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Synthesis, Thermal and Biological Screening of Six co-Ordinate Complex and Heterocyclic Base Adducts of Co (II) derived from N(4) Thiosemicarbazone

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ABSTRACT:Cobalt (II) complex has been synthesized by refluxing $CoCl_2.6H_2O$ with 5-chloro-2-hydroxy acetophenoneN(4) methyl thiosemicarbazone.Adducts were synthesized by refluxing $CoCl_2.6H_2O$,thioemicarbazone in presence of heterocyclic base like pyridine, 2-chloro pyridine,3-chloro pyridine,4-chloro pyridine. Thiosemcarbazone has been characterized by ¹³C-NMR, ¹H NMR,ESI-MS as well as IR, electronic spectra. Octahedral geometry for the six coordinate complex and adducts has been predicted from magnetic and spectral data. The thiosemicarbazone and its Co (II) complexes have been found antibacterial and showed growth inhibitory activity against *Staphylococcus aureu,Bacilus subtilis(Gram+ve), Escherichia Coli,Peudomonas aeruginosa (Gram-ve)* bacterial species.

KEYWORDS: Thiosemicarbazone, N(4) methyl thiosemicarbazone, CoCl₂.6H2O.

I.INTRODUCTION

Thiosemicarbazone ligand loses a proton from the phenolic group and acts as a singly charged tridentate ligand, coordinating through oxygen, the imino-nitrogen and thione sulphur atom. It acts as a doubly negatively charged tridentate by losing proton from mercapto group. Heterocyclic thiosemicarbazones are the compounds having wide spectrum of medicinal properties, antibacterial activity ,tuberculosis ,leprosy ,malaria [1-5]. Thiosemicarbazones have also been investigated for superoxide dismutase-like radical scavengers [6]. The transition metal complexes of ligands containing N/S donor centers constitute the active centers of several metalloenzymes such as hydrogenase,xanthine oxidase and nitrogenase [7].

Co(III) complexes showed wide range of antimicrobial activities. Co(III) complex of untiulcer drug famotidine turned have greater antimicrobial activity against *E. coli* and *M. lysodeikticus* than the metal free drug [8]. The octahedral cobalt complex possessed good activity against both Gram (+) and Gram(-) bacterial strains but not higher than the free ligand [9]. The Co (II) complex of the sodium salt of Monensin A was found active against the Gram(+) bacteria. The mole ratio (M:L) in the complex was 1:2. The cobalt(II) complex was found inactive against Gram(-) bacteria.this might be because of its hydrophobicity and large molecular weight [10]. Cobalt(III) complexes are been hypoxia selective antitumor agents . This concept is based on the the tumor cells develop resistance to chemotherapeutic agents under anaerobic conditions. In hypoxic conditions they may be reduced to Co (II) species by loss of neutral ligand [11-14].

In this work we report the synthesis, spectral characterisation and biological studies of six coordinate complex and adducts of Co (II) with 3,5-dichloro 2-hydroxy acetophenone N(4) methyl thisemicarbazone.

II.MATERIALS AND METHODS

All the chemical used are of A.R.grade.Magnetic measurement was carried out by Faraday method. IR spectra were recorded in the range 4000-200 cm⁻¹ using Jasco spectrometer. NMR spectra were recorded in the mixture of CDCl₃ and DMSO-d₆ (1:1 v/v) with a Bruker AC-300F 300MHz spectrometer. Conductivity measurements were carried out on Systonics conductivity meter-304. UV-Visible spectra were measured on Jasco UV-visible double beam spectrophotometer.Metal in the complex and adducts was estimated by E.D.T.A using xylenol orange as an indicator.



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III.EXPERIMENTAL

Synthesis of ligand:

Thiosemicarbazone was synthesized by refluxing ethanolic solutions of 5-chloro 2-hydroxy acetophenone and N(4)methyl thiosemicarbazide in the mole ratio 1:1 for 3 hours. The product obtained was filtered , washed with cold ethanol and then diethyl ether. Recrystalised from acetic acid and dried over P2O5 in vacuum.



