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Synthesis, Characterization, Crystallite size Determination and Evaluation of Biological Activity of Novel Co(II), Ni(II), Cu(II), Zn(II) Ternary Metal Complexes

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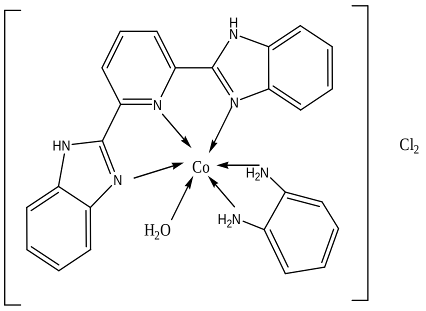
ABSTRACT

Four novel mixed ligand metal complexes namely $[Co(L_1)(L)_2H_2O]Cl_{2,}$*(1)* $[Zn(L_1)(L_2)OAc]OAc$ *(2),* $[Ni(L_1)(L_2)SO_4]$ *(3) and* $[Cu(L_1)(L_2)Cl]Cl$ *(4) where* L_1*=2,6-bis (benzimidazole-2yl) pyridine (BBP),* L_2*=Ortho phenylene diamine (OPDA) have been synthesized and characterized by elemental analysis, molar conductance measurements, magnetic susceptibility measurements, TGA, DTA studies, mass, IR, ESR, electronic, SEM-EDX, powder XRD studies. Based on elemental analysis and spectral studies six coordinated geometries were assigned to the metal complexes. Powder XRD studies proved that the complexes were in nanocrystalline phase. Antibacterial activity of metal complexes was checked against gram positive and gram negative bacterial pathogens such as MRSA, B.cereus, B.subtilis, P.aeruginosa, E.coli, P.vulgaris. The ternary metal complexes inhibited the growth of bacterial strains and exhibited better anti bacterial activity.*

Graphical Abstract



$[Co(BBP)(OPDA)H_2O]Cl_2$

Keywords: Antibacterial activity, Benzimidazole, Nanocrystalline phase, Ternary metal complex.

INTRODUCTION

Benzimidazole and its derivatives are an important class of aromatic heterocyclic compounds with a broad spectrum of biological activities such as antimicrobial, anticancer, antivirus, anti inflammatory and anticonvulsant. Benzimidazole and their metal complexes have variable physico-chemical properties, significant structural diversity and versatile biological properties. Bis (benzimidazole) ligands, which represent a class of aromatic N-donor organic linkers, are still less developed .Transition metal complexes of these ligands have attracted considerable attention as catalytic systems for use in the oxidation of organic compounds. Thus Bis (benzimidazole) ligands containing three N tridentate systems and their metal complexes stimulated wide spread interest in biological activities. Since the benzimidazole unit is the key-building block for a variety of compounds which have crucial role in the functions of biologically important molecules, there is a constant and growing interest over the past few years for the synthesis and biological studies of benzimidazole derivatives [1-3]. In this study, we have concentrated on the synthesis, characterization, antibacterial activity of mixed-ligand Co(II), Ni(II), Cu(II) and Zn(II), 2,6-bis (benzimidazol-2-yl)pyridine complexes.

MATERIALS AND METHODS

Reagents and solvents: All the chemicals and solvents were of analytical reagent grade and were used as received without further purification. 2, 6-pyridine dicarboxylic acid, O-phenylene diamine were purchased from Sigma–Aldrich chemicals. All the experiments involving with the interaction of the ligand and complexes were dissolved in a solvents ethanol, methanol, acetone and dmf. The C, H and N percentage composition of complexes and ligand BBP were determined using micro analytical methods on Perkin Elmer 240C (USA) elemental analyzer. FT-IR spectra of the ligand and its complexes were recorded by using KBr pellets in the range 4000–400 cm^{-1} using FT-IR spectrometer. The UV–Visible spectra of the primary ligand (BBP) and its metal complexes were carried out in DMSO using Elico SL159 spectrophotometer. The mass spectra of the compounds were recorded by the ESI technique on VG AUTOSPEC mass spectrometer. Magnetic measurements were carried out on a Guoy balance model 7550 using Hg [$Co(NCS)_4$] as standard. Diamagnetic corrections were carried out by Pascal’s constant. ESR spectra of metal complexes were recorded on JEOL JES-FA200ESR spectrometer (X-band microwave unit).The conductivity measurements were carried out in DMSO (10^{-3}M) using Digisun Electronic Digital conductivity meter, 0.01 M KCl solution is used for calibration. The melting points of metal complexes, primary ligand and decomposition temperature of complexes were determined on Polmon instrument (Model No. MP-9). The X-ray powder diffraction analysis was carried out by using PAN Analytical X'pert Powder X-ray diffractometer with Cu Kα radiation. The diffraction data integrated by using Match program. Scanning electron microscopy (SEM) was obtained for complexes using Tescan Vega-3 LMU electron microscope.

