

A study on Structural Aspects and Biological Activity of (E)-N'-[2-Hydroxybenzylidene]benzohydrazide and its Metal Complexes

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Abstract: The spectroanalytical studies of VO(II), Mn(II), Co(II), Ni(II) and Cu(II) complexes with the title compound (E)-N'-[2-Hydroxybenzylidene]benzohydrazide, as well as antifungal and antibacterial activity of Cu(II)-HBBH complex were carried out. The complexes were characterized by elemental analysis, IR, NMR, mass, ESR and TGA & DTA. The mode of bonding in these complexes has been suggested on the basis of analytical and spectroscopic data. The ligand coordinates to the central metal ion through oxygen of enolic form of carboxy hydrazone, phenolic oxygen and azomethine nitrogen atoms in VO(II), Mn(II), and Cu(II) complexes through the dissociation of two protons. While in Co(II) and Ni(II) complexes the dissociation of only phenolic proton is observed and hence the donor sites are oxygen of phenolic group, amide carbonyl oxygen and imine nitrogen. The Cu(II) complex exhibits lethal effect on gram negative bacteria *Escherichia coli* and fungal strain *Candida albicans*. It has also been observed that concentration of compounds play a vital role in the degree of inhibition.

Key words: (E)-N'-[2-Hydroxybenzylidene]benzohydrazide(HBBH), VO(II), Mn(II), Co(II), Ni(II) and Cu(II) complex, Biological activity

I. Introduction

There has been considerable interest in the development of novel compounds with antiinflammatory, analgesic, antimicrobial, antitumor, antiviral activities. Hydrazones with various biological activity possessing an azomethine -NHN=CH- proton constitute an important class of compounds for new drug development. Also these ligands and their metal complexes play an important role in the development of coordination chemistry and are good complexing agents. A number of complexes have been reported with amide group ligands, which exhibit diverse coordinating behaviour with different metal ions¹⁻². The amide group offers two potential binding atoms, the oxygen and nitrogen, for complexation with metal ions. The copper (II) complex of 5-bromosalicylaldehyde exhibits antibacterial activity and benzoyl hydrazones of salicylaldehyde exhibits antibacterial, antifungal, antimicrobial activities. The 2-hydroxy-4-chloro benzoyl hydrazone has antimicrobial activity³. Therefore many researchers have synthesized these compounds as target structures and elevated their biological activities. These observations have been guiding for the development of new hydrazones that possess varied biological activities.

Keeping in view the various biological activities, synthesis and characterization of some transition metal complexes with 2-hydroxy benzylidene benzohydrazide (HBBH), and biological activity of its copper complex have been discussed in the present investigation.

II. Experimental Section

I.R spectra were recorded on a Perkin-Elmer 435 Spectrophotometer. ¹H NMR were recorded in CDCl₃/ DMSO-d₆ on a Bruker WH (270 MHz) spectrometer, mass spectra were recorded on Micro Mass V.G70-70H spectrometer operating at 70 eV using direct inlet system. Thermal behavior of complexes studied by thermal gravimetric analysis (TGA) and differential thermal analysis (DTA) techniques were recorded on Mettler Toledo TGA/SDTA in the temperature range of 0-1000⁰ C in nitrogen atmosphere at a heating rate of 20⁰ C/min.

1.1 Materials

All the chemicals and solvents were of Analar grade. Solvents such as water, ethanol and methanol were purified before use by following standard procedures⁴. Nutrient broth (NB), Nutrient Agar (NA), Sabouraud dextrose agar (SDA), Sabouraud dextrose broth (SDB), Peptone water and antibiotics Ciprofloxacin (Bacteria), Ketoconazole (Fungi) were from Hi-media laboratories. DMSO was used from E.Merck.

1.2 Synthesis of ligand

Ligand HBBH is synthesized by the reported procedure ⁵.

1.3 Synthesis of metal complexes

The metal complexes of Cu (II), Ni (II), Co (II) and Mn(II) were prepared by mixing ethanolic solution of the ligand (E)-N'-[2-hydroxy benzylidene] benzohydrazide (HBBH) and corresponding metal chloride solution in 1:2 molar ratio. The resulting mixture was refluxed on water bath for 10-12 hours. The pH of the solution for complexation was maintained by adding few drops of dilute methanolic ammonium hydroxide solution (pH 4-7). The precipitated complexes are then recrystallized in ethanol. The resulting complexes are washed with petroleum ether and dried under pressure over anhydrous CaCl₂ in a desiccator.

The metal complex of vanadium is synthesized by adopting different method. To an equal volume of water concentrated sulphuric acid, solution of vanadium pentoxide dissolved in ethanol is added with constant stirring and the resulting mixture is boiled until the colour changes from green to blue. As a result the solid precipitate separated is collected from the cooled mixture by filtration and HBBH ligand (dissolved in ethanol) is added to the filtrate. The acid reaction mixture is neutralized by careful addition of 10% sodium carbonate solution with stirring. The resulting compound is separated by filtration on the Buchner funnel and is allowed to dry in a desiccator.

