

Full Length Research Paper

Synthesis, characterization and antimicrobial activity of mixed ligand complexes of Co (II) and Cu (II) with N, O/S donor ligands and amino acids

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A series of new mixed ligand complexes of Co(II) and Cu(II) with thiosemicarbazones/semicarbazone (\pm)-5-isopropenyl-2-methylcyclohex-2-enthiosemicarbazone (IPMCHTSC, L₁H), 1,7,7-trimethylbicyclo[2,2,1]heptanethiosemicarbazone (TBHTSC, L₂H) and 1,7,7-trimethylbicyclo [2,2,1]heptanesemicarbazone (TBHSC, L₃H)} and amino acids {glycine (A₁H) or DL-alanine (A₂H)} have been synthesized by the reaction of metal dichloride with ligands IPMCHTSC, TBHSC or TBHTSC and A₁H or A₂H in a 1:1:1 molar ratio in refluxing ethanol. The newly synthesized complexes (1-12) have been characterized by elemental analyses, molar conductance, electronic, IR, FAB mass spectroscopy and thermogravimetric analysis. On the basis of these spectral data, a square pyramidal geometry was proposed for all of these complexes. FAB mass spectroscopic studies of (1), (3) and (4) suggest their monomeric nature. The ligands and their complexes have been screened for their antibacterial and antifungal activities against bacterial strains *E. coli*, *S. aureus*, *P. vulgaris* and fungal strains *A. niger* and *C. albicans*. The results of these studies show the metal (II) complexes to be more antibacterial/ antifungal against one or more species as compared to the uncomplexed ligands.

Keywords: Thiosemicarbazone, semicarbazone, amino acid, FAB mass, thermogravimetric analysis, antimicrobial activity.

INTRODUCTION

The study of transition metal complexes containing biologically important ligands is made easier because certain metal ions are active in many biological processes (Beyer, 1986; Das *et al.*, 1990; Tümer *et al.*, 1999). The fact that transition metals are essential metallic element and exhibit great biological activity when associated with certain metal-protein complexes, participating in oxygen transport, electronic transfer reactions or the storage of ions (Albertin *et al.*, 1975) has created attention in the study of systems containing these metals.

Mixed ligand complexes with metal ion bound to two different and biochemically important ligands have aroused interest as model for metallo-enzymes. The physiologically interesting mixed ligand complexes of transition metals with amino acids play an important role in biological systems and have been a subject of

great interest for researchers (Berthon *et al.*, 1984; Shivankar *et al.*, 2003; Adkhis *et al.*, 2000; Kiss *et al.*, 1985; Hinojosa *et al.*, 1987; Manjula *et al.*, 1990; Çakir *et al.*, 2000). It is also well established that mixed ligand complexes play a decisive role in the activation of enzyme and also storage and transport of active substances (Hughes, 1987; Aull *et al.*, 1980; Freeman, 1973). The interaction of Pd (II) and Pt(II) with salts of amino acids simply or mixed with N-based aromatic ligands to form oligonuclear complexes (Jin *et al.*, 2000; Hollis *et al.*, 1989) which have been used alone or in combination with other chemotherapeutic drugs (Kumer *et al.*, 1985; Wong *et al.*, 1999).

Interests in the thiosemicarbazones and semicarbazones complexes have been stimulated by their biological activity (Padhye *et al.*, 1985; West *et al.*, 1990, 1991). These compounds present a great variety of biological activity ranging from antitumor, fungicide, anti-inflammatory, bactericides and antiviral activities (Nandi *et al.*, 1984, Ali *et al.*, 1984; Scovill *et al.*, 1982; Hossain *et al.*, 1996; Bindu *et al.*, 1998). Recently some Ni(II) and Cu(II) complexes of citronellal thiosemicarba-

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zones have been reported for their cell proliferation inhibition property and apoptosis test on human leukemia cell line U937 (Ferrari *et al.*, 2002).

Our previous work reported on the synthesis, characterization and antimicrobial studies of binary and mixed ligand complexes of some transition metals with semicarbazones/ thiosemicarbazones and amino acids or N-protected amino acids (Sharma *et al.*, 2005, 2006, 2006, 2009; Nagar *et al.*, 2007).

In view of the important biological activity of the thiosemicarbazones/semicarbazones, amino acids and their metal complexes, some mixed ligand metal(II) complexes, 1-12 of the type $[MCl(LH)(A)]H_2O$ (where M = Co(II) and Cu(II); LH = thiosemicarbazone/semicarbazone (IPMCHTSC, TBHTSC and TBHSC); AH = amino acid (glycine, DL-alanine) were formed by a stoichiometric ratio of M: LH: AH as 1 : 1 : 1. These two different ligands incorporated with the metal ion were used in order to study the effect of the presence of two different types of ligands on the biological activity.

Experimental

MATERIAL AND METHODS

All the chemicals and reagents used were of AR grade. Solvents were distilled by conventional methods prior to use. Ligands were prepared by method reported earlier (Sharma *et al.*, 2005, 2009; Brousse *et al.*, 2002). Metal contents were measured by complexometric titrations. Sulfur was estimated gravimetrically as $BaSO_4$ and chloride content was determined by Volhard's method (Vogel, 1989). Elemental analyses were carried out on thermoquest analyzer. The IR spectra were recorded with KBr pellets in the $4000-225\text{ cm}^{-1}$ range. NMR spectra of ligands (IPMCHTSC, TBHSC and TBHTSC) were recorded in $CDCl_3$ solvent using TMS as internal standard on JEOL FX 300 NMR spectrometer at 300.4 and 75.45 MHz frequencies for 1H and $^{13}C\{^1H\}$ NMR respectively, electronic spectra were recorded on Agilent UV/visible spectrophotometer and molar conductivities of 10^{-3} M DMSO solutions were measured on a microprocessor based conductivity meter model 1601/E. Thermogravimetric analysis was performed by PerkinElmer Thermal analyzer with the heating rate $35-900/20^\circ\text{C}$ under nitrogen atmosphere. Mass spectra were recorded on Shimadzu mass spectrometer.

Synthesis of ligands

Carvone thiosemicarbazone (IPMCHTSC), camphor semicarbazone (TBHSC) and camphor thiosemicarbazone (TBHTSC) were prepared according to the reported method (Sharma *et al.*, 2005, 2009; Nagar *et al.*, 2007; Brousse *et al.*, 2002).

IPMCHTSC

Yield: 91% (2.01 g); M. pt. 109°C ; IR (cm^{-1}) 3441s, 3240s, br $\nu(\text{NH}_2)$; 3155s, $\nu(\text{NH})$; 1592, $\nu(\text{C}=\text{N})$; 840m, $\nu(\text{C}=\text{S})$; 1H NMR ($CDCl_3$, ppm) : 9.05 (s, 1H, NH-C=S); 7.32, 7.05 (2s, 2H, NH_2); 6.20-6.77 (m, 1H, =CH-CH₂); 4.83, 4.79 (2s, 2H, =CH₂); 2.79 (dd, 1H, J = 3.0Hz, -CH₂-CH-CH₂-); 2.22-2.69 (m, 2H, CH₂); 2.09-2.18 (m, 2H, CH₂); 1.86 (s, 3H, CH₃); 1.75 (s, 3H, CH₃); ^{13}C NMR ($CDCl_3$, ppm) : 178.7 (C=S); 150.2 (C=N); 146.8 (C-7); 135.3 (C-2); 132.0 (C-3); 110.7 (C-8); 40.6 (C-5); 30.1 (C-4); 29.2 (C-6); 20.6 (C-9); 17.7 (C-10). Anal. Found for $C_{11}H_{17}N_3S$ (223.34): C, 59.40; H, 7.91; N, 18.01; S, 14.40%. Calcd. C, 59.15; H, 7.67; N, 18.81; S, 14.36%.

TBHTSC

Yield: 75% (3.3 g); M. Pt. 139°C ; IR (cm^{-1}): 3425s, 3225s, br $\nu(\text{NH}_2)$; 3195s, $\nu(\text{NH})$; 1595s, $\nu(\text{C}=\text{N})$; 875m, $\nu(\text{C}=\text{S})$; 945, $\nu(\text{N}-\text{N})$; 1H NMR ($CDCl_3$, δ ppm): 9.45 (s, 1H, NH-C=S); 7.27, 7.23 (2s, 2H, NH_2); 2.36-2.44 (1H, H-3 exo); 2.02-2.05 (m, 1H, H-3 endo); 1.87-1.92 (m, 1H, H-4); 1.80-1.85 (m, 1H, H-5 exo); 1.70-1.79 (m, 1H, H-6 exo); 1.33-1.42 (m, 1H, H-6 endo); 1.18-1.26 (m, 1H, H-5 endo); 0.98 (s, 3H, H-9); 0.94 (s, 3H, H-10); 0.74 (s, 3H, H-8); ^{13}C NMR ($CDCl_3$, δ ppm): 177.4 (C=S); 167.2 (C=N); 52.3 (C-1); 47.9 (C-7); 43.9 (C-4); 32.4 (C-3); 33.8 (C-6); 27.1 (C-5); 19.4 (C-8 or C-9); 18.5 (C-8 or C-9); 11.0 (C-10). ; Anal. Found for $C_{11}H_{19}N_3S$ (225.35): C, 58.57; H, 8.43; N, 18.63; S, 14.31. Calcd. C, 58.62; H, 8.49; N, 18.64; S, 14.22 %.

