

## SYNTHESIS, SPECTRO-ANALYTICAL AND BIOLOGICAL STUDIES OF N'<sup>1</sup>[(E)-(4-METHOXYPHENYL)METHYLIDENE]-1,3- BENZOTHIAZOLE-2-CARBOHYDRAZIDE AND ITS COMPLEXES

Kunche Sudeepa<sup>1</sup>, Boinala Aparna<sup>2</sup>, Mudavath Ravi<sup>2</sup>,

Palreddy Ranjithreddy<sup>2</sup>, Karunakar Rao Kudle<sup>3</sup> and CH. Sarala devi<sup>2\*</sup>

<sup>1</sup>Department of Chemistry, Nizam College, Hyderabad -500 001, Telangana, India.

<sup>2</sup>Department of Chemistry, Osmania University,  
Hyderabad -500 007, Telangana, India.

<sup>3</sup>Department of Biochemistry, Osmania University,  
Hyderabad -500 007, Telangana, India.

### ABSTRACT

The synthesis and structural studies of N'<sup>1</sup>[(E)-(4-Methoxyphenyl)methylidene]-1,3-benzothiazole-2-carbohydrazide (MPMBC) and its solid metal complexes were carried out by employing spectroanalytical techniques. The characterization of the title compound MPMBC was carried out by Mass, FT- IR, <sup>1</sup>H- NMR and <sup>13</sup>C- NMR spectral data. The equilibrium studies were carried out in 70% v/v Dioxane-Water medium to enumerate number of dissociable protons in MPMBC at 303 K and 0.1 M (KNO<sub>3</sub>) ionic strength adopting pH-metric technique. These studies indicated dissociation of one proton and also inferred that the same proton is dissociated relatively at low pH in presence of Cu (II) ion due to the formation of corresponding complex. The spectrophotometric studies employed enabled to establish the stoichiometry of metal complexes under investigation. The metal complexes of MPMBC with copper (II), nickel (II) and cobalt (II) have been carried out by employing various spectro-analytical techniques viz; Mass, FT-IR, TGA, ESR, molar conductance and EDS analyses. Further anti-bacterial activity and DNA binding studies were also explored as preliminary investigation to get an insight for in-vitro and in-vivo studies.

**Keywords:** N'<sup>1</sup>[(E)-(4-Methoxyphenyl) methylidene]-1, 3-benzothiazole-2-carbohydrazide (MPMBC).

### INTRODUCTION

Benzothiazole ring possess pharmacological activities such as anti-viral<sup>1</sup>, anti-bacterial<sup>2</sup>, antimicrobial<sup>3</sup> and fungicidal activities<sup>4</sup>. The compounds having benzothiazole moiety were found to be useful as anti-allergic<sup>5</sup>, anti-diabetic<sup>6</sup>, anti-tumour<sup>7</sup>, anti-inflammatory<sup>8</sup> and anthelmintic<sup>9</sup>. Keeping in view the biological relevance of benzothiazoles, we report here the synthesis and characterisation of N'<sup>1</sup>[(E)-(4-Methoxyphenyl)methylidene]-1,3-benzothiazole-2-carbohydrazide (MPMBC) and its metal complexes. Antimicrobial activity and DNA binding studies were also carried out in the present investigation to explore the potential aspects of title compound and its metal complexes in the field of bioinorganic chemistry<sup>10</sup>.

### MATERIALS AND METHODS

All chemicals used were of Analytical Reagent (A.R) grade. The Mass spectral data was collected on Agilent Single Quad Mass Spectrometer using Electrospray Ionization (ESI-MS), IR Spectra using Perkin-Elmer 337 spectrophotometer in KBr pellet in the range 200-4000 cm<sup>-1</sup>, while <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra was recorded on Bruker WH (270 MHz) spectrometer using CDCl<sub>3</sub>. The pH measurements were made using a digital ELICO electronic model LI 120 pH meter in conjunction with a combined glass and calomel electrode and spectrophotometric studies were carried out on SL-171 Minispec spectrophotometer, where 1cm cells were used. The thermal studies were carried out in a dynamic nitrogen atmosphere (20 ml min<sup>-1</sup>)

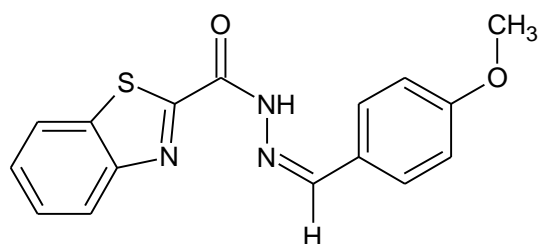
with a heating rate of  $10^{\circ}\text{C min}^{-1}$  using a Shimadzu TGA-50H. ESR spectra were recorded on a Varian E112 spectrometer using L band in air atmosphere at room temperature. The particle size and morphology of title compound and its complex were recorded on Ziess Scanning Electron Microscope. INCA EDX instrument was used to study the elemental analysis and absorption spectra were recorded on Elico SL 159 UV-Visible spectrophotometer using 1 cm quartz microcuvettes. The melting points were determined with Polmon apparatus (Model No.MP-90).

## EXPERIMENTAL

The title compound was synthesised by three step procedure wherein the literature method was employed for first two steps for the formation of Benzothiazole ethyl-2-carboxylate (BTEC) and followed by corresponding hydrazide Benzothiazole-2-carboxyhydrazide (BTCH)<sup>11,12</sup>. In subsequent step the title compound MPMBC was synthesised by following the procedure mentioned below.

### Synthesis Of MPMBC

BTCH (0.5 g; 2.59 mmol) was dissolved in 20 mL of methanol, (0.35 g; 2.58 mmol) of anisaldehyde was added and the reaction mixture was refluxed for 4 hours. The yellow product obtained was filtered, washed with methanol, dried, checked for purity with TLC and then recrystallized from 70:30 (v/v) mixture of methanol and water.(m.p  $193^{\circ}\text{C}$ ).



$\text{N}'[(\text{E})-(4\text{-Methoxyphenyl})\text{methylidene}]-1,3\text{-benzothiazole-2-carbohydrazide (MPMBC)}$

### Synthesis of metal complexes

A solution of Cu (II), Ni (II) and Co (II) chlorides (0.27 g, 1.58 mmol, 0.38g, 1.59 mmol and 0.38 g,1.60 mmol) was added to the hot methanol solution of MPMBC ( 0.5 g, 1.6077 mmol ) in 1:1 molar ratio. The reaction mixture was refluxed for about 8 hours. The metal complexes were filtered and washed with hot methanol, followed by washings with petroleum ether and finally dried. (m.p  $>300^{\circ}\text{C}$ ).

## RESULTS AND DISCUSSION

The spectro-analytical techniques such as FT-IR, Mass, NMR, ESR, and TGA are used for the characterization of MPMBC and its metal complexes.

### Mass Spectrum

The mass spectrum of the MPMBC (Figure - 1) shows a dominant peak at  $m/z$  311, which is in accordance with the expected molecular ion ( $\text{M}^+$ ) peak.

### IR Spectrum

The IR spectrum of MPMBC (Figure -2) shows the presence of a sharp intense band at  $1666\text{ cm}^{-1}$  which corresponds to  $\text{-C=O}$  of  $\text{-CONH}$  group. The bands at  $3284\text{ cm}^{-1}$  and  $3024\text{ cm}^{-1}$  are attributable to aromatic  $\text{-C-H}$  stretching frequencies. The other absorptions bands at  $1603\text{--}1533\text{ cm}^{-1}$  correspond to azomethine and ring  $\text{-C=N}$  stretching frequencies respectively. A band at  $825\text{ cm}^{-1}$  indicates the presence of para disubstituted aromatic ring.

### $^1\text{H NMR}$

The  $^1\text{H NMR}$  spectrum of MPMBC (Figure -3) in  $\text{CDCl}_3$  shows a singlet at  $\delta$  3.84 ppm (s,3H) corresponding to  $\text{-OCH}_3$ . A multiplet in the region  $\delta$  6.91-8.29 ppm (m,9H) is attributable to aromatic protons and azomethine proton. The  $\text{-NH}$  proton is observed as singlet at  $\delta$  10.32 ppm (s,1H).

### $^{13}\text{C NMR}$

$^{13}\text{C NMR}$  spectrum of MPMBC (Figure -4) exhibits signals in the region  $\delta$  114.53-162.93 ppm which has been assigned to aromatic carbons, the signals at  $\delta$  55.41 and  $\delta$  29.72 ppm correspond to carbon atom of thiazole ring and methyl carbon respective

### Equilibrium studies

To know the chelation properties of the title compound, an attempt is made to study its potential donor sites that bind with metal ions. The proton – ligand constants ( $\text{pK}_a$ ) of MPMBC was determined by adopting Irving-Rossetti pH-metric technique, the pH-metric titrations were carried out in 70% Dioxane-Water medium at 303 K and 0.1 M ( $\text{KNO}_3$ ) ionic strength ((Figure - 5).

