IJARSCT

International Journal of Advanced Research in Science, Communication and Technology

International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal

ISSN: 2581-9429

Volume 6, Issue 4, February 2026

Impact Factor: 8.2

Ultrasonic Synthesis, Characterization, and Antibacterial Evaluation of Terephthalaldehydebis(Thiosemicarbazone) and Glycine Mixed-Ligand Complexes

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Abstract: *Mixed-ligand metal complexes offer a versatile platform for developing new therapeutic agents with enhanced biological properties. This study describes the efficient synthesis of a novel series of transition metal complexes utilizing a dual-ligand system. Mixed-ligand complexes were synthesized via an ultrasonication-assistedmethod using terephthalaldehydebis(thiosemicarbazone) (TPTSC) and glycineas second ligand. The Schiff base ligand and its complexes were rigorously characterized through elemental analysis, FT-IR, molar conductance, magnetic moment measurements, mass spectrometry, and XRD studies. Analytical data confirmed a 2:1:2 (M: L_1: L_2) stoichiometry, corresponding to the general formula$[M_2L_1(L_2)_2].nH_2O$ where M = Co(II), Ni(II), Cu(II), Cd(II) and Zn(II), L_1 = TPTSC and L_2 = glycine. Molar conductance measurements indicated the non-electrolytic nature of the complexes. Spectroscopic evidence revealed that the Schiff base coordinates in a bidentate fashion through N and S donor sites, while glycine binds via its amino Nitrogen (N) and carboxylate Oxygen (O) atoms. All complexes were found to adopt a tetrahedral geometry. Biological screening demonstrated that the mixed-ligand complexes possess significant antibacterial activity against a range of pathogens, including S. aureus, E. coli, K. pneumoniae. These findings highlight the potential of ultrasonication as a rapid synthetic route for bioactive coordination compounds.*

Keywords: Mixed-ligand complexes; Schiff base; Ultrasonication; Terephthalaldehyde bis(thiosemicarbazone); Antibacterial activity

I. INTRODUCTION

Preparing new Schiff base ligands with electron donors and electron acceptors is important for developing metal complexes with their properties and new reactivities. Schiff bases and metal complexes have many biological, medicinal, analytical, and industrial applications, in addition to their catalytic properties in organic synthesis. Semicarbazones Schiff base has many activities such as antibacterial, antifungal, antiviral, antitumor,antibacterial, antioxidant etc[1-3]. Metal complexes play an important role in the development of coordination chemistry relevant to catalysis, materials science, and life sciences. Currently, scientists are interested in the coordination of polydentate ligands having N, O, S as donor sites[3a]. Metal complexes with polydentate ligands have been classically reported in many studies. Recently, mixed metal complexes of ligands have been used in rapidly developing biological and chemical chemistry. Chiral mixed ligand metal complexes consisting of 8-hydroxy quinoline and sugar were used for the hydrolysis of metal acetate [3b]. Researchers now attracting more for the combination because of the potential use of Schiff bases in biological modeling, catalysis, molecular magnets, and synthesis of important drugs for antibiotics,

DOI: 10.48175/IJARSCT-31722

antiallergy and anticancer [1-4]. The structure and reactivity of Schiff bases attract medical attention [5]. Intramolecular hydrogen bonds between the Schiff base's OH and C=N hydrogen atoms determine the properties of different molecular systems [6-7]. Today, interest in the chemistry of semicarbazones and thiosemicarbazones and their metal complexes is increasing due to their applications in medicinal chemistry. Besides pesticides, herbicides, nematicides and phytochemicals, they can also be used as plastics and polymerization stabilizers, initiators and antioxidants [8-9]. Schiff base is widely used as ligands in coordination chemistry due to their simple structure and donating ability. Schiff base metal complexes can be used in catalysts, dyes and analytical chemistry [10-11]. Schiff base coordination derived from amino/thiol/phenol, terephthalaldehyde and amiothiols has been extensively studied. Thiosemicarbazone complexes (TSC) are a clinically important class of drugs whose anti-inflammatory properties were first described in the 1960s and are currently under development. TSC is increasingly being investigated for its inhibitory effect on cancer cells. It can work very well with modified and unmodified elements, and the complexes have the ability to define interesting properties and applications(white spectrum of the ligand) based on the thiolatebehaviour of the sulfurcoordination [12]. Thiosemicarbazone is an interesting compound with many medical uses. The biological activity of thiosemicarbazone is related to its ability to chelate with transition metal ions. The chelating ability is a function of the properties of the hard and soft NS donor atoms incorporated into the structure. The structure of thiosemcarbazone provides many free atoms making it a versatile ligand in both neutral and anionic forms[13]. The spectrum of $[Cu(HL_2)H_2O)Cl_2].H_2O$ shows the ν(S-H) band at 2120cm^{-1}. This is good evidence that the HL_2 ligand is easily converted to the thiol form without deprotonation[13], it can be substituted and activated by Cu(II) ion. The ν(CS) and ν(CN) bands in the free ligands HL_1 and HL_2, indicate the participation of the complex's S atom formation. Enabling metals into thiosemicarbazone ligands can alter or enhance their biological activity [14].