All the complexes are quite stable to air and moisture, the physical state of all complexes is amorphous and all are coloured, with good solubility in DMSO.

III. Results and Discussion

1.4 Infrared Spectra

A comparative study of the IR spectrum of ligand with that of the metal complexes showed disappearance of ν_{OH} absorption band which was observed as broad band centered at 3366 cm^{-1} . The appearance of this band as broad band around 3431 cm^{-1} in the spectra of complexes is assigned to associated water molecule. A strong band at 1621 cm^{-1} due to $\nu_{(C=O)}$ amide group and band at 1606 cm^{-1} due to $\nu_{(C=N)}$ azomethine in the ligand HBBH has shifted down to 1605 and to 1570 in the spectra of VO (II), Mn (II), Co (II), Ni (II) and Cu (II) metal complexes " Figs. 1, 2,3,4 & 5". This suggests the involvement of the azomethine group and amide group in coordination with the metal⁶⁻⁸. Another important ligand band at 1368 cm^{-1} due to phenolic-OH deformation is found to be absent in complexes. This indicates the deprotonation of phenolic-OH on coordination with metal ions⁹⁻¹⁰. A band at 1272 cm^{-1} in ligand spectrum due to C-O shifts to higher side in complexes. This substantiates the aforementioned observations¹¹⁻¹². A medium intensity band at $758\text{ cm}^{-1} \pm 4\text{ cm}^{-1}$ in all the metal complexes is assignable to rocking mode due to coordinated water molecule. Some new bands of weaker intensity at $530 \pm 20\text{ cm}^{-1}$ and 462 cm^{-1} are due to $\nu_{(M-O)}$ and $\nu_{(M-N)}$ bonding.