Synthesis of complex

CoCl₂.6H₂O (0.01 mole) was dissolved in absolute ethanol. The ethanolic solution of thiosemicarbazone (0.01 mole) in slight excess over the metal: ligand ratio of 1:1 was added to it dropwise and with constant stirring. The rection mixture was refluxed for an hour, after addition of one gram of sodium acetate. The brown product was filtered and washed with a small portion of hot water, cold ethanol and then ether and dried over P_2O_5 in vacuum.



Synthesis of adducts

The adduct Co.L.B (B-heterocyclic base like pyridine, 2-chloro pyridine, 3-chloro pyridine, 4-chloro pyridine) was synthesized by adding slowly ehanolic solutions of CoCl₂.6H₂O,heterocyclic base to the hot ethanolic solution of thiosemicarbazone in the ratio 1:1:1 and refluxing reaction mixture for 7 hours. The adduct obtained was filtered, washed with hot water, cold ethanol and then ether and dried over P2O5 in vacuum .



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(B = pyridine, 2-chloro pyridine, 3-chloro pyridine, 4-chloro pyridine)

TABLE 1

Compounds	Colour	Empirical Formula	Molar conductance	Magnetic Moment B.M.	
		Ohm ⁻¹ cm ² mole ⁻			
L	Yellow	C ₁₀ H ₁₁ N ₃ OSCl ₂	-	-	
Co-L.(H ₂ O) ₃	Brown	$C_{10}H_{15}N_3O_4SCl_2Co$	50.7	4.50	
Co-L.Py.(H ₂ O) ₂	Brown	$C_{15}H_{18}N_4O_3SCl_2Co$	52.5	4.52	
Co- 2-Cl py.(H ₂ O) ₂	Brown	$C_{15}H_{17}N_4O_3SCl_3Co$	55.9	4.55	
Co- 3-Cl py.(H ₂ O) ₂	Brown	C ₁₅ H ₁₇ N ₄ O ₃ SCl ₃ Co	57.6	4.59	
Co- 4-Cl py.(H ₂ O) ₂	Brown	$C_{15}H_{17}N_4O_3SCl_3Co$	59.7	4.60	

The colours, empirical formula of ligand and its complexes are presented in Table 1. The complex and all adducts are soluble in DMF in which conductivity measurements were made $(30^{\circ}C)$, showing all complexes to be non electrolyte [15]. The magnetic susceptibility of complex and adducts carried out at room temperature $(27^{\circ}C)$ fall in the range of 4.50-4.60 B.M (Table 1). The high spin octahedral complexes show magnetic moment in the range 4.50-4.65 B.M. respectively [16].



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¹H-NMR

Signals at 11.6, 3.30 ppm are assigned to – OH, - CH₃ protons respectively.

¹H-NMR signals at 12.00 and 2.4 ppm are assigned to - OH and - CH₃ protons respectively. The signals at 2.30, 3.01 correspond to ⁴NH and H⁴N-CH₃ respectively. Signal at 10.5 ppm corresponds to ²NH. Aromatic protons show multiplets at 7.0, 7.20, 7.35, ppm.

¹³C-NMR (DMSO-D₆: оррт

125.20 (C = C-Cl), 128.75 (C = C), 127.79 (C = C - Cl), 126.70 (C = C), 129.10 (C = C), 151.20 (C = C - OH), 154.37(C = N), 175.85 (C = S), 35.10 (NH - CH₃).



 $(\textbf{Calcd}) \textbf{ found ESI-MS m/z, ion } M^+ : C_{10}H_{11} N_3 OSCl_2 (292.17) 292.89, C_{10}H_{15}N_3 O_4 SCl_2 Co (403.13) 403.78, \\ C_{15}H_{18}N_4 O_3 SCl_2 Co (464.21) 464.70, C_{15}H_{17}N_4 O_3 SCl_3 Co (498.65) 498.20, C_{15}H_{17}N_4 O_3 SCl_3 Co (498.65) 498.22, \\ C_{15}H_{17}N_4 O_3 SCl_3 Co (498.65) 498.24. \\$

Mass spectral data confirmed the structures of ligand, complex and adducts as indicated by molecular ion peak (M+1) corresponding to their molecular weights.