These findings encouraged us to focus on synthesizing new products to get novel biological active agents. New Schiff base ligand (TPTSC) and their metal complexes were prepared and characterized with elemental analysis and spectroscopic support. Further, the manuscript presents*in vitro*antiproliferative and antibacterial synthesized ligands and their complexes. Thus, our findings will deliver progressive valuable insights for designing drugs for biological use

II. EXPERIMENTAL

2.1. Methods and materials

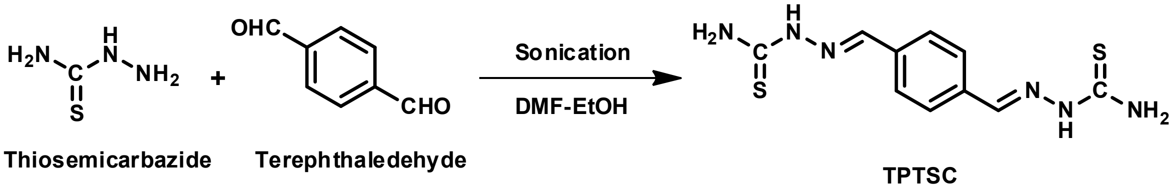
All chemicals and reagents used in the experiment were of analytical grade and were used without further purification. Solvents were purified and dried using standard procedures.

The mixed-ligand metal complexes were synthesized using a Schiff base, a metal halide, and an amino acid as the secondary ligand [15]. The metal salts Nickel(II) chloride, Cobalt(II) chloride, Copper(II) chloride, Zinc(II) chloride, and Cadmium (II) chloride were used as received without further purification. Ethanol and acetone were used as solvents after purification and drying according to standard protocols [3a]. Dimethylformamide (DMF) and Dimethyl sulfoxide (DMSO) were purchased from Merck.Elemental analyses (carbon, hydrogen, and nitrogen) were carried out at IIT Bombay. For metal ion estimation, approximately 250 mg of the metal complex was digested with concentrated nitric acid and a few drops of perchloric acid. The resulting residue was completely dissolved in 0.1 M HCl by gentle heating, and this solution was used for the determination of cobalt by the EDTA titration method [4].

Molar conductivity measurements were performed at 10^{-3} M concentration in DMF using a 180 Elico model digital conductivity meter. The Gouy balance was calibrated using $Hg[Co(SCN)_4]$ as the standard. The UV–Visible absorption spectra were recorded in DMF at 10^{-3} M concentration on a Shimadzu UV-160A spectrophotometer. Infrared spectra were recorded on a PerkinElmer FT-IR Model 1650 spectrophotometer.

2.2.Preparation of terephthalaldehydebis(thiosemicarbazone)