These studies reveal that the title compound has one dissociable proton with  $\text{pK}_a$  value of 13.42 (Figure -6) corresponding to the  $\text{-NH}$  proton. The  $\text{pK}_a$  values thus calculated in 70% dioxane-water medium is very high compared to the value computed using ChemAxon software (9.83). The observed variation between the experimental and computed  $\text{pK}_a$  is due to the effect of 70 % - aquo-organic medium on the basicity of title compound<sup>13-15</sup>.

This is in accordance of the fact that the decrease of dielectric constant with the increase of mole fraction of organic solvent would increase pka value in general<sup>16-19</sup>. The titration curves clearly indicated the release of dissociable proton more easily in presence of Cu<sup>2+</sup> ions.

### Stoichiometry of the complex

The composition of the complex was confirmed by performing spectrophotometric measurements using Jobs and mole ratio methods<sup>20</sup>. In Jobs continuous variation method, the solutions of the MPMBC and Cu<sup>2+</sup> with same molar concentrations (0.001M) are mixed in varying volume ratios, keeping the total volume of the mixture constant. The pH of solutions is maintained constant by addition of Sodium acetate buffer. The absorbance of each solution is measured at wavelength 570 ( $\lambda_{max}$ ). The absorbance is plotted against the mole fraction of the ligand (Figure -7). The resulting curve showed a maximum absorbance value at 0.5 mole fraction of ligand, corresponding 1:1 stoichiometric metal to ligand ratio. While in mole ratio method a series of solutions are prepared, in which the molar concentration of metal ion is kept constant and that of the ligand was varied. The pH of solutions was maintained constant by adding acetate buffer. A plot of the absorbance versus number of moles of the MPMBC per mole of Cu<sup>2+</sup> ion showed two straight lines (Figure -8) with intersection corresponding to 1:1 stoichiometry of metal complex, in agreement with the results obtained from Job's method.

### Characterization of metal complexes of MPMBC

#### IR SPECTRA

The coordination mode of the ligand during the complex formation is revealed by the comparative IR spectral study (Table-1) of the entitled compound and their Cu (II) Ni (II) and Co (II) complexes. On complexation, the bands at 1666 and 1603-1533 cm<sup>-1</sup> which were assigned to the stretching of C=O and C=N were shifted to a lower frequency region (figure 9-11). This shift is probably due to the complexation of the metal to the ligand through oxygen of the carbonyl group. The presence of coordinated water in the spectra of all metal complexes were suggested by broad absorption around 3419-3442cm<sup>-1</sup>, participation of oxygen and nitrogen atoms in the coordination is further confirmed by far IR spectral bands at 570-430cm<sup>-1</sup> range corresponding to M-N and M-O respectively.

### Mass Spectrum

The mass spectra of the metal complexes are presented below (figure 12-14) and the analysed data is presented in (Table 2). All the complexes show m/z peaks which are in good agreement with the theoretical values. The mass spectra confirm that metal complexes are formed in 1:1 ratio.

### Thermal Study of Complexes

Thermo gravimetric analysis (TGA) technique is used to determine the decomposition nature of the complexes. The samples were heated in the temperature ranges from room temperature to 1000° C.

#### Cu (II) - MPMBC

The thermogram of Cu-MPMBC chelate (figure -15) shows three decomposition steps within the temperature ranging from 250°C - 965°C. The first step of decomposition within the temperature range from 200°C -250°C corresponds to the loss of coordinated water. While the second step occurs within the temperature range from 300°C-350°C and corresponds to the partial decomposition of ligand. The final step 500°C - 850°C corresponds to the removal of the organic part of the ligand leaving metal oxide as the residue.

#### Co (II) - MPMBC

Thermogram of Co complex (figure -16) shows the weight loss in the range of 100-1200°C. Some prominent weight loss in the temperature range of 100-120°C may be due to the loss of lattice water. The second step of decomposition within the temperature range of 270-425°C corresponds to the partial decomposition of the ligand. The final stage involves the loss of organic molecule leaving the metal oxide as residue in the temperature range of 680-1200°C.

The presence of water molecules in the complexes as indicated in the IR spectra was ascertained and verified by the thermal analysis. The thermal studies indicate that complexes decompose after dehydration.

### SEM and EDS Analysis

The SEM pictures (figure - 17 (a-d) ) of the ligand and its complexes under investigation gives information about the morphology of the compounds, the compounds under study exhibited different surface morphology. After complexation the change in morphology of the particles was observed.

The ligand and complexes were characterized by EDS (figure 18 (a-d) ) to determine the percentage of C, N, O, S and Metal. The observed and calculated percentages of the

elements are in good agreement (Table No-2). The molar conductivity data of the complexes are consistent with the non-electrolytic nature of the complexes.

### Electronic Spin Resonance Spectra

ESR studies (figure -19) gives information about the distribution of unpaired electrons, the magnitude of covalency and geometrical properties. The room temperature ESR spectrum of Cu (II) - MPMBC recorded at X band exhibited two  $g$  values corresponding to  $g_{\parallel}$  at 2.0237 and  $g_{\perp}$  at 1.934 confirming axial symmetry.

Based on the above data the following structure can be tentatively assigned to the complexes (figure 20-22).

### DNA-Binding studies

#### Absorption titrations

The synthesized complexes were studied for their DNA binding capability using calf thymus DNA by the method of spectrophotometric titrations. The interaction of a specific metal complex with DNA was carried out in tris buffer (50Mm Tris-HCl/1Mm NaCl buffer, pH 7.5) with constant initial concentration of metal complex (40 $\mu$ M). The concentration of calf-thymus (CT) DNA was determined from the absorption intensity at 200nm. Absorption titrations were performed by varying the concentration of CT-DNA from 0-140 $\mu$ M at constant temperature. Binding of complexes to DNA through intercalation results in red shift and hypochromism, due to strong stacking interactions between aromatic chromophore of the complex and base pairs of DNA<sup>21</sup>. A decrease in the absorption intensities (hypochromism) of the complexes was observed evidently on after addition of DNA which indicates the interaction between the complexes and DNA. The binding constants ( $K_b$ ) of the complexes have been determined from the data recorded using the following equation (figure -23)

$$[\text{DNA}]/(\epsilon_a - \epsilon_f) = [\text{DNA}] / (\epsilon_b - \epsilon_f) + 1/K_b (\epsilon_b - \epsilon_f)$$

The extinction coefficient  $\epsilon_a$ , was obtained by calculating  $A_{\text{obs}} / [\text{complex}]$ ,  $\epsilon_f$  and  $\epsilon_b$  correspond to the extinction coefficient for the free (unbound) and fully bound complex respectively. A plot of  $[\text{DNA}] / (\epsilon_a - \epsilon_f)$  vs.  $[\text{DNA}]$  will have a slope equal to  $1/(\epsilon_b - \epsilon_f)$  and intercept equal to  $1/K_b(\epsilon_b - \epsilon_f)$  (fig-17), Binding constant  $K_b$  is given by the ratio of the slope and the intercept (Table -3).

Viscosity measurements were conducted on Ostwald's viscometer at  $30 \pm 0.01^\circ\text{C}$  using fixed concentration of DNA solution (100  $\mu$ M) with increasing concentration of metal

complexes (0–50  $\mu$ M) in phosphate buffer (10 mM, pH 7.0) for flow time measurements (figure - 24). Each sample was measured in triplicate and the average flow time was calculated with a digital stopwatch. Data were presented as  $(\eta/\eta_0)^{1/3}$  versus the ratio of concentration of compound and DNA, where  $\eta$  is the viscosity of DNA in the presence of the complex, and  $\eta_0$  is the viscosity of DNA alone. The sequence of observed increase in values of viscosity correlates the binding affinity to DNA.

### Anti Bacterial Studies

#### Disc diffusion assay

The MPMBC and its metal complexes were individually tested against some microorganisms, Gram positive, namely *Bacillus subtilis*, *Staphylococcus aureus* and Gram negative *Escherichia coli*, and *Klebsiella Pneumonia*.

Single disk diffusion as a qualitative assay was performed, four to five colonies of each organism were inoculated into 4 ml of broth and incubated for 4 to 6 h at  $37^\circ\text{C}$ . Bacteria were cultured on to agar plates using a sterile absorbent cotton swabs. Then plates were incubated at  $35^\circ\text{C}$  and the zones of inhibition were measured after 24 hours (figure - 25). Each organism was tested in duplicate on different days to measure the reproducibility of the test. Ampicillin (10  $\mu$ g/disc) was used as reference antibacterial agent. All assays were performed in duplicate. The inhibition of bacteria at 10  $\mu$ g concentration for the MPMBC and its metal complexes are presented in (Table-4). The MPMBC holds less activity than the metal chelates, this would suggest that the chelation could facilitate the ability of a complex to cross a cell membrane and can be explained by Tweedy's chelation theory<sup>22</sup>. All the test compounds show lesser activity than the standard.

