

Synthesis, Physico-chemical, Spectral, and Antibacterial Activity Studies of Ni(II), Mn(II), and Cu(II) Complexes of Schiff Base Ligand Containing Triazole moiety

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ABSTRACT

A new series of Ni(II), Mn(II), and Cu(II) complexes of tridentate Schiff base derived from condensation of 3-Amino-1,2,4-triazole (3-Amino-s-Triazole) with salicylaldehyde (2-Hydroxybenzaldehyde) were synthesized in a 2:1 molar ratio. Physical (Magnetic measurements, molar conductance, and TG), spectral (IR, UV-Vis, and Mass) and analytical data have established the structures of synthesized Schiff base and its metal complexes. The elemental analysis data suggest the stoichiometry to be 2:1 (L: M). All the complexes are non-electrolytic in nature as suggested by molar conductance measurement. Infra-red spectral data indicate the coordination between the ligand and the central metal ion through deprotonated phenolic oxygen, triazole nitrogen, and azomethine nitrogen atoms. Spectral studies and magnetic susceptibility measurements suggest an octahedral geometry for the complexes. The ligand and its complexes were tested/screened for their antibacterial activity against two Gram-positive bacteria species *Staphylococcus aureus* and *Lactobacillus* and two Gram-negative species *Escherichia coli* and *Pseudomonas aeruginosa*. It is observed that the ligand as well as the complexes showed good activity against all microbes.

Key words: 3-Amino-1,2,4-triazole, Antibacterial activity, Complexes, Schiff base, Spectral studies.

1. INTRODUCTION

3-Amino-1,2,4-triazole, also known as Amitrole (AT), is commonly known as herbicide and it has fungicide activity [1]. AT is adsorbed on soil particles and organic matter by proton association. It has been found to cause cancer of the thyroid in rats and mice. It is also of interest as a ligand which forms complexes both in aqueous solution and in the solid state. It has at least two donor centers available for coordination. Hence, Schiff base metal complexes derived from 3-Amino-1,2,4-triazole were chosen for the present study. It is evident that an imine derivative, (-HC=N) group is an essential structural requirement in biological activity [2,3]. The increasing interest in transition metal complexes containing a Schiff base ligand is derived from their well-established role in biological systems as well as their catalytic and pharmaceutical applications [4,5]. The metal complexes from bidentate ligands have often been studied recently because of their applications in enhancement of drug action [6,7]. Transition metals are essential for normal functioning of living organisms and are, therefore, of great interest as potential drugs [8]. The coordination chemistry of nitrogen donor ligands is an active area of research. Schiff bases derived from Salicylaldehyde are well known for their interesting ligational properties and exclusive applications in different fields [9,10]. The interaction of these donor ligands and metal ions gives complexes of different geometries, and the literature survey reveals that these complexes are potentially biologically more active. Thus, in recent years, Schiff bases and their metal complexes have attained much attraction because of their extensive biological activities [11,12]. Recently, the Schiff base metal complexes derived from Xipamide-Salicylaldimine (L) have been synthesized and studied for their antifungal activities [13,14]. Schiff bases are

widely applicable because of their wide range of applications such as antibacterial [15], antifungal [16], and antiviral [17] activities.

The aim of present paper is to synthesize, characterize, and screen the antibacterial activity of metal complexes of (E)-2-[(1-H-1,2,4-triazol-3-yl-imino)methyl]phenol Schiff base on some pathogenic bacteria.

2. EXPERIMENTAL

All the chemicals used were of AR/GR grade. Pure sample of 3-Amino-1,2,4-Triazole (AT), molecular formula C₂H₄N₄, molecular weight 84.08 g/mol, melting point ~150°C was obtained from Himedia pharmaceuticals Ltd. Metal salts such as NiCl₂·6H₂O, MnCl₂·4H₂O, and CuCl₂·2H₂O were of Merck. Solvents used were ethanol, acetone, DMF, and dimethylsulfoxide (DMSO).