TBHSC

Yield: 90% (5.7 g); M. Pt. 238°C ; IR (cm^{-1}): 3455s, 3260s, br $\nu(\text{NH}_2)$; 3232s, $\nu(\text{NH})$; 1698, $\nu(\text{C}=\text{O})$; 1553s, $\nu(\text{C}=\text{N})$; 960, $\nu(\text{N}-\text{N})$; 1H NMR ($CDCl_3$, δ ppm): 8.40 (s, 1H, NH-C=O); 7.28, 7.22 (2s, 2H, NH_2); 2.37-2.44 (m, 1H, H-3 exo); 2.02-2.05 (m, 1H, H-3 endo); 1.86-1.94 (m, 1H, H-4); 1.80-1.85 (m, 1H, H-5 exo); 1.70-1.79 (m, 1H, H-6 exo); 1.33-1.42 (m, 1H, H-6 endo); 1.18-1.26 (m, 1H, H-5 endo); 0.98 (s, 3H, H-9); 0.94 (s, 3H, H-10); 0.74 (s, 3H, H-8); ^{13}C NMR ($CDCl_3$, δ ppm): 163.5 (C=O); 158.1 (C=N); 52.3 (C-1); 47.9 (C-7); 43.9 (C-4); 32.5 and 33.5 (C-3 and C-6); 27.2 (C-5), 19.4 (C-8 or C-9); 18.6 (C-8 or C-9); 11.0 (C-10). Anal. Found for $C_{11}H_{19}N_3O$ (209.28): C, 63.06; H, 9.07; N, 20.06. Calcd. C, 63.12; H, 9.15; N, 20.07 %.

Preparation of the mixed ligand complexes

To an ethanolic solution (~10 ml) of $CuCl_2 \cdot 2H_2O$ (0.8551 g, 5 mmol), a hot ethanolic solution (~10 ml) of ligand, TBHTSC (1.0162 g, 5 mmol) and ethanolic

Table S1: Antibacterial activity data for ligands and Co(II) and Cu(II) complexes after 24 hours.

Compound	Concentration (mg/disc)	Inhibition zone (mm)		
		<i>S. aureus</i>	<i>P. vulgaris</i>	<i>E. coli</i>
L ₁ H	0.25	0.00	0.00	2.00
	0.50	6.00	4.00	10.00
	0.75	10.00	10.00	11.00
	1.00	12.00	13.00	12.00
L ₂ H	0.25	4.00	0.00	4.00
	0.50	6.00	0.00	14.00
	0.75	9.00	12.00	17.00
	1.00	13.00	16.00	23.00
L ₃ H	0.25	0.00	4.00	3.00
	0.50	10.00	9.00	6.00
	0.75	14.00	13.00	12.00
	1.00	19.00	16.00	19.00
A ₁ H	0.25	0.00	2.00	0.00
	0.50	5.00	6.00	8.00
	0.75	12.00	16.00	11.00
	1.00	18.00	21.00	13.00
A ₂ H	0.25	0.00	0.00	2.00
	0.50	4.00	3.00	7.00
	0.75	8.00	9.00	10.00
	1.00	15.00	13.00	17.00
(1)	0.25	0.00	7.00	15.00
	0.50	10.00	10.00	19.00
	0.75	16.00	18.50	20.00
	1.00	19.00	22.00	21.00
(2)	0.25	15.00	0.00	16.00
	0.50	17.00	0.00	19.00
	0.75	18.00	16.00	20.00
	1.00	21.00	19.00	22.00
(3)	0.25	5.00	0.00	3.00
	0.50	12.00	0.00	7.00
	0.75	18.00	6.00	9.00
	1.00	21.00	11.00	22.00
(4)	0.25	0.00	0.00	4.00
	0.50	8.00	9.00	10.00
	0.75	16.00	13.00	16.00
	1.00	18.00	18.00	21.00
(6)	0.25	14.00	0.00	16.00
	0.50	16.00	0.00	18.00
	0.75	18.00	17.80	20.00
	1.00	19.00	24.00	24.00
(7)	0.25	5.40	5.00	0.00
	0.50	6.00	8.00	7.00
	0.75	10.00	16.00	8.00
	1.00	12.00	18.00	12.00
(9)	0.25	0.00	0.00	0.00
	0.50	10.00	0.00	4.00
	0.75	12.00	12.00	7.00
	1.00	18.00	13.00	19.00

Table S1 Continues

Compound	Concentration (mg/disc)	Inhibition zone (mm)		
		<i>S. aureus</i>	<i>P. vulgaris</i>	<i>E. coli</i>
(11)	0.25	4.00	6.00	0.00
	0.50	9.00	7.00	12.00
	0.75	10.00	9.00	18.00
	1.00	13.00	16.00	20.00
(12)	0.25	12.00	0.00	18.00
	0.50	16.00	0.00	20.00
	0.75	18.00	20.00	21.00
	1.00	20.00	28.00	22.50
Ampiciline(standard)	0.03	20.00	45.00	16.00
Tetracyclin e(standard)	0.03	22.00	42.00	27.00

solution of DL-alanine (0.4470 g, 5 mmol) containing 5 mmol of NaOH were added dropwise with constant stirring. After complete addition the reaction mixture was refluxed for 4 h and cooled to room temperature. The resulting precipitate was filtered and washed with distilled water for the removal of NaCl and dried in vacuum to give light gray coloured solid.

Similar route have been employed for the preparation of other complexes.

Antimicrobial Study

Evaluation of antibacterial activity by inhibition zone technique

The ligands and the mixed ligand Co (II) and Cu (II) complexes were screened for their antibacterial activity against three bacterial strains *Staphylococcus aureus* Gram (+ve), *Protius vulgaris* and *Escherichia coli* Gram (-ve), using the paper disc method (Bauer *et al.*, 1966). Antibacterial screening was carried out using the standard disc diffusion test. Different concentrations of compounds (0.25-1.0 mg/disc) were incorporated in 6 mm diameter sterile discs (Himedia, India) and dried. Four discs were placed on Nutrient Agar (NA) plate (Himedia, India) seeded with test bacterial, including ampiciline and tetracycline standard antibiotic disc. After overnight incubation at 37°C, the agar plates were observed for zones of inhibition (bactereostatic diameter in mm) (Table S1).

Evaluation of antifungal activity by agar well diffusion method

The ligands and complexes were screened for their antifungal activity against two fungal strains, *Aspergillus*

niger and *Candida albicans* using agar well diffusion method (Parez *et al.*, 1990). Sabouraud Dextrose Agar (SDA) was poured into petri dishes. After solidification 0.25 ml of test fungal strains were inoculated by cotton swab in the media. After the sterile borer (6 mm) the test compound (0.25-2.0 mg) was introduced into the well and plates were incubated at 37°C for 48 h. All samples were tested in duplicates. Fungal growth was determined by measuring the diameter of zone of inhibition (Table S2).

Determination of minimum inhibitory concentrations (MICs) of compounds by agar plate serial dilution method.

MIC values can be determined by a number of standard test procedures. The most commonly employed method is the agar dilution (agar plate serial dilution) (Van Dyck *et al.*, 1994). Serial dilutions are made of the products in bacterial growth media. The test organism are then added to the dilutions of the products, incubated and scored for growth. This procedure is a standard assay for antimicrobials. In this method nutrient agar (NA) (Himedia, India) plate is inoculated and antimicrobial diffuses from a disk or from a paper strip into the agar. Mark the plates so that the orientation is obvious. Transfer diluted test compound suspensions to the wells of an inoculum replicating apparatus. Use the apparatus to transfer the inoculum to the series of agar plates, including a control plate without antimicrobial agent. Allow the inoculum spots to dry at room temperature before inverting the plates for incubation. After inoculation, incubate plates at 35-37°C in air for 18h. If the agar dilution MICs were determined at first appearance of control growth, as is commonly done, small variations in the time of reading (as could occur by variation in observer perception of when initial

Table S2. Antifungal activity data for ligands and Co(II) and Cu(II) complexes after 48 hours.

