	278±2	44.20 (43.55)	3.30 (3.44)	7.59 (8.02)
[Cu ₃ (C ₁₈ H ₁₄ N ₂ O ₄)Cl ₂ (H ₂ O) ₂] ⁺² .2Cl ⁻	690.5 ^t	42	Green brown	283±2	31.28 (32.13)	2.32 (2.49)	4.05 (4.42)
[Zn ₂ (C ₁₈ H ₁₄ N ₂ O ₄)Cl(H ₂ O)] ⁺ .Cl ⁻ .4H ₂ O	613.8 ^t	82	Light yellow	>300	35.19 (34.83)	3.58 (3.89)	4.56 (5.06)

Note: t is calculated molecular weight, e is experimental molecular weight

4.2. Results of AAS

Table 2. AAS data of the complexes

Compound	Metal in the complex (%)	
	Calculated	Found
Ni(C ₁₈ H ₁₄ N ₂ O ₄).H ₂ O	14.72	15.56
[Cu ₃ (C ₁₈ H ₁₄ N ₂ O ₄) ₂] ⁺² .2Cl ⁻ .4H ₂ O	19.50	20.27
[Cu ₃ (C ₁₈ H ₁₄ N ₂ O ₄)Cl ₂ (H ₂ O) ₂] ⁺² .2Cl ⁻	28.99	30.10
[Zn ₂ (C ₁₈ H ₁₄ N ₂ O ₄)Cl(H ₂ O)] ⁺ .Cl ⁻ .4H ₂ O	14.25	14.93

Atomic Absorption Spectroscopy (AAS) analysis of the complexes was carried out by direct method which gave

total metal content. The calculated and experimental values of metal percentage in each complex are in fair agreement. These results are very supportive of the proposed formulae of the complexes (Table 2).

By using the relation $\Lambda_M = K/C$, the molar conductance of the complexes (Λ_M) has been calculated, where C is the molar concentration (1.0×10^{-3}) of the metal complex solutions in methanol. Molar conductance values of the complexes are depicted in Table 3. Λ_M values of the chelates reveal non-electrolytic nature of the Ni(II) complex, 1:2 electrolytic nature of the Cu(II) complex and 1:1 electrolytic nature of the Zn(II) complex.

Table 3. Data of conductivity measurements

Complex	Conc. of complex (mol/L)	Cond. (Λ) $\times 10^6 \Omega \text{cm}^{-1}$	Specific cond. ($L = k\Lambda$) $\times 10^6 \Omega \text{cm}^{-1}$	Molar cond. (Λ_M) 1000L/C $\Omega^{-1} \text{cm}^2 \text{mol}^{-1}$	Elect. nature
Ni(C ₁₈ H ₁₄ N ₂ O ₄).H ₂ O	0.0020	82.1	78.76	39.69	Non-elect.
[Cu ₃ (C ₁₈ H ₁₄ N ₂ O ₄) ₂] ⁺² .2Cl ⁻ .4H ₂ O	0.0010	134.7	130.66	159.65	1:2 elect.
[Cu ₃ (C ₁₈ H ₁₄ N ₂ O ₄)Cl ₂ (H ₂ O) ₂] ⁺² .2Cl ⁻	0.0012	190.8	185.07	154.22	1:2 elect.
[Zn ₂ (C ₁₈ H ₁₄ N ₂ O ₄)Cl(H ₂ O)] ⁺ .Cl ⁻ .4H ₂ O	0.0013	112.4	109.03	86.46	1:1 elect.

Note: Cell constant (k) = 0.97, Conductance of methanol (solvent) = $1.04 \times 10^{-4} \Omega \text{cm}^{-1}$

All the synthesized compounds were stable in air and higher melting points of the complexes than that of the ligand (Table 1) reveal that the complexes are much more stable than the ligand. The synthesized ligand was soluble in methanol, propanol, ethanol and acetone but insoluble in benzene and toluene whereas all the complexes were soluble in methanol, ethanol, propanol and insoluble in benzene.

From the elemental analysis (C, H, N and metal) data and the molar conductance values, molecular formulae of the ligand and its complexes have been derived as noted in Tables 1-3.