2.1. Synthesis of Schiff base

2.2.1. (E)-2-[(1-H-1,2,4-triazole-3-yl-imino)methyl]phenol

The compound was synthesized from salicylaldehyde and 3-amino-1,2,4-triazole by adding 1000 ml of salicylaldehyde ethanolic solution

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ISSN NO: 2320-0898 (p); 2320-0928 (e)

DOI: 10.22607/IJACS.2022.1002002

Received: 11th January 2022;

Revised: 20th January 2022;

Accepted: 22th January 2022

(1.22 g; 0.01 mol) to same volume of ethanol solution/same solvent of 3-amino-1,2,4-triazole (0.8144 g; 0.01 mol), the mixture was refluxed for 2 h and kept overnight at room temperature. The resulting solution was evaporated to 20% of its original solution, and the product was collected by filtration, washed several times with ethanol, recrystallized from hot ethanol, and then dried. The melting point of yellow crystals was found to be 185°C.

2.2. Stoichiometry by Monovariation Method

For the synthesis of complexes, ligand-metal ratio was determined by conductometric titration using monovariation method on systronics conductivity meter using dip type electrode. 20 ml of the ligand (0.01 M) was diluted to 200 ml using pure ethanol and titrated against the respective metal salts (0.02 M) solution prepared in the same solvent. Conductance was recorded after each addition of metal salt solution. Graph is plotted between corrected conductance and volume of metal salt added. From the equivalence point in the graph, it has been concluded that the complex formation of the ligand with the metals takes place in the ratio 2:1 (L: M).

2.3. Synthesis of Complexes

The solid complexes were prepared by mixing ethanolic solutions of the ligand (HL) (0.18 g and 0.01 mol) with ethanolic solution of chlorides of Ni(II) (0.059 g and 0.005 mol), Mn(II) (0.049 g and 0.005 mol), and Cu(II) (0.043g and 0.005 mol) separately. The resulting solutions were checked for pH and pH was adjusted by adding few drops of N/10 NaOH solution. The solutions were refluxed for 4 h and the refluxed solutions were kept for some days, solid crystalline complexes appeared in the solution which were filtered off, washed thoroughly with same solvent and finally with acetone, vacuo dried, and weighed. Melting points of the complexes were recorded.

2.4. Analysis and Instrumentation

Elemental analysis was carried out on VarioMICRO V2.20 ElementarAnalysenSysteme GmbH, from IIIM, Jammu. Metal contents were determined gravimetrically [18]. The infrared spectra were recorded on FT-IR Spectrophotometer Model RZX (Perkin Elmer) using KBr pellets from SAIF, Panjab University, Chandigarh. Molar conductance measurements were made in 10^{-3} M DMF solution on a Systronics direct reading Conductivity Meter (Model 303). The melting points of the ligand and the complexes were recorded in open capillaries on a capillary melting point apparatus. Electronic spectra were recorded on a UV-VIS-NIR-Spectrophotometer Model Lambda 750 Perkin Elmer from SAIF, PU, Chandigarh. The magnetic susceptibility measurements were carried out on a Vibrating Sample Magnetometer from IIT, Roorkee. The thermogravimetric analysis was carried out in nitrogen atmosphere (0.00 l/min.) with a heating rate of $10^{\circ}\text{C min}^{-1}$ at ambient pressure using Perkin Elmer TG Analyser within a temperature range from room temperature to 1000°C at Department of Chemistry, University of Jammu, Jammu.

2.5. Antibacterial Activity

In vitro antibacterial activity of the newly synthesized compounds was investigated against two Gram-positive bacteria species *Staphylococcus aureus* and *Lactobacillus* and two Gram-negative species *Escherichia coli* and *Pseudomonas aeruginosa* by the agar well diffusion method [19,20]. Nutrient agar (Hi Media, India) was used as the bacteriological medium. The extracts were dissolved in 10% aqueous DMSO to a final concentration of $50\mu\text{g}/\mu\text{L}$. Pure DMSO was taken as the control. $100\mu\text{L}$ of inoculums was aseptically introduced on to the surface of sterile agar plates and sterilized cotton swabs were used for even distribution of the inoculums. Wells were prepared in the agar plates using a sterile cork borer of 6.0 mm diameter. $50\mu\text{L}$ of test and control compound was introduced in the well. The same procedure was used for all the strains. The plates were incubated aerobically at 35°C and examined after 24 h. The diameter of the zone of inhibition produced by each agent was measured with a ruler.

3. RESULTS AND DISCUSSION

Through a condensation reaction, an amino group available in the pure compound was allowed to react with salicylaldehyde to form a Schiff base ligand (L) which was subsequently, reacted with metal ions to form Schiff base metal complexes. The ligand and the metal(II) complexes were isolated pure from EtOH in good yields and they are of various colors. The ligand is yellow, nickel complex is pale green, manganese complex is grass green colored while copper complex is light green in color.

