

ANTIMICROBIAL EVALUATION OF NOVEL CU(II), NI(II) AND ZN(II) TRANSITION METAL COMPLEXES OF NNO FUNCTIONALIZED SCHIFF BASE LIGANDS

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Abstract

Schiff base ligand obtained by the condensation of Glutaraldehyde with L-valine were complexed with Cu(II), Ni(II) and Zn(II) transition metal ions. Structural features were obtained from their elemental analyses, magnetic susceptibility measurements, molar conductance data, IR, UV-Vis, ¹H-NMR spectral studies and X-ray diffraction study. The UV-Vis and magnetic susceptibility studies suggest an octahedral geometry around the central metal ions. IR data show that the ligand behaves as tetradentate ligand coordinating through both carbonyl oxygen and terminal nitrogen atoms. The molar conductance measurements indicate that the complexes are non-electrolytic in nature. The powder XRD data shows that the complexes are microcrystalline. The antimicrobial activities of ligand and its complexes screened by Disc Diffusion method shows that the metal complexes were more potent than the parent Schiff base ligand against one or more bacterial and fungal species.

Key words: Magnetic Susceptibility; Octahedral; Spectral studies; Disc Diffusion.

1. INTRODUCTION

Bioinorganic chemistry usually deals the interaction of inorganic elements with the organism at the molecular level ^[1]. The interaction between small molecules and biological macromolecules has become an important research topic in bioinorganic chemistry; especially the interaction between transition metal complexes and DNA has aroused the widespread interest ^[2,3]. This helps us not only to understand the life processes at the molecular level but also to promote the development of chemistry discipline itself. Amino acid Schiff bases are usually composed of amino acids with different aldehyde or ketone carbonyl groups. Schiff base is a multidentate ligand, which plays an important role in medicinal and pharmaceutical areas ^[4,5]. Schiff bases, whose metal complexes are of great interest, have often been employed as chelating ligands in the field of coordination chemistry. They are able to coordinate metals through imine nitrogen and another group, more often than not, linked to aldehyde or ketone. In recent years, studies on the amino acid Schiff bases and their metal complexes are very active. Synthesis, characterization, structure, and thermodynamic and kinetic properties of this kind of compounds have been reported, and their antibacterial, anti-inflammatory, and anticancer activities have been widely studied ^[6]. Schiff base metal complexes also find diverse applications in industry and daily life. There exist myriad reports on the biological activities of Schiff base ligands and their metal complexes, including their use for enzyme modeling, catalytic activity and their function as antimicrobial, antifungal and antitumor agents. It has been demonstrated that biological activities of these compounds are influenced significantly by metal coordination. Schiff bases facilitate the process of inducing substrate chirality, tuning the metal-centered electronic factor, enhancing solubility and performing either homogeneous or heterogeneous catalysts ^[7]. Copper, Nickel and Zinc are essential trace elements for the human body, and the metalloenzymes play important physiological functions in the organisms. They have been reported to act as anticonvulsant and antiepileptic agents or vitamins and presented antibacterial, antifungal, antimicrobial, antioxidant, and antiproliferative/anticancer activities. Therefore, the research on complexes has attracted more and more attention and become more and more important in the field of bioinorganic and coordination chemistry. Furthermore, recent studies have witnessed a great deal of interest in the synthesis and characterization of transition metal complexes containing Schiff bases as ligands due to their

applications in pharmaceutical fields. Therefore, it is an important research topic to study the relationship among structure, property and biological activity of these amino acid Schiff base complexes^[8].

In view of the above considerations, we have synthesized three new metal(II) complexes of Schiff base derived from the reactions of L-valine and glutaraldehyde. These complexes have been characterized by elemental analysis, conductance measurement, magnetic susceptibility, UV spectra, infrared spectra, ¹H NMR and Powder X-ray diffraction. Furthermore, the interactions of these complexes with bacterial and fungal species have been studied.

2. MATERIALS AND METHODS

2.1. Materials

All the glassware used for the preparation were cleaned with chromic mixture, detergent and finally with distilled water and then dried in Oven at 110°C. The materials used in the investigation were of reagent grade, purchased commercially and used as received. Reagents such as Glutaraldehyde, L-Valine, metal (II) chloride and nitrate were of Merck products. Double distilled water was used throughout the experimental work.