Compound	Concentration (mg)	Inhibition zone (mm)	
		<i>A. niger</i>	<i>C. albicans</i>
L ₁ H	0.25	0.00	0.00
	0.50	0.00	0.00
	1.00	2.00	4.00
	1.50	4.00	6.00
	2.00	6.00	9.00
L ₂ H	0.25	0.00	0.00
	0.50	0.00	0.00
	1.00	5.00	4.00
	1.50	8.00	7.00
	2.00	14.00	12.00
L ₃ H	0.25	0.00	0.00
	0.50	0.00	0.00
	1.00	7.00	8.00
	1.50	11.00	10.00
	2.00	16.00	14.00
A ₁ H	0.25	0.00	0.00
	0.50	2.00	0.00
	1.00	4.00	2.00
	1.50	8.00	4.00
	2.00	10.00	12.00
L ₁ H	0.25	0.00	0.00
	0.50	0.00	0.00
	1.00	2.00	4.00
	1.50	4.00	6.00
	2.00	6.00	9.00
L ₂ H	0.25	0.00	0.00
	0.50	0.00	0.00
	1.00	5.00	4.00
	1.50	8.00	7.00
	2.00	14.00	12.00
L ₃ H	0.25	0.00	0.00
	0.50	0.00	0.00
	1.00	7.00	8.00
	1.50	11.00	10.00
	2.00	16.00	14.00
A ₁ H	0.25	0.00	0.00
	0.50	2.00	0.00
	1.00	4.00	2.00
	1.50	8.00	4.00
	2.00	10.00	12.00
A ₂ H	0.25	0.00	0.00
	0.50	0.00	4.00
	1.00	2.00	7.00
	1.50	8.00	10.00
	2.00	11.00	13.00
(1)	0.25	0.00	0.00
	0.50	0.00	16.50
	1.00	0.00	16.00
	1.50	18.00	18.00
	2.00	22.00	20.00

Table S2 Continues

Compound	Concentration (mg)	Inhibition zone (mm)	
		<i>A. niger</i>	<i>C. albicans</i>
(2)	0.25	0.00	0.00
	0.50	0.00	11.00
	1.00	0.00	15.00
	1.50	14.00	20.00
	2.00	17.00	22.00
(3)	0.25	0.00	0.00
	0.50	0.00	4.00
	1.00	0.00	7.00
	1.50	10.00	10.00
(4)	2.00	17.00	14.00
	0.25	0.00	0.00
	0.50	6.00	0.00
	1.00	8.00	4.00
	1.50	10.00	5.00
(6)	2.00	12.00	15.00
	0.25	0.00	0.00
	0.50	0.00	12.00
	1.00	0.00	18.00
	1.50	10.00	20.00
(7)	2.00	14.00	24.00
	0.25	0.00	0.00
	0.50	0.00	7.00
	1.00	2.00	9.00
	1.50	6.00	10.00
(9)	2.00	12.00	14.00
	0.25	0.00	0.00
	0.50	0.00	2.00
	1.00	0.00	6.00
	1.50	4.00	9.00
(10)	2.00	8.00	12.00
	0.25	0.00	0.00
	0.50	0.00	10.00
	1.00	0.00	12.00
	1.50	7.00	14.00
(11)	2.00	9.00	19.00
	0.25	0.00	0.00
	0.50	0.00	0.00
	1.00	7.00	6.00
	1.50	9.00	9.00
(12)	2.00	13.00	12.00
	0.25	0.00	0.00
	0.50	0.00	9.00
	1.00	2.00	12.00
	1.50	4.00	15.00
Fluconazole (standard)	2.00	5.00	19.00
	1.00	32.00	38.00

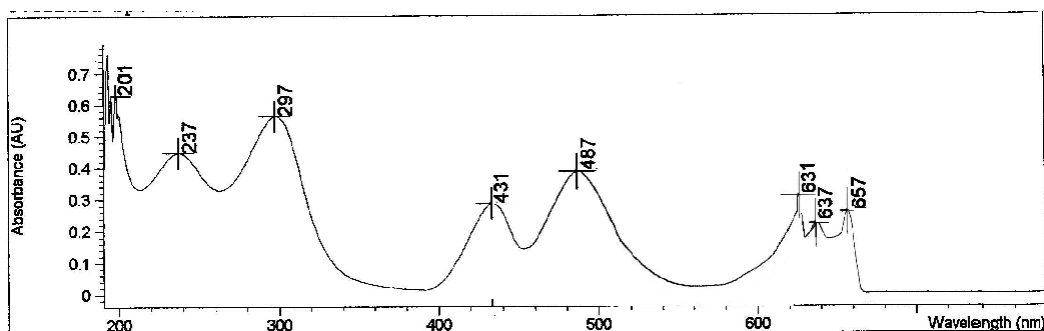


Figure 1. UV/Vis spectra of (A) [CoCl(TBHTSC)(Gly)]H₂O

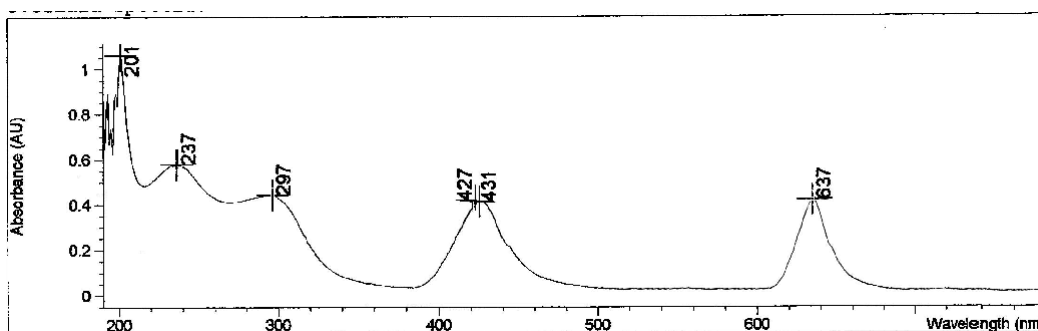
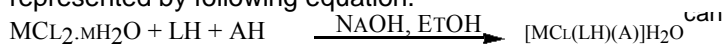


Figure 1. (B) [CuCl(TBHTSC)(DL-ALA)]H₂O

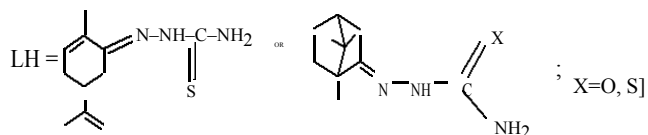
growth appears) induced large MIC variations, particularly with rapidly growing strains (Table 5).

RESULTS AND DISCUSSION

Reactions of Co(II) and Cu(II) chloride with thiosemicarbazones/semicarbazone (LH) and amino acids (AH) in 1:1:1 molar ratio in refluxing ethanol in presence of NaOH, yielded the complexes of type [MCl(LH)(A)]H₂O (Figure 1). The general reaction can be represented by following equation.



[WHERE M = Co(II), Cu(II); M = 2 FOR Cu(II) AND 6 FOR Co(II); AH = HOOC-CHR-NH₂; R = H, CH₃, TBHSC and TBHTSC



Several analytical techniques were used to characterize the complexes including microanalysis (CHN), IR, FAB mass and electronic spectra, thermogravimetric analysis (TGA) and conductometric measurements. Analytical data for the newly synthesized complexes are given in Table 1. All the metal complexes are non hygroscopic in nature, stable at room temperature and

soluble in DMSO.

The molar conductivity shows that all the complexes are non-electrolytes with $\lambda = 17.1-30.0 \Omega^{-1} \text{ cm}^2 \text{ mol}^{-1}$ in DMSO (10^{-3} M) solution at room temperature.

IR spectra

The main IR spectral vibrations of mixed ligand Co(II) and Cu(II) complexes are given in Table 2. The spectra show characteristics band positions, shifts and intensities in comparison with the free ligands which be correlated to bidentate thiosemicarbazone/semicarbazone binding and amino acid chelation. In The IR spectra of ligand IPMCHTSC, CH₃TBHSC and TBHTSC, the highest frequency bands observed in the 3425-3455 cm⁻¹ and 3225-3260 cm⁻¹ region are assigned respectively to the asymmetric and symmetric stretching of terminal NH₂ group, the next high energy band in the 3155-3232 cm⁻¹ region due to the imino group vibration, in the complexes these bands are not affected indicating non participation of amino and imino nitrogen atoms in coordination. In the spectrum of TBHSC, amide (C=O) band observed at 1698 cm⁻¹ is shifted to lower frequency region in the corresponding mixed ligand complexes due to the participation of amido oxygen in chelation. The thiosemicarbazones show medium intensity band in the 840-875 cm⁻¹ region due to the $\nu(\text{C}=\text{S})$ and the downw-