The nickel and manganese complexes did not melt/decompose when heated up to 300°C whereas copper complex gets decomposed at $240\text{--}250^{\circ}\text{C}$. The analytical data of the complexes correspond with 1:2 (metal: ligand) stoichiometry. Thus, the general formula $[\text{M}(\text{L}_2)]$ where $[\text{M}(\text{II}) = \text{Ni, Mn, and Cu}]$, has been assigned to the metal complexes, respectively. They are very air stable solids at room temperature without decomposition for a long time. The complexes are non-hygroscopic, insoluble in water, and other common organic solvents but soluble in DMF and DMSO. The molar conductance value of the complexes (measured in 10^{-3} M DMF) is in the range $35\text{--}49\text{ Scm}^2\text{mol}^{-1}$, indicating/revealing the non-electrolytic nature [21] of the complexes. The magnetic moment data indicated all the complexes to be paramagnetic in nature. Thermograms of the metal complex indicate the presence of lattice water molecules and are stable complexes. The analytical data and molar conductance values are given in Table 1.

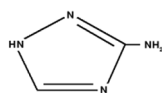
3.1. Magnetic Measurements

The magnetic moment data are presented in Table 1. The Mn(II) complex showed a value of 5.38 B.M., which is slightly lower than the spin only value of 5.92 B.M. for high spin octahedral Mn(II) complexes [22]. Ni(II) complex showed magnetic moment values of 3.18 B.M., slightly higher than the spin only (2.83 B.M.) value, indicating an octahedral environment around Ni(II) ion [23]. The

Table 1: Analytical and Physicochemical data of Schiff base and its complexes

Ligand/Complexes	Mol. Wt.	Elemental Analysis (Found [calcd.] [%])				Colour (yield%)	M.Pt.($^{\circ}\text{C}$)	Scm ² mol ⁻¹	μ_{eff} (B.M.)
		C	H	N	M				
(C ₉ H ₈ N ₄ O)	188.18	58.20 (57.91)	4.00 (4.28)	28.7 (29.76)	---	Walnut (61)	185	----	
[Ni (C ₁₈ H ₁₈ N ₈ O ₄)]	469.08	45.03 (46.09)	4.00 (3.87)	25.70 (23.9)	12.51	Pale green (54)	>300	35.2	3.18
[Mn (C ₁₈ H ₁₈ N ₈ O ₄)]	465.32	48.70 (46.46)	4.03 (3.90)	25.70 (24.08)	11.80	Grass green (43)	>300	32.43	5.38
[Cu (C ₁₈ H ₁₈ N ₈ O ₄)]	473.93	46.15 (45.61)	3.98 (3.83)	25.35 (23.65)	13.4	Light green (50)	240 (Decomposition)	48.20	1.83

Cu(II) complex exhibited a value of 1.83 B.M., which suggests a distorted octahedral geometry [24] around the central metal ion.



(3-Amino-1,2,4-Triazole)

Figure 1: Structure of pure compound.

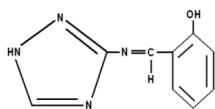


Figure 2: Structure of Schiff base (At-S).

Table 2: Important IR spectral bands (cm⁻¹) of the ligand and its complexes

Ligand/ Complexes	^ν C-O	^ν C=N	^ν OH	^ν N-N	^ν M-O	^ν M-N	^ν NH
[(C ₉ H ₈ N ₄ O)]	1277s	1613s	3129s	981s			3235b
[Ni (C ₁₈ H ₁₈ N ₈ O ₄)]	1275s	1636m	---	975s	647m	422s	3210b
	1283s	1640m	---	974s	637s	442s	3201s
[Mn (C ₁₈ H ₁₈ N ₈ O ₄)]	1271m	1643s	---	992m	635s	463s	3163bs
[Cu (C ₁₈ H ₁₈ N ₈ O ₄)]							

Table 3: TG results of the metal complex

Complex	Steps	Temperature (°C)	TG Mass%		Assignments
			Calcd.	Found	
[Ni (C ₁₈ H ₁₈ N ₈ O ₄)]	1 st	100–150	7.27	6.7	–H ₂ O ₂ (Water molecules), Exotherm (broad)
	2 nd	150–540	15.4	13.6	–C ₂ H ₂ N ₃ (Triazole ring), Exotherm
	3 rd	540–650	17.01	16.2	–C ₆ H ₄ loss of protonated benzene Exotherm (sharp)
	4 th	650–900	38.0	36.9	–C ₉ H ₆ N ₄ (loss of Schiff base moiety), Endotherm
	5 th	900–1000	10.57	11.4	–NO ₂ (organic moiety) Endotherm (broad)
			15.17	15.2	–NiO (Residue)