2.2. Preparation of Schiff Base

Schiff base from L-Valine under investigation were prepared according to the following procedure. In a 50 ml conical flask, separate solutions were prepared by dissolving (1 g) of glutaraldehyde and (0.75 g) of L-Valine in 15 ml of ethanol. Mix the glutaraldehyde solution with the amino acid solution. The mixture was stirred for 8 hours on a water bath at 70°C. The obtained precipitate was filtered off and washed with cold ethanol. The solid products were recrystallized from hot ethanol to give yellow crystals, dried in vacuo over silica gel.

2.3. Preparation of Metal Complexes

The preparation of the metal complexes under study were prepared according to procedure previously described in the literature, thus the complex $[\text{CuL}_2]_n\text{H}_2\text{O}$ have been prepared by the reaction of (2 mmole) of ligands with (1 mmole) of Copper (II) nitrate dissolved in (20 ml) absolute ethanol and stirring for 24 hours. The obtained complexes were collected after evaporation of ethanol and was left in the desiccator to be dried under P_2O_5 . Similarly the other complexes were also prepared according to the same procedure.

2.4 Physical measurements

Microanalytical data of the compounds were recorded using a Vario EL III elemental analyzer. Ultraviolet spectra were recorded using Shimadzu double beam visible spectrophotometer in the visible region. The molar conductance of the metal complexes were determined in DMSO on SYSTRONICS digital conductivity meter. Magnetic susceptibility of the complexes were measured by Guoy balance using Copper sulphate as calibrant. IR spectra in the range of 4000 to 400 cm^{-1} were recorded on a Perkin Elmer FT-IR spectrometer MODEL 1600 as KBR discs. ¹H NMR spectra (300 MHz) of the samples in DMSO- d_6 were recorded by employing TMS as internal standard. Powder XRD were recorded on a computer controlled X-ray diffractometer system JEOL JDX 8030.

2.5 Antimicrobial Activity

In vitro antimicrobial tests were carried out by an adapted agar-disc diffusion technique using 0.5 McFarland suspension of bacteria obtained from 24 h cultures. The antimicrobial activities of the synthesized compounds were determined against *Klebsiella sps*, *Escherichia coli*, *Staphylococcus aureus* (bacteria) and *Candida sps*, *Aspergillus niger* and *Aspergillus fumigates* (fungi) microbial strains. The compounds were solubilised in dimethylsulfoxide to a final concentration of 1 mg/mL. A volume of 10 μL of each tested compounds solution was distributed directly on the solid medium previously seeded with the microbial inoculums. The inoculated petri plates were incubated for 24 h at 37°C. Antimicrobial activity was assessed by measuring the growth inhibition zones diameters. Standard discs of chloramphenicol served as positive controls for antimicrobial activity but filter disc impregnated with solvent were used as a negative control.

3. RESULTS AND DISCUSSION

3.1. Physical Properties and Elemental Analyses

The physical properties and results obtained from C.H.N. analyses and metal contents of the prepared compounds are described in Table 1. The analytical data were almost agreeable with calculated values with some deviations attributed to incomplete combustion or technical errors. The metal complexes were formed in 1:1 stoichiometric ratio, normally stable at room temperature and hygroscopic in nature. The Schiff base ligands are soluble in common organic solvents like ethanol and methanol and the corresponding complexes are soluble in DMSO. The molar conductivity of the metal complexes lies in the range of 10-20 ($\Omega^{-1} \text{mol}^{-1} \text{cm}^2$), indicating that all the complexes were non-electrolytes.

Table 1 :Analytical and physiochemical data of Schiff base and its metal complexes

Ligand/ Metal Chelate	Empirical Formula	Colour	Molar Cond. (Ωm^{-1} $\text{cm}^2 \text{mol}^{-1}$)	Elemental analysis % Found (cal)			
				C Found (Cal)	H Found (Cal)	N Found (Cal)	M Found (Cal)
Glu-val ligand	$\text{C}_{15}\text{H}_{30}\text{N}_2\text{O}_6$	Yellow	-	63.43 (62.11)	8.9 (9.3)	8.7 (9.5)	-
[Cu(glu)(val). 2H ₂ O]	$\text{C}_{15}\text{H}_{30}\text{N}_2\text{O}_6\text{Co}$	Green	12.15	44.54 (45.26)	7.2 (7.6)	6.9 (7.1)	15.01 (15.96)
[Ni(glu)(val).2 H ₂ O]	$\text{C}_{15}\text{H}_{30}\text{N}_2\text{O}_6\text{Ni}$	Pale green	11.5	45.64 (44.35)	7.7 (8.0)	6.9 (7.3)	14.86 (15.20)
[Zn(glu)(val). 2H ₂ O]	$\text{C}_{15}\text{H}_{30}\text{N}_2\text{O}_6\text{Zn}$	Colourless	10.05	49.68 (48.74)	8.14 (8.05)	6.58 (6.47)	13.89 (13.45)