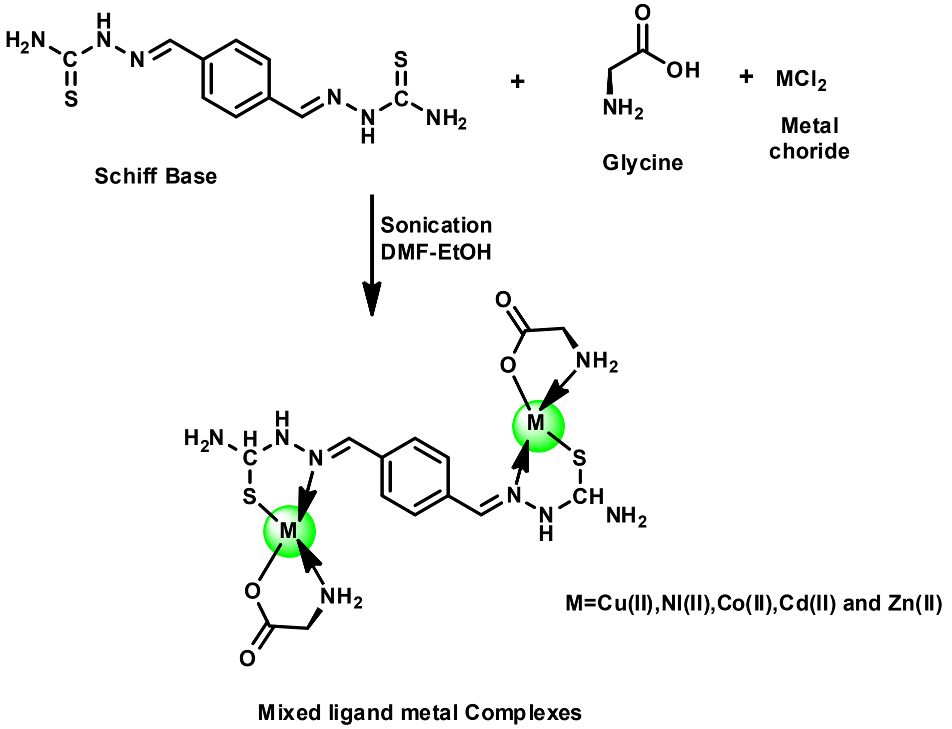
An ethanolic solution of terephthalaldehyde (1.15g, 0.01mol, 20mL) was mixed with an ethanolic solution of thiosemicarbazide (0.270g,0.02mol, 20mL) stirred in a sonicator for 20min. The yellow precipitate formed was filtered and recrystallized from hot alcohol to get a chromatographically (TLC) pure compound[16].



Scheme 1: General synthetic route for obtaining Schiff Base ligand (TPTSC).

2.3.Preparation of mixed ligand transition metal complexes

The complexes were synthesized by DMF-ethanol (1:1, 20mL) of solution of ligand (5mmol) with an ethanolic solution (20mL) corresponding metal salt(10mmol) and ethanolic solution of an amino acid as glycine (10mmol) were mixed and kept in sonicator for 30minutes, the coloured complex precipitate out [17]. The metal salts used in this study were metal (II) chloride (0.02mol) was added to a DMF solution of the ligand TPTSC(0.01 mol, 20mL) and the mixture was stirred with a second ligand amino alcohol (0.02 mol, 20mL) for 30min in a sonicator. The precipitated mixed ligand metal complex was filtered, washed with water, then ethanol, and dried in a vacuum[16].



Scheme 2: Synthetic route for the preparation of Mixed ligand transition metal complexes.

2.4. Antibacterial activities

The antibacterial activity of the terephthalaldehydebis(thiosemicarbazone) ligand and mixed ligand complexeswerestudied as per the cup-plate method using free strains of bacteria viz.,*Staphylococcus aureus, Proteus vulgaris,* and *Escherichia coli*. The solution of the test compounds is prepared in DMSO at 500µg/mL. The bacterial strains are inoculated in 100 mL of the sterile nutrient broth and incubated at 37 ± 1 °C for 24 h [18]. The density of the bacterial suspension is standard. A well-uniform diameter (6mm) is made on agar plates, after inoculating them separately with the test organisms aseptically. The standard drug and the test compounds are introduced with the help of a micropipette and the plates are placed in the refrigerator at 8-10 °C for proper diffusion of the drug into the media [1]. After two hours of cold incubation, the petri dishes are transferred to the incubator and maintained at 37 ± 2 °C for 18-24 hrs. Then the petri dishes are observed for the zone of inhibition by using the vernier scale. The results are reported by comparing the zone of inhibition shown by the test compounds with the standard drug Tetracycline. The results are the mean value of the zone of inhibition of three sets measured in millimeters.