In order to determine the binding modes (coordination sites) of the ligand in the complexes, IR spectrum of the

ligand was compared with the spectrum of the complexes. Phenolic group stretching vibration observed at 3458 cm^{-1} in the free ligand spectrum disappeared in the spectrum of the complexes. The appearance of a new band in the range of $488-554 \text{ cm}^{-1}$ attributed to M-O groups, clearly reveals an involvement of phenolic group of the ligand in coordination with the metal ions. Another band in the range of $453-473 \text{ cm}^{-1}$ corresponds to M-O-M bridged vibration in Cu(II) and Zn(II) complexes. Considerable disturbance in oscillations of phenolic C-O groups of the ligand during complexation also supports the coordination of phenolic group of the ligand. The azomethine groups stretching vibrations, displayed in the ligand spectrum at 1613 cm^{-1} , appeared at low value in complexes indicating presence

of this group in coordination zone of the metal complexes. A new band in spectra of the complex at 465-482 cm^{-1} characteristic of M-N groups, confirms coordination of the azomethine group of ligand with metal ions [47].

The band at 247 cm^{-1} exhibits a chlorine bridged Cu-Cl-Cu structure of the monoligand trinuclear Cu(II) complex, whereas a band at 303 cm^{-1} in Zn(II) complex shows coordination of chlorine at terminal position.

Two to three bands displayed in the range of 832-1044 cm^{-1} of the monoligand trinuclear Cu(II) and monoligand binuclear Zn(II) complexes correspond to wagging, twisting and rocking vibrations of H_2O molecules. These

indicate presence of water molecules in the coordination zone of the metals. The low energy bands at 332 cm^{-1} and 267 cm^{-1} respectively, being attributed to oscillations of M-OH₂ groups, support the coordination of water molecules into these complexes. The lattice water displays in the spectrum of the complexes the symmetric & antisymmetric stretching and bending vibrations in the ranges of 3215-3493 cm^{-1} and 1601-1646 cm^{-1} , respectively.

The gram magnetic susceptibility values and the magnetic moment in BM were calculated as presented in Table 4.

Table 4. Data of magnetic measurements

Complexes	Gram susceptibility (X_g) $\times 10^6$	Molar susceptibility (X_M) $\times 10^6$ C.G.S	Diamagn. correction 10^6 C.G.S	Effective susceptibility (X_M) $\times 10^6$	Effective magnetic moment (μ_{eff}) (B.M.)	Hybridization
Ni(C ₁₈ H ₁₄ N ₂ O ₄).H ₂ O	1.287	513.13	-147.88	661.01	1.25	$\text{dsp}^2 \rightleftharpoons \text{sp}^3 \text{d}^2$
[Cu ₃ (C ₁₈ H ₁₄ N ₂ O ₄) ₂] ²⁺ .2Cl ⁻ .4H ₂ O	0.412	410.00	-233.68	644.65	1.23	Sp ² d
[Cu ₃ (C ₁₈ H ₁₄ N ₂ O ₄)Cl ₂ (H ₂ O) ₂].2Cl ⁻	0.505	348.70	-247.88	596.58	1.19	Sp ² d
[Zn ₂ (C ₁₈ H ₁₄ N ₂ O ₄)Cl(H ₂ O)] ⁺ .Cl ⁻ .4H ₂ O	-	-	-240.08	-	Diamag.	Sp ³

T=22 °C, 295 °K

The band splitting pattern in the absorption spectrum of Ni(II) complex is consistent with square planar geometry of its spin-paired d⁸ configuration whereas magnetic moment of 1.25 B.M., lower than spin free value (2.83 BM) could be accounted for the presence of some octahedral spin free state of this complex along with the square planar spin paired system in the solid state (Table 4).

Magnetic moments of the two Cu(II) complexes, which are 1.23 BM and 1.19 BM respectively are lower than the expected spin free value of 1.73 BM. This may be attributed to antiferromagnetic interactions of the metal atoms in these trinuclear paramagnetics.

