

Synthesis, Characterization And Antibacterial Analysis Of Benzocaine Schiff Base Metal Complexes

Reena Bhatt^{1*}, Shweta Mishra¹, and Niharika Shivhare²

¹*Shri Vaishnav Vidyapeeth Vishwavidyalaya, Indore (M.P.) India*

²*Prestige Institute of Management and Research, Indore (M.P.) India*

Transition metal complexes containing Schiff base ligands represent a large class of compounds that might be used to develop new metal-based antibacterial medicines. It was demonstrated that the antibacterial activity of the metal (II) complexes was higher than that of the free Schiff base ligand. For this experiment, a unique set of metal (II) complexes involving 3-(2-O-tolylhydrazono)pentane-2,4-dione were prepared in an alcoholic medium. The Schiff base is produced by a condensation process between ortho toluidine and acetyl acetone. The synthesised compounds were characterised using a variety of analytical techniques, such as elemental analysis, scanning electron microscopy (SEM) for structural clarity, FT-IR, UV-Vis, and antibacterial examination against *Staphylococcus aureus* and *Escherichia coli*. Since schiff base ligands and their complexes exhibit a broad range of biological actions, their value in medicine has been demonstrated.

Keywords: Schiff base, Metal (II) complex. Analytical analysis, Antibacterial study, etc.

INTRODUCTION

Complexes of Transition Metals with Schiff Base Ligands

Metal complexes of Schiff base, containing Ni(II), Cu(II), Fe(II), Co(II), and Zn(II) ions, have been essential in the advancement of coordination chemistry. Because of their physiological DNA binding and cleavage capabilities, transition metal complexes have piqued interest. Current research is on using metal complexes as chemical nucleases. It has been shown that the present emphasis of study is on in-organic complexes as chemical nucleases. Inorganic complexes have been shown to be useful as sequence-specific DNA binding agents in footprinting investigations, as diagnostic agents in medical applications, and for genomic research [1].

In order to comprehend the structure of biomolecules and biological processes, the Schiff base transition metal complexes have also been utilised as biological models [2]. Essential metallic elements for life, manganese, cobalt, nickel, copper, and zinc show increased biological activity when linked to certain metal protein complexes, where they take part in electron transfer processes, oxygen transport, or ion storage [3]. The investigation of systems containing these metals has seen a huge surge in interest as a result.

Because copper compounds selectively permeate cancer cell membranes, copper complexes have shown to be one of the most metallic species with broad anticancer action [4, 5]. Cu^{2+} 's capacity to bind with single-strand DNA by chelation with adjacent phosphate groups has been documented in the provisos study [6]. As a result, there is growing interest in creating novel Schiff base complexes containing a distinct transition metal, which may function as anticancer drugs by cleaving the DNA of cancer cells in a physiological setting [7, 8]. Recent years have seen a rise in interest in magnetic nanoparticles (MNPs) because of their special qualities and range of uses [9–11].

Transition Metal Complex with Schiff Base Ligands Has Antibacterial Activity

Bacteria with multiple antibiotic resistance are directly linked to the rise in mortality brought on by infectious illnesses. There is a pressing medical need to produce novel antibacterial medications with inventive and more potent modes of action [12].

In organic chemistry, Schiff bases' biological importance is utilised in a wide range of synthetic applications. Attacks at the nitrogen atom start the acylation of Schiff bases by acid anhydrides, acid chlorides, and acyl cyanides, which results in a net addition of the acylation agent to the carbon-nitrogen double bond. Such reactions have found useful applications in the synthesis of natural products [13].

In a number of enzymatic processes where an enzyme interacts with an amino or carbonyl group of the substrate, schiff bases seem to represent a significant intermediate. The biochemical process, which includes the condensation of a main amine in an enzyme, often a lysine residue, with a carbonyl group of the substrate to generate an imine or Schiff base, is one of the most significant forms of catalytic mechanism.

A charge transfer between the peptide groups and the oxygen atoms of the Schiff bases can occur when the Schiff base formed between methylglyoxal and the amino group of the lysine side chains of proteins is bent back towards the N atom, according to a stereochemical investigation using a molecular model [1]. In this regard, pyridoxal Schiff bases have been created and examined from a biological perspective. These bases are generated from pyridoxal and amino acids. Numerous investigations have demonstrated that salicylaldehyde may be condensed with various heterocyclic compounds to produce a derivative [14] that has strong antibacterial and antifungal action. An active example of combating *Mycobacterium tuberculosis* is shown in Figure 1 below [15].