Synthesis of ligand and complexes: The primary ligand BBP was prepared according to the procedure reported earlier [4].

Synthesis of Complexes 1, 2, 3 and 4: The complexes 1, 2, 3 and 4 were prepared by mixing $CoCl_2.6H_2O$ (0.238 gm, 1 mM), $Zn(OAc)_2.2H_2O$(0.219 gm,1mM), $NiSO_4.XH_2O$(0.290 gm,1 mM) and $CuCl_2.2H_2O$ (0.170 gm, 1 mM) respectively in 50 ml methanol, BBP (0.312 gm, 1 mM) in15 mL methanol, and the mixture was refluxed for 2 h . To this O-phenylene diamine (0.108 gm, 1 mM) in 15 mL methanol was added and refluxed at refluxing temperature for 3 h. The resulting solution was slowly evaporated. After 5 days, poly crystalline yellow to brown precipitates were obtained, they were washed thoroughly with acetone, and then evaporated in vacuum over anhydrous $CaCl_2$. The color of the Co, Ni complexes was coffee brown, Cu complex was black and Zn complex was colorless and the yield of the metal complexes was in between 70-76%.

Complex-1 Analytical data: IR (ν_{max})3419–3385 cm^{-1} (νO–H),3140 cm^{-1} ($\nu_{N–H}$)3048 cm^{-1} ($\nu_{C–H}$) 1610 cm^{-1} ($\nu_{C,N}$Im), 1319 cm^{-1} ($\nu_{C,N}$Py), 1454 cm^{-1} (υ_{N-H}Imd),1116 cm^{-1} ($\nu_{C,N}$Imd),428 cm^{-1} ($\nu_{M–N}$), 582 cm^{-1} ($\nu_{M–O}$); Anal. Calc. for $C_{25}H_{23}N_7Cl_2OCo$ Cal: C 52.91, H 4.09, N 17.29; found: C 52.11, H4.10, N 17.40%. Molar conductance (10^{-3} M DMF) 141S cm^2 mol^{-1}, μ_{eff}: 4.8 BM; UV-Vis (nm) 233, 331, 449, 712; ESI-MS: 496 $[Co(BBP)(OPDA)H_2O]^{+2}$.

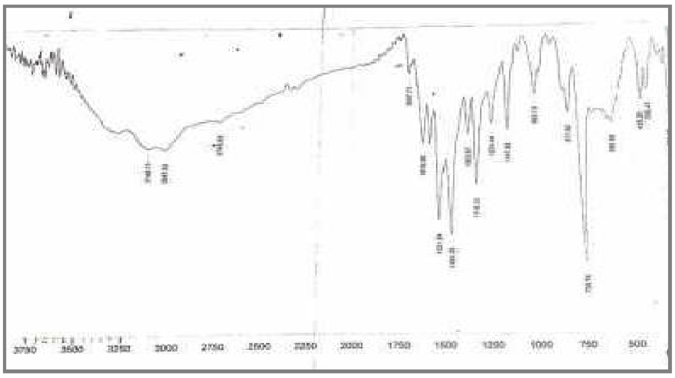


Figure 1. IR spectrum of complex 1.