TG: Thermoanalytical

Table 4: Antibacterial activity of ligand and its metal complexes, MIC showing inhibition zone in µg/mL

Ligand/Complexes	Gram-positive		Gram-negative	
	<i>Staphylococcus aureus</i> µg/mL	<i>Lactobacillus</i> µg/mL	<i>Pseudomonas aeruginosa</i> µg/mL	<i>Escherichia coli</i> µg/mL
[(C ₉ H ₈ N ₄ O)]	23	21	18	21
[Ni (C ₁₈ H ₁₈ N ₈ O ₄)]	17	12	12	20
[Mn (C ₁₈ H ₁₈ N ₈ O ₄)]	18	14	18	12
[Cu (C ₁₈ H ₁₈ N ₈ O ₄)]	20	16	18	18
Streptomycin	27	26	24	23

MIC: Minimum inhibitory concentration

3.2. Electronic Spectra

The electronic spectra of the complexes were recorded in the solution state. The energies of the observed spin allowed bands in all the complexes agreed with the octahedral geometry. The electronic spectrum of Mn(II) complex shows four weak bands at 16000, 19810, 20746, and 26740 cm⁻¹, which can be assigned to ⁶A_{1g}→⁶T_{1g}, ⁶A_{1g}→⁴T_{2g}, ⁶A_{1g}→⁴E_g and ⁶A_{1g}→⁴T_{1g}, respectively, for an Mn(II) ion in an octahedral field [25].

The electronic spectrum of the Ni(II) complex displayed three bands at 12500, 18900, and 26400 cm⁻¹, assigned to ³A_{2g}(F)→³T_{2g}(F), ³T_{1g}(F) and ³T_{1g}(P) transitions, respectively, which indicate octahedral geometry [26] of the Ni(II) complex. The electronic spectrum of the Cu(II) complex showed two ligand field bands at 13700 and 18000 cm⁻¹, assigned to the transitions ²E_g→²T_{2g} and charge transfer, respectively. The electronic spectrum of the Cu(II) complex suggests a distorted octahedral geometry [27].

3.3. Infrared Spectra

The IR spectrum of the Schiff base showed a sharp band near 1613 cm⁻¹ which may be due to the azomethine linkage [28], which was shifted to higher frequencies in the metal complexes, indicating coordination of the metal ions through the azomethine nitrogen [29]. The ligand showed a strong band at 3129 cm⁻¹ due to the phenolic –OH group [30]. This band was absent in the spectra of all the complexes, indicating involvement of this group in the formation of the complexes [31]. The appearance of the M–N bands at 422, 442, and 463 cm⁻¹ and the M–O bands at 647, 637, and 635 cm⁻¹ in the complexes indicates that At-S was coordinated through an O and an N atom [32,33]. The weak bands observed at 1277, 1275, 1283, and 1271 cm⁻¹ are characteristics of ^νC–O (phenolic) [34] in the Schiff base and the metal complexes, respectively. The FT-IR spectrum of ligand shows a broad band at 3235 cm⁻¹ due to ^ν(NH) stretching vibrations [35]. This band gets shifted to lower frequency side or shows decrease in intensity on complexation [36]. The shift of about 2–6 cm⁻¹ of ^ν(C–O) band to lower region in

complexes was observed indicating participation of the carbonyl oxygen in coordination [37]. The appearance of bands for ν cyclic (C-N) at 1315cm^{-1} in ligand which gets shifted to higher frequencies by $30\text{--}55\text{cm}^{-1}$ in the complexes indicates coordination of the triazole nitrogen to the metal in the complex formation. The IR spectral data and their tentative assignments are given in Table 2 and Figures 1-6.