The three bands displayed in the electronic spectrum of both Cu(II) complexes in 24390-25000 cm^{-1} , 26316-27027 cm^{-1} and 28986-30303 cm^{-1} regions are characteristic of ${}^2\text{B}_{1g} \rightarrow {}^2\text{A}_{1g}$, ${}^2\text{B}_{1g} \rightarrow {}^2\text{B}_{2g}$ and ${}^2\text{B}_{1g} \rightarrow {}^2\text{E}_g$ transitions respectively of square planar geometry. The high energy bands spreading in 34483-43478 cm^{-1} range are owing to ligand to metal charge transfer. The two bands displayed in the electronic spectrum of Ni(II) complex at 23529 and 28572 cm^{-1} are characteristic of ${}^1\text{A}_{1g} \rightarrow {}^1\text{B}_{1g}$ and ${}^1\text{A}_{1g} \rightarrow {}^1\text{A}_{2g}$ transitions respectively of square planar Ni(II) complex [12,27].

Table 5. Electronic spectrum of ligand and M-L complexes

Complex	Band Frequency (cm^{-1})	Assignment	Geometry
Ni(C ₁₈ H ₁₄ N ₂ O ₄).H ₂ O	23529	${}^1\text{A}_{1g} \rightarrow {}^1\text{B}_{1g}$	Square planar
	28572	${}^1\text{A}_{1g} \rightarrow {}^1\text{A}_{2g}$	
	33898	LMCT	
	37736	LMCT	
	45456	LMCT	
[Cu ₃ (C ₁₈ H ₁₄ N ₂ O ₄) ₂] ²⁺ .2Cl ⁻ .4H ₂ O	24390	${}^2\text{B}_{1g} \rightarrow {}^2\text{A}_{1g}$	Square planar
	26316	${}^2\text{B}_{1g} \rightarrow {}^2\text{B}_{2g}$	
	28986	${}^2\text{B}_{1g} \rightarrow {}^2\text{E}_g$	
	35714	LMCT	
	43478d	LMCT	
	25000	${}^2\text{B}_{1g} \rightarrow {}^2\text{A}_{1g}$	
[Cu ₃ (C ₁₈ H ₁₄ N ₂ O ₄)Cl ₂ (H ₂ O) ₂]. 2Cl ⁻	27027	${}^2\text{B}_{1g} \rightarrow {}^2\text{B}_{2g}$	Square planar
	30303	${}^2\text{B}_{1g} \rightarrow {}^2\text{E}_g$	
	34483	LMCT	
	40000	LMCT	
	43478	LMCT	
	27727	LMCT	
[Zn ₂ (C ₁₈ H ₁₄ N ₂ O ₄)Cl(H ₂ O)] ⁺ .Cl ⁻ .4H ₂ O	27727	LMCT	Tetrahedral

The electronic spectrum of the Zn(II) complex (Table 5) shows an absorption band at 440 nm (27727cm^{-1}) attributed to the L→M (charge transfer) transition, which is compatible with this complex having a tetrahedral structure [39].

4.3. Structures of Ligands and Its Complexes

On collaborating structural interferences arrived from all the aforesaid studies following structures of the ligands and their complexes are proposed (Fig. 3-7).