3.2 Infra-red Spectra

Infrared spectra (IR) are highly useful in identifying the nature of coordination sites, particularly in Schiff base complexes involving multidentate ligands and valuable information regarding the nature of functional group attached to the metal ion^[9]. The IR spectra of the free ligand was compared with its complexes to describe the coordination sites of the metals that may be involved in chelating. The IR spectra of the ligand showed a broad band in the region 3550 cm^{-1} , assignable to intramolecular hydrogen bonded -OH groups. This band disappears on complexation suggesting chelation of the oxygen of the Schiff base ligand and with different metals. The spectrum of the ligand showed $\text{C}=\text{N}$ band in the region 1660 cm^{-1} which was shifted to lower frequency in the spectra of the corresponding metal complex indicating the involvement of $\text{C}=\text{N}$ nitrogen in coordination to the metal ions. Accordingly, the ligand acts as a tetradentate chelating agent, bonded to the metal ion through the nitrogen atoms of the Schiff base. The medium bands at $550\text{-}500 \text{ cm}^{-1}$ and $428\text{-}486 \text{ cm}^{-1}$ was attributed to M-N and M-O frequency. The IR spectra of the ligand and its complexes are described in Table 2.

Table 2. IR Spectral data of glu –val Schiff base Complexes (in cm^{-1})

Sl.No	Complex	V(M-O)	V(M-N)	V(C=N)	V(OH)	V(COO ⁻)
1	[Cu(glu)(val).2H ₂ O]	544	428	1598	3318	1353
2	[Ni(glu)(val).2H ₂ O]	500	435	1607	3200	1365
3	[Co(glu)(val).2H ₂ O]	524	486	1638	3122	1394

3.3 Electronic Spectra Studies

The electronic spectra and magnetic measurements were conducted in order to obtain the geometry of the complexes. The electronic absorption spectra of the ligands and their M(II) complexes were recorded in solid state at room temperature. The electronic spectral measurements are used for assigning the stereochemistry of metal ions in the complexes based on the positions and number of d-d transition peaks. The electronic spectra of the metal complexes were recorded at room temperature in the UV region using DMSO solvent which exhibit intraligand and charge transfer transitions. The Schiff base ligand shows the absorption band within 275nm region which is assigned $\pi \rightarrow \pi^*$ transition of the C=N chromophore. On complexation, this band was shifted to lower wavelength region at 240-260 nm and suggesting the co-ordination of azomethine nitrogen with Cu(II) Ni(II) and Zn(II) ions. The spectra also shows the other transition is in the range of 325-335 nm in all the complexes which can be assigned to $n \rightarrow \pi^*$ transition. Another transition is in the range of 350-360 nm which may be due to the charge transfer transition. The spectra also shows certain absorption band at 600 and 630 nm which is in accordance with d-d transition. The Zn(II) complexes doesnot exhibit d-d electronic transition due to the completely filled 'd' orbital and found to be diamagnetic in nature. The observed values of magnetic moment for complexes are generally diagnostic of the coordination geometry about the metal ion. The observed magnetic moment of the Cu(II) complex is 1.86 B.M., which confirms the octahedral structure of the complexes. Ni (II) has the electronic configuration $3d^8$ and should exhibit a magnetic moment higher than that expected for two unpaired electrons in octahedral 4.5 B.M.