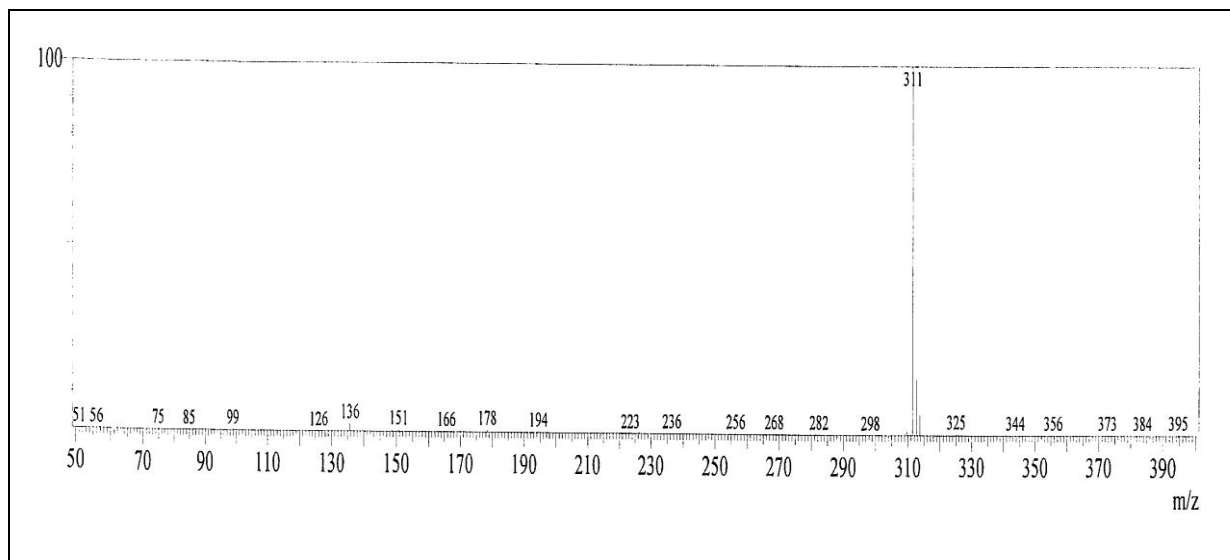
### CONCLUSION

The entitled compounds were synthesised and characterized by various spectro-analytical studies. The analytical data of the complexes show that the metal ligand stoichiometry is 1:1. Their structures have been established on the basis of spectral studies. All the complexes were coloured, thermally stable and non-electrolytic in nature. The ligand and the metal complexes have been screened for their antimicrobial activities. A comparative study of the ligand and its complexes indicates that the complexes exhibit slightly higher activity than the free ligand. The absorption and viscosity studies for DNA binding with synthesized complexes inferred high binding with copper complex.

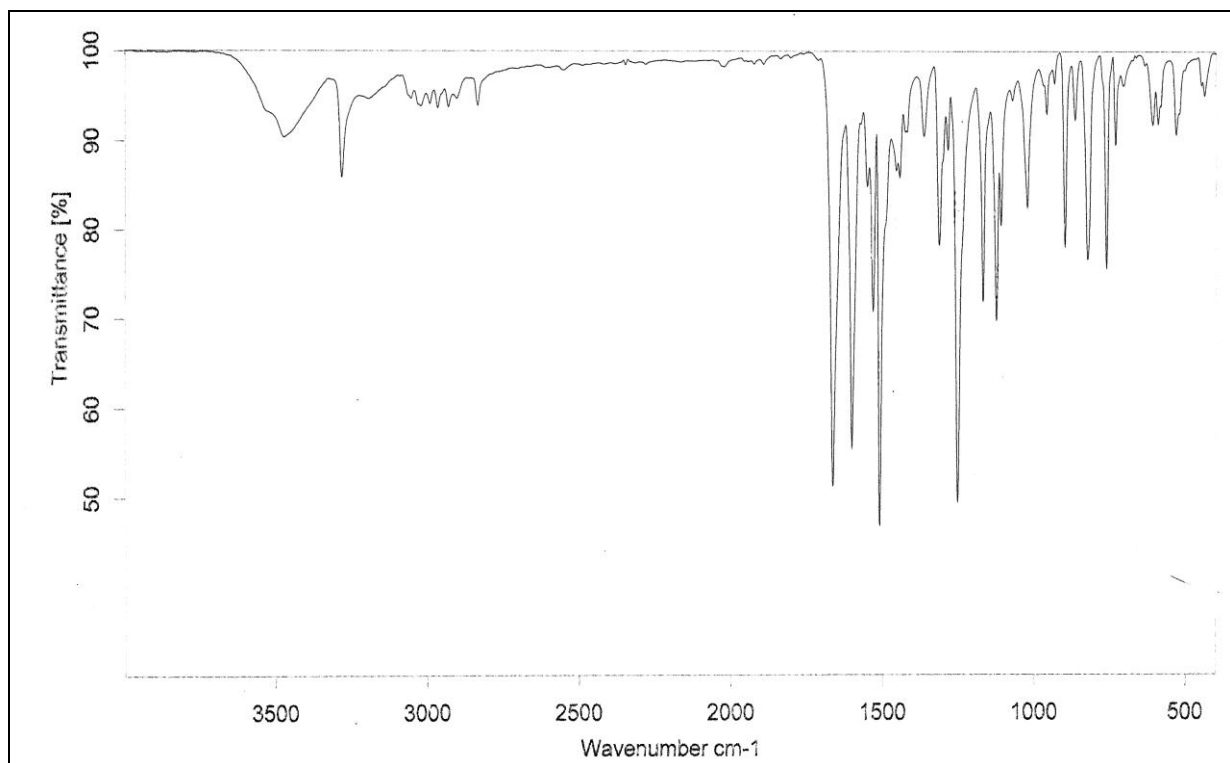
**ACKNOWLEDGEMENTS**

We are thankful to Instrumentation Lab Facilities Department of Chemistry, Osmania University for providing necessary facilities, the

Central Facilities for Research & Development (CFRD), and Department of Microbiology, Osmania University for providing necessary facilities.