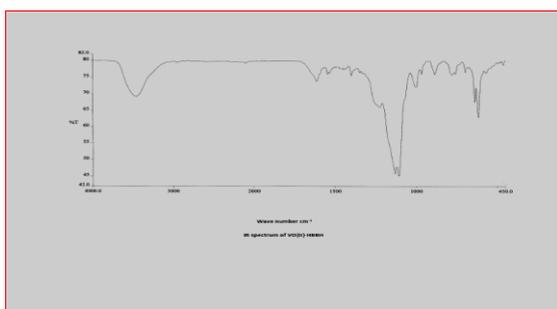


Fig.1 IR spectrum of VO (II)-HBBH

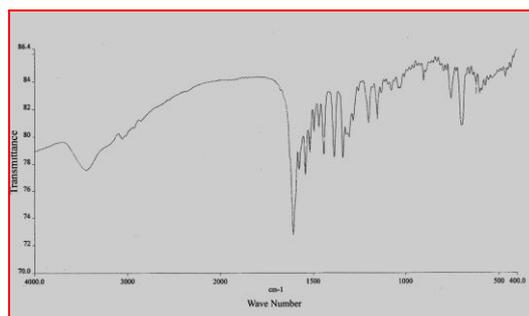


Fig.2 IR spectrum of Mn (II)-HBBH

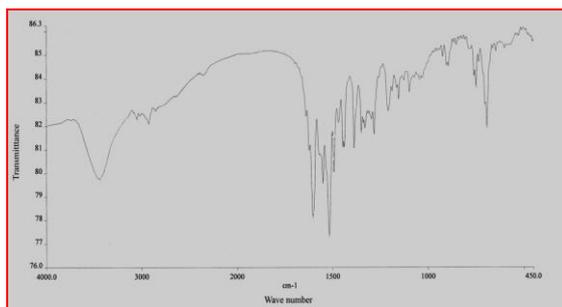


Fig.3 IR spectrum of Co (II)-HBBH

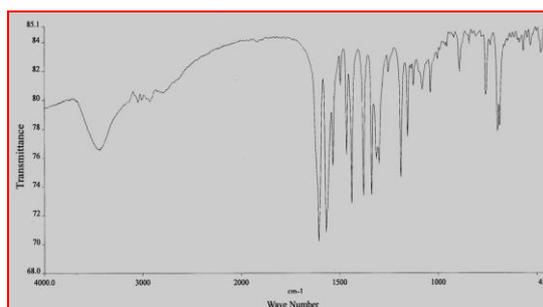


Fig.4 IR spectrum of Ni (II)-HBBH

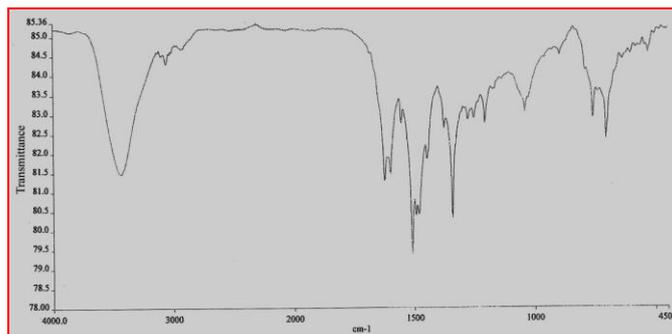


Fig.5 IR spectrum of Cu (II)-HBBH

1.5 $^1\text{H-NMR}$ spectrum of VO(II)-HBBH complex

On comparison with the ligand spectrum of HBBH⁵, the $^1\text{H-NMR}$ spectrum of vanadium complex exhibits a peak around δ 8.9 ppm. The appearance of this peak is assigned to azomethine proton which is in downfield region due to deshielding of the proton. This suggests the involvement of azomethine group in coordination with the metal ion. The disappearance of chemical shift due to phenolic proton and amide proton in the proton NMR spectrum of the vanadyl complex further gives evidence for the involvement of phenolic oxygen and carbonyl oxygen in coordination with the metal ion. The complex spectrum retains the aromatic protons appearing around the region δ 6-7.5 ppm as in the ligand spectrum. Thus the above information gives evidence for the formation of metal complex involving phenolic-O, carbonyl-O and azomethine nitrogen in coordination with the vanadyl ion "Fig6" .

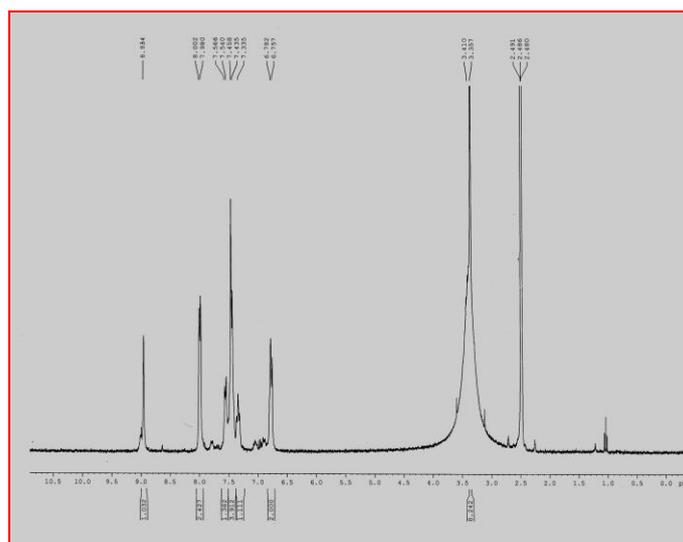


Fig.6 $^1\text{H-NMR}$ spectrum of VO (II)-HBBH

1.6 Mass Spectra

The mass spectra of all metal complexes shows a fragment ion peak at m/z 241, which is consistent with the expected protonated ion $(\text{M}+\text{H})^+$ as observed in the ligand mass spectrum⁵. The molecular ion peak occurs at m/z = 293 in the mass spectrum of Mn(II)-HBBH complex "Fig.7". The mass spectrum of this complex also shows fragment ion of lower intensity at m/z = 294 that corresponds to carbon isotope. The peak at m/z = 293 gives inference about the existence of the metal complex in 1:1 ratio.

The mass spectrum of Co(II)-HBBH "Fig.8" shows a molecular ion peak $(\text{M})^+$ at m/z = 537 suggesting the complex to be dimeric. Analysis by mass spectroscopy gave the molecular ion $[\text{M}^+]$ peak of complex Ni(II)-HBBH "Fig.9" at desired position m/z = 534 indicating dimeric nature of metal complex. The mass spectrum of Cu(II) – HBBH complex "Fig.10" also contains ions at m/z 263, some 23 mass units higher than the ligand mass. These are identified as sodium adduct peaks .