Table 1. Analytical data for complexes

Compound	Colour	Yield (%)	M.P	% Analysis Found (Calcd.)						Molar conductance ($\Omega^{-1} \text{mol}^{-1} \text{cm}^2$)
				C	H	N	S	M	Cl	
[CoCl(IPMCHTSC)(Gly)]H ₂ O [CoCl(C ₁₁ H ₁₇ N ₃ S)(C ₂ H ₅ NO ₂)]H ₂ O (1)	Dark brown	95	110	37.01 (38.00)	6.17 (5.88)	13.34 (13.63)	7.60 (7.80)	13.80 (14.34)	8.60 (8.62)	17.1
[CoCl(IPMCHTSC)(DL-ala)]H ₂ O [CoCl(C ₁₁ H ₁₇ N ₃ S)(C ₃ H ₇ NO ₂)]H ₂ O (2)	Dim gray	71	159	38.10 (39.58)	5.75 (6.16)	12.75 (13.18)	7.12 (7.54)	13.44 (13.87)	8.10 (8.34)	18.5
[CuCl(IPMCHTSC)(Gly)]H ₂ O [CuCl(C ₁₁ H ₁₇ N ₃ S)(C ₂ H ₅ NO ₂)]H ₂ O (3)	Aquamarine	74	142	36.53 (37.58)	5.99 (5.82)	13.10 (13.48)	6.99 (7.71)	14.92 (15.29)	8.05 (8.53)	18.9
[CuCl(IPMCHTSC)(DL-ala)]H ₂ O [CuCl(C ₁₁ H ₁₇ N ₃ S)(C ₃ H ₇ NO ₂)]H ₂ O (4)	Dark-khaki	81	255	37.75 (39.15)	5.73 (6.10)	11.99 (13.04)	6.97 (7.46)	14.30 (14.79)	7.79 (8.25)	19.2
[CoCl(TBHSC)(Gly)]H ₂ O [CoCl(C ₁₁ H ₁₉ N ₃ O)(C ₂ H ₅ NO ₂)]H ₂ O (5)	Light blue	72	245	39.05 (39.35)	6.10 (6.60)	14.10 (14.12)	-	14.78 (14.85)	8.35 (8.93)	21.2
[CoCl(TBHSC)(DL-ala)]H ₂ O [CoCl(C ₁₁ H ₁₉ N ₃ O)(C ₃ H ₇ NO ₂)]H ₂ O (6)	Thistle	89	252	41.53 (40.93)	6.05 (6.87)	13.01 (13.63)	-	13.79 (14.34)	8.75 (8.63)	22.4
[CuCl(TBHSC)(Gly)]H ₂ O [CuCl(C ₁₁ H ₁₉ N ₃ O)(C ₂ H ₅ NO ₂)]H ₂ O (7)	Medium aquamarine	79	255	37.93 (38.90)	5.75 (6.52)	12.96 (13.95)	-	15.30 (15.83)	8.54 (8.83)	26.5
[CuCl(TBHSC)(DL-ala)]H ₂ O [CuCl(C ₁₁ H ₁₉ N ₃ O)(C ₃ H ₇ NO ₂)]H ₂ O (8)	Aquamarine	75	230	41.02 (40.48)	5.96 (6.79)	13.40 (13.48)	-	15.19 (15.29)	8.15 (8.53)	24.9
[CoCl(TBHSTSC)(Gly)]H ₂ O [CoCl(C ₁₁ H ₁₉ N ₃ S)(C ₂ H ₅ NO ₂)]H ₂ O (9)	Dark brown	80	210	37.10 (37.82)	6.30 (6.34)	12.46 (13.57)	7.20 (7.76)	14.02 (14.27)	8.20 (8.58)	28.1
[CoCl(TBHSTSC)(DL-ala)]H ₂ O [CoCl(C ₁₁ H ₁₉ N ₃ S)(C ₃ H ₇ NO ₂)]H ₂ O (10)	Pale violet-red	78	245	39.01 (39.39)	7.01 (6.61)	13.01 (13.12)	7.03 (7.51)	13.20 (13.80)	7.93 (8.30)	29.4
[CuCl(TBHSTSC)(Gly)]H ₂ O [CuCl(C ₁₁ H ₁₉ N ₃ S)(C ₂ H ₅ NO ₂)]H ₂ O (11)	Silver	90	254	37.63 (37.40)	6.10 (6.27)	12.75 (13.42)	7.09 (7.68)	14.96 (15.22)	8.40 (8.49)	27.5
[CuCl(TBHSTSC)(DL-ala)]H ₂ O [CuCl(C ₁₁ H ₁₉ N ₃ S)(C ₃ H ₇ NO ₂)]H ₂ O (12)	Light gray	70	250	38.10 (38.97)	6.23 (6.54)	11.95 (12.98)	6.94 (7.43)	14.30 (14.72)	8.03 (8.21)	29.9

Table 2: Main IR spectral bands for complexes

Compound	LH moiety					AH moiety		Non ligand bands			
	$\nu(\text{NH}_2)$	$\nu(\text{NH})$	$\nu(\text{C=O})$	$\nu(\text{C=N})$	$\nu(\text{C=S})$	$\nu(\text{NH}_2)$	$\nu(\text{COO})$	$\nu(\text{M-O})$	$\nu(\text{M-N})$	$\nu(\text{M-S})$	$\nu(\text{M-Cl})$
(1)	3440 as 3242 s	3155	-	1580	830	3240 as 3050 s	1625 as 1375 s	480	440	372	325
(2)	3441 as 3240 s	3152	-	1565	825	3200 as 3040 s	1610 as 1375 s	492	436	350	345
(3)	3438 as 3238 s	3150	-	1572	835	3222 as 3045 s	1620 as 1370 s	445	420	378	375
(4)	3435 as 3241 s	3155	-	1560	829	3245 as 3035 s	1615 as 1375 s	455	442	345	340
(5)	3450 as 3260 s	3230	1680	1545	-	3255 as 3042 s	1622 as 1380 s	485	447	-	320
(6)	3455 as 3262 s	3235	1678	1540	-	3265 as 3050 s	1619 as 1390 s	465	450	-	315
(7)	3452 as 3265 s	3232	1675	1538	-	3272 as 3039 s	1624 as 1380 s	455	425	-	345
(8)	3455 as 3261 s	3230	1685	1541	-	3262 as 3042 s	1615 as 1375 s	450	435	-	330
(9)	3425 as 3220 s	3190	-	1582	850	3260 as 3055 s	1629 as 1390 s	475	438	390	342
(10)	3420 as 3225 s	3197	-	1579	852	3275 as 3070 s	1622 as 1385 s	472	444	340	337
(11)	3422 as 3226 s	3192	-	1585	860	3267 as 3062 s	1618 as 1390 s	456	430	345	325
(12)	3424 as 3223 s	3195	-	1575	865	3230 as 3079 s	1625 as 1380 s	465	432	335	317

ard shifting in this band in the corresponding complexes suggests coordination of metal ion through the C=S group. The spectra of IPMCHTSC, TBHSC and TBHTSC exhibit a strong band in the 1553-1595 cm^{-1} region due to the C=N mode of azomethine linkage. In the complexes this band is shifted to lower frequency region suggesting that the unsaturated nitrogen of azomethine linkage is coordinated to the metal.

The N-H asym and N-H sym vibrations observed at 3040 and 2960 cm^{-1} , respectively, in the free amino acids are shifted to higher wave numbers i.e. in the range 3220–3174 cm^{-1} and 3080–3050 cm^{-1} respectively, in the spectra of the complexes, suggesting coordination of the amino group through nitrogen with the metal ion. The $\nu_{\text{asym}}(\text{COO}^-)$ band of the free amino acids i.e. 1590 cm^{-1} is shifted to higher wave number, i.e. in the range 1640–1630 cm^{-1} and the $\nu_{\text{sym}}(\text{COO}^-)$ mode observed at 1400 cm^{-1} in the spectra of free amino acids is found to be shifted to lower wave number i.e. 1375-1390 cm^{-1} , in the spectra of the complexes indicating the coordination of the carboxylic acid group via oxygen with the metal ion.

Nakamoto, Morimoto, and Martell showed that for a given ligand, the difference ($\nu_{\text{asym}} - \nu_{\text{sym}}$) would increase

as the M–O bond becomes more covalent, since the caboxylate stretching becomes correspondingly more asymmetrical (Abdel-Rahman, 2001). In the present investigation, this difference being in the range 228–250 cm^{-1} indicates that the M–O bond is purely covalent (Nakamoto *et al.*, 1961; Hamrit *et al.*, 2000).

Thus it has been concluded that in these complexes IPMCHTSC, TBHSC and TBHTSC, act as neutral bidentate ligand coordinating through the carbonyl oxygen or thiol sulfur and azomethine nitrogen and amino acids act as monobasic bidentate ligand and coordinate through the amino nitrogen and carboxylate oxygen. The non ligand bands occurring in the 420-450, 442-492, 335-390 and 315-375 cm^{-1} regions are tentatively assigned to $\nu(\text{M-O})$, $\nu(\text{M-N})$, $\nu(\text{M-S})$ and $\nu(\text{M-Cl})$ modes respectively.

A band around 3500-3600 cm^{-1} indicates the presence of lattice water in these complexes (Nakamoto, 1986).