**Fig. 1: Mass spectrum of MPMB**



**Fig. 2: IR Spectrum of MPMB**

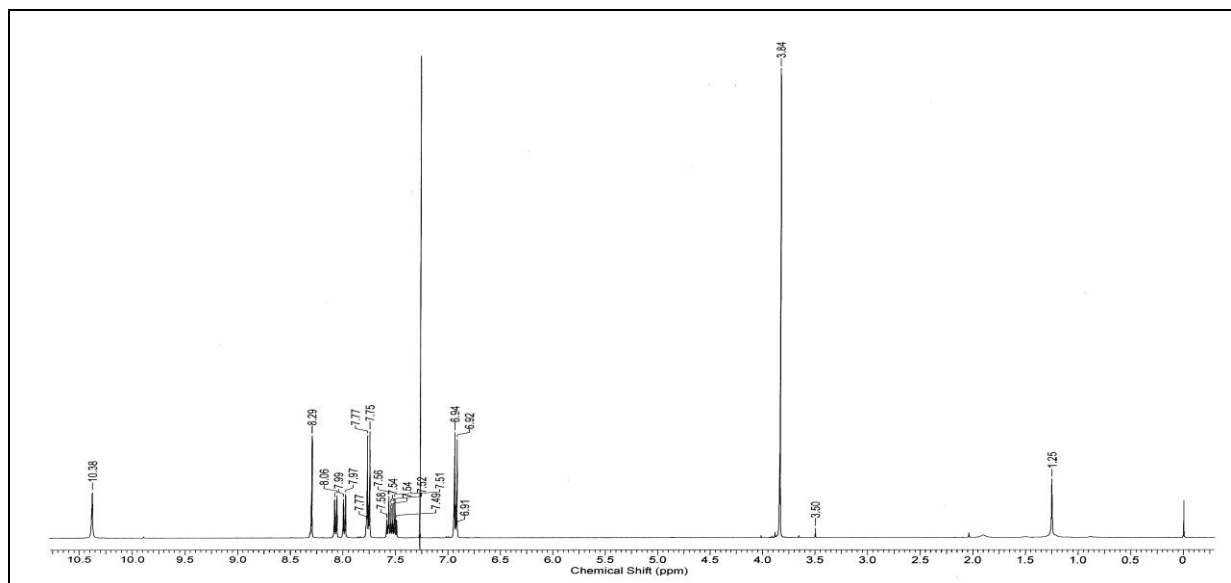


Fig. 3: <sup>1</sup>H NMR Spectrum of MPMBC

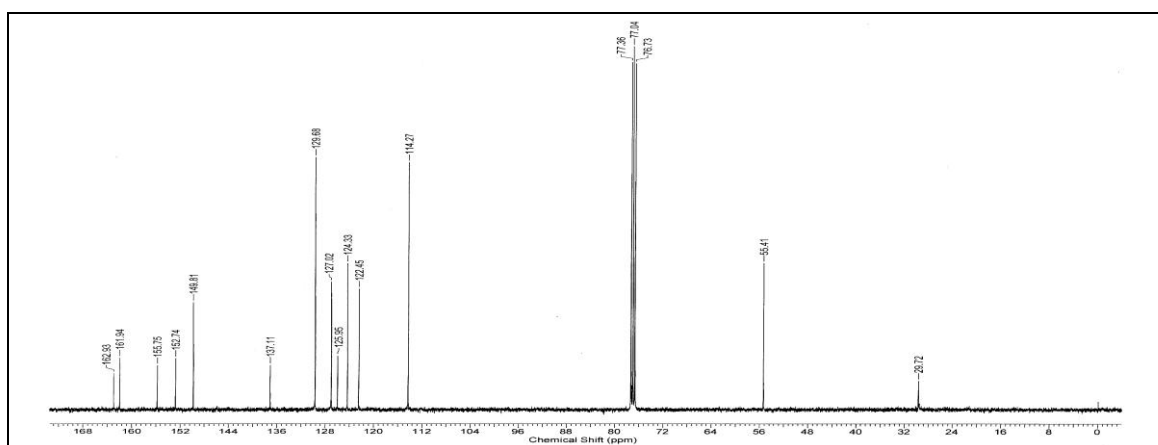


Fig. 4: <sup>13</sup>C NMR Spectrum of MPMBC

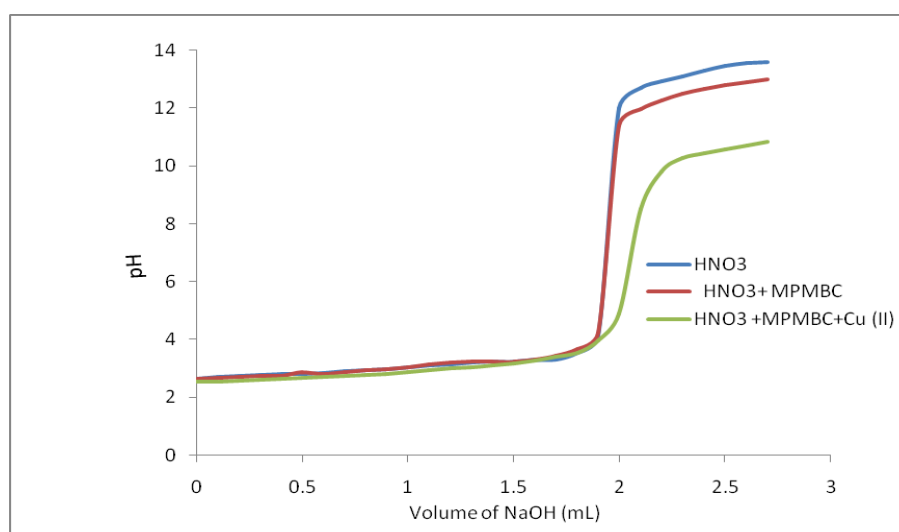


Fig. 5: pH titration curves of MPMBC system in Dioxane-Water medium at 303 K and 0.1 M (KNO<sub>3</sub>) ionic strength

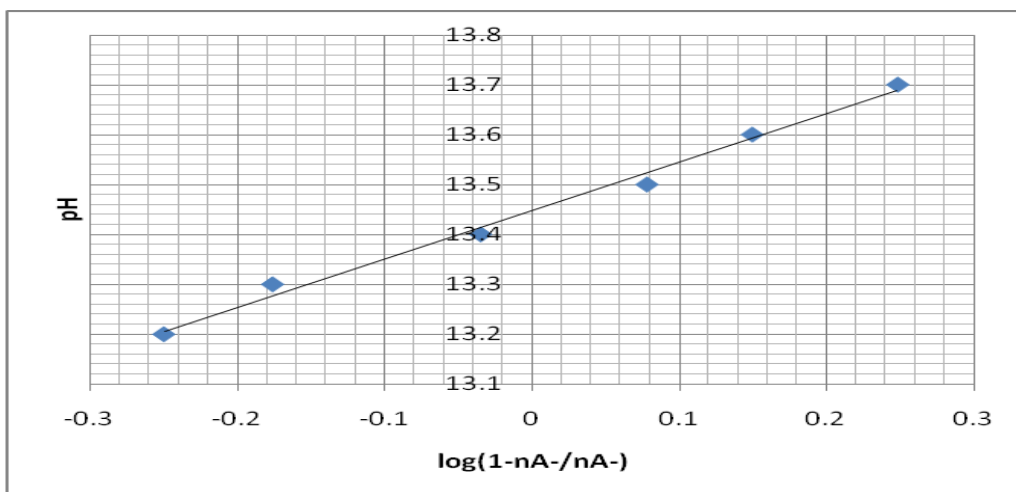


Fig. 6: Plot of Log (1-n̄A) / (n̄A) vs pH of MPMBC

Figure 7: Plot of absorbance versus mole fraction of MPMBC at 303 K in Dioxane medium

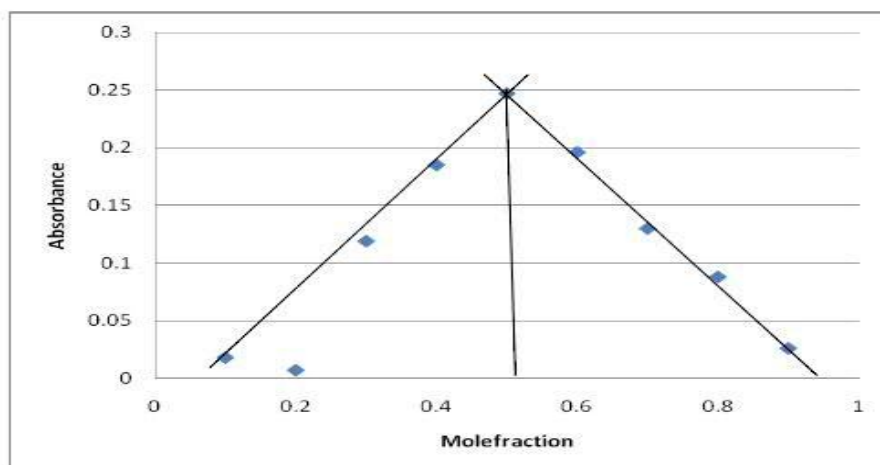
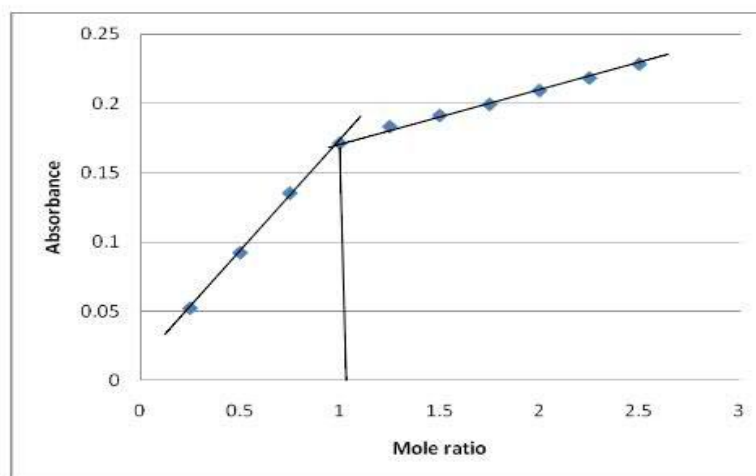


Figure 8 : Plot of absorbance versus mole ratio of MPMBC at 303 K in Dioxane medium