3.4. Mass Spectra

The mass spectrum of $[\text{Mn}(\text{C}_{18}\text{H}_{18}\text{N}_8\text{O}_4)]$ and $[\text{Cu}(\text{C}_{18}\text{H}_{18}\text{N}_8\text{O}_4)]$ shows molecular ion peaks at m/z values of 432.3 and 440.10 due to $[\text{Mn}(\text{L})_2]$ and $[\text{Cu}(\text{L})_2]$, which are in accordance with the proposed formula of the complexes. The other peaks at m/z values of 172.1, 133.12, 83.03, and 120.21 may be due to the radical ions ($\text{C}_9\text{H}_7\text{N}_4\text{O}$), ($\text{C}_8\text{H}_5\text{NO}$), ($\text{C}_2\text{H}_2\text{N}_4$), and ($\text{C}_7\text{H}_5\text{NO}$) of the

complexes. The base peaks at m/z 226.1 and 237.92 may be due to the metals (Mn and Cu) linked to the donor atoms of the ligand. Such type of fragmentation pattern has been reported by many workers [38,39] [Figure 7].

3.5. Thermal Studies

The TG for the complex was carried out within a temperature range from room temperature to 1000°C . The TG studies indicate that water is present only as lattice water outside the coordination sphere and does not involve in bonding as this water gets lost below 150°C . The decomposition temperature ranges, percent mass losses, and the residue leaving behind are given [40-42]. The Ni(II) complex of the formula $[\text{Ni}(\text{AT-SAL})_2] \cdot 2\text{H}_2\text{O}$ gives a five-stage decomposition process. The first stage from 100°C to

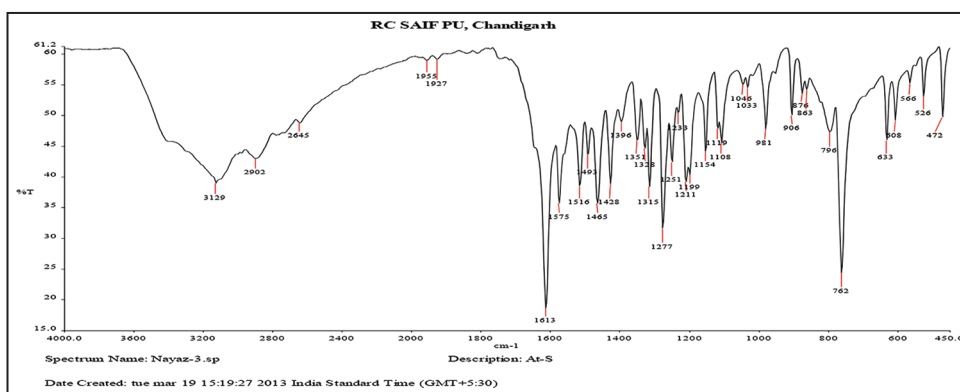


Figure 3: IR spectra of Schiff base.

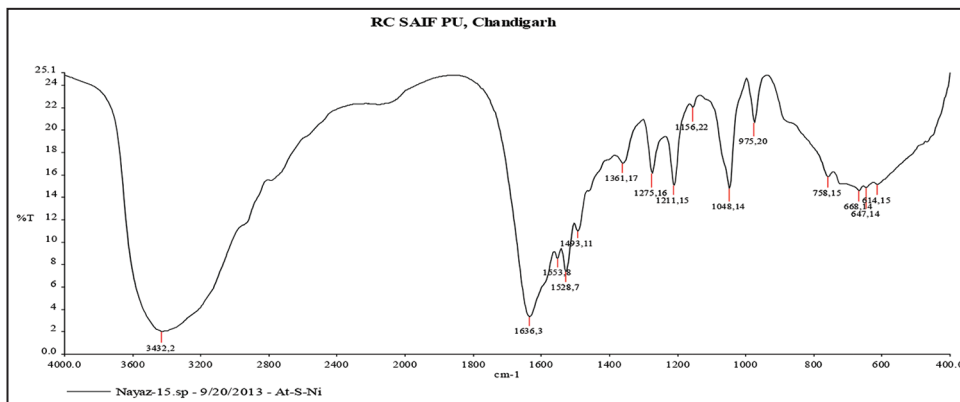


Figure 4: IR spectra of Ni^{II} complex.