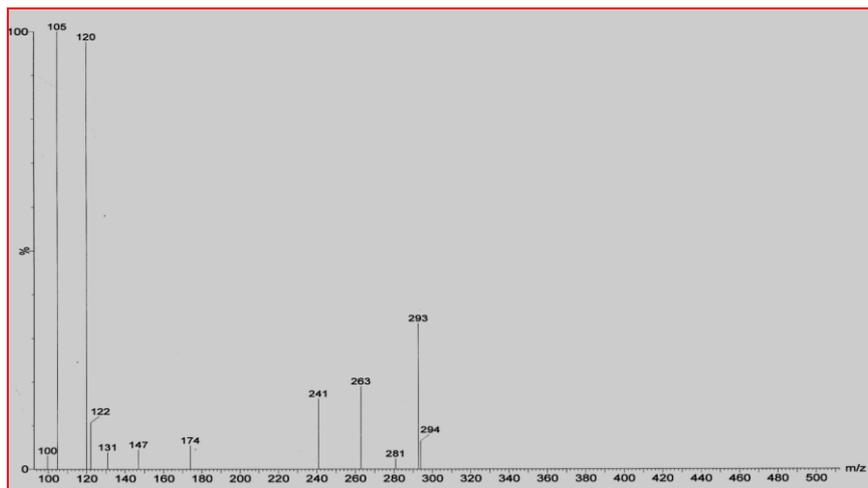


Fig.7 Mass spectrum of Mn (II) – HBBH

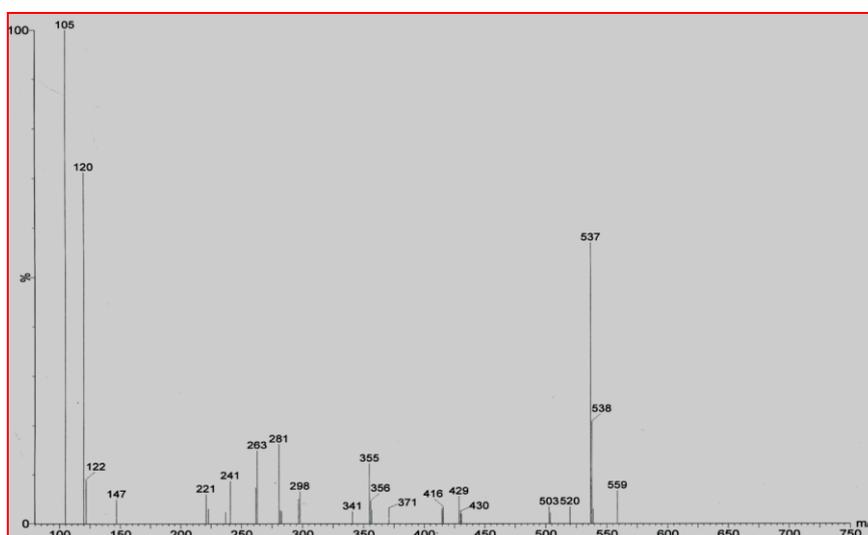


Fig.8 Mass spectrum of Co (II) – HBBH

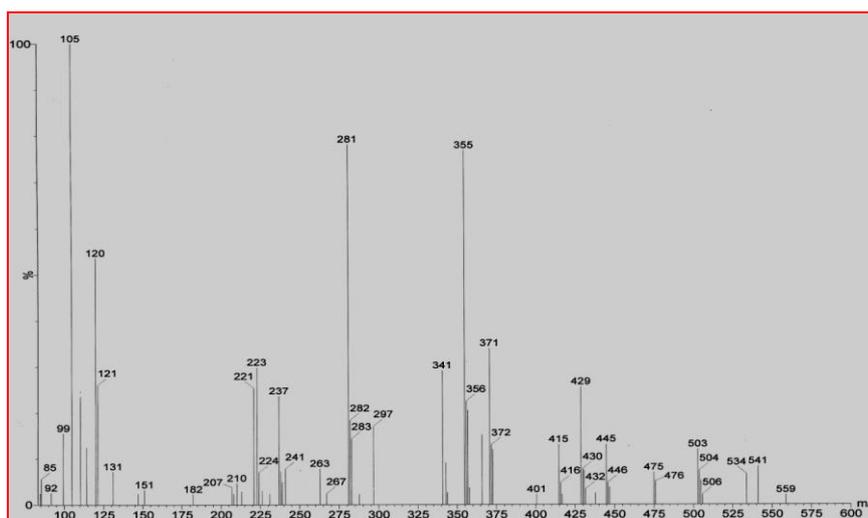


Fig.9 Mass spectrum of Ni (II) – HBBH

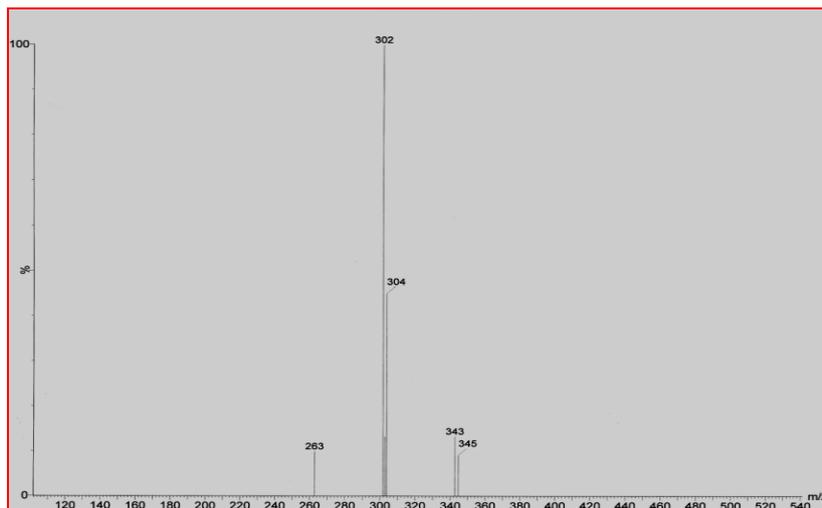


Fig.10 Mass spectrum of Cu (II) – HBBH

1.7 Electron Spin Resonance Spectra

The ESR spectra of metal complexes were recorded in liquid nitrogen at room temperature.