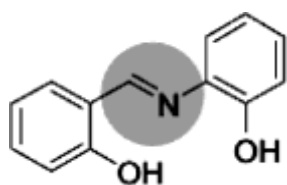


Figure 1. N-(Salicylidene)-2-hydroxyaniline as the example of bioactive Schiff Base

Additionally contributing to the efficient suppression of bacterial growth are schiff bases containing 2,4-dichloro-5-fluorophenyl moieties [16]. However, the compounds derived from p-toluidine and furylglyoxal have antibacterial activity against *Proteus vulgaris*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli*. Benzimidazole, thiazole, pyridine,

glucosamine, pyrazolone, hydrazine, thiazolidiones, indole, thiosemicarbazone, and p-fluorobenzaldehyde are examples of Schiff base derivatives with antibacterial activity [17].

MATERIAL AND METHODS

Synthesis

In the present case the synthesis of Schiff base metal complex required separate synthesis for three major compounds (A, B and C). Compound A was synthesized by condensation reaction of acetyl acetone and o-toluidine in alcoholic medium. This compound A was then treated with benzocaine to obtain Compound B. This reaction also proceeds in alcoholic medium. The compound B thus obtained is treated with metal (II) ions to get different complexes. The different metals used are Ni(II), Cu(II), Fe(II), Co(II), and Zn(II). The metal complexes thus synthesized are Compound C. Figure 2 below depicts the chemical reaction taking place during the synthesis of different compounds.

The preparation of **Compound A** takes place in following steps:

Step 1: Mixture of Acetyl acetone (0.01M) + Sodium acetate (0.01M) + 25 ml ethanol is refrigerated at 0 – 5 °C for few hours.

Step 2: Mixture of ortho toluidine (0.01) + 10 ml HCl + 10 ml distilled water is kept in ice bath.

Step 3: Paste of Sodium nitrite (0.01) in minimum distilled water is kept in ice bath.

Step 4: Mixture in step 2 and paste of sodium nitrite in step 3 are mixed with constant stirring.

Step 5: The mixture obtained in step 4 is then added to the refrigerated mixture in step 1.

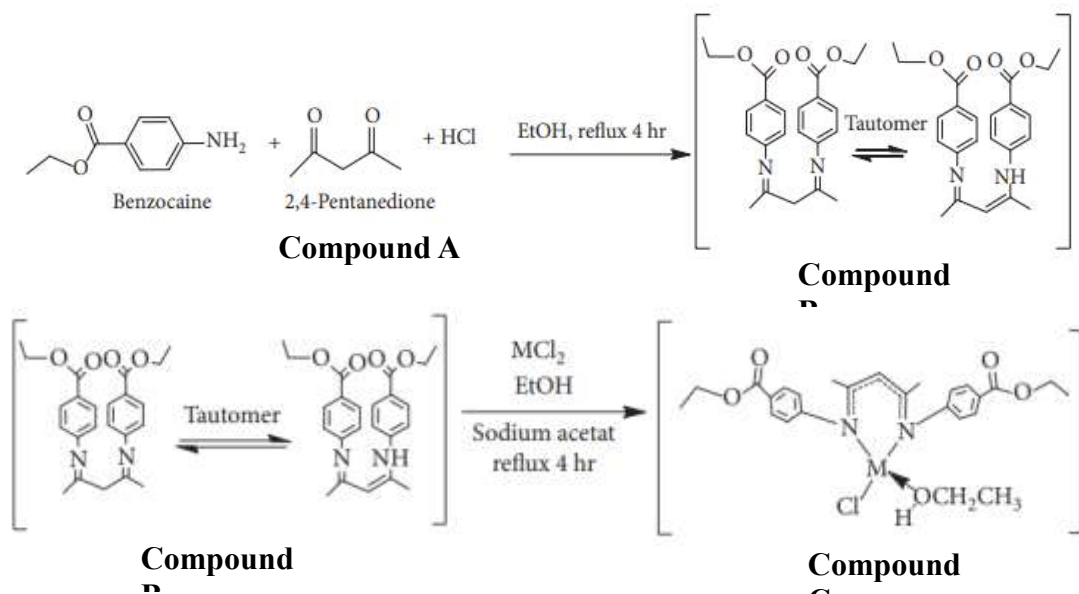


Figure 2.: General preparation for compound B and compound C

The synthesis of Schiff base metal complex for Ni(II), Co(II) and Cu(II) metals has been done. Which involved preparation of 3-(2-O-tolylhydrazono)pentane-2,4-dione (**Compound A**), (3-(2-O-tolylhydrazono)pentane-2,4-dione) benzoate (**Compound B**) and series of metal complexes of (3-(2-O-tolylhydrazono)pentane-2,4-dione) benzoate (**Compound C**).