Complex-2 Analytical data: IR υ_{max}: 3373 cm^{-1} (ν_{N-H}), 3062 cm^{-1} (ν_{C-H}),1573cm^{-1} (ν_{C-N} imd), 1500 cm^{-1} ($\nu_{sym}CH_3COO$) 1427 cm^{-1} ($\nu_{C=C}$imd), 1319 cm^{-1} ($\nu_{C=N}$Py), 1234 cm^{-1}1(ν Asym CH_3COO), 1149 cm^{-1} ($\nu_{C,N}$Imd), 745 (νOCO), 584 cm^{-1} (ν_{M-O}); 426 cm^{-1} (ν_{M-N}); Anal.Calc. for $C_{29}H_{27}O_4N_7Zn$: Cal: C 57.76, H 4.51, N 16.26; found: C 57.65, H 4.57,N 16.47%. Molar conductance (10^{-3}M DMF) 80 S cm^2 mol^{-1} μ_{eff}:0BM;UV–Vis(nm): 231, 275, 324, 356; 432, 450 ESI-MS:544[Zn(BBP)(OPDA)OAc]+

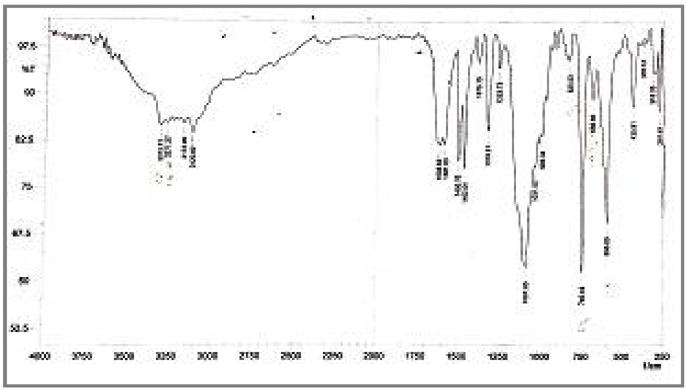


Figure 2. IR spectrum of complex 3.

Complex-3 Analytical data: IR υ_{max}: 3313 cm^{-1} (ν_{N-H}), 1608 cm^{-1}, (ν_{C-N}imd), 1581cm^{-1} (ν_{C-N}imd), 1462 cm^{-1} ($\nu_{C=C}$Py), 1319 cm^{-1} ($\nu_{C=N}$Py), 1087 cm^{-1} ($\nu_{C,N}$Imd),825, 686cm^{-1} (SO_4), 596 cm^{-1} (ν_{M-O}); 435 cm^{-1} (ν_{M-N}); Anal. Calc. for $C_{25}H_{21}O_4N_7Ni$: Cal: C 52.28, H 3.69, N 17.07; found: C 52.14, H 3.60, N 17.26 %. Molar conductance (10^{-3}M DMF) 16 Scm^2 mol^{-1} μ_{eff}: 3.3 BM; UV–Vis (nm): 256, 275, 314, 465, 762, 914; ESI-MS: 596 $[Ni(BBP)(OPDA)SO_4]+Na^+$

Complex-4 Analytical data: IR υ_{max}: 216 cm^{-1} (ν_{N-H}), 3042 cm^{-1} (ν_{C-H}), 1578 cm^{-1}, (ν_{C-N}imd), 1475 cm^{-1} ($\nu_{C=C}$Py), 1318cm^{-1} ($\nu_{C=N}$Py), 1082 cm^{-1} ($\nu_{C,N}$Imd), 435 cm^{-1} (ν_{M-N}), 324((ν_{M-Cl}) ; Anal. Calc. for $C_{25}H_{21}Cl_2N_7Cu$: Cal: C 52.28, H 3.69, N 17.07; found: C 52.14, H 3.60, N 17.26 %. Molar conductance (10-3M DMF) 88 Scm^2mol^{-1}, μ_{eff}:1.74 BM; UV–Vis (nm): 274, 384, 415, 637; ESI-MS:519.5 $[Cu(BBP)(OPDA)Cl]^+ + H^+$

Antibacterial Assay:

Microbial strains: Gram positive Strains Methcillin- resistant Staphylococcus *aureus* (MRSA, NC TC 13616), *Bacillus subtilis* (ATCC 6633), *Bacillus cereus*, (ATCC 14579) and Gram negative strains *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 43816), *Escherichia* *coli* (ATCC 8739), *Proteus vulgaris* (ATCC 13315), *Salmonella typhi* (ATCC 19430) were procured from American Type Culture Collection, USA. Methcillin resistant *Staphylococcus aureus* was purchased from Culture Collections, UK. All bacterial strains stored at -80°C were streaked on Luria Bertani (LB) agar plates (Hi-media Laboratories, Mumbai, India) and incubated at 37°C for 20 to 24 h. From each a few isolated colonies were selected and suspended in 5 ml of LB broth in sterile culture vessel. This vessel was plugged with cotton and incubated with gentle shaking (140 rpm) at 37°C for 20 h.

Culture media: Nutrient Agar (NA) containing bromo cresol purple was used for the activation of Bacillus species while NA was used for the other antibacterial activity. Mueller Hinton Agar (MHA) was used for minimum inhibitory concentration (MIC).

Inoculation preparation: The bacterial strains were inoculated into sterilized nutritive broth and incubated at 35 ± 2°C for 24 h. The turbidity of the resulting suspensions are diluted with same nutritive broth to obtain a transmittance of 25% at 580 nm, this percentage was calculated spectrophotometrically using Bausch and Lomb spectrophotometer comparable to McFarland turbidity standard. This level of turbidity is equivalent to approximately 3.0×108 CFU mL^{-1} (a stock standard from which a working standard was drawn with concentration of 1×108 CFU mL^{-1}).

Agar well diffusion assay: The antibacterial activity of selected medicinal plant extracts was performed based on the guidelines of Clinical and Laboratory Standard Institute. The selective medium was inoculated with the test organism and once the agar was solidified, the wells were created using a six millimeters diameter bork corer. The wells were filled with 25 µL of the compounds of 50, 75 and 100 µg mL^{-1} concentrations. ciproflaxacin (10 µg mL^{-1}) is used as positive control. The test was carried out in triplicate. The plates were incubated at 35 ± 2°C for 24 h.

Minimum inhibitory concentration (MIC): The MIC of the fractions was determined by diluting the varied concentrations (0.0-1000 µg mL^{-1}) of compounds. Equal volume of the fractions and nutrient broth were mixed in the test tube. Specifically 0.1 mL of standardized inoculums of 1to 2X 10^{7} cfu mL^{-1} was added to each tube. The tubes were incubated aerobically at 37°C for 18-24 h. 2 control tubes were maintained for every test batch. That is one tube with extracts and the growth medium without inoculums (antibiotic control) and the other tube containing the growth medium, physiological saline and the inoculums (organism control). MIC was determined as the lowest concentration of the extracts permitting no visible growth (no turbidity) when compared with the control tubes.MIC of the prepared metal complexes was given in the table 1.

Table 1. Minimum Inhibitory Concentrations (µg mL^{-1}) of complexes 1, 2, 3 and 4

Compounds	**MRSA**	***B. subtilis***	***B. cereus***	***P. aeruginosa***	***E.coli***	***P.vulgaris***
Complex 1	>200	>300	>250	>300	>200	--
Complex 2	>150	>150	>200	>100	93.5	>200
Complex 3	>50	75.6	>150	86.9	>150	>200
Complex 4	>50	85.2	>150	88.7	>150	>200

RESULTS AND DISCUSSION

Characterization by TGA/DTA: The thermo gravimetric analysis (TGA) and differential thermal analysis (DTA) of complexes 1, 2, 3 and 4 were carried out at temperature ranges from 0 to 800°C by using alumina as reference compound. A small weight loss in the temperature range of 200–250°C for the Co(II) complex indicates the loss of coordinated water molecule from the complex. The weight loss in the ranges of 200–600°C is attributed to the decomposition of complexes.