The ESR spectra of the vanadyl (II) complex " Fig.11" shows a typical eight-line pattern. This eight-line pattern indicates that the unpaired electron interacts with the nuclear spin of single vanadium V ($I=7/2$)¹³⁻¹⁴. The result is seen as hyperfine splitting of the ⁵¹V nucleus and the values suggests the complex to be square pyramidal.

The spectrum of the Mn (II)-HBBH complex " Fig.12" at LNT shows a six fold splitting indicating symmetrical six coordination of the Mn(II) ion. In this spectrum the g values are isotropy with small anisotropy due to increasing magnitude of the applied magnetic field. The interaction of nuclear spin of copper with unpaired electrons is seen in the spectrum by a six fold (2I+1) splitting. As the g values are isotropic the metal ion is in cubic environment.

The ESR spectrum of Cu(II)-HBBH complex " Fig.13" shows four well resolved peaks in $g_{||}$ region indicating the monomeric form of complex due the hyperfine interactions. While in g_{\perp} region the resolution is poor. The trend $g_{||} > g_{\perp}$ for this complex indicates that unpaired electron is localized in the $d_{x^2 - y^2}$ orbital of Cu (II) ion and is characteristic for the axial symmetry. These results suggests that Cu (II)-HBBH complex has tetragonal elongation along the z-axis.