The three compound (A, B and C) are characterized by FTIR, UV and XRD analysis. The different graphs interpret that the compounds prepared are in the good agreement with the expected values.

Characterisation

Numerous analytical methods, including elemental analysis, FT-IR, XRD, and SEM for structural clarification and antibacterial analysis against *Staphylococcus aureus* and *Escherichia coli*, were used to characterise the synthesised compounds. The analytical data for the ligand and the metal complexes are shown in table 1 below.

Table 1: The analytical data for the ligand and the metal complexes

Sr. No.	Compound	Molecular formula	Molecular weight	Colour	Yield (%)
1	A	$C_{12}H_{14}N_2O_2$	218	Yellow	76
2	B	$C_{30}H_{32}N_4O_4$	512	Yellow	72
3	C-Ni complex	$C_{32}H_{40}N_4O_5NiCl$	654.5	Light green	71
4	C-Cu complex	$C_{32}H_{40}N_4O_5CuCl$	659.0	Blueish	69
5	C-Zn complex	$C_{32}H_{40}N_4O_5ZnCl$	661.0	Light yellow	66
6	C-Co complex	$C_{32}H_{40}N_4O_5CoCl$	654.5	Light yellow	73
7	C-Fe complex	$C_{32}H_{40}N_4O_5FeCl$	651.5	Light yellow	65

FTIR Spectra

The FTIR spectra for all the five metal complexes are shown below in figure 3.