3.4 ^1H NMR Spectra

Proton nuclear magnetic resonance spectral analysis was performed for the synthesized ligand and metal complexes which provided further evidence for the structural characteristics of the metal complexes. All the proton of Schiff base ligands were found as to be in their expected regions. The ^1H NMR spectral data of the Schiff base ligands is in accord with the proposed structures and confirms the nature of bonding which discussed in their IR spectra. The ^1H -NMR spectra of the Schiff base in DMSO resonated at 8.35 ppm for $-\text{CH}=\text{N}$. The absence of this peak noted in the complexes proves the loss of the $-\text{OH}$ proton due to complexation. The azomethine proton signal in the spectrum of the complexes moved downfield compared to the free ligand, suggesting deshielding of the $>\text{C}=\text{N}$ group due to coordination with metal ion. The multisignals within 3.53, 3.4, 2.4 and 1.5 ppm are assigned to the $-\text{CH}_3$, $-\text{CH}_2$ and $-\text{CH}$ groups respectively. The azomethine proton signal in the spectra of the corresponding complexes is shifted downfield compared to the free ligand, suggesting deshielding of the azomethine group due to the coordination with the metal ion. There is no appreciable change in all other signals of the complexes.

3.5 Powder XRD study

X-ray Diffraction data was recorded by using $\text{Cu K}\alpha$ radiation (1.5406 Angstrom). The intensity data was collected over a 2θ range of $4-60^\circ$. All the diffraction peaks in the X-ray diffraction pattern of the Schiff base metal complexes agreed with the reported standard data and no characteristic peaks were obtained. The mean grain size of the particles were determined from the XRD line broadening measurement using Scherrer's equation (1)

$$d_{\text{XRD}} = 0.9 \lambda / \beta \cos \theta \quad (1)$$

Where λ is the wavelength ($\text{Cu K}\alpha$), β is the full width at half maxima (FWHM) and θ is the diffraction angle. A definite line broadening of the diffraction peak is an indication that the synthesized material is microcrystalline. The diffractogram and associated data depicted the 2θ value for each peak, relative intensity and inter-planar spacing. XRD diffractogram shows that $[\text{Cu}(\text{glu})(\text{val}).2\text{H}_2\text{O}]$, $[\text{Ni}(\text{glu})(\text{val}).2\text{H}_2\text{O}]$ and $[\text{Zn}(\text{glu})(\text{val}).2\text{H}_2\text{O}]$ complexes has the crystallite size of 41 nm, 39 nm and 46 nm suggesting the microcrystalline nature.

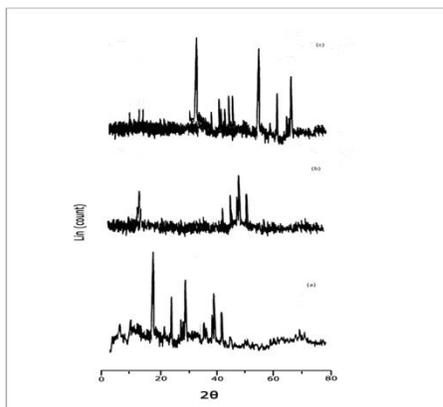


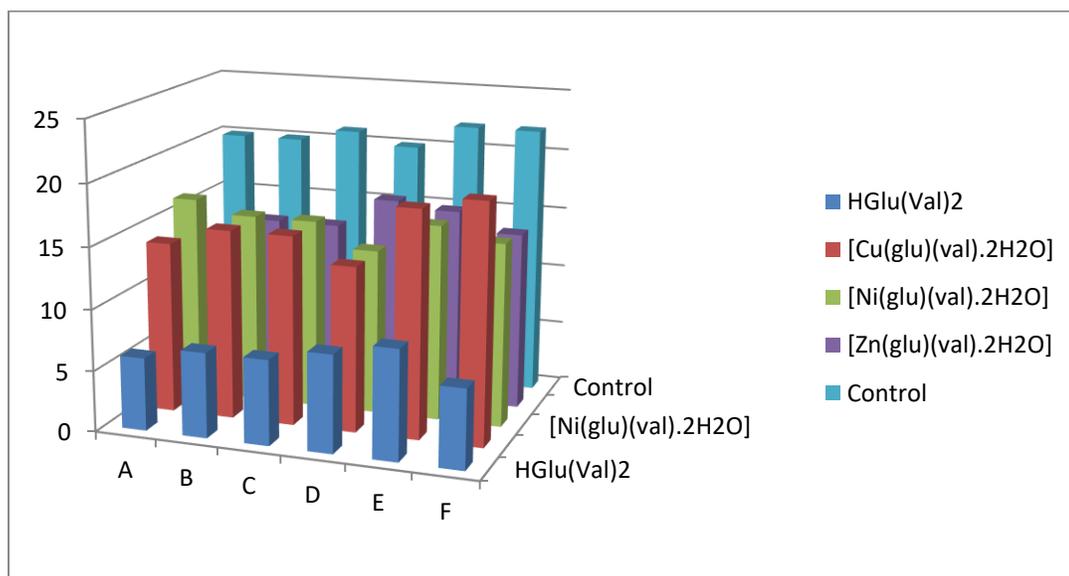
Fig 1 : Powder XRD pattern of Schiff base complexes

