



## SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITIES OF MERCAPTOBENZIMIDAZOLE, THIOSEMICARBAZIDE AND THIOCYANATE MIXED LIGANDS COBALT(II) COMPLEXES

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### ABSTRACT

Mixed ligand Cobalt(II) complexes with mercapto benzimidazole, thiosemicarbazide and thiocyanate have been synthesized and analyzed. Characterization by elemental analysis, conductivity, melting point, solubility, Uv-visible and IR-spectroscopic studies showed the formation of mono and di- nuclear complexes. Spectral studies results suggest a tetrahedral geometry for the cobalt (II) thiosemicarbazide complex and octahedral arrangement for the mercapto benzimidazole mixed ligand complexes. A dimerized structure was proposed for the mercaptobenzimidazole cobalt(II) complex. In these complexes, the metal ion coordinates to the ligand through the nitrogen atom. The thiocyanate ion attaches via the sulphur atom. The results of conductivity revealed that the complexes were all non –electrolytes. The metal complexes were screened for their antimicrobial activities using agar disc diffusion technique and the results revealed that they were active against both bacteria and fungi species tested.

**Keywords:** Cobalt complexes, mixed ligand, mercapto benzimidazole, thiosemicarbazide, antimicrobial activities.

### INTRODUCTION.

It has been known that metal ions are involved in biological processes of life and have been subject of interest (Bolos et al; 2002). The mode of action of these metal ions are often complex but are believed to involve bonding to the heteroatoms of the heterocyclic residues of biological molecules such as protein, enzymes, nucleic acids, and others. (Fadmideh *et al*; 2008). Complexes of cobalt have shown biological activities and these activities are attributed to their interactions with enzymes such as ribonucleotide reductase, the RNA – dependent DNA polymerase, the formation of oxygen active species and the reaction with cell thiols (Garcia *et al*; 2001). Cobalt complexes are structurally prototypical systems that are

biologically relevant in vitamin B12 function and biomedical applications of cancer therapy. A common synthetic form of the vitamin, cyanocobalamin, does not occur in nature, but is used in many pharmaceuticals, supplements and as food additive, due to its stability and lower cost (Jaouen *et al*, 2006). Ligands having oxygen and nitrogen as donor atoms are by far the most extensively studied. Interest in sulfur donor chelating agents has grown over the years and the number of chemical studies in the area has increased considerably. Interest in complexes of these ligand systems now covers several areas, ranging from general consideration of the effect of sulphur and electron delocalization in transition metal complexes to potential biological activity and

practical application (Sathisha *et al*; 2008). Mixed ligand complexes are also well known to play significant roles in biological system. Synthesis, structural studies and some other properties of the mixed ligand transition metal complexes formed with histine and adenine or guanine and other amino acids have been reported (Singh *et al*, 2000). Synthesis and characterization of mixed Co (II) complexes formed with histidine and N-(2-acetamidoiminodiacetic) acid have been studied. The synthesis and characterization of some bivalent simple and mixed ligand transition metal complexes of hippuric acid and nitrilotriacetic acid and iminodiacetic acid have been reported (Kumar, 2009)

Thiosemicarbazides and their complexes have been found to possess a wide variety of biological activities against bacteria, fungi and certain type of tumors (Aly, 2011). Some works have been done on the transition metal complexes of substituted thiosemicarbazides particularly the 1-aryl-4-aryl derivatives. 1,4-Dibenzyl-3-thiosemicarbazide contains oxygen, sulphur and nitrogen as potential donor atoms, and is liable to form deprotonated complexes by loss of hydrazinic proton(s) via enolisation or thioenolisation (Singh *et al*; 2001). Thiosemicarbazides are interesting because they form highly stable and intensely colored complexes which are used for spectrophotometric determination of metal ions in different media (West *et al*; 1993; Akl *et al*; 2006; Saad *et al*; 2007). They show catalytic activity (Hamada *et al*; 2006) and are potentially beneficial as antibacterial and anticancer agents (Seleem *et al*; 2005). Among the ligands attracting the largest interest for their role in binding metals in metalloproteins are the imidazole derivatives. Inspection of mercaptobenzimidazole indicates that it can exist in two tautomeric forms with potential metal binding sites between the N and S atoms (Ravikumar *et al*; 1995). The present work aims to synthesize and characterize complexes formed between mercaptobenzimidazole, thiosemicarbazide, thiocyanate and cobalt (II) salts and determine their anti fungal and antibacterial activities

## MATERIALS AND METHODS

### Materials

All reagents involved in the synthesis were of analytical grade and were used without further purification.