III. RESULTS AND DISCUSSION

All of the complexes are non-hygroscopic stable solids. They are colored and thermally stable indicating a strong metal-ligand bond. The elemental analysis data of metal complexes (Table 1) is consistent with the general formulation of mixed ligand metal complexes [20]. The conductance values in the DMF at the concentration 10^{-3} mole /dm^3 fall in the range of11-8.42μScm^{-1}, thus indicating the non-electrolytic nature of the complexes. The results clearly demonstrate that the Co(II), Zn(II), Cd(II) and Ni(II) complexes have tetrahedral geometry, while the Cu(II) complex has square planar geometry [21].The solubility of the ligand and the complexes was checked in water and many organic solvents. Ligands were soluble in Ethanol, DMF and DMSO while complexes were soluble in DMF and DMSO [22].The melting point of mixed ligand metal complexes was determined and found higher.

Table 1 : Physical and Analytical Data

Compd.	Molecular formula	Mole. Wt.	Elemental analysis, calcd. (found%)					
			C	H	N	S	O	M
1	$C_{10}H_{12}N_6S_2$	280.4	42.84 (42.74)	4.31 (4.28)	29.97 (29.88)	22.87 (22.86)	-	-
2	$Cu_2C_{14}H_{18}N_8O_4S_{2.}2H_2O$	599.12	30.60 (31.08)	3.27 (3.24)	20.38 (20.50)	11.64 (11.59)	11.64 11.72)	15.28 (15.12)
3	$Co_2C_{14}H_{18}N_8O_4S_{2.}$ H_2O	586.0	31.08 (31.78)	3.33 (3.53)	20.72 22.08	11.84 (12.02)	11.84 (11.98)	21.79 (21.69)
4	Ni_2 $C_{14}H_{18}N_8O_4S_2.H_2O$	596.67	31.91 (32.11)	3.31 (3.38)	20.60 (20.80)	11.77 (11.82)	11.77 (11.78)	21.72 (22.04)
5	Cd_2 $C_{14}H_{18}N_8O_4S_{2.}$ H_2O	657.0	34.27 (34.18)	2.75 (2.68)	25.70 (26.87)	17.13 (17.24)	17.13 (17.65)	34.27 (34.32)
6	Zn_2 $C_{14}H_{18}N_8O_4S_2$ H_2O	581.0	30.40 (30.42)	3.25 (3.30)	20.27 (20.32)	11.58 (11.68)	11.58 (11.62)	23.66 (23.76)

3.1. Magnetic susceptibility and electronic spectra

The Ni(II) complex shows a magnetic moment 4.32 B.M and 3.7 B.M, expected for tetrahedral Ni (II) and Co(II) complexes. At room temperature magnetic moment of the Cu(II) complexis 1.80 B.M approaching square planar or tetrahedral geometry [23]. The electron spectra of free Schiff Base ligands and mixed ligand metal complexes show 256-280 nm band indicates $\pi \rightarrow \pi^*$ transitions and can be assigned to aromatic rings, while the 282-296 nm band is attributed to $n \rightarrow \pi^*$ transitions of the CH=N chromophore, corresponding to the $n \rightarrow \pi^*$ transition of the ligand [22]. The band in the 325-390 nm range assigned to the $n \rightarrow \pi^*$ electronic transition of the thiosemicarbazone group (C=S) indicates the redshift and the change of this band with changing color supports the formation of the complex.

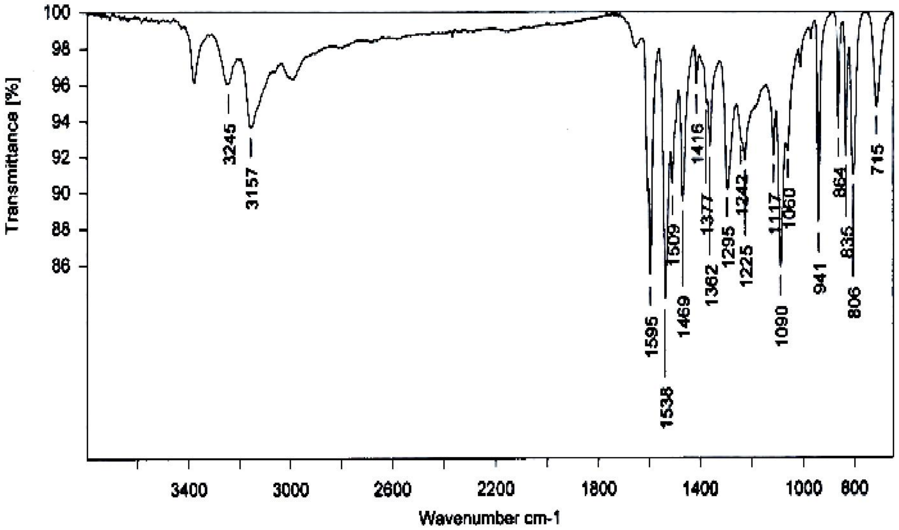
3.3.FT-IR spectra:

The FTIR spectrum of the mixed ligand metal complexes is recorded as a KBr disc. Some significant frequencies are distributed (Table 2). In the FT-IR spectrum, the peak of the free ligand thiosemicarbazone exhibited high peaks at 3397-3264 cm^{-1} (two peaks), 3193, and 1590 cm^{-1}, which were attributed to symmetric and asymmetric stretching.

NH_2,-NH and C=N respectively [17]. The absence of bands in the region 2000-2500 cm^{-1} depending on the S-H group in the complex spectrum shows that the ligands are mainly in the thionine form[24]. The new absorption of 1530-1585 cm^{-1} indicates the formation of a complex [22] with the metal ion form the nitrogen of the HC=N bond and the sulfur of the thiosemicarbazonegroup [15]. The thiosemicarbazone complex displays a cis configuration as a bidentate ligand bonded in a bidentate manner with a thion/thiolsulfur atom and an azo nitrogen atom [25]. The C=N vibration signal is seen at 1544cm^{-1} and C=S signal is seen in the thiosemicarbazone region at 1362-1359 cm^{-1}. The signal shifts to higher values in the complex, revealing the thiosemicarbazone site. It works with urea thiosemicarbazone ion center. Since C=S, C-S and N-C-N become N-C=N when complexed, these bands are not present in the spectrum of the complex [26]. Additionally, the symmetric and symmetric stretching peaks of the free amino group (-NH_2) are shown at 3561-3245cm $^{-1}$. On the other hand, the new vibration of the -C=N stretching peak of the imine group was observed at 1599-1538cm^{-1}. As expected, these results show positive. For the synthesized compound (1-6), the vibration peak of the amine group (-NH) was found to be 3368-3369 cm^{-1}, and the C=S signal of the thiosemicarbazone region was found to be 1462-1409cm^{-1} and -C-N group vibration was observed at 1222-1152 cm^{-1}.Another important vibration occurs in the spectra of compounds consisting of -C-O, -CH_2 functional groups. The COO-symmetric stretching peak was observed at 1362-1080 cm^{-1} in the complex;attributed to the coordination of the carboxyl group of the amino acid glycine with ion as shown in Figure 1, a new band emerges. The attribution of the 3151-3250 cm^{-1} frequency in the complex to the ν(-NH_2) amino acid group shows that the nitrogen atom of the -NH_2 group is in relationship with the metal ion [27].

Table 2: IR spectra terepthalaldehydebis(thiosemicarbazone) Ni glycine complex [$Ni_2L_1(L_2)_2$]

IR	N-H cm^{-1}	ν (C=N) cm^{-1}	C=S cm^{-1}	ν(C-N) cm^{-1}	C-H cm^{-1} Aromatic	COOcm^{-1} Aliphatic	ν (CS) cm^{-1}
Ligand(L_1)	3250	1612	1255	1465	3062	1080	864
$Ni_2.L_1.(L_2)_2$	3183	1587	1282	1463	3066	1076	802
$Cu_2.L_1.(L_2)_2$	3142	1592	1276	1503	3089	1099	797
$Co_2.L_1.(L_2)_2$	3188	1587	1309	1463	3073	1077	802
$Cd_2.L_1.(L_2)_2$	3157	1587	1284	1465	3079	1098	805
$Zn_2.L_1.(L_2)_2$	3180	1587	1275	1494	3050	1078	804