Infrared spectral characterization: In the IR spectrum of primary ligand (BBP) the bands observed in the range of 3325-3385 cm^{-1} are assigned to $v_{(N-H)}$ stretching vibrations [5]. The peak observed in the range of 3100 cm^{-1} is assigned to $v_{(C–H)}$ stretching frequency, the band at 1635 cm^{-1} is assigned to $v_{(C=N)}$, 1455cm^{-1} for $v_{(N-H)}$ of the imidazole moiety [6] and a peak at 1492 cm^{-1} is assigned to $v_{(C=C)}$ stretching frequencies. In the IR spectra of complexes the $v_{(N-H)}$ is replaced by a new absorption in the region 3140-3373 cm^{-1}, confirming the coordination of the metal ions through N-atom of the imidazole ring (Table 2). In Co(II) complex a new absorption band in the region of 3400–3600 cm^{-1} is attributed to the $v_{(O-H)}$ of the coordinated H_2O to the metal ion. The IR spectra of the complexes also showed, shifted imidazole in plane resonance at 993-1083 cm^{-1}, pointing to further coordination of imidazole N atom to the metal. Stretching frequency $\upsilon(_{N-H})$ typically observed at 3351 cm^{-1} indicating the coordination of NH_2 of ortho phenylenediamine to the metal ion. In the far IR spectra of the complexes reveals υ $(_{M-N})$ stretching vibrations in the region 426-435 cm^{-1} and $\upsilon(_{M-O})$ stretching vibrations are in the region of 582-596 cm^{-1} confirm the formation of metal complexes.

Table 2. FT-IR spectral studies of BBP and Co (II), Zn(II), Ni(II), Cu(II) complexes

Compound	$\nu_{O–H}$	$\nu_{N–H}$	$\nu_{C–H}$	$\nu_{C=C}$	$\nu_{C=N}$	$\nu_{M–N}$	$\nu_{M–O}$	$\nu_{M–Cl}$
BBP	--	3325-3384	3100	1492	1635	--	--	--
Complex 1	3419	3140	3048	1454	1610	428	582	--
Complex 2	--	3373	3062	1427	1573	426	584	--
Complex 3	--	3313	3120	1462	1608	435	596	--
Complex 4	--	3216	3042	1475	1606	430	--	324

Electronic spectra, Mass, ESR Spectra and magnetic moment: The electronic absorption studies are often very helpful in the evaluation of results furnished by other methods of structural investigation. The electronic spectral measurements provide quick and reliable information regarding the geometry of metal complexes based on the position and number of d–d transition peaks. The electronic absorption spectra of the BBP and its metal complexes were recorded at room temperature using DMSO as solvent. The two absorption bands at 449 nm and 712 nm observed in the electronic spectrum of the Co(II) complex assigned to high spin d–d transitions which is in conformity with distorted octahedral geometry (Figure 3). Only one broad band is observed at 637 nm in the electronic spectrum of the Cu(II) complex assigned to d–d transitions ($^2E_g \rightarrow {}^2T_{2g}$) which is in conformity with distorted octahedral geometry [7]. The Complexes 2 and 3 were assigned octahedral geometry from their infra red, absorption spectra and conductivity measurements. The strong bands in the UV region 300 nm to 400 nm are assigned to metal to ligand charge transfer (MLCT) transitions [8]. The ESI-Mass spectra of the complexes 1, 2, 3 and 4 gave peaks at m/z: 496, 544, 596 and 520 were assigned to $[M]^+$ for complexes 1, 2 and $[M+Na,]^+$, $[M+H]^+$ ions respectively for the complexes 3,4. Colors of the complexes 1, 3 and 4 are due to d-d transition of unpaired electrons. Complex 2 was found to be color less which was in agreement with absence of d-d transitions in its absorption spectra. The magnetic moment (μ_{eff}) of the complexes 1 and 3 was in the range of 4.3-5.0 and 3.3-3.5 BM respectively at room temperature, which is expected range for octahedral [9] cobalt, nickel complexes. Whereas Zn(II) complex has zero magnetic moment indicates its diamagnetic nature. Cu(II) complex has magnetic moment of 1.74 BM indicates the presence of one unpaired electron [10]. These values also indicate that the complexes are monomeric in nature.