### Synthesis of metal complexes

Cobalt (II) complexes were synthesized by adding pink solution of 0.475g (2mmol.) of  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  in 10ml methanol to 0.182g (2mmol.) thiosemicarbazide in methanol to give a deep wine coloured solution. The mixture was agitated on a magnetic stirrer for 3 hours after which the mixture was allowed to stand overnight until a deep brown precipitate was formed. This was washed with distilled water followed by diethyl ether, the residue kept and dried over KOH in a dessicator. Similarly mercapto benzimidazole complexes were synthesized by reacting equimolar concentrations of cobalt (II) chloride and the ligand in methanol. Mixed ligand complexes were prepared by adding 2mmol potassium thiocyanate in 10ml methanol to the metal/ligand solutions in ratio 1:1:1.

### Characterization of metal complexes

Melting points of the complexes were determined in open-tube delivery apparatus, chloride analyses were carried out by Mohr methods, sulphate contents of the complexes were analysed by gravimetric method; and metal contents were determined via complexometric titration. The molar conductance of the solid complexes in dimethylformamide was measured by using a model WPACM35 conductivity meter, Solutions of the complexes were run on UV-2500PC UV- visible series (8000-200nm). Solid state FTIR spectra of the metal complexes were recorded on a Shimadzu FTIR spectrophotometer using KBr pellets in the range of  $400\text{-}4000\text{cm}^{-1}$ .

### Evaluation of antibacterial activity of the metal complexes

The bacteria used for this experiment included *Escheriochia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhimurium*. All bacteria were cultured in peptone water for 18-24hrs at  $37^\circ\text{C}$  and were seeded on the sterile nutrient agar (NA) plates containing 8mm wells. A concentration of each of the complexes

(0.02g/mL) was prepared and 0.5ml of each sample was introduced into the bore agar well and incubated for 24hrs at 37°C. Control plate was also set up using standard Streptomycin sulphate at 0.02g/mL. Zones of inhibition around the wells were measured and results were quoted as the diameter (mm) of the zones of inhibition around the wells (Onifade, 1998).

**Evaluation of antifungal activity of the metal complexes**

The test fungi for this experiment were *Fusarium solani* and *Cercospora cucurbitarium*. solution of each metal complex (0.02g/ml) were prepared and 2ml aseptically mixed with 15ml of sterile molten potato dextrose agar (PDA) that had been cooled to 45°C before pour plating and allowed to solidify at ambient temperature. The fungi were inoculated at the centre of the plates with the aid of sterile 4mm cork borer. Benlate was used as control. All plates were incubated at 27°C for 144 hours, while mycelia growth was measured at 24 hours interval (Mistra *et al*; 1995).

**RESULTS AND DISCUSSION**

Metal complexes of thiosemicarbazide, mercaptobenzimidazole with cobalt (II) were obtained in powdery and crystalline forms. They were all stable to air all soluble in dimethyl formamide. The elemental analyses of the complexes presented in table 1 showed different stoichiometry for the isolated complexes. The

sharp melting points achieved within 172-392°C show that the complexes are pure

The reaction of cobalt (II) chloride with thiosemicarbazide at an equimolar ratio in methanol, gave a brown coloured complex in 18% yield. This complex assumes a tetrahedral arrangement based on the results of elemental analysis and the electron spectra analysis as presented in table II. Reaction mechanism for this complex indicates coordination through dative bonding and the elimination of two molecules of water.



From the proposed structure for this complex (figure 1) it is obvious that bonding of the ligand to the central metal is through the sulfur atom.