Electronic spectra

The significant electronic absorption bands in the spec-

Table 3. Characteristic absorption bands in electronic spectra

Compound	Spectral bands (nm)
(1)	652; 637; 631; 487; 432; 298; 236; 201
(2)	656; 635; 632; 485; 435; 297; 237; 201
(3)	637; 432; 427; 297; 238; 201
(4)	635; 435; 425; 296; 236; 201
(5)	654; 637; 631; 484; 437; 297; 237; 201
(6)	656; 639; 634; 487; 433; 297; 237; 201
(7)	635; 430; 428; 295; 237; 201
(8)	637; 435; 426; 298; 237; 201
(9)	657; 637; 631; 487; 431; 297; 237; 201
(10)	657; 638; 634; 489; 432; 297; 237; 201
(11)	636; 436; 427; 297; 237; 201
(12)	637; 431; 427; 297; 237; 201

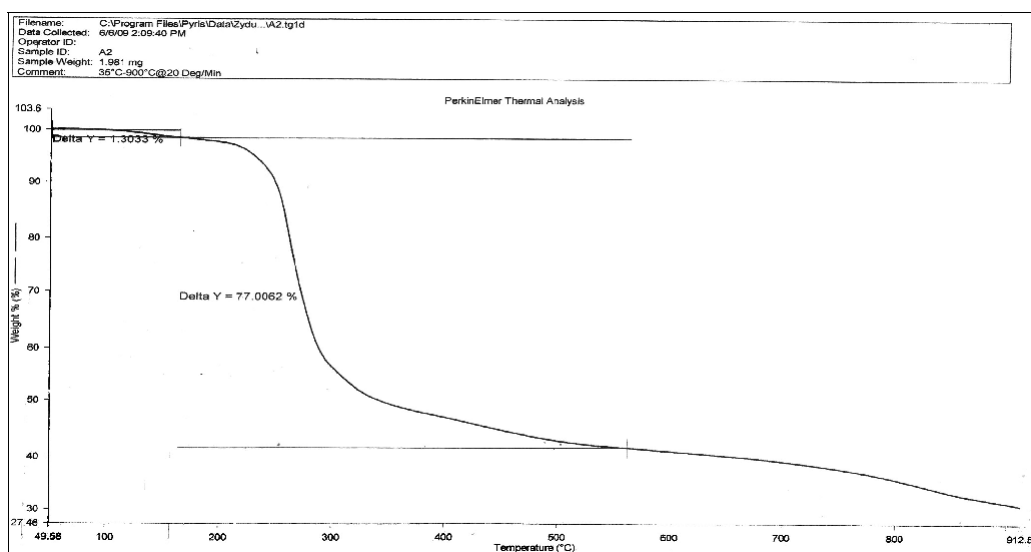


Figure 2. TGA curve of {weight (%) vs temperature (°C)}[CuCl(C₁₁H₁₇N₃S)(C₂H₅NO₂)]H₂O

tra of the Co(II) complexes recorded in DMSO. The thiosemicarbazone, semicarbazone and amino acids and its complexes show π -* and n -* bands at 201 and 295-298, 237 nm respectively (Figure 1A). The d-d bands are observed at 654-657, 635-639 and 630-634 nm. Two bands at 484-489 and 431-437 nm are assigned to ligand-to-metal (LM) charge transfer transitions. The spectra of the cobalt (II) complexes resemble the spectra of other five coordinate cobalt (II) complex (Roy *et al.*, 1984; Lever, 1984), and square pyramidal structure may be assigned for these complexes.

The electronic spectra of the Cu(II) complexes shows band 635-639, 430-437, 425-428 and 294-298 nm (Figure 1B) which are assigned to d-d transitions, ligand-to-metal charge transfer transitions and ligand internal transitions. Absorption bands have been assigned to Cu(II) complexes having square pyramidal structure with considerable distortions towards t_{bp}

(trigonal bipyramidal) (McLachlan *et al.*, 1985; Adhikary *et al.*, 1994; Bhattacharya *et al.*, 1996) (Table 3).

Thermal studies

The thermogravimetric analysis was undertaken for [CuCl(C₁₁H₁₇N₃S)(C₂H₅NO₂)]H₂O (**3**) [Figure. 2]. The thermogram for complex shows two weight loss steps due to pyrolysis of organic byproduct. In first step slight weight loss upto 180°C of sample is due to the presence of water. The thermogram exhibits completion of the decomposition at 900°C. The residual was 24.05% (Cal.), a value of CuS being the final product 24.00% (obs.).

Mass spectra

The FAB mass spectral studies of three of the representative compounds [CoCl(C₁₁H₁₇N₃S)(C₂H₅NO₂)]

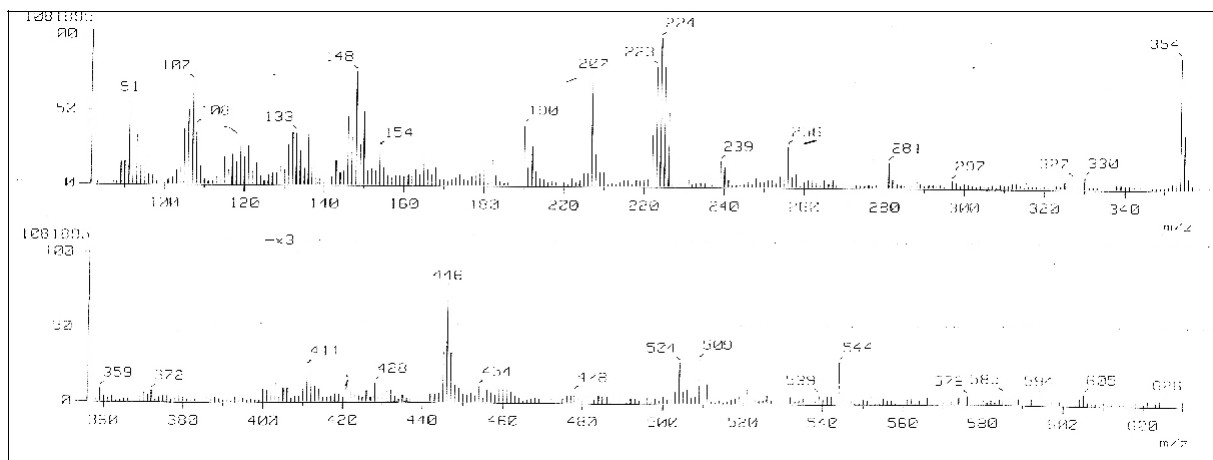


Figure 3. (a) Splitting patterns of FAB mass of [intensity vs m/z](a) $[\text{CoCl}(\text{C}_{11}\text{H}_{17}\text{N}_3\text{S})(\text{C}_2\text{H}_5\text{NO}_2)]\text{H}_2\text{O}$

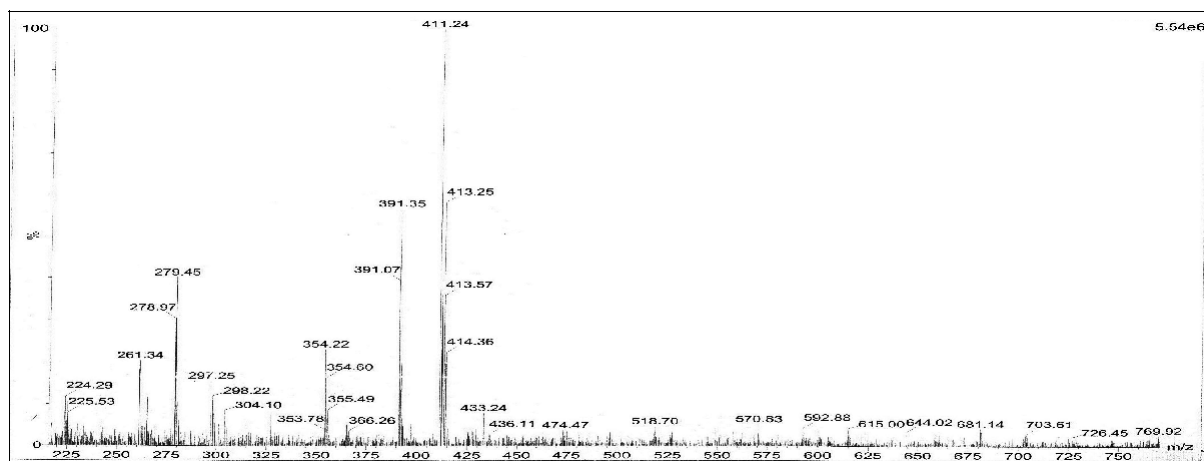


Figure 3. (b) $[\text{CuCl}(\text{C}_{11}\text{H}_{17}\text{N}_3\text{S})(\text{C}_2\text{H}_5\text{NO}_2)]\text{H}_2\text{O}$

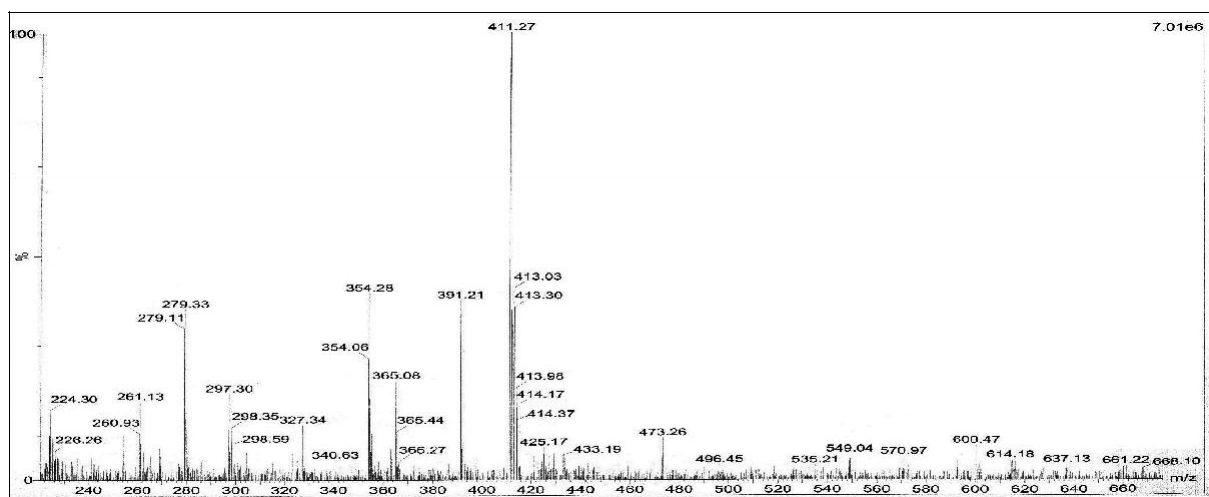


Figure 3. (c) $[\text{CuCl}(\text{C}_{11}\text{H}_{17}\text{N}_3\text{S})(\text{C}_3\text{H}_7\text{NO}_2)]\text{H}_2\text{O}$