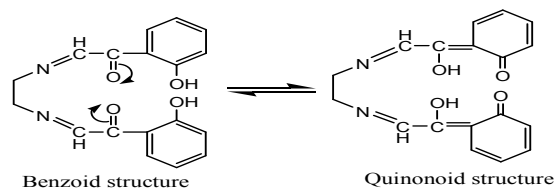


Figure 3. Proposed tautomeric structures of the ligand

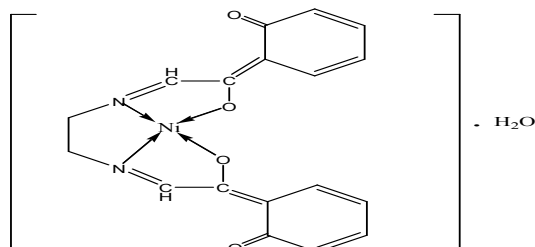


Figure 4. Suggested Structure of Square Planar Nickel(II) complex

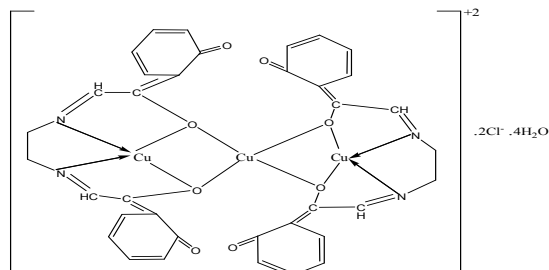


Figure 5. Suggested Structure of trinuclear Copper(II) complex with two ligands

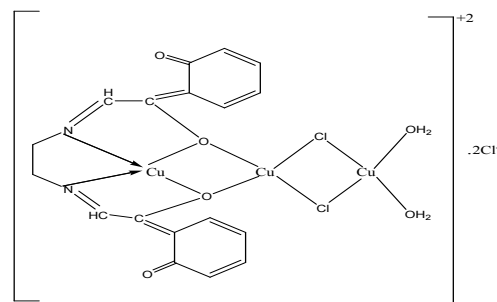


Figure 6. Suggested Structure of trinuclear Copper(II) complex with monoligand

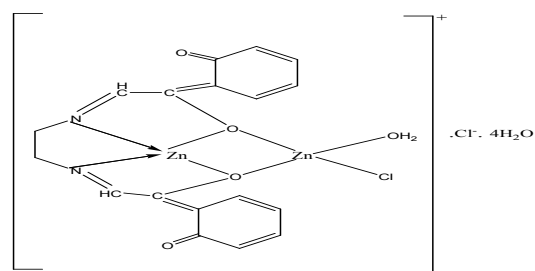


Figure 7. Suggested Structure of Zinc(II) Complex

4.4. Antimicrobial Screening of the Ligands and Their Metal Complexes

All the products including ligands and complexes were screened against *Staphylococcus aureus* and *Xanthomonas holcicola* bacterial strains, as well as *Aspergillus niger* and *Fusarium oxysporum* fungal strains, in order to assess their potential as antimicrobial agents by disc diffusion method. The zones of inhibition based upon zone size around the discs were measured (Fig. 8-11).

The bactericidal activity of the complexes was higher than ligands. It reveals a positive impact of coordination of metals with ligands in enhancing the activity [36]. However among all the four complexes, the Zn(II) complex has shown the highest activity against *Aspergillus niger* and *Fusarium oxysporum* fungus. The Ni(II) complex was equally active as a standard drug. The Zn(II) and biligand trinuclear Cu(II) complexes were close to the Ni(II) complex in their inhibition action against this fungus. It is quite obvious from the results that antimicrobial activities dependent on the dose (Table 6)

Table 6. Antimicrobial activity of the ligands and their complexes

No	Compound	Zone of inhibition (mm)							
		Bacterial strains				Fungal strain			
		<i>Staphylococcus aureus</i>		<i>Xanthomonas holcicola</i>		<i>Aspergillus niger</i>		<i>Fusarium oxysporum</i>	
		10µL	20µL	10µL	20µL	10µL	20µL	10µL	20µL
1	Ni(C ₁₈ H ₁₄ N ₂ O ₄).H ₂ O	15	18	13	22	15	22	15	20
2	[Cu ₃ (C ₁₈ H ₁₄ N ₂ O ₄) ₂] ⁺² .2Cl ⁻ .4H ₂ O	17	19	25	31	8	15	13	20
3	[Cu ₃ (C ₁₈ H ₁₄ N ₂ O ₄)Cl ₂ (H ₂ O) ₂].2Cl ⁻	17	20	22	27	10	15	8	12
4	[Zn ₂ (C ₁₈ H ₁₄ N ₂ O ₄)Cl(H ₂ O)] ⁺¹ .Cl ⁻ .4H ₂ O	21	22	25	32	19	31	12	18
5	Ligands	7	9	0	0	7	10	0	0
6	DMSO (Solvent)	0	0	0	0	0	0	0	0
7	Bavistin (Standard)	-	-	-	-	20	30	15	20
7	Chloramphenicol (Standard)	18	21	25	30	-	-	-	-

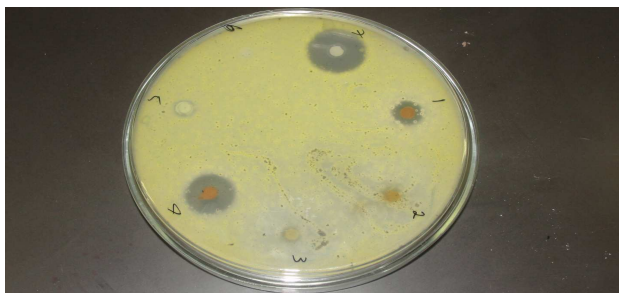


Figure 8. Effects of ligands and their complexes on *Aspergillus niger* fungal strain