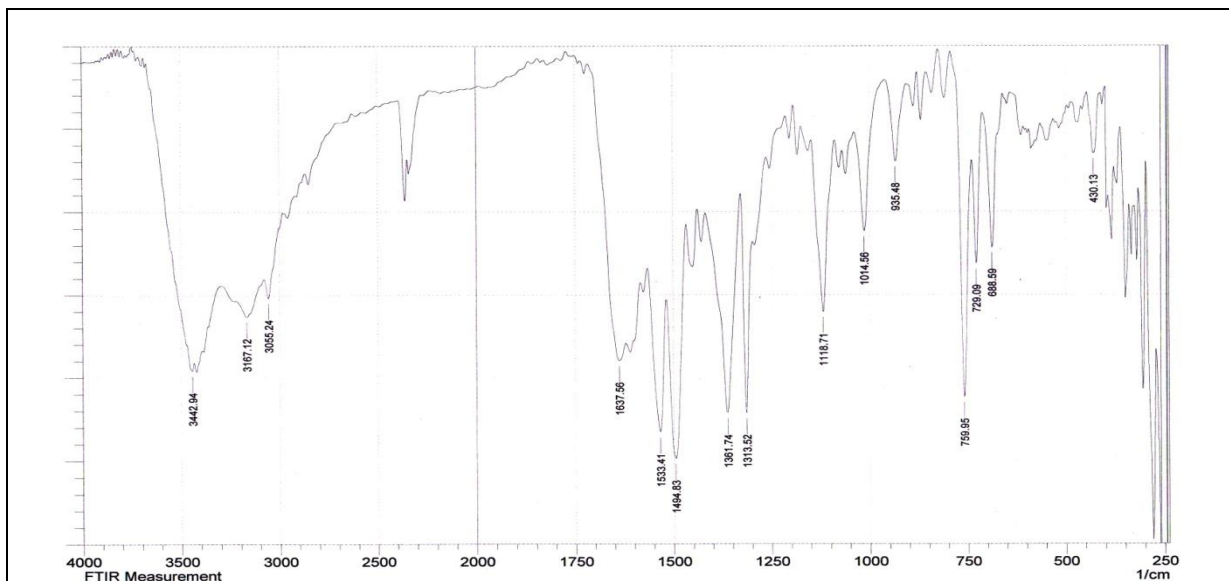


Fig. 9: IR Spectrum of Cu(II)- MPMBC

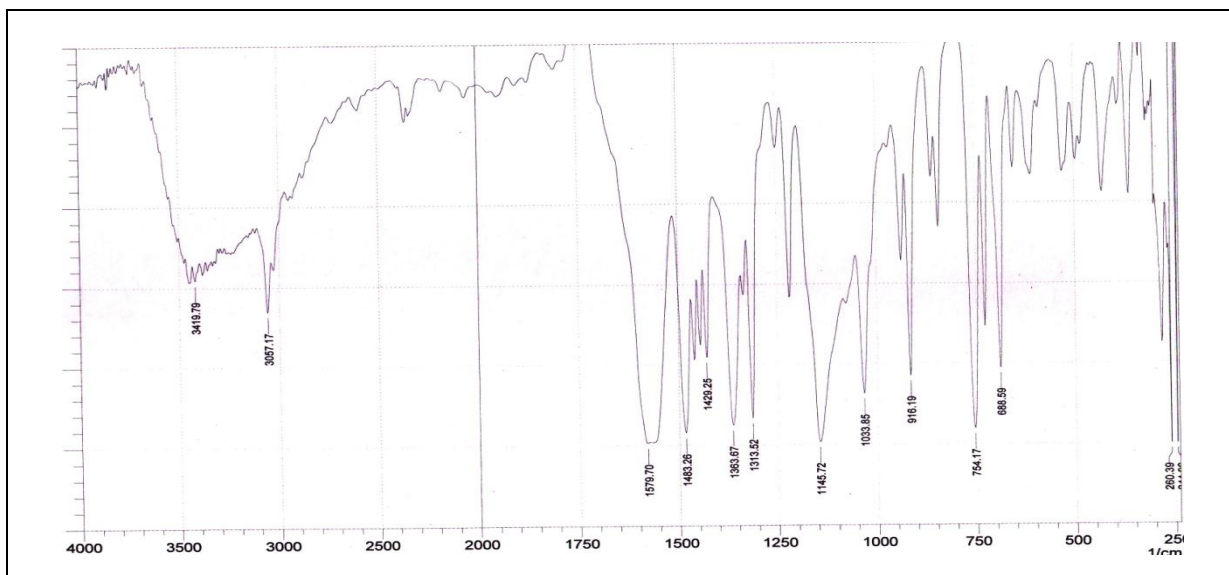


Fig. 10: IR spectrum of Ni (II)- MPMBC

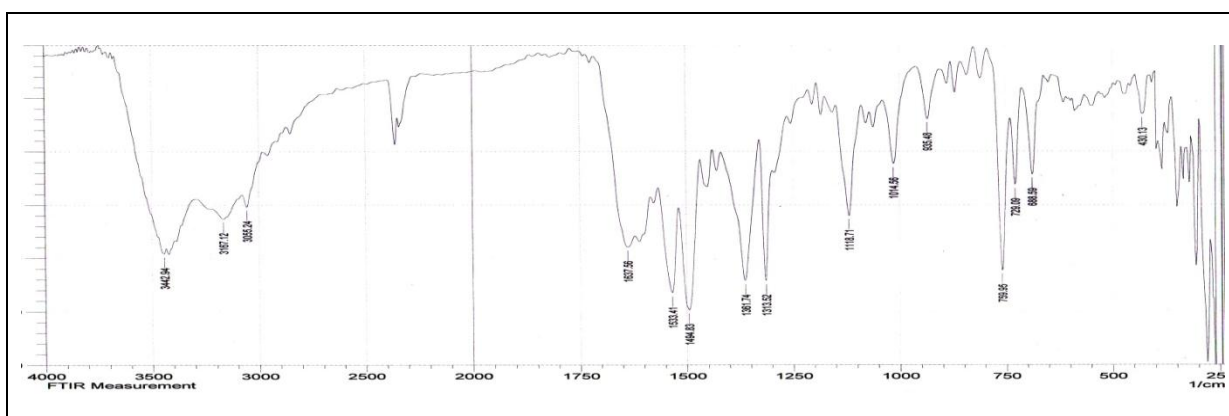


Fig. 11: IR Spectrum of Co(II)- MPMBC



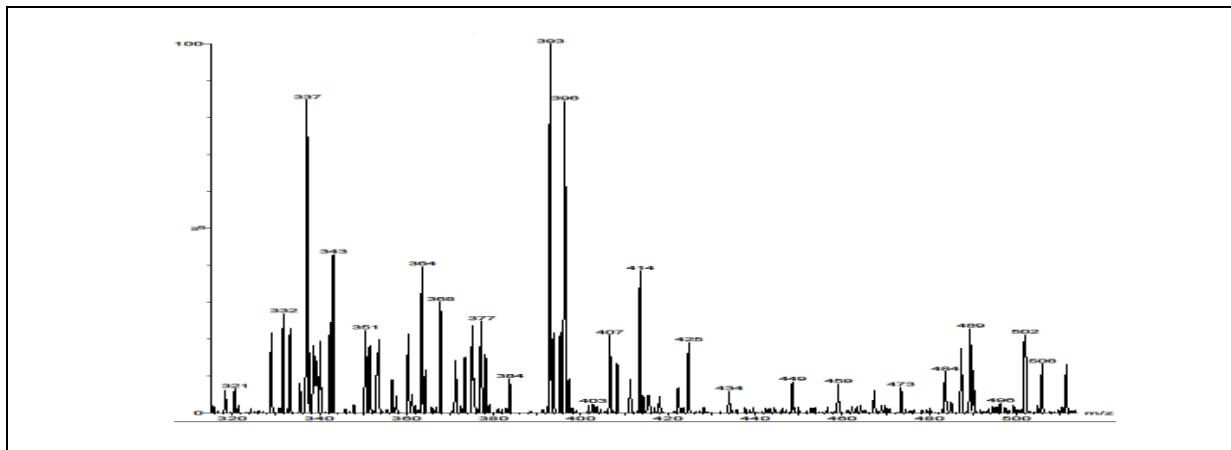


Fig. 12: Mass Spectrum of Cu(II)-MPMBC