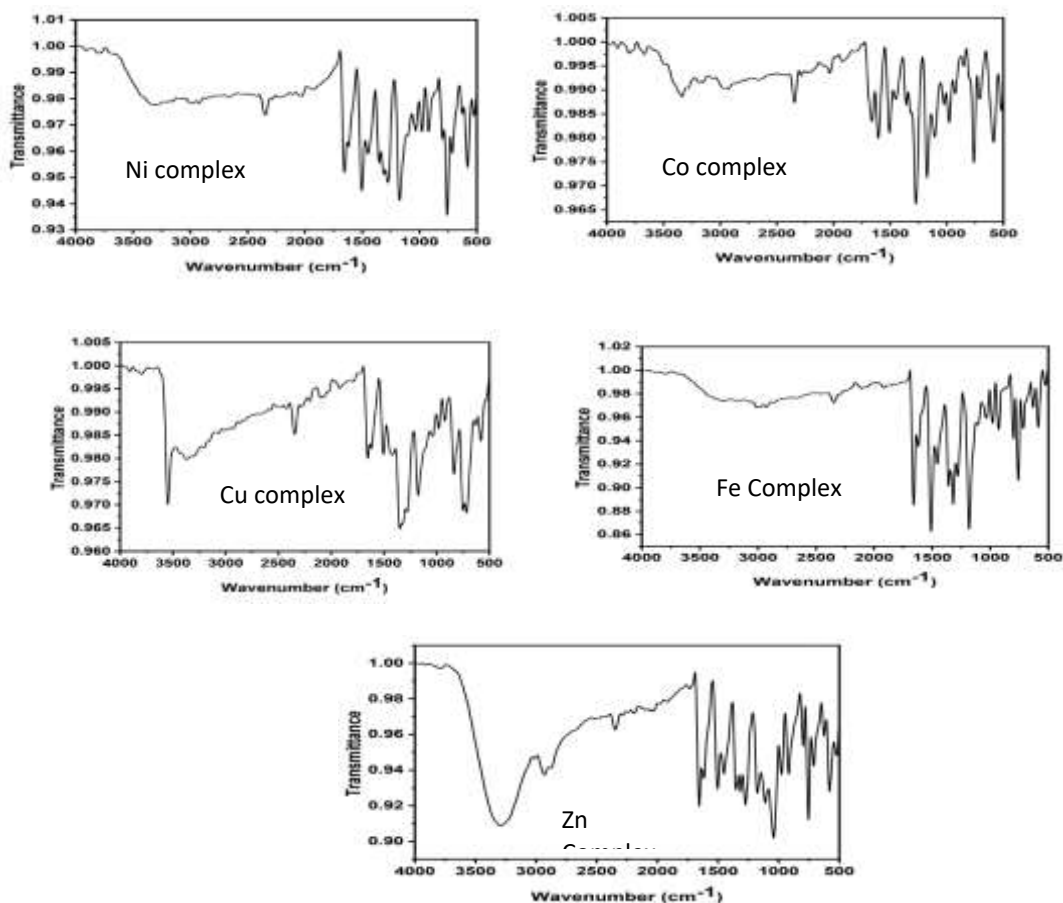


Figure 3: FTIR spectra for Compound C

The FTIR spectra of the ligands and all the complexes show that the ligands are coordinated to the metal ions through the nitrogen atom. This is demonstrated by the shift of the $\nu(\text{C}=\text{N})$ band at 11629 cm^{-1} to a lower frequency by about 10 and 20 cm^{-1} in the complexes: $\nu(\text{N}-\text{H})$ at 2240 cm^{-1} , $\nu(\text{N}=\text{C})$ at 1629 cm^{-1} , $\nu(\text{NC}(\text{Me}))$ at 2998 cm^{-1} , and $\nu(\text{NC}(\text{Ar}))$ at 2120 cm^{-1} . Only the spectra of the transition metal complexes at 512 and 519 cm^{-1} showed the presence of new bands, which are caused by the nitrogen-metal stretching vibrations and not the ligand. According to the FTIR measurements, the Schiff base ligand therefore forms a bidentate connection with the metal. We can verify that the theoretical data is acceptable by comparing the experimental and theoretical data, which we find to be identical.

XRD Graph

The XRD analysis of the ligand and the different metal complexes are shown in figure 4 below.

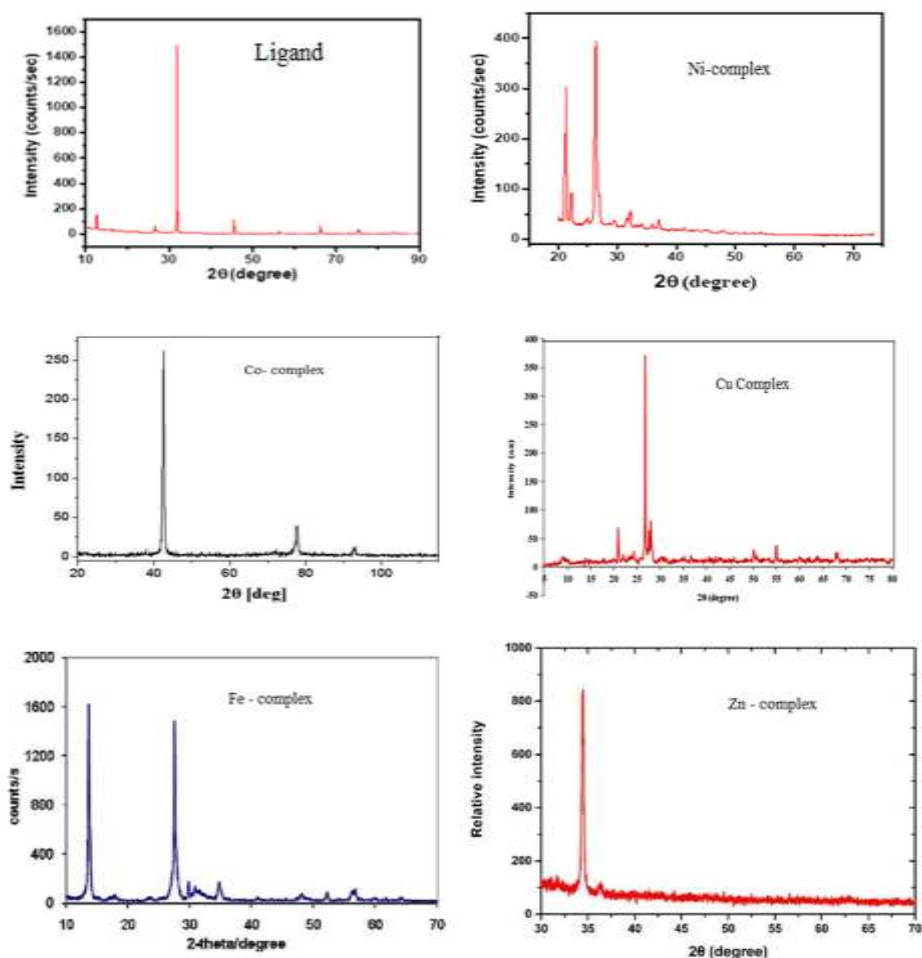


Figure 4: XRD graph for Compound B (ligand) and Compound C (metal complexes)