Uv-Visible spectra of the metal complex in dimethylformaldehyde in the range of 400-800nm, showed characteristic bands indicating that all the cobalt (II) complexes absorbed in the visible region showing the presence of chromophore -C≡N of larger wavelength, since the longer the conjugated system, the longer the wavelength of the absorption maxima (dudley *et al*;1987). The absorption bands found for all the cobalt (II) complexes in the visible regions (400-800nm) showed promotion of the non-bonding electron in a π-bond to a higher energy level and an indication of an extended conjugation system. (Nakamoto, 2009)

**Table 1: Colour, melting point and elemental analysis of isolated complexes**

No.	Compound	Colour/ % Yeild	M.p(°C)	% Calculated				% Found			
				M	Cl	S	N	M	Cl	S	N
1	[Co(CH <sub>5</sub> N <sub>3</sub> S) <sub>2</sub> Cl <sub>2</sub> ].4H <sub>2</sub> O	Brown 18	172	15.14	18.49	16.67	10.97	15.16	18.01	15.20	10.10
2	[Co(C <sub>7</sub> H <sub>6</sub> N <sub>2</sub> S) <sub>2</sub> Cl <sub>2</sub> 2H <sub>2</sub> O]. 2H <sub>2</sub> O	White 25	270	11.57	14.14	12.75	5.59	11.75	13.73	11.98	5.35
3	[Co(CH <sub>5</sub> N <sub>3</sub> S) <sub>2</sub> (SCN) <sub>2</sub> .2H <sub>2</sub> O]	Ash 53	190	15.01	-	32.56	17.85	14.86	-	32.09	17.62
4	[Co <sub>2</sub> (C <sub>7</sub> H <sub>6</sub> N <sub>2</sub> S) <sub>2</sub> Cl <sub>2</sub> ].4H <sub>2</sub> O	Blue 80	392	21.70	12.70	11.45	5.01	20.85	11.56	10.15	4.85
5	[CoL <sub>2</sub> Cl <sub>2</sub> (CH <sub>5</sub> N <sub>3</sub> S) <sub>2</sub> ]. 4H <sub>2</sub> O	Brown 67	202	8.63	10.38	18.70	20.49	9.02	10.05	17.30	21.02

L=(C<sub>7</sub>H<sub>6</sub>N<sub>2</sub>S)

**Table 2: Infrared and electron spectra data of ligand and isolated solid complexes**

Compound	Conductivity (ohm <sup>-1</sup> cm <sup>2</sup> mol <sup>-1</sup> )	IR cm-1	Uv.λ <sub>max</sub> .nm
[Co(CH <sub>5</sub> N <sub>3</sub> S) <sub>2</sub> Cl <sub>2</sub> ].4H <sub>2</sub> O	1.6 X10 <sup>-4</sup>	577(Co-Cl),1241(C=S),1407,1628, 3137(- NH), 3292(-OH)	400
[Co(CH <sub>5</sub> N <sub>3</sub> S) <sub>2</sub> (SCN) <sub>2</sub> .2H <sub>2</sub> O]	0.9 X10 <sup>-4</sup>	606(Co-L), 722(H <sub>2</sub> O), 1178(C=S), 1367, 1488, 1628, 2077(-SCN),3146 (-NH)	650
[Co(C <sub>7</sub> H <sub>6</sub> N <sub>2</sub> S) <sub>2</sub> Cl <sub>2</sub> .2H <sub>2</sub> O].2H <sub>2</sub> O	0.2 X10 <sup>-4</sup>	420,482(Co-Cl), 593, 720(H <sub>2</sub> O), 1005Co- L), 1166, 1267(C=S),1366,3148(NH), 3325(-OH)	600
[Co <sub>2</sub> (C <sub>7</sub> H <sub>6</sub> N <sub>2</sub> S) <sub>2</sub> Cl <sub>2</sub> ].4H <sub>2</sub> O	0.1 X10 <sup>-4</sup>	409,595(Co-Cl)-,720(H <sub>2</sub> O),1006(Co-L), 1168(C=S), 1362,1486,1617,3166(NH), 3177	600
[Co(C <sub>7</sub> H <sub>6</sub> N <sub>2</sub> S) <sub>2</sub> Cl <sub>2</sub> (CH <sub>5</sub> N <sub>3</sub> S) <sub>2</sub> ].4H <sub>2</sub> O	0.7 X10 <sup>-4</sup>	411, 585, 1180 (C=S), 1472 (NH), 3178 (NH), 3435 (OH)	630
CH <sub>5</sub> N <sub>3</sub> S		510,610,810,1175,1295(C=S)1310,1460(NH),1622 (C=C),3195(NH)	
C <sub>7</sub> H <sub>6</sub> N <sub>2</sub> S		600,750,980,1185,1275(C=S),1470(NH),1510,1622 (C=C),3175(NH)	