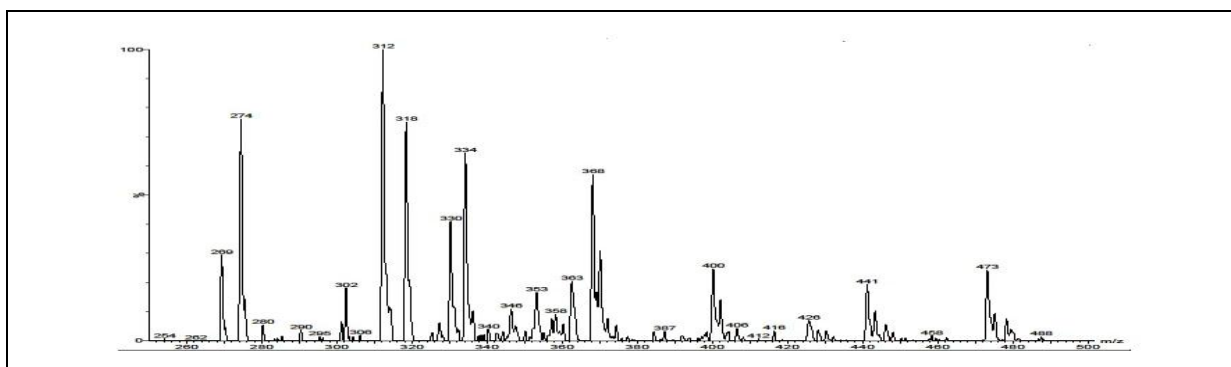


Fig. 13: Mass Spectrum of Ni(II)-MPMBC

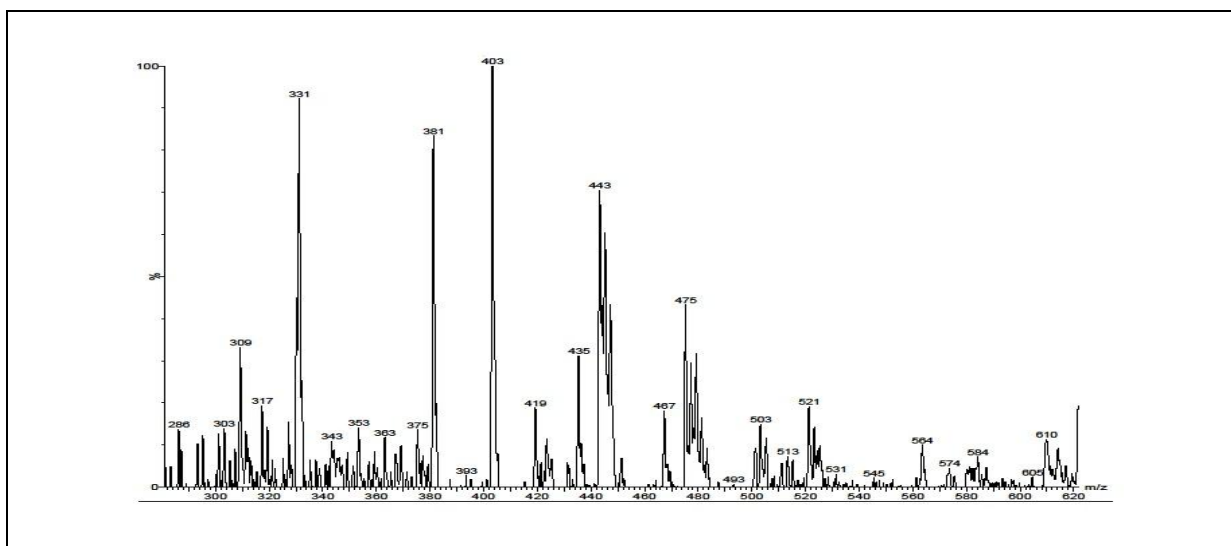


Fig. 14: Mass Spectrum of Co(II)-MPMBC

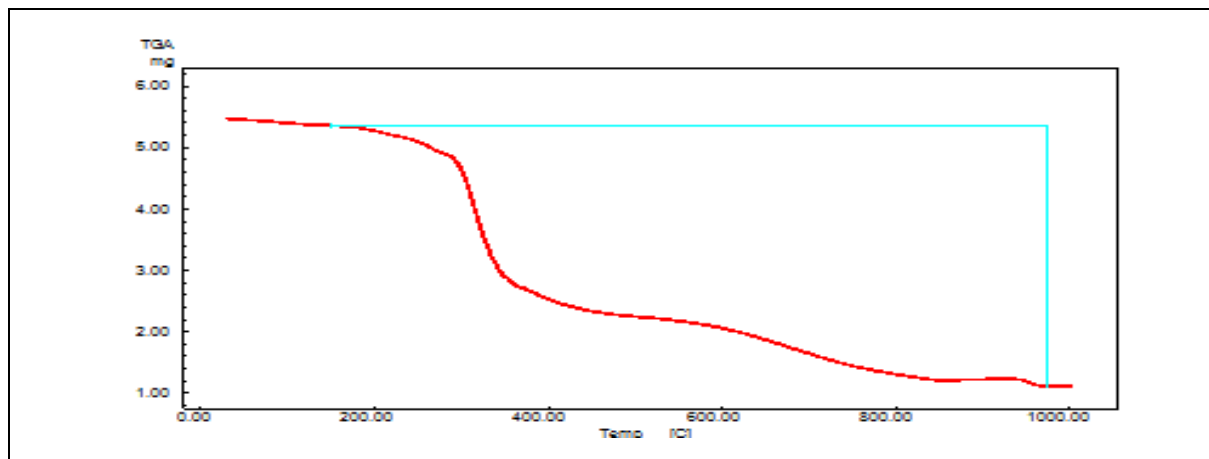


Fig. 15: TGA curve of Cu-MPMBC

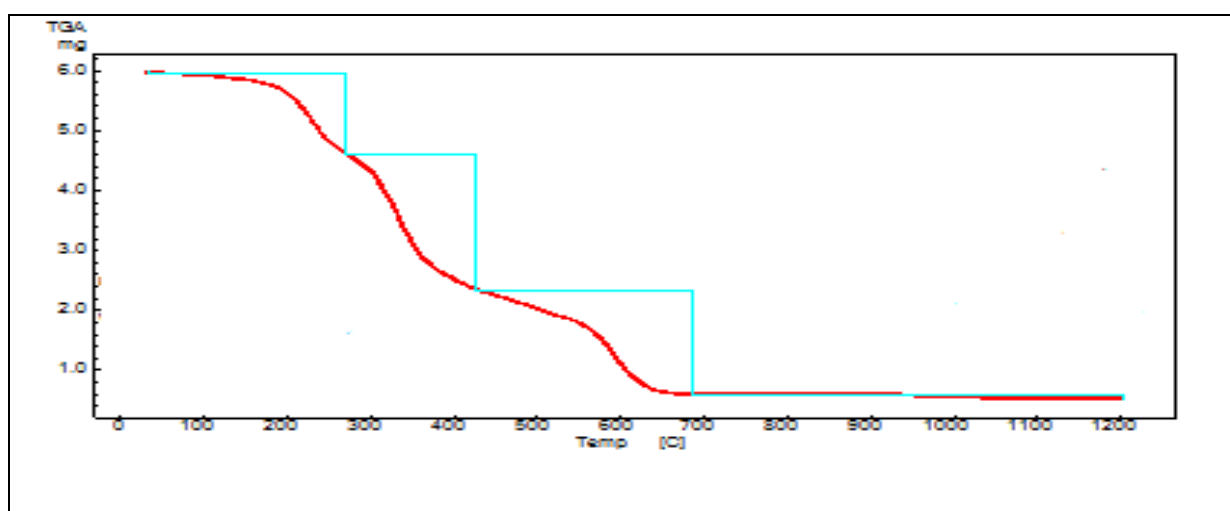


Fig. 16: TGA Curve of Co-MPMBC

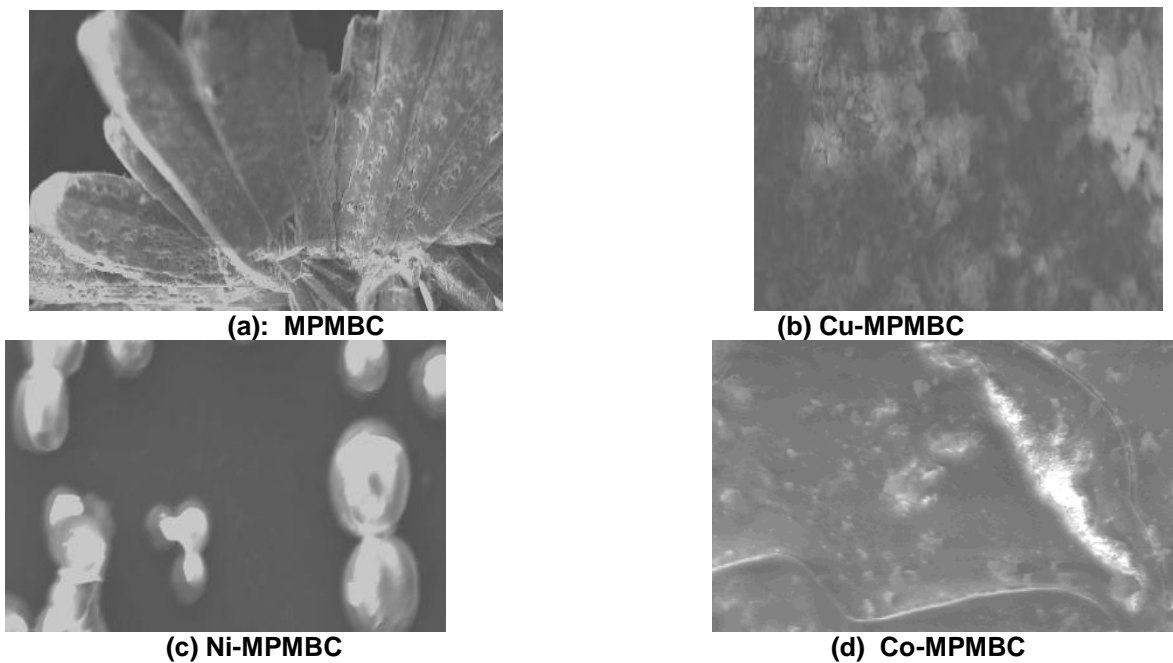


Fig. 17:

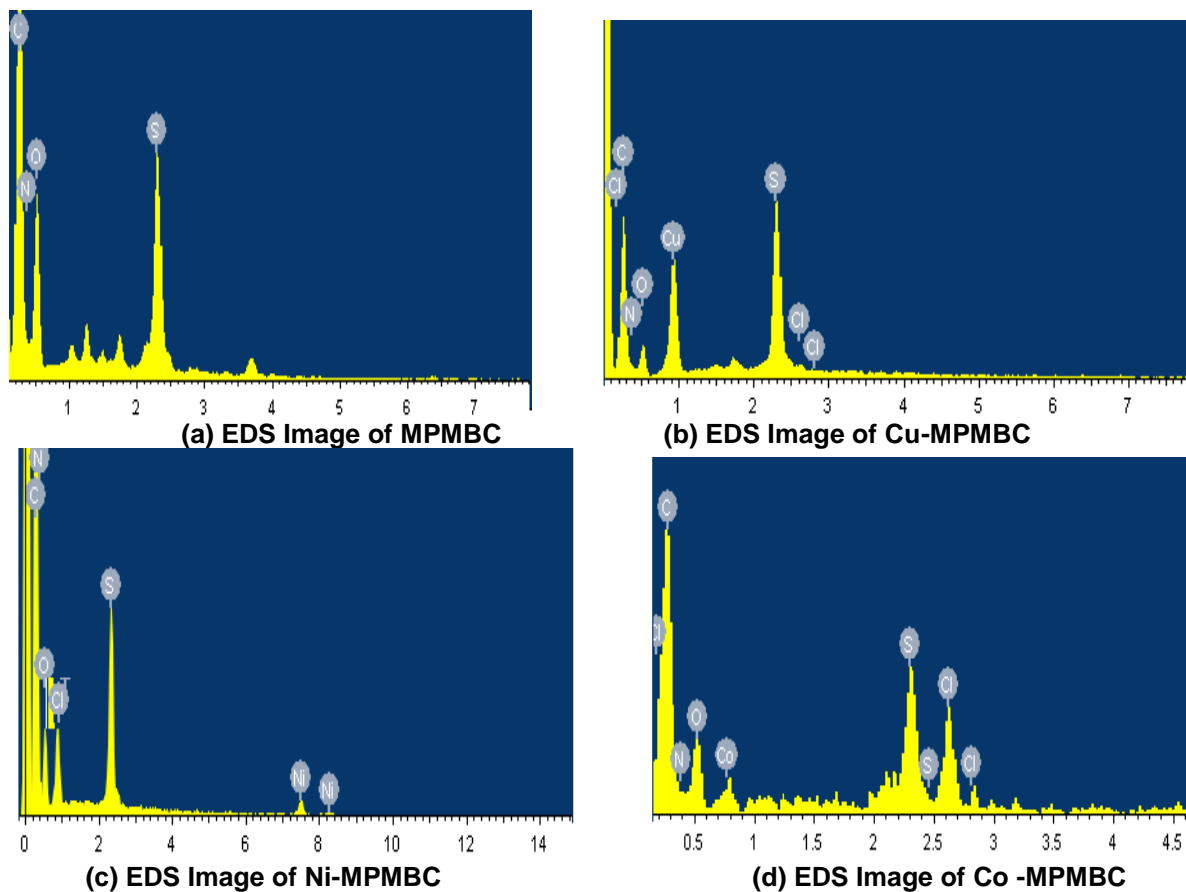


Fig. 18:

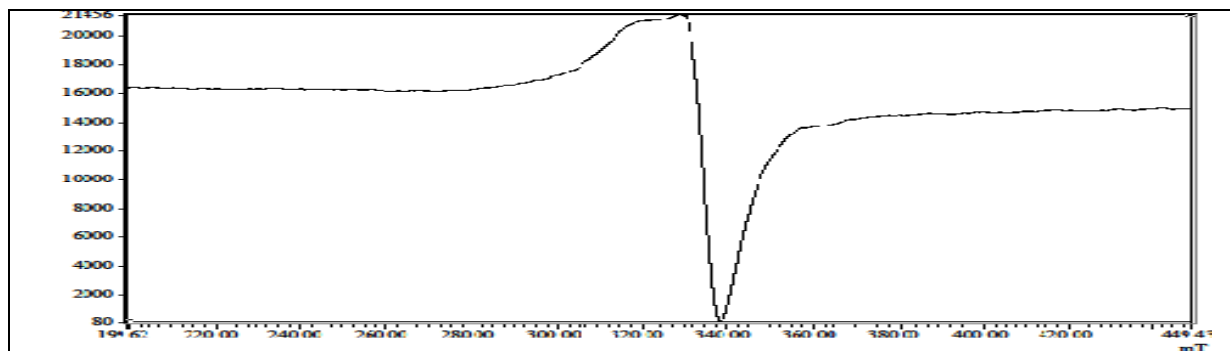


Fig. 19: ESR of Cu-MPMB

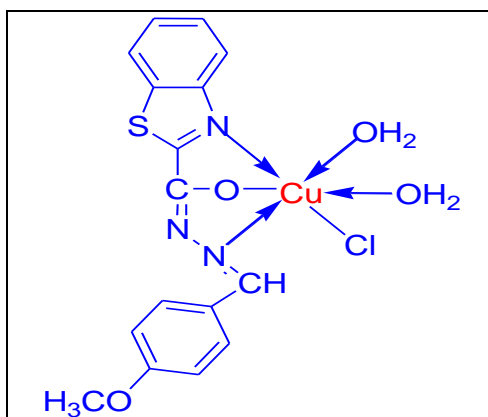


Fig. 20: Tentative structure of Cu-MPMB

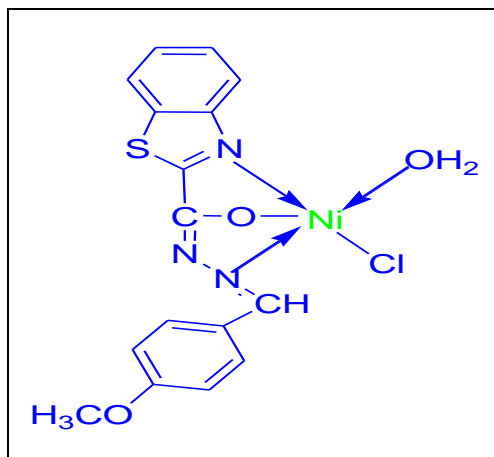


Fig. 21: Tentative structure of Ni-MPMBBC

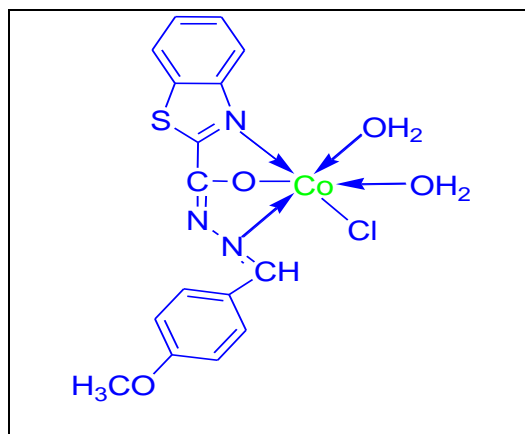
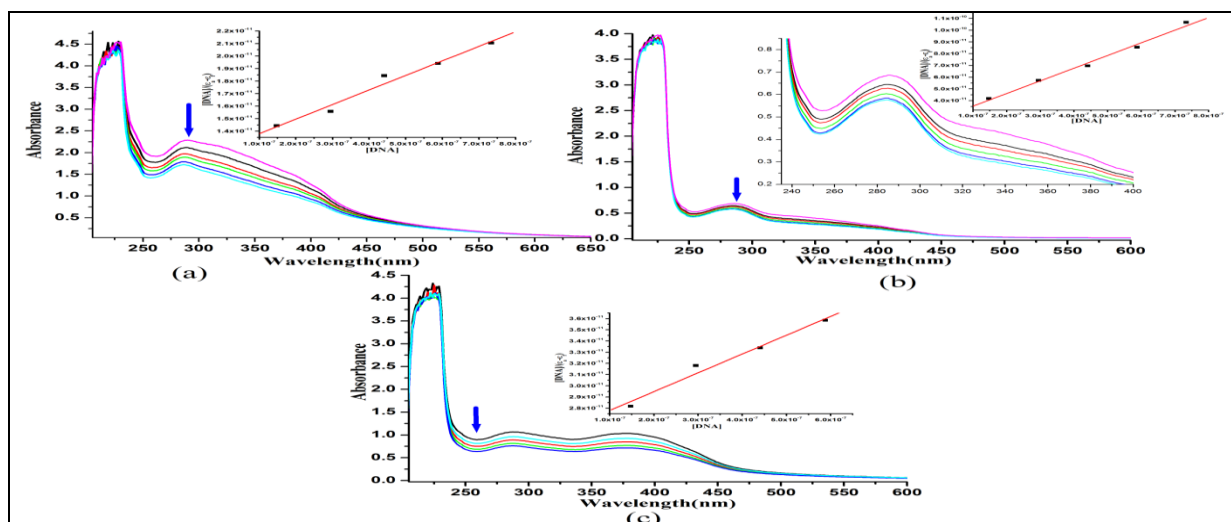


