



## Biological Screening of Some New Metal Oxalato Complexes

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### ABSTRACT

It is now a truism that, in recent years that chemistry has experienced an impressive renaissance. Academic and industrial research in chemistry is flourishing and the field of co-ordination chemistry is growing exponentially, especially the complexation of transition metals with organic ligands. This paper describes the preparation and characterization of mixed ligand transition metal complexes of Cobalt (II) ions with di basic oxalic acid as primary ligand and heterocyclic amines as secondary ligands. Their conventional physical and chemical properties analyses have been done. On the basis of physico-chemical analysis and spectral studies metal complexes may be formulated as  $[M(OA)_2L_2]$ , where M stands for bivalent cobalt metal ion, OA for oxalate ion and L for heterocyclic amines, viz. pyridine, quinoline, iso-quinoline and 4-picoline. The complexes are quite stable coloured solid but sparingly soluble in water. Magnetic moment values and spectral studies reveal the tetrahedral geometry to all the prepared complexes. The synthesized cobalt oxalate complexes with pyridine ( $C_1$ ), quinoline ( $C_2$ ), isoquinoline ( $C_3$ ) and with 4-picoline ( $C_4$ ) were screened by the disc diffusion method against the multi drug resistant fungal pathogen *Fusarium oxysporum* f. sp. *lycopersici* and *Alternaria solani* causing diseases in vegetable crops. In vitro antifungal screening indicates that the complexes show enhanced antifungal activities against the fungal strains as compared with parental compounds. Complex with 4- picoline ( $C_4$ ) as secondary ligand showed the highest antifungal activity.

**KEYWORDS :** Mixed ligand, transition metal complexes, In vitro antifungal screening, *Fusarium oxysporum* f. sp. *lycopersici*, *Alternaria solani*.

### Introduction:

An exhaustive survey of the existing literature reveals that a very little study has been conducted on the metal complexes of dibasic acid. The magnetic properties of some carboxylic acid derivatives of cobalt (II) and iron (III)<sup>[1]</sup>. Malonato-bridge cobalt (II) complexes were prepared and characterized by elemental analyses, electronic spectra, magnetic susceptibilities, and X-ray analysis<sup>[2]</sup>. Synthetic chemicals constitute important sources of various bioactive compounds such as antibacterial<sup>[3]</sup>, antifungal<sup>[4]</sup> and anticancer<sup>[5]</sup> compounds. The metal complexes of phthalic acid have been studied both from pharmacological<sup>[6]</sup> and industrial point of view as indicated by available literature. Some complexes of transition metal ions with malonic acid<sup>[7]</sup> phthalic acid<sup>[8]</sup> and maleic acid<sup>[9]</sup> have been found medicinally important.

Considering these facts, the present work describes the synthesis, characterization and antimicrobial screening of mixed ligand complexes of copper (II) with oxalic acid as primary ligand and heterocyclic amines, viz. pyridine, quinoline, iso-quinoline and 4-picoline as secondary ligands on the basis of various physico-chemical techniques.

### Experimental

#### Materials and methods:

All the chemicals used in the synthesis of transition metal complexes were of analytical grade. Cobalt salt used in the present study was cobalt dichloride. Methanol, ethanol and benzene were further purified by double distillation. Oxalic acid was used as primary ligand. Used oxalic acid was of highest purity. Secondary ligands heterocyclic amines (pyridine, quinoline, iso-quinoline and 4-picoline) were used as received.

#### Preparation of Co (II) complexes:

Mixed ligand transition metal complexes were prepared as reported earlier<sup>[10]</sup>. The freshly prepared cobalt (II) chloride salt and oxalic acid were mixed in 100 ml of absolute ethanol and refluxed on a water bath for an hour and then the calculated amount of an alcoholic solution of hetero amine bases was added. The mixture was again refluxed for an hour and then cooled. The precipitate formed were filtered, washed several times with ethanol and then dried in vacuum over phosphorus pentoxide ( $P_2O_5$ ).

#### Physical measurements:

Carbon, hydrogen and nitrogen were estimated microanalytically on Elementar Vario EL III elemental analyzer at CDRI, Lucknow. Conduc-

tivity measurements were made on ELICO EQ 660 conductivity bridge using DMF as a solvent. Metal contents were estimated by standard methods<sup>[11]</sup>. Magnetic susceptibility measurements were made at room temperature by Gouy's method using  $Hg[Co(SCN)_4]$  as calibrant. The diamagnetic correction of metal-ligand system was calculated using Pascal's constant. The purity of metal complexes was checked by TLC method along with standard ligands. IR spectra in the range 4000–400  $cm^{-1}$  were recorded at CDRI, Lucknow on a Shimadzu FTIR 8201 P C spectrometer where as spectra in the range 4000–250  $cm^{-1}$  were recorded on a Perkin Elmer infra red spectrophotometer 521 at the Department of Chemistry, IIT Roorkee. The electronic spectra of the compounds were recorded at CDRI, Lucknow on a Shimadzu UV 1601 spectrophotometer. The antifungal activities were carried out at Microbiological Lab, IFTM University, Moradabad.