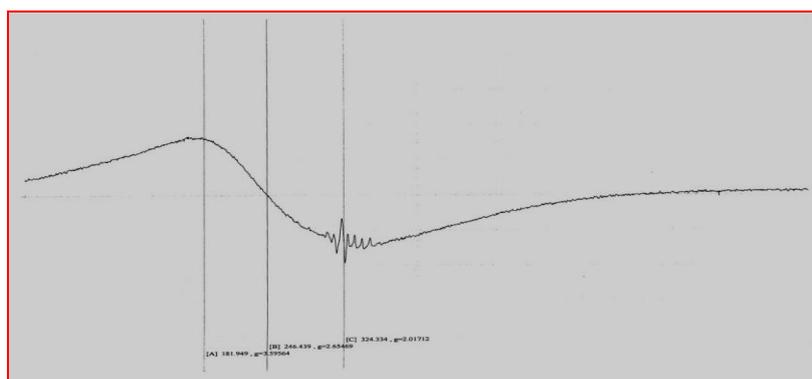


Fig.11 ESR of VO(II) –HBBH

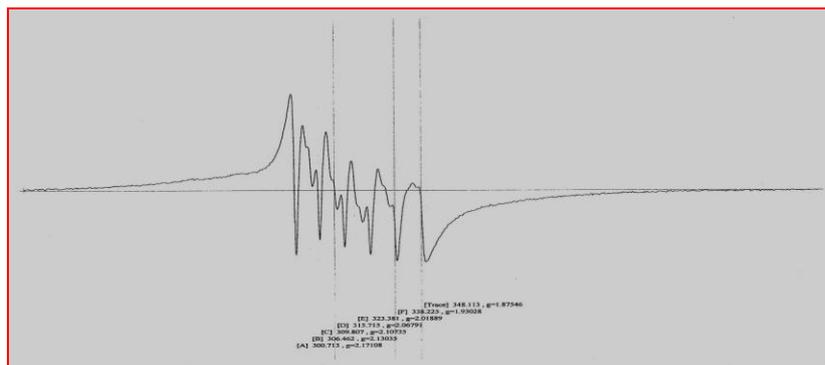


Fig.12 ESR of Mn(II)-HBBH

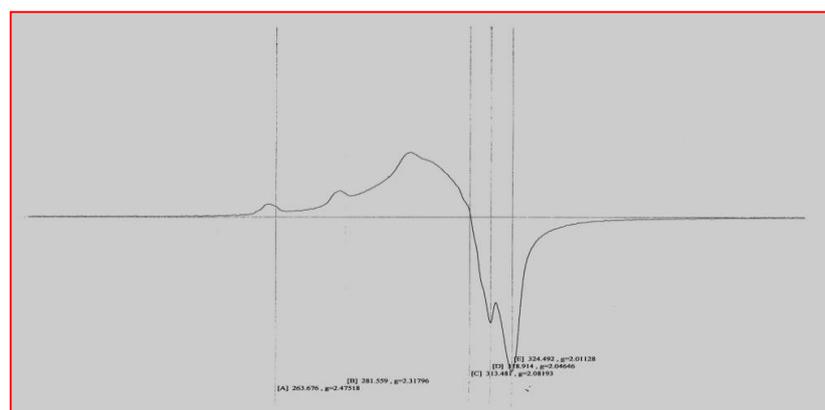


Fig.13 ESR of Cu(II)-HBBH

1.8 Thermal Studies of M (II) -HBBH complexes

Thermal gravimetical analysis (TGA) and differential thermal analysis (DTA) of the complexes VO(II), Mn(II), Co(II), Ni(II) and Cu(II) –HBBH complexes " Figs.14,15,16,17 & 18 " shows a gradual weight loss in the temperature range 114⁰ C to 200⁰ C and an endothermic DTA peak in the region around 300⁰ C in all the metal complexes indicates loss of coordinated water molecule. The second step of decomposition from 280⁰ C to 800⁰ C in all the metal complexes corresponds to decomposition of ligand moiety. An exothermic peaks $t_{max}= 600^0$ C on DTA curve occurs for VO(II) –HBBH complex " Fig.14 " during the second stage of decomposition. The mass of the final residue in all the metal complexes corresponds to stable metal ion or metal oxide.