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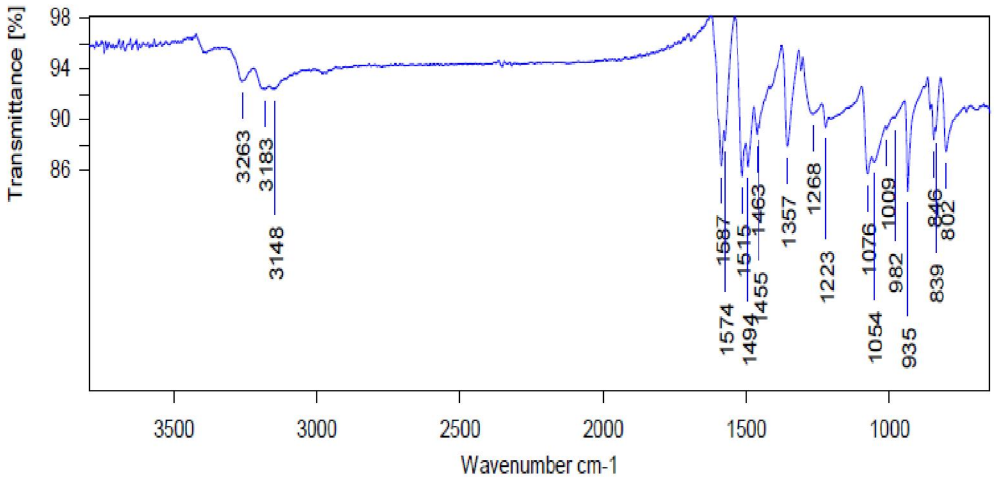
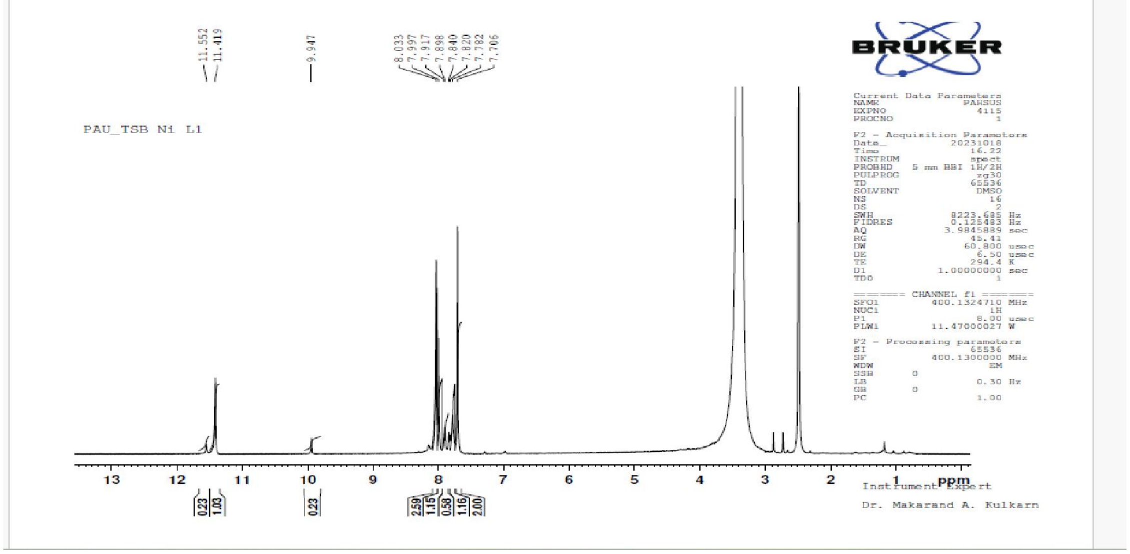


Fig. 1 Infra-Red spectrum of Co_2 L_1 $(L_2)_2$

3.4. NMR analysis:

The ^{1}H-NMR spectra of the ligand were recorded in DMSO solution asshown in Fig.2**.** The NMR spectra of the ligand showed a singlet in the regionδ8.60- 8.64 for the hydrazine NH proton. The -CH=N proton displayed a sharp singlet at δ11,67-11.79 ppm in the spectra of ligands [28]. The aromatic protons observed in the region δ7.39-8.40 ppm.NMR peak at δ 1.43 in ligand and δ1.41 for the complex is for =N-NH-, H2N-CS- δ3.55, NH-CS. δ8.63, NH_2 at 8.05[32]



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PULPROG              zg30
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SOLVENT              DMSO
NS                     16
DS                      2
SWH              8223.685 Hz
FIDRES           0.125483 Hz
AQ              3.9845889 sec
RG                  45.41
DW                 60.800 usec
DE                   6.50 usec
TE                  294.4 K
D1             1.00000000 sec
TD0                     1