Figure 9. Effect of ligands and their complexes on *Fusarium oxysporum* fungal strain



Figure 10. Effect of ligands and their complexes on *Staphylococcus aureus* bacterial strain

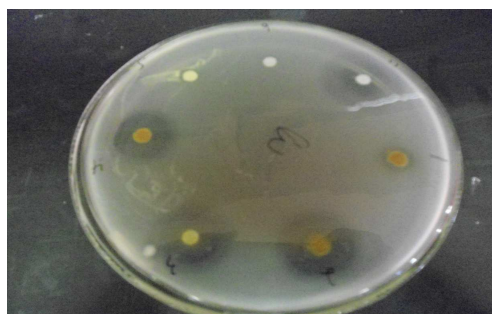


Figure 11. Effect of ligands and their complexes on *Xanthomonas holcicola* bacterial strain

5. Conclusion

In the present research a new Schiff's base ligands, obtained by the coordination of ethylenediamine with orthohydroxyphenylglyoxal, have been used for preparation of Ni(II), Cu(II) and Zn(II) complexes. All the

new products were characterized by spectroscopic data (UV-visible, FT-IR and AAS), molecular weight, elemental analysis, conductance and magnetic measurements. FT-IR studies have showed the coordination of azomethine nitrogen and phenolic oxygen atoms to the metal ions. Magnetic measurements data in conjugation with electronic spectrum data revealed square planar geometry of Ni(II) and Cu(II) complexes, tetrahedral stereochemistry of diamagnetic Zn(II) complex. Molar conductance values showed 1:2 and 1:1 electrolytic nature of both Cu(II) and Zn(II) complexes respectively whereas Ni(II) complex was non-electrolytic. Elemental and metal analysis results are consistent to the molar formulae of the complexes.

Antimicrobial studies revealed the highest antimicrobial activities of the Zn(II) complex against all used microorganisms. The Schiff's base ligands showed smaller activity than complexes of the metals.

Recommendations

On the bases of recent reports that Schiff's bases and their complexes are getting enhanced importance in their applications in treatment of diverse infections including HIV and in other areas like in making light emitting diodes, water purification etc. I therefore, propose and recommend with confidence that the present Schiff's base ligand and other Schiff's bases could be synthesized to prepare complexes with transition metals of 3d,4d and 5d series and products should be characterized for their structures and explored for their applications in different fields of interests.

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Appendices

Appendix 1: Electronic spectrum of Ni(II) and the two Cu(II) complexes

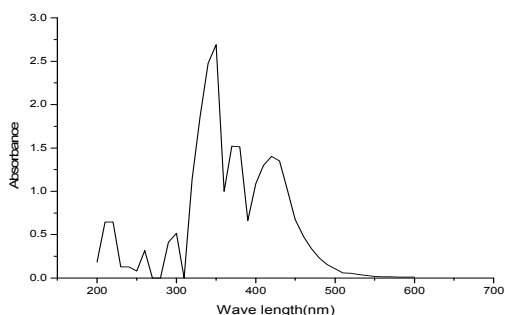


Figure 12. Electronic spectrum of Ni(II) complex, $Ni(C_{18}H_{14}N_2O_4) \cdot H_2O$

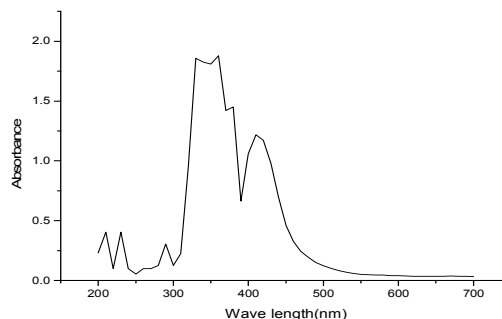


Figure 13. Electronic spectrum of Cu(II) Complex, $[Cu_3(C_{18}H_{14}N_2O_4)_2]^{+2} \cdot 2Cl \cdot 4H_2O$