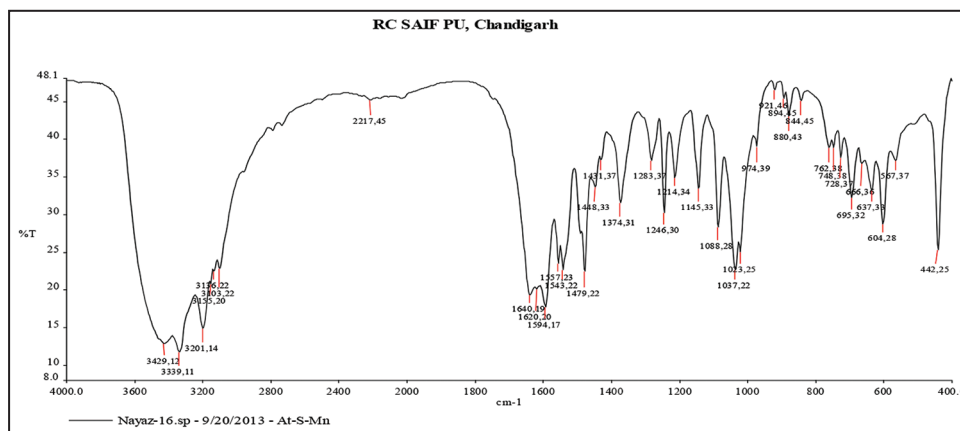


Figure 5: IR spectra of Mn^{II} complex.

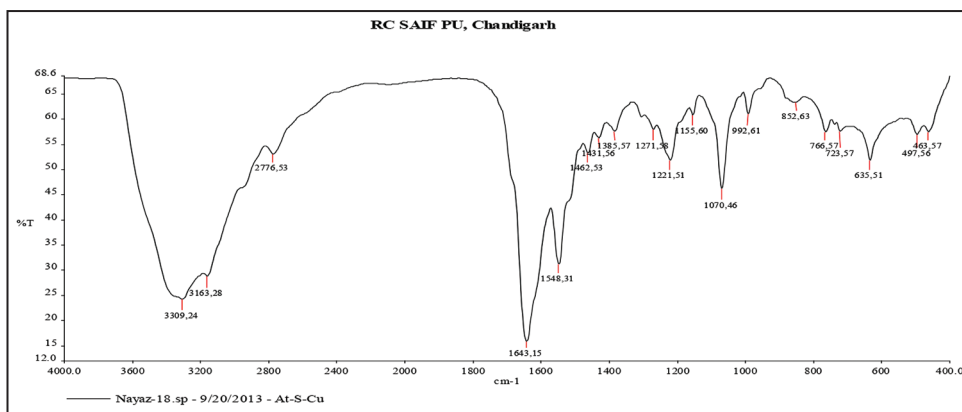


Figure 6: IR spectra of Cu^{II} complex.

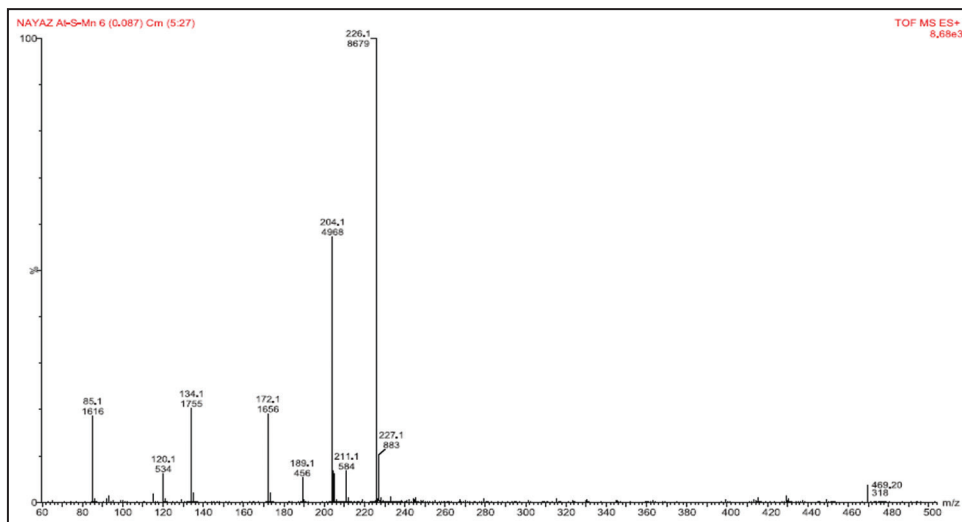


Figure 7: Mass spectrum of Mn^{II} Complex.