**Table 3: Evaluation of antibacterial activity of the metal complexes on tested organisms**

Complexes	E.Coli	S.Aureus	S.typhi	Candida albica
[Co(CH <sub>5</sub> N <sub>3</sub> S) <sub>2</sub> Cl <sub>2</sub> ].4H <sub>2</sub> O	9.5	5.0	9.0	7.0
[Co(CH <sub>5</sub> N <sub>3</sub> S) <sub>2</sub> (SCN) <sub>2</sub> ].H <sub>2</sub> O	11	7.0	9.5	4.5
[Co(C <sub>7</sub> H <sub>6</sub> N <sub>2</sub> S) <sub>2</sub> Cl <sub>2</sub> ].4H <sub>2</sub> O	9.2	5.0	7.0	4.5
[Co <sub>2</sub> (C <sub>7</sub> H <sub>6</sub> N <sub>2</sub> S) <sub>2</sub> Cl <sub>2</sub> ].4H <sub>2</sub> O	8.2	4.5	6.5	6.5
Streptomycin(Control)	21	15	15	16
Ligand (CH <sub>5</sub> N <sub>3</sub> S)	3.3	3.0	2.2	2.4
(C <sub>7</sub> H <sub>6</sub> N <sub>2</sub> S)	3.6	3.4	2.9	2.0

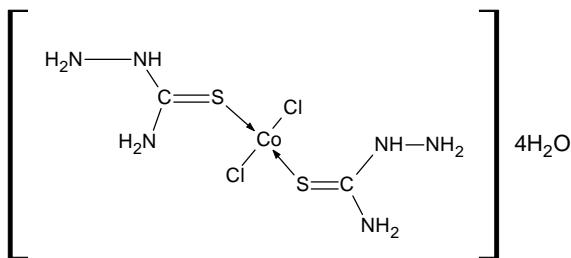


Figure 1. Structure of dichloro dithiosemicarbazide cobalt (II) tetra hydrate

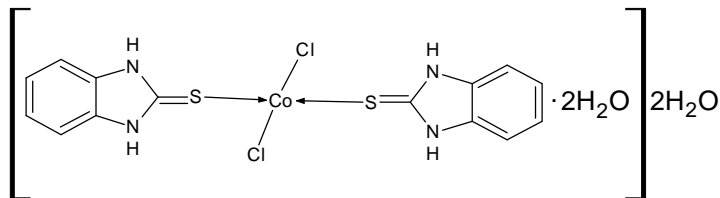


Figure 2. Structure of diaqua dichloro dimercaptobenzimidazole cobalt (II) dihydrate

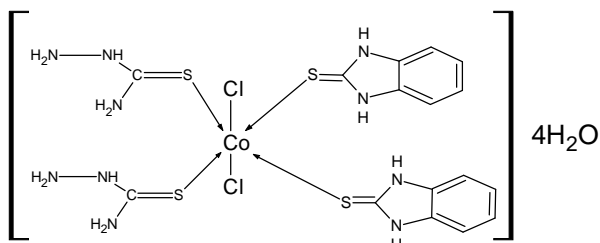


Figure 3. Structure of dichloro dithiosemicarbazide dimercaptobenzimidazole cobalt (II) tetra hydrate

From the spectral data presented in table 2, the metal complex showed the bands in the region  $557\text{cm}^{-1}$  and  $600\text{cm}^{-1}$  not originally found in the spectrum of the ligand confirming the presence of chloride ion a broad band at  $3137\text{cm}^{-1}$  is ascribed to the  $\nu\text{NH}$  vibration. The appearance of this band is an indication that bonding was through the sulfur atom, and not through the N-H. Equally some molecules of  $\text{H}_2\text{O}$  were found at the outer sphere of the complex and these appeared at  $3292\text{cm}^{-1}$  as broad band.