ESR Spectral students of paramagnetic transitions metal (II) complexes provide information about the distribution of the unpaired elections and hence about the nature of the bonding between the metal ion and its ligands. The Cu (II) complex exhibited well resolved anisotropic signals in the parallel and perpendicular regions. The observed data showed that gP=2.3 and $g^{\wedge}$=2.04. The gP value are greater than $g^{\wedge}$ suggesting major distortion from octahedral symmetry in the Cu(II) complex. The gP is a moderately sensitive function for indicating covalency. The observed gP value for the complex showed gP > 2.3, which is characteristic of anion environment. The trend gP > g^ >2.0023 observed for the complex indicates that unpaired election is localized in dx^2-y^2 orbital of the Cu(II) ion. Thus a tetragonal distortion is proposed for the complex. The Cu(II) complex showed axial symmetry parameter value <4 indicating the considerable exchange interaction in the complex [11]. From all the spectral data a distorted octahedral geometry has been proposed for the complexes 1, 4 and octahedral geometry for the complexes 2, 3.

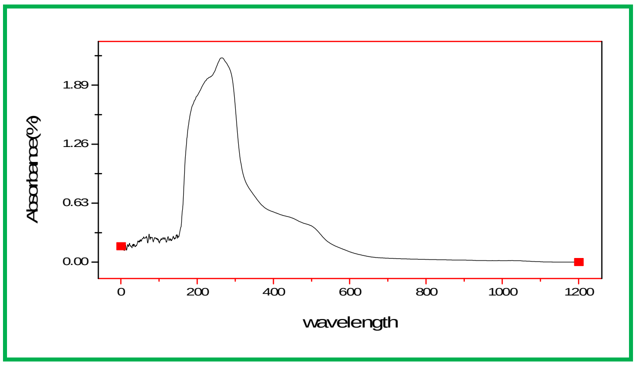


Figure 3. Absorption spectrum of Co(II) complex.

Powder XRD and SEM Analysis: The powder X-ray diffraction data for the complexes were obtained and used for structural characterization. The powder XRD pattern of Co(II) and Zn(II) complexes are shown in (figure 4 and 5) respectively. All the four complexes were crystalline in nature. Co(II) complex was crystalline with triclinic(anorthic) structure. The space group is P1.The unit cell parameters of the complex a=5.6337A^0, b=5.6337A^0, c=17.6466A, αβγ=90^0. Crystallite sizes of the complexes 1 and 4 are of 57.55 and 81.67 which were determined by Debye scherrer's formula [12] indicating that the complexes were in nano crystalline phase.

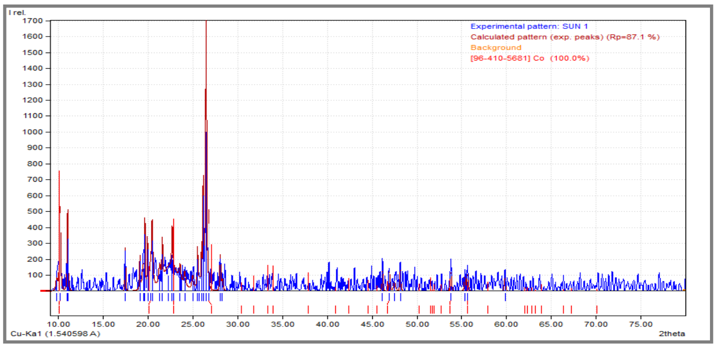


Figure 4. Powder XRD spectrum of Co(II) complex.

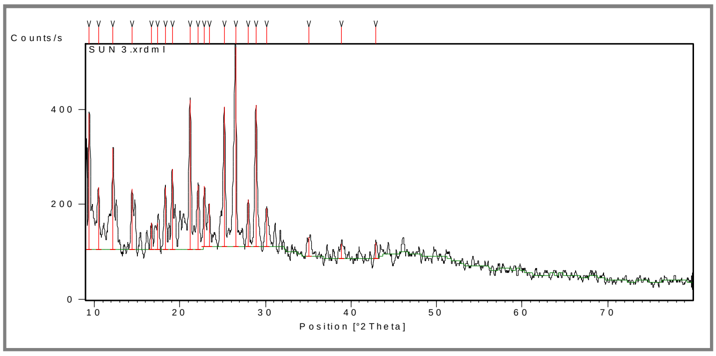


Figure 5. Powder XRD spectrum of Zn(II) complex.