Appendix 2: IR spectrum of the ligands and complexes

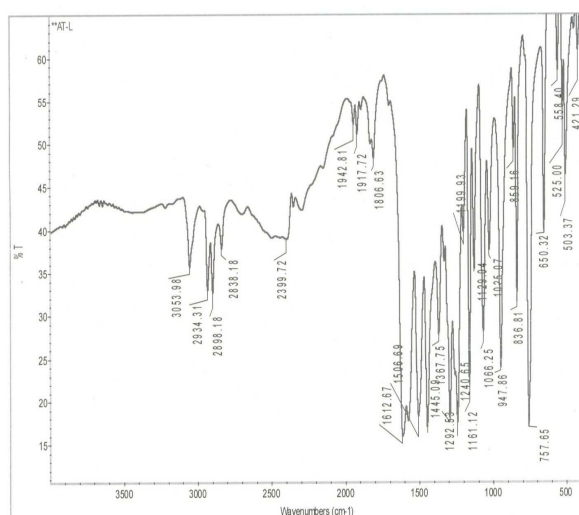


Figure 14. IR spectrum of ligands $C_{18}H_{16}N_2O_4$ ($500-4000\text{ cm}^{-1}$)

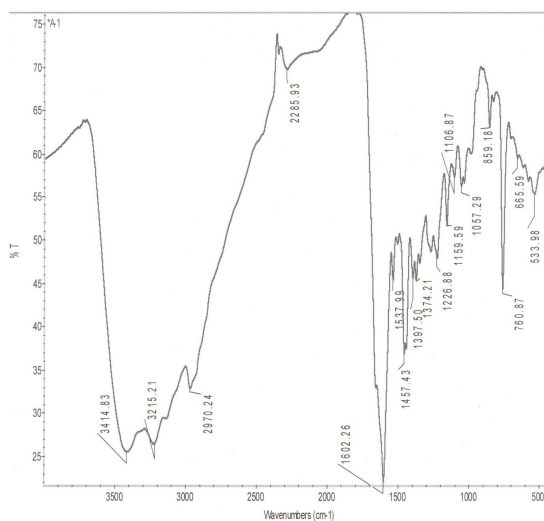


Figure 15. IR spectrum of Cu(II) complex $[Cu_3(C_{18}H_{14}N_2O_4)_2]^{+2} \cdot 2Cl \cdot 4H_2O$ ($500-4000\text{ cm}^{-1}$)

Appendix 3: Atomic absorption spectrum of the complexes

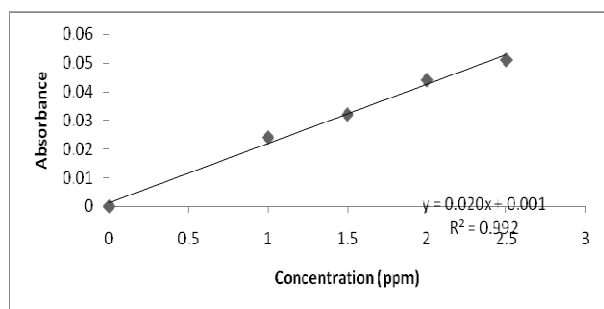


Figure 16. Atomic absorption spectrum of $[Zn(C_{18}H_{14}N_2O_4)Cl(H_2O)] \cdot Cl \cdot 4H_2O$

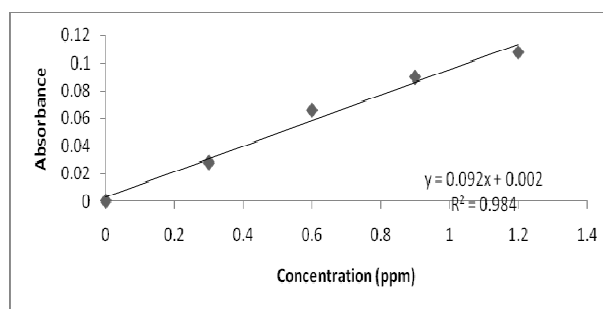


Figure 17. Atomic absorption spectrum of $[Cu_3(C_{18}H_{14}N_2O_4)_2]^{+2} \cdot 2Cl \cdot 4H_2O$

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