H_2O (1), $[\text{CuCl}(\text{C}_{11}\text{H}_{17}\text{N}_3\text{S})(\text{C}_2\text{H}_5\text{NO}_2)]\text{H}_2\text{O}$ (3) and $[\text{CuCl}(\text{C}_{11}\text{H}_{17}\text{N}_3\text{S})(\text{C}_3\text{H}_7\text{NO}_2)]\text{H}_2\text{O}$ (4) [Figure.3 (a-c)]

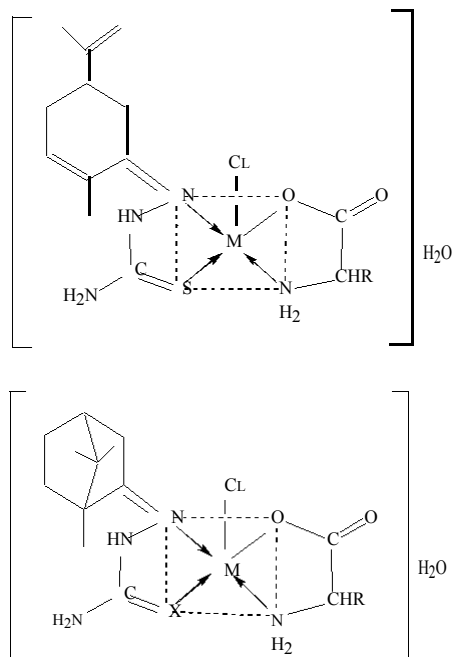
indicate their monomeric nature. The splitting patterns of mass spectra of compounds are shown in Table 4.

Table 4. Fragmented molecular ions vs m/z values of [CoCl(C₁₁H₁₇N₃S)(C₂H₅NO₂)]H₂O (1), [CuCl(C₁₁H₁₇N₃S)(C₂H₅NO₂)]H₂O (3) and [CuCl(C₁₁H₁₇N₃S)(C₃H₇NO₂)]H₂O (4).

Compound	Fragmented ions	m/z values
[CoCl(C ₁₁ H ₁₇ N ₃ S)(C ₂ H ₅ NO ₂)]H ₂ O (1)	[CoCl(C ₁₁ H ₁₇ N ₃ S)(C ₂ H ₅ NO ₂)]H ₂ O	411
	[CoCl(C ₁₁ H ₂₄ N ₄ SO)] ⁺	354
	[Co(C ₉ H ₂₄ N ₃ SO)] ⁺	281
	[Co(C ₇ H ₂₃ N ₃ SO)] ⁺	256
	[Co(C ₇ H ₂₂ N ₃ S)] ⁺	239
	[Co(C ₇ H ₂₁ N ₂ S)] ⁺	224
	[Co(C ₇ H ₂₀ N ₂ S)] ⁺	223
	[Co(C ₇ H ₁₈ NS)] ⁺	207
	[Co(C ₆ H ₁₃ NS)] ⁺	190
	[Co(C ₃ H ₇ NS)] ⁺	148
	[Co(C ₂ H ₄ NS)] ⁺	133
	[Co(H ₃ NS)] ⁺	108
	[Co(H ₂ NS)] ⁺	107
	[(CoS)] ⁺	91
	[CuCl(C ₁₁ H ₁₇ N ₃ S)(C ₂ H ₅ NO ₂)]H ₂ O (3)	[CuCl(C ₁₁ H ₁₇ N ₃ S)(C ₂ H ₅ NO ₂)]H ₂ O
[CuCl(C ₁₁ H ₁₇ N ₃ S)(C ₂ H ₅ NO ₂)HO] ⁺		414.41
[CuCl(C ₁₁ H ₁₇ N ₃ S)(C ₂ H ₅ NO ₃)] ⁺		413.40
[CuCl(C ₁₁ H ₁₇ N ₃ S)(C ₂ H ₃ NO ₃)] ⁺		411.38
[CuCl(C ₁₁ H ₁₅ N ₃ S)(NO ₂)] ⁺		366.32
[CuCl(C ₁₀ H ₁₅ N ₃ S)(NO ₂)] ⁺		354.30
[CuCl(C ₁₀ H ₁₄ N ₃ S)(NO ₂)] ⁺		353.29
[CuCl(C ₁₀ H ₁₁ N ₃ S)] ⁺		304.29
[CuCl(C ₁₀ H ₅ N ₃ S)] ⁺		298.23
[CuCl(C ₁₀ H ₄ N ₃ S)] ⁺		297.22
[CuCl(C ₈ H ₂ N ₂)] ⁺		225.10
[CuCl(C ₈ H ₂ N ₂)] ⁺		224.10
[CuCl(C ₁₁ H ₁₇ N ₃ S)(C ₃ H ₇ NO ₂)]H ₂ O (4)		[CuCl(C ₁₁ H ₁₇ N ₃ S)(C ₃ H ₇ NO ₂)]
	H ₂ O	
	[CuCl(C ₁₁ H ₁₇ N ₃ S)(C ₃ H ₇ O ₂)]	414.44
	HO] ⁺	
	[CuCl(C ₁₁ H ₁₇ N ₃ S)(C ₃ H ₇ O ₃)] ⁺	413.43
	[CuCl(C ₁₁ H ₁₇ N ₃ S)(C ₃ H ₅ O ₃)] ⁺	411.41
	[CuCl(C ₁₁ H ₁₇ N ₃ S)(C ₃ HO ₂)] ⁺	391.38
	[CuCl(C ₁₁ H ₁₇ N ₃ S)(CO ₂)] ⁺	366.35
	[CuCl(C ₁₁ H ₁₇ N ₃ S)(O ₂)] ⁺	354.34
	[CuCl(C ₁₁ H ₁₇ N ₂ S)(O ₂)] ⁺	340.33
	[CuCl(C ₁₀ H ₁₆ N ₂ S)(O ₂)] ⁺	327.31
	[CuCl(C ₁₀ H ₁₅ S)(O ₂)] ⁺	298.29
	[CuCl(C ₁₀ H ₁₄ S)(O ₂)] ⁺	297.28
	[CuCl(C ₁₀ H ₁₂ S)(O)] ⁺	279.27
	[CuCl(C ₁₀ H ₁₀ S)] ⁺	261.26
	[CuCl(C ₁₀ H ₉ S)] ⁺	260.25
	[Cu(C ₁₀ H ₈ S)] ⁺	224.80

The molecular ion peak of **(1)** appears at m/z 411.0, thus confirming the formation of a metal complex in 1:1:1 ratio. Appearance of some molecular ion peaks at higher m/z than molecular ion peak in the FAB mass

spectra may be due to re-association of fragments. On the basis of above spectral data, the five-coordinated geometry has been suggested for these complexes (Figure 4).



Where M= Co(II), Cu(II); X = O, S; R = H, CH₃
Figure 4. Proposed structural formula for the complexes [MCl(LH)(A)]H₂O

Table 5. Minimum inhibitory concentration (mg/ml) for ligands and mixed ligand Co(II) and Cu(II) complexes

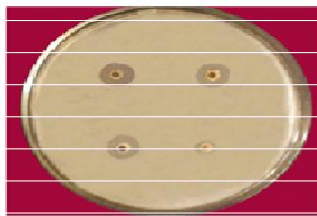
Compound	Antibacterial activity			Antifungal activity	
	<i>S. aureus</i>	<i>P. vulgaris</i>	<i>E. coli</i>	<i>A. niger</i>	<i>C. albicans</i>
L ₁ H	0.42	0.83	0.69	1.80	1.06
L ₂ H	0.82	0.78	0.44	1.29	0.99
L ₃ H	0.46	0.79	0.47	1.09	1.08
A ₁ H	0.52	0.62	0.54	0.60	0.42
A ₂ H	0.59	0.81	0.71	0.56	0.51
(1)	0.46	0.23	0.23	0.97	0.38
(2)	0.20	0.61	0.19	1.28	0.44
(3)	0.32	0.46	0.40	0.98	0.94
(4)	0.49	0.48	0.45	1.16	1.22
(6)	0.20	0.58	0.25	1.29	0.41
(7)	0.22	0.22	0.34	1.40	0.49
(9)	0.49	0.88	0.52	1.52	1.98
(10)	0.24	0.21	0.22	1.47	0.48
(11)	0.84	0.77	0.49	1.49	1.18
(12)	0.19	0.67	0.21	1.76	0.48

Antimicrobial activity

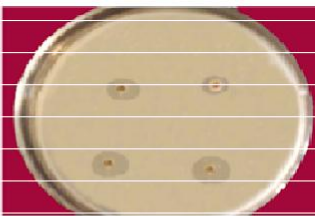
The ligands and mixed ligand complexes were screened for their antibacterial activity against *S. aureus*, *P. vulgaris* and *E. coli* and for antifungal activity against *A. niger* and *C. albicans*. The compounds were tested at different concentrations and zone of inhibition have been measured in mm. The antibacterial and antifungal activity results, presented in Tables S1 and

S2 respectively. The minimum inhibitory concentrations of all the compounds have been given in Table 5.