The SEM micro graphs of the complexes were analysed for their morphology and grain size the SEM–EDS micro graph of the Co(II) complex is shown in (Figure 6). It is observed from SEM EDS micrographs that the complexes showed agglomerated thin plates, ice granular and ice plates like morphologies with average grain size of 0.54 to 0.85 µm.

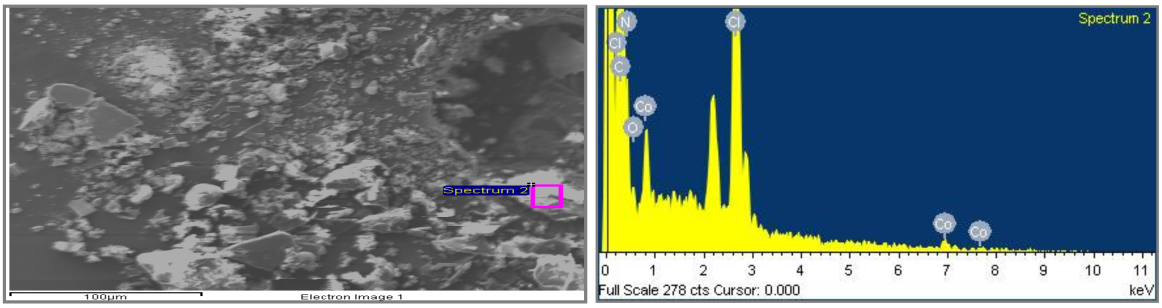


Figure 6. SEM micrograph and EDS of Co(II) complex.

Estimation of particle size for the complexes 1 and 4: The estimation of particle size has been made following Hall-Williamson method [13]. In the present work, the particle size has been determined by measuring the integrated widths of the diffraction peaks rather than half-widths. After eliminating the instrumental broadening effects, the particle size (*D*) and lattice strain (*J*) have been determined using the equation (1).

$$Br \cos\theta = k\lambda / D + J \sin\theta \qquad (1)$$

Where *Br* is the peak broadening due to crystallite size and *J* the lattice strain, *k* the shape factor usually taken as 1.0 and *D* the particle size in nanometers, θ and λ are the Bragg angle and the wavelength of incident X-ray beam in nm.

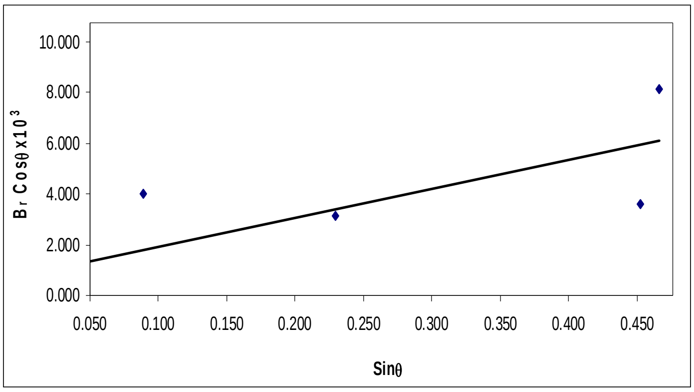


Figure 7. Hall-Williamson plot for the complex 1.

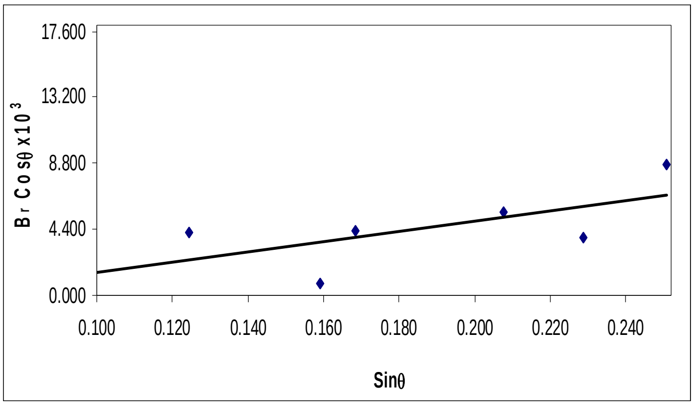


Figure 8. Hall-Williamson plot for the complex 4.