3.6. Antimicrobial study

The qualitative screening of the susceptibility spectra of various microbial strains to newly synthesized compounds showed that all tested compounds exhibited antimicrobial effect quantified by the occurrence of a growth inhibition zone Fig .2. For all tested complexes, the diameters of the inhibition zones were superior to those exhibited by the ligand. The lowest antimicrobial spectrum was noticed for the $[\text{Zn}(\text{glu})(\text{val}).2\text{H}_2\text{O}]$ complex, while the largest inhibitions zones were exhibited by the $[\text{Cu}(\text{glu})(\text{val}).2\text{H}_2\text{O}]$ complex. Tweedy's chelation theory offers an explanation for the increased antimicrobial activity of the metal complexes. In the chelated complex, the positive charge of the metal ion is partially shared with the donor atoms of the ligand and electron delocalization occurs over the whole chelate ring. In this way, the lipophilic character of the metal chelate is increasing and favouring its permeation through the lipid layers of the bacterial membranes and blocking the metal binding sites in the microorganism.

Table 3: Antimicrobial data of the ligand and their complexes

Schiff base/ Complex	Zone of inhibition in mm					
	A	B	C	D	E	F
HGlu(Val) ₂	6	7	7	8	9	6.5
$[\text{Cu}(\text{glu})(\text{val}).2\text{H}_2\text{O}]$	14.0	15.5	15.5	13.5	18.5	19.5
$[\text{Ni}(\text{glu})(\text{val}).2\text{H}_2\text{O}]$	16.5	15.5	15.5	13.5	16	15
$[\text{Zn}(\text{glu})(\text{val}).2\text{H}_2\text{O}]$	11.0	14.0	14.0	16.5	16.0	14.5
Control	20	21	21	21	22	20



A-Escherichia coli
 B-Staphylococcus aureus
 C-Klebsiella sps

D-Aspergillus niger
 E-Aspergillus fumigates
 F-Candida sps

Fig .2:Antimicrobial activities of Schiff base ligands and its complexes by disc diffusion method (Zone inhibition in mm)

Based on the spectral and analytical data,the structure of the synthesized compounds are given below.

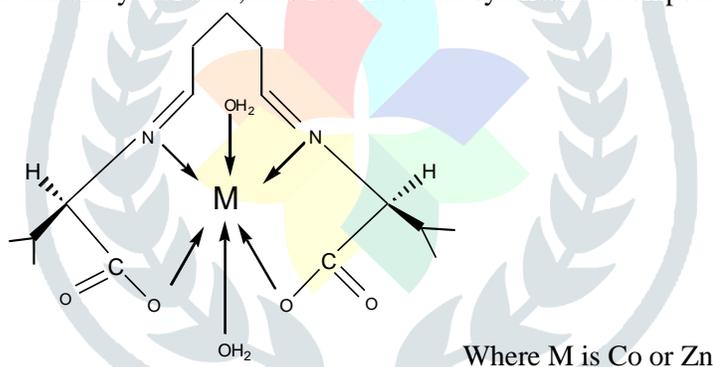


Fig .3: Structure of $[M(glu)(val).2H_2O]$ complexes

4. CONCLUSION

The synthesized Schiff bases derived by the condensation of L-valine with glutaraldehyde act as bidentate ligand coordinated with some transition metal Cu (II),Ni (II)and Zn(II), via the carboxylate oxygen and the nitrogen of azomethine group. Octahedral geometry have been proposed for all the complexes with the help of different spectral studies IR,UV, 1H NMR, Powder XRD, CHN analysis, molar conductance and magnetic susceptibility measurements. The low molar conductance of the complexes indicates that they are non-electrolyte and neutral. The antimicrobial activity of Schiff bases ligands and their complexes were tested against different microbes and it was found that the complexes exhibit better activity than corresponding ligands.

5. ACKNOWLEDGMENT

We are grateful to UGC for rendering financial support for undergoing this part of research.

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