Compounds	Elemental Analysis Found (Calculated) %								
	Metal%	%C	%H	%N	%S				
L	-	41.74	3.13	14.88	10.21				
		(41.11)	(3.79)	(14.38)	(10.97)				
Co-L.(H ₂ O) ₃	14.07	29.13	3.24	10.74	7.08				
	(14.62)	(29.79)	(3.75)	(10.42)	(7.95)				
$Co-L.Py.(H_2O)_2$	12.30	38.21	3.32	12.87	6.30				
	(12.69)	(38.81)	(3.91)	(12.07)	(6.91)				
Co- 2-Cl py.(H ₂ O) ₂	11.82	36.73	3.84	11.84	6.94				
	(11.03)	(36.13)	(3.44)	(11.24)	(6.43)				

TABLE.2 ANALYTICAL DATA



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Co- 3-Cl py.(H ₂ O) ₂	11.77	36.52	3.55	11.72	6.06
	(11.03)	(36.13)	(3.44)	(11.24)	(6.43)
Co- 4-Cl py. $(H_2O)_2$	11.88	36.15	3.88	11.88	6.77
	(11.03)	(36.13)	(3.44)	(11.24)	(6.43)

Elemental analysis data are consistent with 1:1 ratio of metal ion, thiosemicarbazone for complex and 1:1:1 ratio for metal, thiosemicarbazone and heterocyclic base for all adducts.Metal in the complexes was estimated by using E.D.T.A.

IV.ELECTRONIC SPECTRAL DATA

Compound	Mode	d-d	L→M	n→π*	$\pi \rightarrow \pi^*$
L	DMF	-	-	25652	41865
				28500	
Co-L.(H ₂ O) ₃	DMF	17554	27155	30855	44800
			25241	35405	
$Co-L.Py.(H_2O)_2$	DMF	17744	27627	31106	44500
			25600	34450	
Co- 2-Cl py.(H ₂ O) ₂	DMF	17255	27041	30980	42553
			25989	34650	
Co- 3-Cl py.(H ₂ O) ₂	DMF	17634	27907	31110	43475
			25585	34260	
Co- 4-Cl py.(H ₂ O) ₂	DMF	17938	27500	31650	44660
			25727	34055	

Table 3 .Electronic spectral data (cm⁻¹)

The Co (II) complexes are usually found in tetrahedral and octahedral environments and less frequently in planer environment. The ground term of Co (II) is ${}^{4}T_{1g}$ or ${}^{4}Eg$ in octahedral coordination depending on whether the complex is high spin or low spin. Three bands have been observed due to spin allowed transitions in the range 30,000-32,000 cm⁻¹ ${}^{1}34,000-36,000$ cm⁻¹ and 17,000-18,000 cm⁻¹ which correspond to ${}^{4}T_{1g}$ (F) $\rightarrow {}^{4}T_{2g}$ (F) (v₁), ${}^{4}T_{1g}$ (F) $\rightarrow {}^{4}A_{2g}$ (F) (v₂) and ${}^{4}T_{1g}$ (F) $\rightarrow {}^{4}T_{1g}$ (P) (v₃) respectively expected for d⁷ system in octahedral field. The absorption bands at 25,000 – 28,000 cm⁻¹ range are assigned to the L \rightarrow M transitions respectively. The appearance of these bands indicated octahedral geometry around Co (II) [17].

V. INFRARED SPECTROSCOPIC DATA (cm⁻¹)

1.L: v (- OH) 3200; v (C = N) 1642; v (- C - S) 790 (s), 1365 (m); v (N - N) 1060; v (²N-H) 3250; v (C - O) 1295.

2.[CoL(H₂O)₃]: v (C = N) 1575; v (C = N-N=C) 1520, v (C-S) 650, 1250,v (N-N) 1130, v (M - N) 420, v (M-O) 505, v (M-S) 330, v (C - O) 1201, v(H₂O) 3542,3560.

3.[Co-L.Py.(H₂O)₂]: v (C = N) 1580; v (C = N-N=C) 1525, v (C-S) 655, 1255; v (N-N) 1132, v (M - N) Base 270, v (M - N) 425, v (M - O) 510, v (M-S) 335, v (C - O) 1210, Band due to HB 1275, v(H₂O) 3522,3560.