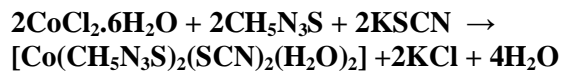
However, strong bands at  $1175$  and  $1295\text{cm}^{-1}$  which were attributed to the  $\text{C}=\text{S}$  bond but shifted to  $1241$  and  $1310\text{cm}^{-1}$  (higher frequencies) also indicated the participation of the sulphur atom in the coordination to the metal complexes.

Similarly, Cobalt(II) chloride reacted with mercaptobenzimidazole to give diaqua dichloro dimercaptobenzimidazole cobalt (II) dihydrate in 25% yield. Uv visible spectra of this complex

suggest an octahedral arrangement (Figure 2.) FTIR data of the complex revealed that there was a shift in the bands  $1185\text{cm}^{-1}$  to  $1166\text{cm}^{-1}$  ( $-\text{C}=\text{S}$ ) showing the coordination of the metal to the ligand through sulfur atom. Prominent band at  $420\text{cm}^{-1}$  confirmed the presence of chloride ions ( $\text{Co}-\text{Cl}$ ), while  $593$  and  $595\text{cm}^{-1}$  bands exhibited by the mercaptobenzimidazole cobalt(II) have revealed the coordination of metal to the ligand ( $\text{Co}-\text{L}$ ). Water molecules are believed to have bonded to the metal atom and this was shown by the appearance of bands at  $720\text{cm}^{-1}$  assigned to coordinated  $\nu(\text{OH})$ . Equally some molecules of  $\text{H}_2\text{O}$  were found at the outer sphere of the complex and these appear at  $3325\text{cm}^{-1}$  as broad band.

Mixed ligand reaction involving thiosemicarbazide and thiocyanate resulted in the formation of an ash coloured complex. The presence of thiocyanate ion in this complexes is reflected in a prominent band at  $2077\text{cm}^{-1}$

which was attributed to  $-\text{SC} \equiv \text{N}$  group from the potassium thiocyanate (Kemp,1989). The reaction mechanism of this compound shows the loss of chloride ions from the metal centre and their subsequent replacement by the thiocyanate group.



Electron spectra of this complex showing a band at 650nm also suggest an octahedral complex. The interaction of mercaptobenzimidazole and thiosemicarbazide in a mixed ligand reaction yielded brown coloured complex. This complex is suggested to be octahedral in shape (Figure 3) and the coordination of the ligands is through the sulfur atoms of both mercaptobenzimidazole and thiosemicarbazide. FTIR spectra of the complex show that the bands assigned to (-NH) are unchanged at  $3178\text{cm}^{-1}$ , while uncoordinated water molecules appear with a broad band at 3435nm.

The results of the screening of the cobalt complexes against selected bacteria species: *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, and *Candida albican*, revealed that metal complexes were more active than the free ligands (Table 3). The increased activity of the metal complexes could be explained on the basis of the overtone concept of cell permeability whereby the lipid membrane that surrounds the cell wall favours the passage of only lipid-soluble materials in which liposolubility is an important factor that control the antibacterial activity (Anjaneyulu and Rao, 1986). On chelation the polarity of the metal ions will be reduced to a greater extent due to overlap of ligand orbitals and partial sharing of the positive charge of the metal ion with donor groups. This increases the delocalization of  $\pi$  – electrons over the whole chelate ring and enhances the lipophilicity of complexes. It is likely that the increased liposolubility of the ligand upon complexation contributes to its facile transport into the bacterial cell which blocks the metal binding sites in enzymes of microorganisms. These complexes also disturb the respiration process of the cell and thus block

the synthesis of proteins which restricts further growth of the organisms (Saeed, 2009).

The effect of the cobalt (II) complexes appear more prominent in all the fungi species within the exposure periods under investigation with a 100% inhibition. It has been observed that metal complexes of ligands containing sulphur are biologically active on complexation (Iqbal et al, 2006).

## CONCLUSION

Five Cobalt(II) Complexes of thiosemicarbazide and mercaptobenzimidazole have been generated and characterized. The bidentate ligands (Thiosemicarbazide and Mercaptobenzimidazole) coordinate both datively and covalently with Cobalt (II), forming tetrahedral, octahedral and dimerized cobalt(II) complexes with good anti fungal and antibacterial activities.

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