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NUC1                   1H
P1                   8.00 usec
PLW1          11.47000027 W

F2 - Processing parameters
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WDW                    EM
SSB       0
LB                   0.30 Hz
GB        0
PC                   1.00

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Fig.2 NMR spectrum of Ligand and its mixed ligand metal complex

3.6. Mass Spectral analysis:

The mass spectrum of the metal complexes is in good agreement with the proposed molecular structure and mass spectrum of the complex shown in Fig.**3**.The molecular ion peakappears at

DOI: 10.48175/IJARSCT-31722

M/Zconfirming the stoichiometry of the complexes respectively[28].The final peak confirms the proposed formula and other peaks are assigned to various fragments. In the mass spectrum of Ni complex, the first peak at m/e 596.87 represents the molecular ion peak of the complex. The primary fragmentation of the complex is due to the loss of -OH_2 from complex species (a) to give species (b) with peak at m/e 558.83. The species (b) further degrades with the subsequent loss of amino acid to give species (c) with peak of 419.03. The species (c) further degrades with the subsequent loss to give a peak at 279.79 the stable species(d) with 100% abundance. This species(d) further degrades forming (e) with m/e 245.

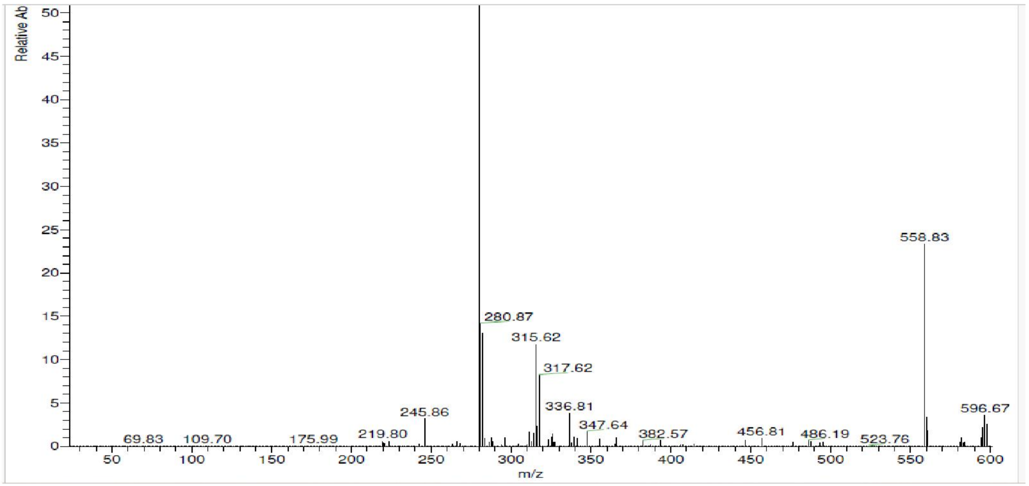

Fig.3 Mass spectra of terephthalaldehydebis(thiosemicarbazone) glycine$Ni_{2.}L_{1.}(L_2)_2$

3.7. XRD studies:

X-ray diffraction is a powerful technique used to study the crystallography structure of materials. It provides in lattice and formation about the arrangement of atoms in a crystal. Terephthalaldehydebis(thiosemicarbazone) and glycine mixed ligand complex, appears to be a coordination complex involvingions and ligands. The XRD analysis of this complex would yield information about the arrangement of atoms in its crystal lattice. XRD shows the purity andthe absence of impurities (fig.4).