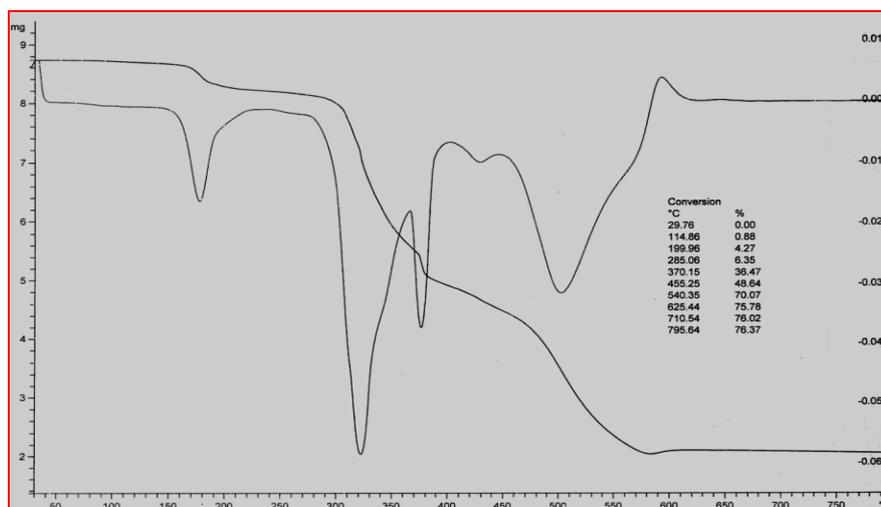


Fig.14 Thermal Gravimetical Analysis of VO (II) – HBBH

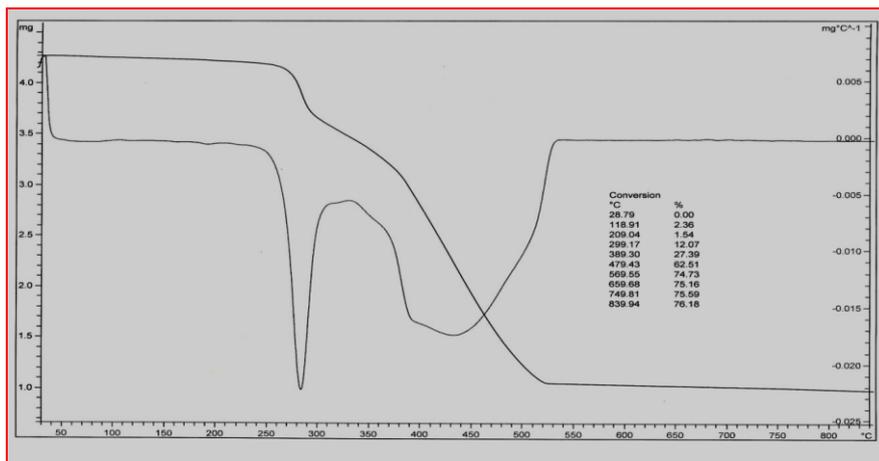


Fig.15 Thermal Gravimetric Analysis of Mn (II) – HBBH

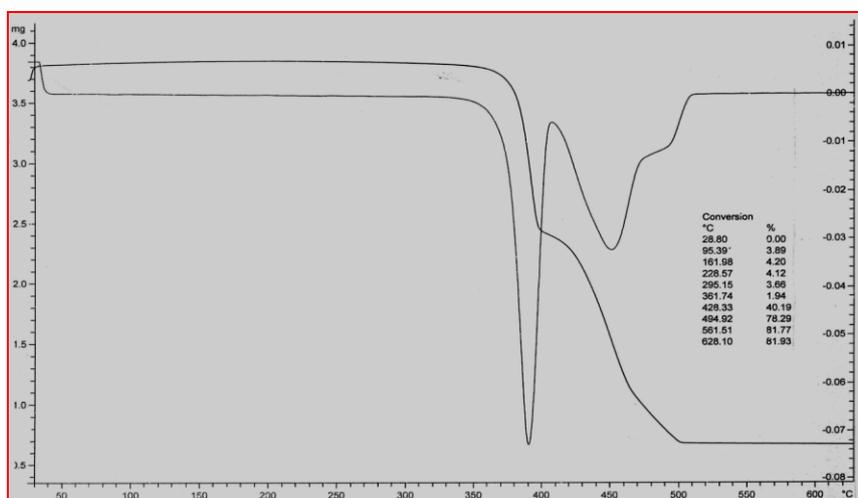


Fig.16 Thermal Gravimetric Analysis of Co (II) – HBBH

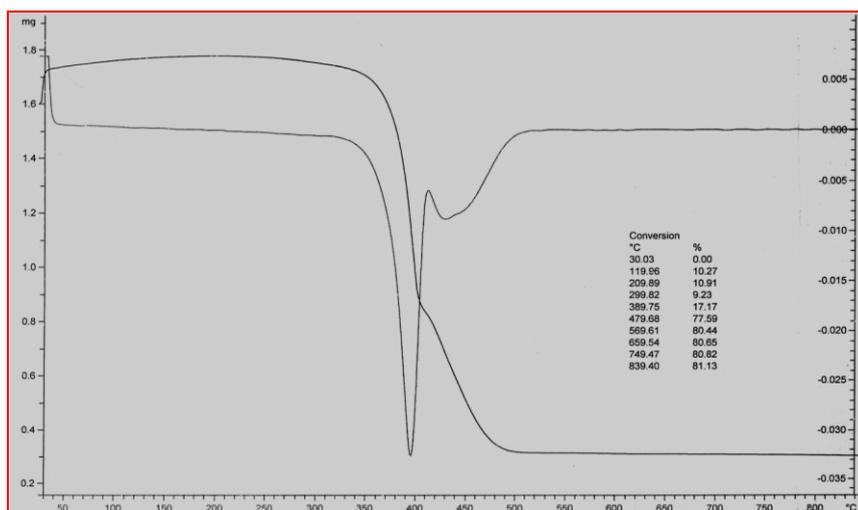


Fig.17 Thermal Gravimetric Analysis of Ni (II)-HBBH

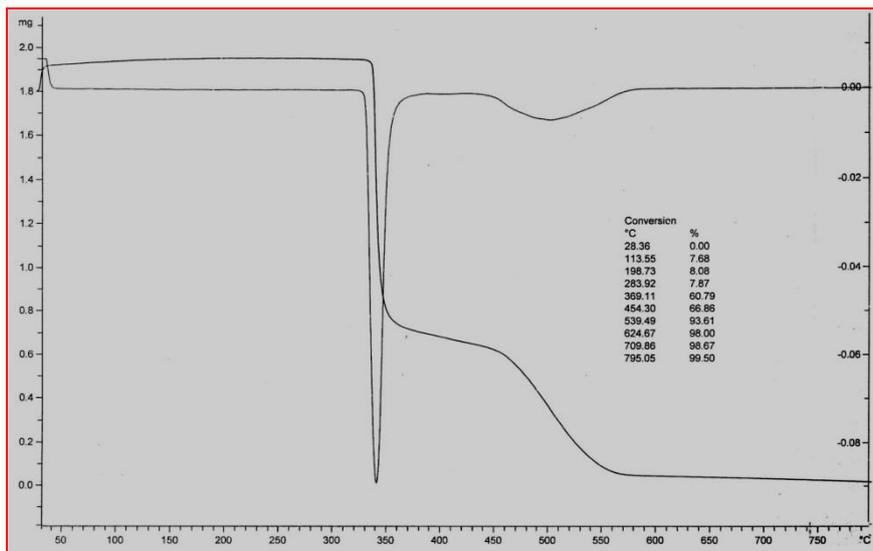


Fig.18 Thermal Gravimetric Analysis of Cu (II) – HBBH

The spectral data and Thermal studies¹⁵⁻¹⁷ supported the composition of VO (II) –HBBH as 1:1 Fig.19, Mn (II) and Cu (II)-HBBH complexes as 1:1 with coordinated water molecules "Fig. 20" and Ni (II) and Co (II)-HBBH complexes as 1:2 "Fig. 21".