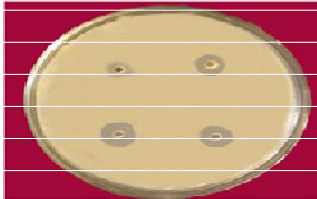
The results show that the mixed ligand complexes are more active than their parent ligands against the same microorganism. The increase in the antimicrobial activity of the mixed-ligand complexes may be due to the effect of the metal ion on the normal cell processes. A possible mode for the activity increase may be considered in light of Tweedy's chelation theory



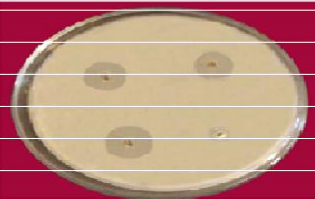
Antibacterial activity of IPMCHTSC against *E. coli*



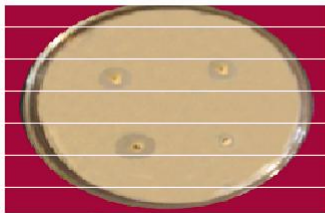
Antibacterial activity of TBHTSC against *E. coli*



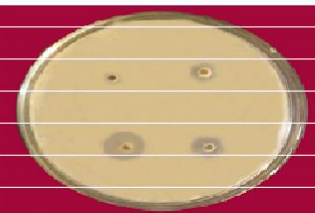
Antibacterial activity of TBHSC against *S. aureus*



Antibacterial activity of TBHSC against *E. coli*

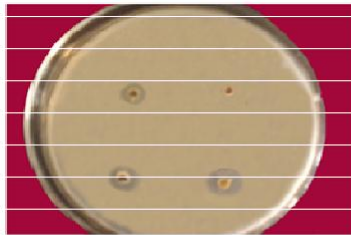


Antibacterial activity of [CoCl (TBHTSC) (Gly)]H₂O against *S. aureus*

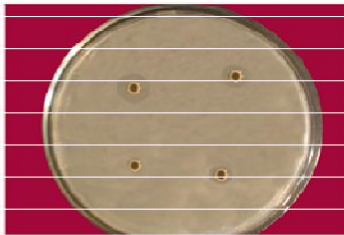


Antibacterial activity of [CoCl (TBHTSC) (Gly)]H₂O against *E. coli*

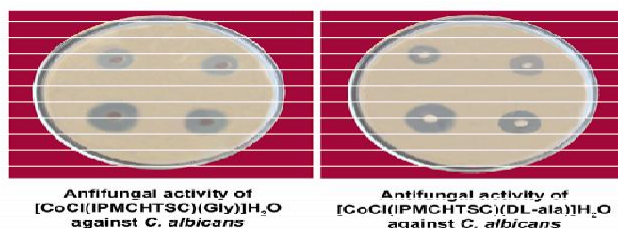
Antibacterial activity of newly synthesized compounds against *S. aureus*, *P. vulgaris*, *E. coli* after 24 hrs at different concentrations



Antifungal activity of IPMCHTSC against *A. niger*



Antifungal activity of IPMCHTSC against *C. albicans*



Antifungal activity of newly synthesized compounds against *A. niger*, *C. albicans* after 48 hrs at different concentrations

(Tweedy, 1964). Chelation considerably reduced the polarity of the metal ion because of the partial sharing of its positive charge with the donor groups and the π -electron delocalization over the whole chelate ring. Such chelation could enhance the lipophilic character of the central metal atom, which subsequently favours its permeation through the lipid layer of the cell membrane. All the mixed ligand complexes showed enhanced antimicrobial activity than the parent ligands.

As shown in table 5, the MIC value of $[\text{CoCl}(\text{C}_{11}\text{H}_{17}\text{N}_3\text{S})(\text{C}_3\text{H}_7\text{NO}_2)]\text{H}_2\text{O}$ (**2**) was observed against *E. coli* at a concentration of 0.19 mg/ml, where no bacterial growth was observed. In case of *P. vulgaris*, the growth of this organism was not observed at a concentration 0.21 mg/ml, indicating that the MIC value of compound $[\text{CoCl}(\text{C}_{11}\text{H}_{19}\text{N}_3\text{S})(\text{C}_3\text{H}_7\text{NO}_2)]\text{H}_2\text{O}$ (**10**) was 0.21 mg/ml against *P. vulgaris*. The MIC value of compound $[\text{CuCl}(\text{C}_{11}\text{H}_{19}\text{N}_3\text{S})(\text{C}_3\text{H}_7\text{NO}_2)]\text{H}_2\text{O}$ (**12**) against *S. aureus* at a concentration 0.19 mg/ml, where no bacterial growth was observed, so the MIC value of compound (**12**) was 0.19 mg/ml against *S. aureus*.

In case of fungal strains, *A. niger* and *C. albicans*, the growth, the growth of these organisms was not observed at concentrations 0.97 and 0.38 mg/ml, respectively, indicating the MIC value of compound $[\text{CoCl}(\text{C}_{11}\text{H}_{17}\text{N}_3\text{S})(\text{C}_2\text{H}_5\text{NO}_2)]\text{H}_2\text{O}$ (**1**) was 0.97 and 0.38 mg/ml against *A. niger* and *C. albicans*, respectively.

CONCLUSION

The mixed ligand Co (II) and Cu (II) complexes isolated during the present study demonstrated that the interaction of metal (II) chloride with thiosemicarbazone/semicarbazone of carvone or camphor and amino acids leads to complexes with 1:1:1 stoichiometry and are found to be mononuclear. The bidentate nature of both type of ligands have been suggested on the basis of spectral evidences. Although there is a sufficient increase in the antibacterial and antifungal activities of the mixed-ligand complexes as compared to the free ligands, they cannot attain the effectiveness of the conventional bacteriocides (ampiciline and tetracycline) and fungicide (fluconazole).

The results show that the mixed ligand complexes of

carvone and camphor thiosemicarbazones have been found to be more active than the corresponding semicarbazones derivatives. The greater activity in the former case may be due to $-\text{N}=\text{C}=\text{S}$ group. The results of antimicrobial activity of mixed ligand complexes, indicates that the Co(II) complexes are more active than the corresponding Cu(II) complexes (Table 5).

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REFERENCES

- Abdel-Rahman LH (2001). N-Phthaloylglycinate ternary complexes with cobalt (II), nickel(II), copper (II), zinc (II), and cadmium (II), metal ions and aromatic mono, bi and tridentate amines. *Trans. Met. Chem.* 26: 412-416.
- Adhikary B, Lucas CR (1994). Copper (II) Complexes of N_2S_3 Ligands Involving Aromatic Nitrogen and Thioether Donors and Having High Redox Potentials. *Inorg. Chem.* 33: 1376-1381. (b)
- Bhattacharyya S, Kumar SB, Dutta SK, Tiekink Edward RT, Chaudhary M (1996). Zinc (II) and Copper (II) Complexes of Pentacoordinating (N_4S) Ligands with Flexible Pyrazolyl Arms: Syntheses, Structure, Redox and Spectroscopic Properties. *Inorg. Chem.* 35(7): 1967-1973.
- Albertin G, Bordigon E, Orio AA (1975). Five-coordinate copper(II) complexes. Synthesis and properties of $[\text{Cu}(\text{tren})\text{L}]^{2+}$ cations. *Inorg. Chem.* 14: 1411-1413.
- AliMA, Chowdhary DA, Naziruddin M (1984). Four- and Five-coordinate Copper (II) complexes containing mixed ligands. *Polyhedron* 3(5): 595-598.
- Aull JL, Daron HH, Friedman ME, Melius P (1980). Interactions of anticancer drugs with enzymes. *Metal Ions Biol. Syst.* (H. Sigel, Ed.), 11: 337-377. Marcel Dekker, New York.
- Bauer AW, Kirby WMM, Sherris JC, Turk M (1966). Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol.* 45: 493-496.
- Berthon G, Blais MJ, Piktas M, Hougbossa K (1984). Trace metal requirements in total parenteral nutrition (TPN): 5. Formation constants for the copper (II)-histidine ternary complexes with threonine, lysine, glycine, phenylalanine, valine, and cystine, and discussion of their implications regarding the copper distribution in blood plasma during TPN and the evaluation of the daily dose of copper. *J. Inorg. Biochem.* 20: 113-130. (b)
- Shivankar VS, Vaidya RB, Dharwadkar SR, Thakkar NV (2003).