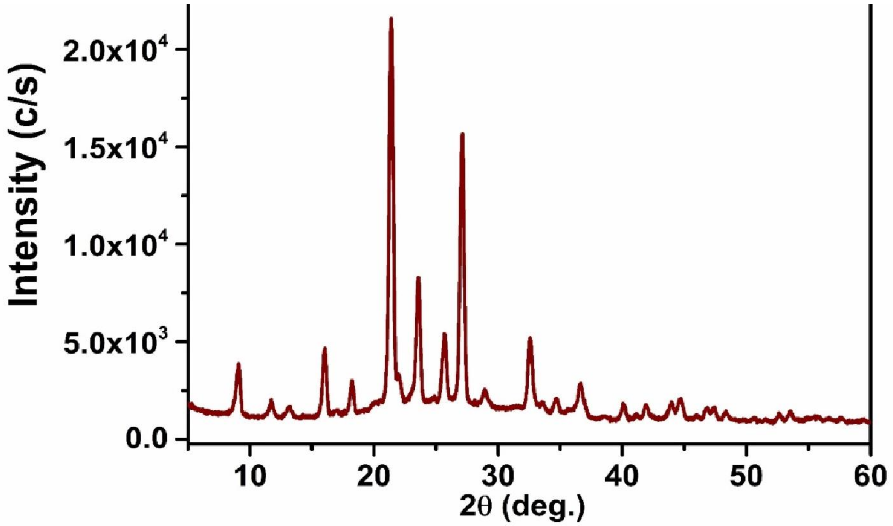

Fig.4.XRD spectrum for $Ni_2(L_1)(L_2)_2$

DOI: 10.48175/IJARSCT-31722

3.8.Thermogravimetric analysis:

To know the thermal stability of these new complexes, the thermogravimetric analysis (TGA and DTA) of the Schiff base ligand and their complexes was used. The thermal analysis shows the presence of water molecules in the inner coordination sphere of the central metal ion. From TGA weight loss data and DTA peak temperature indicate weight loss equivalent and one or two molecules of water 3.42 to 7.28% in the temperature range 100 to 200°C. From 200 to 600°C, a sharp decrease in weight shows loss due to one Schiff base and amino acid moiety with estimated mass loss of all complexes.[23].

3.9.*In vitro antibacterial studies*

The antibacterial bioactivity of Schiff bases and their metal-ligand complexes was tested. The bacteria used in this study include Gram-positive and Gram-negative bacteria[23]. The antibacterial properties of synthetic ligands and their mixed ligand complexes were evaluated using the agar diffusion technique. The biological test results of the compounds are summarized in Table 3. The activity of Schiff base ligands and mixed ligand complexes increases with increasing concentration and plays an important role in increasing inhibition levels[30-31].

Table 3 Antibacterial activities of the ligand and its mixed ligand complexes (diameter of zone of inhibition (mm)

Sr. No.	Test sample	Schiff baseL_1	$Cu_2L_1(L_2)_2$ complex	$Co_2L_1(L_2)_2$ complex	$Ni_2L_1(L_2)_2$ complex	$Zn_2L_1(L_2)_2$ complex	$Cd_2L_1(L_2)_2$ complex	DMF
1	Klebsiella Pneumonia	9.5	11.8	12.2	10.4	11.3	12.4	0.0
2	Escherichia Coli	9.6	10.8	10.2	11.1	13.2	12.6	0.0
3	Staphylococcus aureus	9.3	12.2	12.7	11.3	13.4	12.7	0.0
4	Enterococcus Faccalis	9.7	14.4	14.7	11.3	13.2	12.2	0.0
5	Salmonella typhi	8.8	11.6	10.1	12.1	12.3	11.2	0.0

IV. CONCLUSION

Mixed-ligand metal complexes were synthesized using a simple sonication method and characterized by UV–Vis, FT-IR, XRD, magnetic susceptibility, and molar conductivity measurements to confirm their structures. The Schiff base ligand, derived from terephthalaldehydebis(thiosemicarbazone), and its mixed-ligand complexes with amino acids were obtained in high yields under ultrasonic conditions.Structural studies suggest that the mixed-ligand Ni(II), Co(II), Cd(II), and Zn(II) complexes possess tetrahedral geometries. In contrast, the Cu(II) complex exhibits a distorted tetrahedral geometry. Coordination occurs through the imine nitrogen and thiolatosulfur atoms of the Schiff base ligand, along with the amino nitrogen and carboxylate oxygen atoms of the secondary ligand. None of the complexes contain coordinated water molecules; however, some complexes include lattice water molecules.The Schiff base ligand and its mixed-ligand metal complexes exhibit enhanced antibacterial activity against selected microorganisms.

Acknowledgment: Author L.V. Gavali is thankful to the Principal, Rayat Shikshan Santha'sM.P.A.S.C. College Panvelfor providing research facilities.I also express sincere thanks to QbDResearch&Development Lab Pvt. Ltd., IIT Bombay, and Analytical Laboratory for providing spectral data.

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DOI: 10.48175/IJARSCT-31722