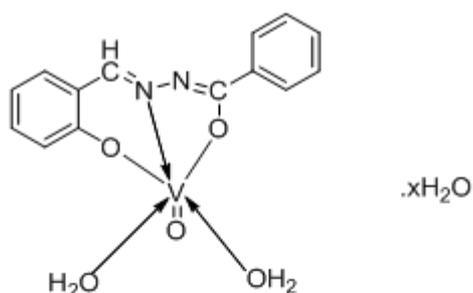


Fig.19 Tentative structure of VO (II)-HBBH complex

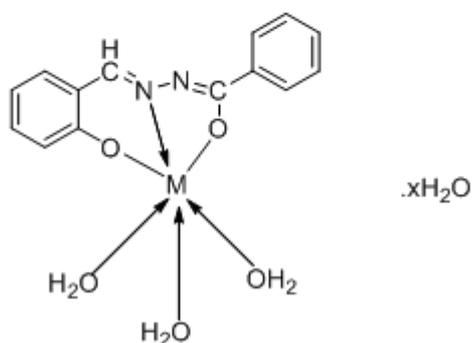


Fig.20 Tentative structure of M (II)-HBBH complex [M(II) = Cu(II) or Mn(II)]

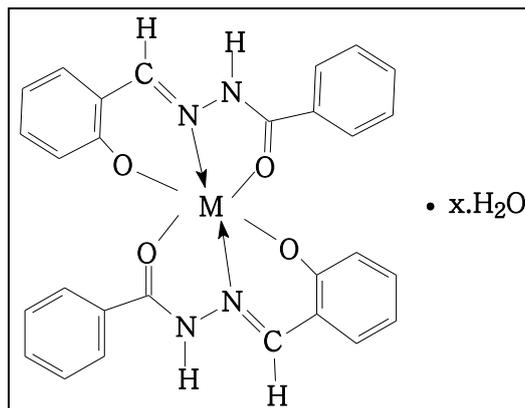


Fig.21 Tentative structure of M (II)-HBBH complexes [M(II) = Ni(II) or Co(II)]

IV. Biological activity

The Biological activity of Cu(II) – HBBH complex is studied against gram negative (*Escherichia coli*) bacteria and the fungal strain *Candida albicans*.

1.9 Preparation and Standardization of Stock cultures

Cultures on receipt were sub cultured in NA/SDA plates and further stored in slants as stock cultures. For the experiments, stock culture was prepared by inoculating each culture from slants to flask in sterile NB and incubated at 37⁰ C for 24 hr. The stock culture was adjusted to 0.5 McFarland standard turbidity and used for assay, in case of fungus SDA and SDB are used instead of NA and NB. Sterile NA plates were prepared and 0.1 ml of the inoculum from standardized culture of test organism was prepared uniformly. Wells were prepared by using a sterile borer of diameter 10mm and 100µl (To get the final concentration of 1000 and 500 µg/well) of the test substance, standard antibiotic were added in each well separately. A standard antibiotic, ciprofloxacin (100 µl) was tested against fungi. The plates were placed at 4⁰ C for 1h to allow the diffusion of test solution into the medium and plates were incubated at a temperature optimal for the test organism and for a period of time sufficient for the growth of at least 10 to 15 generations (usually 24h at 37⁰ C) for bacteria and (usually 48h at room temperature) for fungi. The zone of inhibition of microbial growth around the well was measured in mm.

2.0 Results

The Cu(II)-HBBH complex showed inhibitory activity against the fungal strain *Candida albicans* and gram negative *Escherichia coli* bacteria. All the results are tabulated in the below table.

Table. I Showing zone of inhibition of bacteria and fungi

Sample Name	Zone of inhibition in mm bacteria(<i>Escherichia coli</i> -ve)		Zone of inhibition in mm fungi (<i>Candida albicans</i>)	
	Concentration		Concentration	
	1000µg	500 µg	1000µg	500 µg
Cu - HBBH	16	15	12	11
Standard	Ciprofloxacin (10 µg) 30		Ketoconazole (20 µg) 20	



Samples at different concentrations showing zone of inhibition against *Escherichia coli*



Samples at different concentrations showing zone of inhibition against *Candida albicans*

The above results of biological studies proved that the concentration of compounds played a vital role in increasing the degree of inhibition. As the concentration of Cu(II)-HBBH complex increases the activity also increased.(Table.I)

V. Conclusions

The IR spectra gave information regarding coordinating sites and complex formation. The ¹H-NMR spectra showed evidence for the formation of metal complexes involving phenolic-O, carbonyl-O and azomethine nitrogen in coordination with the metal ion. The mass spectral data provided information regarding metal complex composition. The ESR spectral data gave information about the geometry of the metal complexes. The thermogravimetric analysis gave information regarding the presence of coordinated water molecule. The biological studies proved that the concentration of compounds played a vital role in increasing the degree of inhibition, as the concentration increases the activity also increased.

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