Powder X-ray diffraction analysis was used to determine the crystalline size and structure of the generated Schiff base ligand and metal complexes. The X-ray powder patterns for ligand and metal complexes are displayed in the graphs above. This investigation confirms that the freshly synthesised metal complexes are semi-crystalline, as evidenced by the distinct and well-defined Bragg Peaks at certain 2θ angles. Using the Debye-Scherrer formula and the intensity of the line with the highest intensity relative to the other lines, the particle sizes of the complexes were determined.

SEM Analysis

The SEM analysis for all the synthesized metal complexes are shown in figure 9 below.

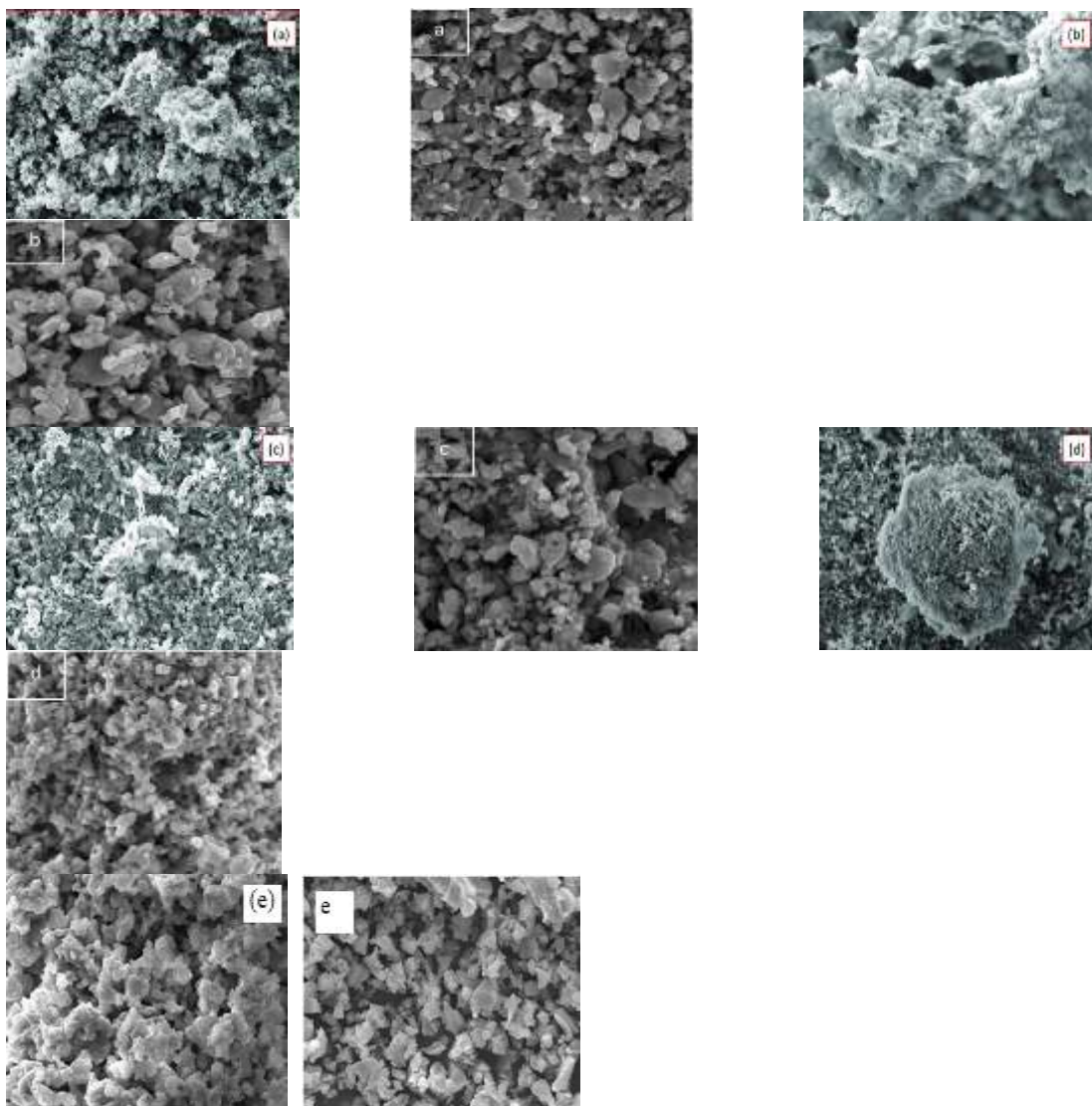


Figure 5: SEM images for Bulk synthesized and nano synthesized Compound C
a) Ni-Complex b) Cu-Complex c) Zn-Complex d) Co-Complex e) Fe-Complex

Utilising scanning electron microscopy (SEM), the micro morphology of the produced nanoparticles (NPs) was examined. SEM offers useful details on the density, geometric characteristics, and structural organisation of solid state materials. The morphology of the synthesised bulk and nanoparticles differs. The structure of the complex affects the nanoparticles' shape. Particle size, shape, and texture are the three aspects of particle characterisation that are covered by morphological analysis. In Figure 5, the bulk and nanoparticle SEM images are shown. Particle characterisation due to form, texture, and other

factors is the focus of the surface inquiry. The bulk and nanoparticle SEM images of the metal complexes of Co, Ni, Cu, and Zn reveal semi-circular, flake, and needle-like morphologies, respectively. The scale of SEM images measured in nanometer or micrometer shown in Figure above.

Antibacterial Analysis

The antibacterial study for the ligand and four synthesized metal complexes (bulk and nano both) by disk diffusion method, the details of the analysis is shown in table 3.

Table 3: Antibacterial activity against gram +ve and gram –ve bacteria at different dosages

Parameter	Dosage		Ligand	Cu-Complex	Co-Complex	Zn-Complex	Ni-Complex