In the present investigation, particle sizes have been determined as 57.55 nm and 81.67 nm for complexes 1 and 4 by using Hall-Williamson method as shown in (Figure 7 and 8).

APPLICATION

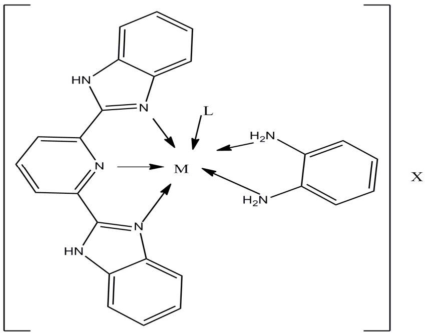
The four metal complexes inhibited the growth of bacterial strains and exhibited antibacterial activity. The results were found in concentration dependent manner. The results shown in table 1 and 3 are minimum inhibitory concentrations and zone of inhibitions values respectively. The anti bacterial activity results of the complexes were compared with reference drug ciprofloxacin at 10 mg mL^{-1}. According to our results, it is found that, the complex 3 is more active than complex 1, 2 and 4. Among the Gram negative strains *P. vulgaris* was found to be least susceptible and *S. typhi* was resistant to all the compounds. The complex 3 exhibited highest zone of inhibition at 20 mm against *P. aeruginosa* at 100 µg mL^{-1}. This value is nearer to the value recorded against standard drug ciprofloxacin (Table 3).

Table 3.0 Biological activity of complexes 1, 3 in the form of zone of inhibitions
Diameter of growth of inhibition zone (mm)

Compounds	**Concentration µg mL^{-1}**	**MRSA**	***B. subtilis***	***B. cereus***	***P. aeruginosa***	***E. coli***	***P. vulgaris***	***S. typhi***
Complex 1	50	15	07	11	09	11	08	--
	75	07	09	13	12	13	10	--
	100	09	10	15	14	15	12	--
Complex 3	50	11	14	14	15	14	09	--
	75	15	17	15	18	16	12	--
	100	17	19	17	20	18	15	--
Ciprofloxacin	10	28	30	26	32	28	26	30

CONCLUSION

Primary ligand (BBP) and the four novel complexes of Co(II), Ni(II),Cu(II), Zn(II) were synthesized and characterized by elemental analysis, electrical conductance, magnetic moment and various spectral techniques. The primary ligand BBP (L_1) acts as a tridentate coordinating through 3 nitrogen atoms confirmed by spectral, analytical, thermal and magnetic studies. Acetate, chloride, sulfate ion, water and OPDA molecules are also coordinated to the metal ions. Six coordinated geometries are assigned to all the four complexes. They inhibited the growth of bacterial strains and exhibited antibacterial activity. The results were found in concentration dependent manner. The anti bacterial activity results of the complexes were compared with reference drug ciprofloxacin at 10 mg mL^{-1} According to our results, the metal complexes showed moderate activity. The Ni(II) complex is more active than complexes 1, 2 and 4. Among the Gram negative strains *P. vulgaris* was found to be least susceptible and *S. typhi* was resistant to all the compounds. The Ni complex exhibited highest zone of inhibition at 20 mm against *P. aeruginosa* at 100 µg mL^{-1}. This value is nearer to the value recorded against standard drug ciprofloxacin.



Complex1: M=Co, L=H_2O, X=Cl_2, Complex 2: M=Zn, L and X=OAc,
Complex 3: M=Ni, L=SO_4, Complex 4: M=Cu, L and X=Cl

Figure 9. Proposed geometries of complexes1, 2, 3, 4

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