- Synthesis, Characterization, and Biological Activity of Mixed Ligand Co (II) Complexes of 8-Hydroxyquinoline and Some Amino Acids. *Synth. React. Inorg. Met.-Org. and Nano-Met. Chem.* 33: 1597-1622. (c)
- Adkhis A, Benali-Baitich O, Khan MA, Bouet G (2000). Synthesis, Characterization and Thermal Behaviour of Mixed-Ligand Complexes of Cobalt (III) with Dimethylglyoxime and Some Amino Acids. *Synth. React. Inorg. Met.-Org. and Nano-Met. Chem.* 30(10): 1849-1858..
- Beyer WG (1986). Manganese in metabolism and enzyme function. Academic Press, New York.
- Bindu P, Kurup MRP, Satyakeerty TR (1998). EPR, cyclic voltametric and biological activities of copper (II) complexes of salicylaldehyde N(4)-substituted thiosemicarbazone and heterocyclic bases. *Polyhedron* 18(3-4): 321-331.
- Brousse BN, Moglioni AG, Alho MM, Álvarez-Larena Á, Moltrasio GY, D'Accorso NB (2002). Behavior of thiosemicarbazones derived from some terpenes under acetylating conditions. *ARKIVOC X* pp 14-23.
- Çakir S, Biçer E, Çakir O (2000). Binary and ternary complexes of Cu (II) ions with saccharin and cysteine. *Electrochem. Comm.* 2: 124-129.
- Das S, Srivastava MN (1990). Mixed ligand complexes of copper (II), nickel (II), cobalt (II) and zinc(II) with nitrilotriacetic acid and iminodiacetic acid as primary ligands and 2-aminophenol as secondary ligand. *Indian J. Chem.* 29A: 707-709.
- Ferrari MB, Bisceglie F, Pelosi G, Sassi M, Tarasconi P, Cornia M, Capacchi S, Albertini R, Pinelli S (2002). Synthesis, characterization and X-ray structures of new antiproliferative and proapoptotic natural aldehyde thiosemicarbazones and their nickel (II) and copper(II) complexes. *J. Inorg. Biochem.* 90: 113-126.
- Freeman HC (1973). Metal complexes of amino acid and peptides. *Inorg. Biochem.* Eichhorn GL (ed). Elsevier, Amsterdam, The Netherlands, 121-166.
- Hinojosa M, Ortiz R, Perallo L, Borrás J (1987). Ternary complexes of Cu (II) ion with cimetidine and L-alanine. *J. Inorg. Biochem.* 29: 119-129.
- Hollis LS, Amundsen AR, Stern EW (1989). Chemical and biological properties of a new series of cis-diammineplatinum (II) antitumor agents containing three nitrogen donors: cis-[Pt(NH₃)₂(N-donor)Cl]⁺. *J. Med. Chem.* 32: 128-136.
- Hossain ME, Alam MN, Begum J, Akbar Ali M, Nazimuddin M, Smith FE, Hynes RC (1996). The preparation, characterization, crystal structure and biological activities of some copper(II) complexes of the 2-benzoylpyridine Schiff bases of S-methyl- and S-benzylthiocarbamate. *Inorg. Chim. Acta* 249 (2): 207-213.
- Hughes MN (1987). Coordination compounds in biology. *Comprehensive Coord. Chem.* 6: 541-754.
- Jin VX, Ranford JD (2000). Complexes of platinum(II) or palladium(II) with 1,10-phenanthroline and amino acids. *Inorg. Chimica. Acta.* 304 : 38-44.
- Kiss T, Gergely A (1985). Copper(II) and nickel(II) ternary complexes of L-dopa and related compounds. *J. Inorg. Biochem.* 25: 247-259.
- Kumer L, Kandasamy NR, Srivastava TS, Amonkar AJ, Adwankar MK, Chitrus MP (1985). Synthesis and spectroscopic studies of potential anticancer [platinum(II)(2,2'- bipyridine)(amino acid)]ⁿ⁺ (n = 1 or 2) complexes. *J. Inorg. Biochem.* 23: 1-11.
- Lever ABP (1984). *Inorganic Electronic Spectroscopy* (2nd ed.). Elsevier, in Amsterdam, New York.
- Manjula V, Chakraborty D, Bhattacharya K (1990). Ternary complexes of Cu(II) involving histidine and another amino acid or dipeptide. *Indian J. Chem.* 29A: 577.
- McLachlan GA, Fallon GD, Martin RL, Spiccia L (1995). Synthesis, Structure and Properties of Five-Coordinate Copper(II) Complexes of Pentadentate Ligands with Pyridyl Pendant Arms. *Inorg. Chem.* 34: 254-261.
- Nagar M, Rawat, S, Sharma R, Sharma H, Agarwal M (2007). Synthesis, characterization and antifungal activity of mixed ligand complexes of Co^{II}, Ni^{II} and Cu^{II} with cis-3,7-dimethyl-2,6-octadienemicarbazone and amino acids. *J. Ind. Chem. Soc.* 84: 341-345.
- Nakamoto K (1986). Lattice water and aquo and hydroxo complexes. In *Infrared and Raman Spectra of Inorganic and Coordination Compounds* (4th ed.). John-Wiley and Sons: New York, pp 227-231.
- Nakamoto K, Morimoto Y, Martell AE (1961). Infrared spectra of aqueous solutions. I. Metal chelate compounds of amino acids. *J. Am. Chem. Soc.* 83: 4528-4532; (b)
- Hamrit H, Djebbar-Sid S, Benali-Baitich O, Khan MA, Bouet G (2000). Potentiometric studies, synthesis, and characterization of mixed ligand complexes of copper(II), nickel(II), cobalt(II), and manganese(II) with N-(2-acetamidoiminodiacetic) acid as the primary ligand and histidine as the secondary one. *Synth. React. Inorg. Met.-Org. Chem.* 30 (10): 1835-1848.
- Nandi AK, Chaudhri S, Mazumdar SK, Gosh S (1984). Effect of chlorine substitution on the structure and activity of 4-phenylthiosemicarbazide: crystal and molecular structure of 4-(4-chlorophenyl) thiosemicarbazide. *J. Chem. Soc. Perkin Trans. 2* (11): 1729-1733.
- Padhye SB, Kauffman GB (1985). Transition metal complexes of semicarbazones and thiosemicarbazones. *Coord. Chem. Rev.* 63: 127-160.
- Parez C, Paul M, Bazerque P (1990). Antibiotic assay by Agar well diffusion method. *Acta Biol. Med. Exp.* 15: 113-115.
- Roy R, Paul P, Nag K (1984). Metal complexes of sulfur-nitrogen chelating agents. Part 12. The chemistry of nickel (II), palladium(II), cobalt(II) and copper(II) complexes of N₂S₂ and N₃S₂ donor systems. *Trans. Met. Chem.* 9: 152-155.
- Scovill JP, Klayman DL, Franchino CF (1982). 2-acetylpyridine thiosemicarbazones complexes with transition metals as antimalarial and antileukemic agents. *J. Med. Chem.* 25 (10): 1261-1264.
- Sharma R, Agarwal SK, Rawat S, Nagar M (2006). Synthesis, characterization and antibacterial activity of some transition metal cis-3,7-dimethyl-2,6-octadienemicarbazone complexes. *Trans. Met. Chem.* 31: 201-206.
- Sharma R, Bansal AK, Nagar M (2005). Transition metal complexes of cis-3,7-dimethyl-2,6-octadienethiosemicarbazone: Synthesis, characterization and biological studies. *Indian J. Chem.* 44A: 2255-2258.
- Sharma R, Nagar M (2006). Synthesis, structural and antibacterial studies of some mixed ligand complexes of Zn(II), Cd(II) and Hg(II) derived from citral thiosemicarbazone and N-phthaloyl amino acids. *Phosphorus, Sulfur Silicon* 181: 2863-2875.
- Sharma R, Nagar M, Agarwal M, Sharma H (2009). Synthesis, characterization and antimicrobial activities of some mixed ligand complexes of Co(II) with thiosemicarbazones and N-protected amino acids. *J. Enz. Inhib. Med. Chem.* 24(1): 197-204.
- Tümer M, Köksal H, Sener MK, Serin S (1999). Antimicrobial activity studies of the binuclear metal complexes derived from tridentate Schiff base ligands. *Trans. Met. Chem.* 24: 414-420.
- Tweedy BG (1964). Possible mechanism for reduction of elemental sulfur by *Monolina fructicola*. *Phytopath.* 55: 910-914.
- Van Dyck E, Smet H, Piot P (1994). Comparison of E test with agar dilution for antimicrobial susceptibility testing of *Neisseria gonorrhoeae*. *J. Clin. Microbiol.* 32: 1586-1588.
- Vogel AI (1989). A text book of quantitative inorganic analysis (5th ed.). London, ELBS: Longman.
- West DX, Padhye SB, Sonawane PB, Chikate RC (1990). Copper (II) complexes of tridentate (O, N, S) thiosemicarbazones. *Asian J. Chem. Rev.* 4(1): 125-137.
- West DX, Padhye SB, Sonawane PB, Chikate RC (1991). Structural and physical correlation in the biological properties of transition metal N-heterocyclic thiosemicarbazones and S-alkyldithiocarbamate complexes. *Structure and bonding* 76: 1-50.
- Wong E, Giandomenico CM (1999). Current Status of Platinum-Based Antitumor Drugs. *Chem. Rev.* 99: 2451-2466.