Zone of Inhibition (in mm)	For gram +ve bacteria Staphylococcus aureus						
	Bulk	100 µg/mL	15.1	15.3	14.8	13.8	8.0
		150 µg/mL	15.4	15.6	15.4	14.0	8.2
		200 µg/mL	16.3	15.7	15.8	14.6	8.3
	Nano	100 µg/mL	15.3	15.1	14.6	13.2	8.1
		150 µg/mL	15.1	15.6	15.8	14.2	8.2
		200 µg/mL	16.5	15.9	15.7	14.6	8.5
	For gram -ve bacteria Escherichia coli						
	Bulk	100 µg/mL	15.8	15.8	8.0	19.7	8.0
		150 µg/mL	15.5	15.5	8.1	18.8	8.0
		200 µg/mL	14.8	15.0	8.0	18.4	7.8
	Nano	100 µg/mL	15.6	15.7	8.2	19.3	8.1
		150 µg/mL	15.2	15.7	8.1	18.5	8.0
		200 µg/mL	14.3	14.8	8.0	18.1	8.2

The antimicrobial properties of the Schiff base ligands and their soluble Metal (II) complexes are shown against *Escherichia coli* and *Staphylococcus aureus*. Chelation hypothesis provides an explanation for the metal chelates' antibacterial effect. The ligands typically operate as more effective bacterial agents as a result of the chelation, killing more bacteria than the ligand itself.

In complexes, the ligand's donor atoms share some of the metal's positive charge, and the entire chelate ring may experience π -electron delocalisation.

Using the well diffusion methods, the antibacterial activity of the free ligand and its associated metal complexes against two different bacterial species, gram-positive *S. aureus* (*Staphylococcus aureus*) and gram-negative *E. coli* (*Escherichia coli*), was evaluated at two different doses (100, 150, and 200 $\mu\text{g/mL}$). Higher doses of the free ligand and its metal complexes (200 $\mu\text{g/mL}$) were shown to have high inhibitory activity against the bacterial species *E. coli*. It was discovered that the Schiff base metal complexes of Co, Ni, Cu, Fe, and Zn were effective against the *S. aureus* and *E. coli* species at lower concentrations (100 $\mu\text{g/mL}$), respectively.

The ligand and the complexes created varying diameters of inhibitory zones against the tested microorganisms, and the findings were compared with typical medications, such as ciprofloxacin, against bacteria at the same doses. The outcomes are displayed in Table 3.