Fig. 22: Tentative structure of Co-MPMBBC



(a). Cu (II) - MPMBBC (b). Co (II) - MPMBBC (c). Ni (II) -MPMBBC

Fig. 23: Absorption Spectrum of Complexes

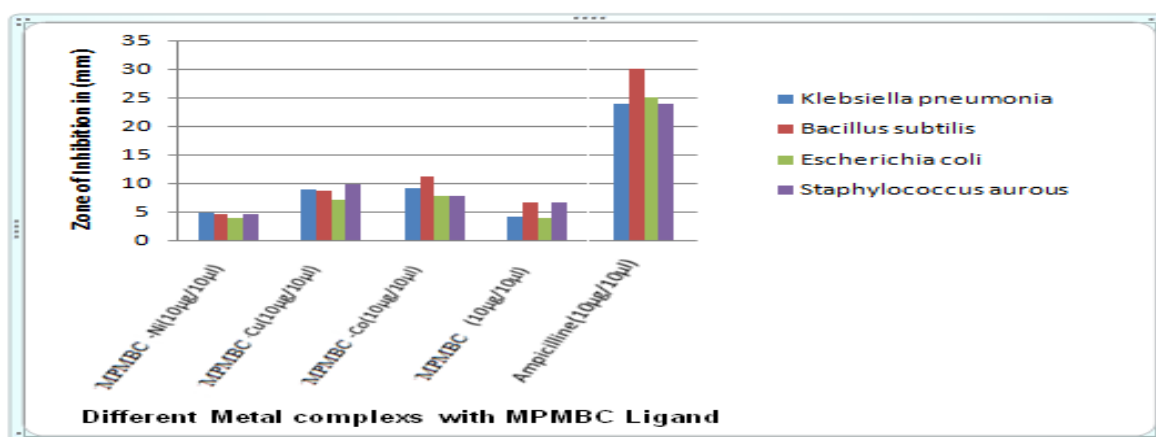
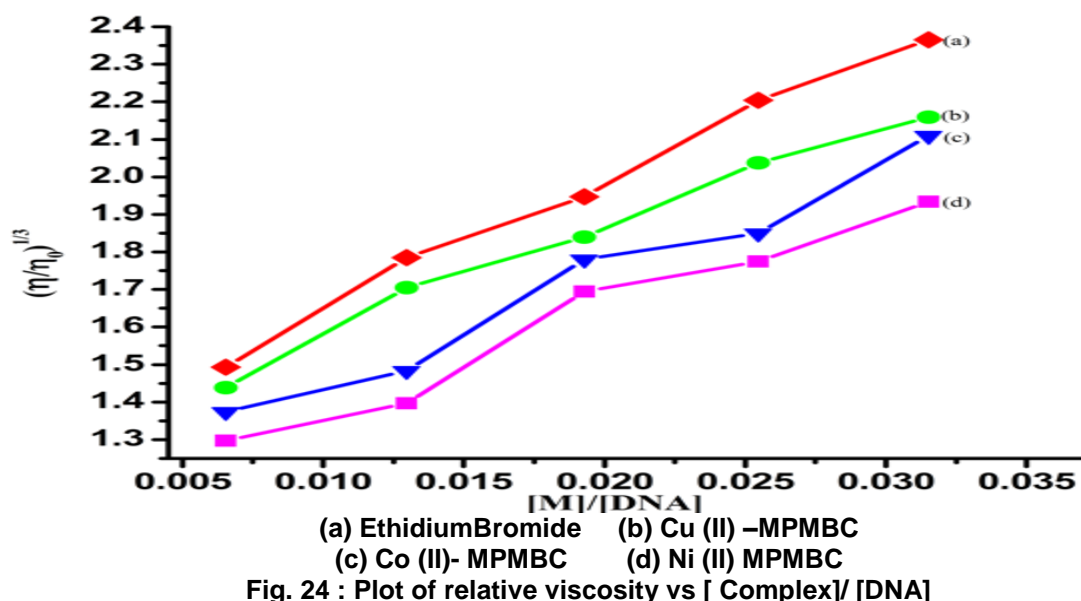


Fig. 25: Zone of Inhibition study of MPMBC and metal complexes in mm

Table 1: IR Frequencies (ν) for some of the functional groups exhibited by the ligand and its complexes

S.No	Compound	ν(C=O) cm <sup>-1</sup>	ν(H <sub>2</sub> O) cm <sup>-1</sup>	ν(C=N) cm <sup>-1</sup>	ν(M-N) cm <sup>-1</sup>	ν(M-O) cm <sup>-1</sup>
1	MPMBC	1666	-	1603	-	-
2	Cu –MPMBC	1598	3419	1579	540	430
3	Ni –MPMBC	1554	3425	1554	534	430
4	Co- MPMBC	1637	3442	1533	570	430

Table 2: Analytical and physical data for MPMBC and its metal complexes

S.No	Compound	Molecular Formula	Mass	Colour	Λ <sub>max</sub> Ω <sup>-1</sup> cm <sup>2</sup> mol <sup>-1</sup>	Elemental analysis % Cal (Obs)				
						C	N	O	S	M
1	MPMBC	C <sub>16</sub> H <sub>13</sub> N <sub>3</sub> O <sub>2</sub> S	311	Yellow	-	61.73 (51.97)	13.50 (14.00)	10.28 (11.91)	10.28 (11.45)	-
2	Cu-MPMBC	Cu- C <sub>16</sub> H <sub>12</sub> N <sub>3</sub> O <sub>2</sub> S.Cl.2H <sub>2</sub> O	449	Brown	6.7	43.14 (50.43)	9.43 (10.18)	7.19 (10.46)	7.19 (8.78)	14.26 (16.93)
3	Ni-MPMBC	Ni-C <sub>16</sub> H <sub>12</sub> N <sub>3</sub> O <sub>2</sub> S.Cl. H <sub>2</sub> O	426	Green	10.54	45.07 (49.90)	9.85 (8.08)	11.26 (11.03)	7.51 (6.37)	13.77 (10.40)
4	Co-MPMBC	Co-C <sub>16</sub> H <sub>12</sub> N <sub>3</sub> O <sub>2</sub> S.Cl.2H <sub>2</sub> O	443	Marrow	4.07	43.34 (44.37)	10.34 (9.48)	14.44 (14.26)	7.88 (8.51)	13.30 (14.91)

**Table 3: DNA binding constants ( $K_b$ ) of metal complexes**

S.no	Compound	$K_b M^{-1}$
1.	MPMBC-Cu	$4.6 \times 10^5$
2.	MPMBC-Ni	$2.9 \times 10^5$
3.	MPMBC-Co	$3.8 \times 10^5$

**Table 4: Zone of Inhibition study of ligand and metal complexes in (mm) on Gram Positive and Negative Bacteria**

S. No	Name of Bacteria	MPMBC (10 $\mu$ l)	MPMBC-Cu (10 $\mu$ l)	MPMBC-Ni (10 $\mu$ l)	MPMBC-Co (10 $\mu$ l)	Ampicillin (10 $\mu$ g/10 $\mu$ l) (AMP)
1	Klebsiella pneumonia	4.3	9	5	9.2	24
2	Bacillus subtilis	6.8	8.7	4.8	11.3	30
3	Escherichia coli	4.1	7.3	4.1	8	25
4	Staphylococcus	6.7	10	4.8	8	23.9

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