4.[Co- 2-Cl py.(H₂O)₂]: v (C = N) 1585; v (C = N-N=C) 1515, v (C-S) 660, 1220, v (N-N) 1120, v (M - N) Base 278, v (M - N) 430, v (M - O) 520, v (M-S) 338, v (C - O) 1225, Band due to HB 1420, v(H₂O) 3550,3572.

5.[Co- 3-Cl py.(H_2O_2]: v (C = N) 1590; v (C = N-N=C) 1525, v (C-S) 665, 1235; v (N-N) 1128, v (M - N) Base 280, v (M - N) 435, v (M - O) 530, v (M-S) 340, v (C - O) 1240, Band due to HB 1450, v(H₂O) 3560,3570.

6.[Co- 4-Cl py.(H₂O)₂]: v (C = N) 1598; v (C = N-N=C) 1535, v (C-S) 670, 1240,v (N-N) 1130, v (M - N) Base 290, v (M - N) 440, v (M - O) 550, v (M-S) 350, v (C - O) 1270, Bands due to HB 1460, v(H₂O) 3570,3588.



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The infrared spectra of Co (II) complexes is useful to detect the bonding sites of all ligand molecules interacted with the metal. The coordination of azomethine nitrogen shifted v ($^{7}C = {}^{1}N$) to lower wave numbers. The band shifted from 1642 cm⁻¹ in uncomplexed thiosemicarbazone to about 1575-1598 cm⁻¹ in the spectra of complexes. The shifting of v (N-N) to higher wave numbers in the spectra of complexes confirms the coordination of azomethine nitrogen. The appearance of new band at 420 - 435 cm⁻¹ confirms the coordination of azomethine nitrogen [18]. The loss of ${}^{-2}N$ -H proton on coordination via thiolate sulphur decreases v (C = S) bands found at 790, 1365 cm⁻¹ in L. The new band at 330 - 350 cm⁻¹ is assignable to v (Co-S) [19]. The presence of new band in the range 505 - 550 is assignable to v (Co-O) [20]. The coordination of N atom (s) of heterocyclic base is confirmed by v (Co-N) band in 270-290 cm⁻¹ range [19,21-25]. The bands due to co-odinated water molecules and heterocyclic bases are also observed [25].

VI.TGA ANALYSIS DATA

The TGA curves of complexes were recorded between the temperatures 30 °C to 800 °C

- 1.[**Co.L.(H₂O)₃**]: First step, 115 °C, Mass loss 8.30 % second step, 138 °C, Mass loss, 13.54 % Third Step 240 °C, Mass loss, 58.02 % Fourth Step, 365.14 °C, Mass loss 65.5 %, Residue 800 °C, % of CoO, 14.23 (14.62).
- 2.[**CoL.py.(H₂O)**₂]: First step, 113 °C, Mass loss 7.70 % second step,140 °C, Mass loss, 25.70 % Third Step 248 °C, Mass loss, 55.02 % Fourth Step, 360 °C, Mass loss, 65.01 %, Residue, 790 °C, % of CoO, 12.10 (12.69).
- 3.[Co- 2-Cl py.(H₂O)₂]: First step, 115 °C, Mass loss 7.84 % second step, 145 °C, Mass loss, 29.00 % Third Step 245 °C, Mass loss, 56.02 % Fourth Step, 361 °C, Mass loss 68.5 %, Residue 800 °C, % of CoO, 15.74 (15.03).
- 4.[Co- 3-Cl py.(H₂O)₂]: First step, 117 °C, Mass loss 7.50 % second step, 142 °C, Mass loss, 27.52 % Third Step 250 °C, Mass loss, 57.02 % Fourth Step, 366 °C, Mass loss, 65 %, Residue, 778 °C, % of CoO, 14.70 (15.03).
- 5.[Co- 4-Cl py.(H₂O)₂]: First step, 116 °C, Mass loss 7.19 % second step, 150 °C, Mass loss, 28.00 % Third Step 238 °C, Mass loss, 58.02 % Fourth Step, 370 °C, Mass loss 66 %, Residue 800 °C, % of CoO, 15.87 (15.03).