The metal complexes exhibit the following sequence of action against the gram-negative bacteria *E. coli*:

Cu-complex > Ligand > Co-complex > Zn-complex > Ni-complex.

Zn(II) complex has a moderate level of activity against *E. coli*, while Cu(II), Co(II), and Ni(II) complexes have a high level of activity.

The Schiff base ligand metal complexes exhibit the following sequence of action against the gram-positive bacteria *S. aureus*:

Zn-Complex > Cu-Complex = Ligand > Co-Complex > Ni-Complex.

Complexes of Zn(II), Cu(II), and Ni(II) exhibit significant activity whereas complexes of Co(II), Ni(II), and Zn(II) exhibit moderate activity against *S. aureus*.

CONCLUSION

Through the use of a reflux reaction, new Schiff base ligand and metal complexes were successfully created. The supporting evidence derived from spectroscopic and analytical data is capable of maintaining the octahedral geometry of any metal complex. The nanoparticles were created by breaking down various metal complexes, and SEM pictures were used to determine their size and form. All the metal complexes with good potential activity against the microorganisms were tested using antimicrobial agents.

The coordination of the ligand to the appropriate metal ion bidentately through β -ketiminato functionality is confirmed by the theoretical data of the ligand and its metal complexes.

References:

1. Xavier, N. Srividhya. Synthesis and Study of Schiff base Ligands. IOSR Journal of Applied Chemistry Volume 7, Issue 11 Ver. I. (Nov. 2014), PP 06-15.
2. Chohan Z. H., Wardell J. L., Low J. N., Meehan P. R., and Ferguson G., "Tetraethylammonium bromo(1,3-dithiol-2-one-4,5-dithiolato)diethylstannate(1-)," *Acta Crystallographica Section C Crystal Structure Communications*, vol. 54, no. 10, 1998.
3. K. Shoaib, W. Rehman, B. Mohammad, and S. Ali, "Proteomics and bioinformatics synthesis, characterization and biological applications of transition metal complexes of [no] donor schiff bases," *Journal of Proteomics & Bioinformatics*, vol. 6, no. 7, pp. 153–157, 2013.
4. C. Metcalfe, J.A. Thomas, *Chem. Soc. Rev.* 32 (2003) 215–224.

5. K. Jiao, Q.X. Wang, W. Sun, F.F. Jian, J. Inorg. Biochem. 99 (2005) 1369–1375.
6. T. Lee, W.A. El-Said, J.-H. Min, J.-W. Choi, Biosens. Bioelectron. 26 (2011) 2304–2310.
7. V. Rajendiran, R. Karthik, M. Palaniandavar, H.S. Evans, V.S. Periasamy, M.A. Akbarsha, B.S. Srinag, H. Krishnamurthy, Inorg. Chem. 46 (2007) 8208–8221.
8. Barve, A. Kumbhar, M. Bhat, B. Joshi, R. Butcher, U. Sonawane, R. Joshi, Inorg. Chem. 48 (2009) 9120–9132.
9. Sun S., Zeng H., J. Am. Chem. Soc. 124 (2002) 8204.
10. Aslam M., Li S., Dravid V.P., J. Am. Ceram. Soc. 90 (2007) 950.
11. Okawa K., Sekine M., Maeda M., Tada M., Abe M., Matsushita N., Nishio K., Handa H., J. Appl. Phys. 99 (2006) 08H102.
12. Katarzyna BRODOWSKA, Elżbieta ŁODYGA-CHRUŚCIŃSKA, Schiff bases - Interesting range of applications in various fields of science. CHEMIK 2014, 68, 2, 129–134
13. Rice L. B.: Unmet medical needs in antibacterial therapy. Biochem Pharmacol., 2006, 71, 7, 991–995.
14. Desai K.R, Patel R.B, Desai P.S.And Chikhali K.H, J Indian Chem Soc,80, 138(2003)
15. Chohar Z.H, Humayun P.A, Khan K.M, J.Enzyme Inhibition and Med Chem, 19,161(2004).
16. Silva da C., Silva da D., Modolo L., Alves R.: Schiff bases: A short review of their antimicrobial activities. J. Ad. Res., 2011, 2, 1–8.
17. Yang X., Wang Q., Huang Y., Fu P., Zhang J., Zeng R.: Synthesis, DNA interaction and antimicrobial activities of copper (II) complexes with Schiff base ligands derived from kaempferol and polyamines. Inorg. Chem. Com., 2012, 25, 55–59.