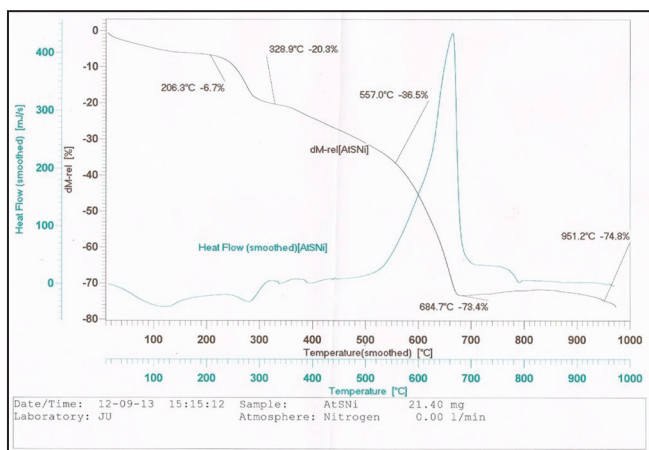


Figure 8: TGA spectrum of At-S-Ni complex.

150°C may represent loss of water molecules of hydration (calcd. mass loss 7.27%, found 6.7%). The second stage from 150°C to 540°C, the third from 540°C to 650°C, the fourth from 650°C to 900°C, and fifth from 900°C to 1000°C stages may be due to the decomposition of two ligand molecules leaving nickel oxide as the residue. All the constituents that are lost during thermal analysis are stable one. The mass losses for these temperature ranges are 15.4% (found 13.6%), 17.01% (found 16.2%), 38.0% (found 36.9%), and 10.57% (found 11.4%), respectively. The complete result of thermal analysis is shown in Figure 8 and compiled in Table 3.

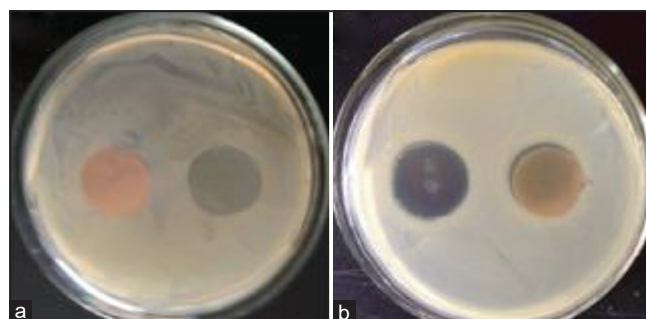


Figure 9: (a and b) Antibacterial activity of ligand and [Ni^{II} and Cu^{II}] Complexes.

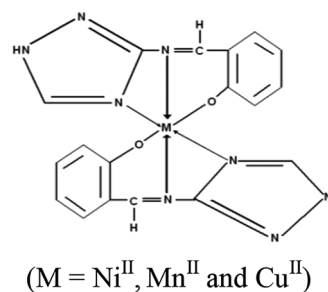


Figure 10: Structure of metal complexes.

3.6. Antibacterial Activities

Antibacterial activity of 3-Amino-1,2,4-Triazole-Salicylaldehyde and its metal complexes was tested *in vitro* against representative Gram-positive bacterial species such as “*S. aureus*” and “*Lactobacillus*” and Gram-negative bacteria species such as “*E. coli*” and “*P. aeruginosa*” by agar well diffusion method. Compounds inhibiting growth of one or both microorganisms were further tested for their minimum inhibitory concentration (MIC) of the compound. At-S ligand is more active against almost all microbes. Furthermore, all the complexes showed very good activity against all organisms (MIC = 25 µg/mL). However, the synthesized compounds showed relatively higher or lower activity than the standard drug, Streptomycin. It may be due the nature of the metal ion, the nature of the ligand, and orientation of the ligand around the metal ion. The photographs are presented in Figure 9, the metal complex is shown in Figure 10 and results are summarized in Table 4.

4. CONCLUSION

Based on stoichiometry and analytical data, it is concluded that the ligand is neutral, tridentate coordinating through the “N,” “N,” and “O” of the azomethine group, triazole ring, and phenolic group, respectively. All the complexes possess 2:1 (L: M) stoichiometry based on analytical and spectral data and octahedral structures have been proposed for the complexes. The ligand and the complexes showed very good activity against all bacteria.

Hence, the proposed tentative structure for the metal complexes is given as below:

5. ACKNOWLEDGMENTS

The authors are thankful to Principal, Sadhu Vaswani Autonomous College, Bhopal for providing necessary facilities for research work, DST for granting FIST program to the college, and UGC for sanctioning UGC Research Award to Dr. Suman Malik, one of the coauthors.

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Author Queries???

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