The coordinated water molecules were eliminated from their complexes at relatively higher temperature than lattice water molecules. The two coordinated water molecules in complex and adducts were removed in one step at less than 120 °C The TGA data of complex and adducts indicated that the decomposition proceeded in several steps. There are three steps after the removal of water molecules in complex and adducts. First at a temperature less than 160 °C, second less than 250 °C and third less than 370 °C. In these steps decomposition of the complexes took place. The decomposition was complete and CoO formed at a temperature > 760 °C.

VII.BIOLOGICAL ACTIVITY (AGAR PLATE DIFFUSION METHOD) TABLE.4 MINIMUM INHIBITORY CONCENTRATION L , CO (II) COMPLEXES AND STANDERED

Compound	Staphylococ aureu	cus	Bacilus subtil		Escherichia Coli		Peudomonas aeruginosa	
	Gram positive				Gram negative			
	2	2.5	2	2.5	2	2.5	2	2.5
	µg/ml	µg/ml	µg/ml	µg/ml	µg/ml	µg/ml	µg/ml	µg/ml
L	0.75	0.42	0.72	0.45	0.77	0.48	0.78	0.47
Co-L.(H ₂ O) ₃	0.68	0.35	0.67	0.21	0.69	0.24	0.61	0.23



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Co-L.Py.(H ₂ O) ₂	0.62	0.34	0.63	0.25	0.63	0.22	0.63	0.26
Co-2-Cl.py.(H ₂ O) ₂	0.63	0.32	0.64	0.24	0.65	0.23	0.65	0.28
Co-3-Cl py.(H ₂ O) ₂	0.65	0.30	0.66	0.23	0.68	0.24	0.66	0.27
Co-4-Cl py.(H ₂ O) ₂	0.68	0.31	0.67	0.25	0.67	0.26	0.64	0.26
CoCl ₂ .6H ₂ O	0.22	0.18	0.23	0.17	0.12	0.24	0.12	0.25
Standard	0.16	0.12	0.17	0.15	0.28	0.13	0.19	0.15

(Std-Amphiciline)

The bacterial assay was carried out by the agar plate diffusion method. Activity was measured by measuring the diameter of inhibition zone in mm. The adducts showed good activity against bacterial species than free ligand. The minimum inhibitory concentration was determined by liquid dilution method [26]. The solutions of ligand and complexes with 2 μ g/ml,2.5 μ g/ml and 3 μ g/ml contrations were prepared in the solvent DMF. The solution of standard drug ampicilin was also prepared in the same concentration. Inoculums of the overnight culture were prepared.0.2 ml of the inoculums was added to the test tubes containing the solutions of the compounds of different concentrations. After the addition of sterile water to each of the test tubes, these were incubated for 24 hours and observed for turbidity. The absorbance of the turbid solutions was measured at 520 nm. The same method was used for standard [27]. It was found that at 2 μ g/ml the absorbance is more. Less absorbance was observed at 2.5 μ g/ml and no absorbance at 3 μ g/ml. The inhibition is more at 2.5 μ g/ml. The metal salt solution showed better inhibition than ligand and complexes. The inhibitory concentration is 2.5 μ g/ml. Thus increase in coordination number in complexes and coordination of metal ion to ligand increases microbial activity.

FIG NO.1 BAR GRAPH (MIC)



The bar graph indicates the minimum inhibitory concentration. The absorbance is less at 2.5µg/ml concentration. The metal salts are found more active than compounds. The inhibitory activity is more in case of standared. The absobances



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at 2µg/ml concentration are in the range0.62-0.68.Inhibition is more at 2.5 µg/ml against the species *Bacilus subtilis*, *Escherichia Coli*, *Peudomonas aeruginosa*.

VIII.CONCLUSION

The complexes and adducts synthesised are thermally stable upto 200^{0} C. The complexes are paramagnetic and the spectral data indicate octahedral geometry. The complexes have been found active against bacterial species. They showed growth inhibitory activity against the species. The minimum inhibitory concentration is 2.5μ g/ml. The activity is more against the species *Bacilus subtilis, Escherichia Coli, Peudomonas aeruginosa*.

EXPECTED STRUCTURES





(**B** = pyridine, 2- chloro pyridine, 3-chloro pyridine, 3-chloro pyridine)

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