#### Antifungal screening:

The *in vitro* antifungal activity on the radial growth of the pathogens was evaluated by food poison technique<sup>[12]</sup> against the fungi *Fusarium oxysporum* f. sp. *lycopersici* and *Alternaria solani* as reported earlier<sup>[13, 14, 15]</sup>

### Results and discussion

#### Elemental analyses:

The elemental analyses data for the cobalt oxalate complexes with pyridine ( $C_1$ ), quinoline ( $C_2$ ), isoquinoline ( $C_3$ ) and with 4-picoline ( $C_4$ ) are as follows-

#### Percentage of different elements in metal complexes, calculated (found)

**Carbon percentage**  $C_1$ -47.23(47.78),  $C_2$ -59.25(58.92),  $C_3$ -59.25(59.12) and  $C_4$ -50.46(50.68)

**Hydrogen percentage**  $C_1$ -3.30(3.56),  $C_2$ -3.48(3.62),  $C_3$ -3.48(3.21) and  $C_4$ -4.24(4.98)

**Nitrogen percentage**  $C_1$ -9.18(8.90),  $C_2$ -6.92(6.57),  $C_3$ -6.92(6.43) and  $C_4$ -8.41(7.99)

**Cobalt percentage**  $C_1$ -19.31(19.82),  $C_2$ -14.55(14.70),  $C_3$ -14.55(14.23) and  $C_4$ -17.69(18.23)

#### Spectral studies:

The infra red spectra of the synthesized complexes ( $C_1$ ,  $C_2$ ,  $C_3$  and  $C_4$ ) were studied and compared with the spectra of oxalic acid and the heterocyclic ligands. The change in the spectral bands gave the

information about the bonding between metal ion and the ligands. The association of carboxylic acid is well known to take place through -COOH group(s). It is therefore expected that mainly -OH and -C=O bands of the monomeric molecules would be affected. The asymmetric carbonyl stretching frequency of carboxylic acid has been noted at 1690-1710 cm<sup>-1</sup>. When carboxylate ion is formed the characteristic carbonyl frequency is replaced by two bands in the region 1500-1600 cm<sup>-1</sup> and 1300-1420 cm<sup>-1</sup> corresponding to asymmetric and symmetric vibrations of carboxylate ion. In the case of oxalato complexes these vibrations become IR active. The infra red frequencies associated with oxalate group in the complexes reported here are in good agreement with those normally associated with the bidentate chelating oxalate group. The -OH stretching absorption appearing at 3175- 3030 cm<sup>-1</sup> in oxalic acid disappears in the complexes and the appearance of new bands in the region 440-490 cm<sup>-1</sup> confirmed the co-ordination of oxygen of -OH group after deprotonation. These may be assigned as ν M-O bands. The disappearance of ν O-H frequency of free oxalic acid on complexation also showed the metal- oxygen linkage. The change in characteristic ring vibrations of heterocyclic amines could not be distinguished due to overlapping with ν C-O and ν C=O stretching bands. The plane and out of plane ring deformation modes<sup>[16-18]</sup> of these ligands were observed at about 510 and 715 cm<sup>-1</sup>. In the complexes new band observed in the region 390-400 cm<sup>-1</sup> indicates the bonding in amines through nitrogen atom and may be assigned as ν M-N mode.

The electronic spectra of cobalt complexes exhibit three bands in the region 4240- 4336 cm<sup>-1</sup>(v<sub>1</sub>), 6255- 6270 cm<sup>-1</sup>(v<sub>2</sub>) and 13408-13500 cm<sup>-1</sup>(v<sub>3</sub>). These may be assigned to three spin allowed transitions <sup>4</sup>A<sub>2</sub>→<sup>4</sup>T<sub>2</sub>, <sup>4</sup>A<sub>2</sub>→<sup>4</sup>T<sub>1</sub>(F) and <sup>4</sup>A<sub>2</sub>→<sup>4</sup>T<sub>1</sub>(P) respectively.

**Magnetic moments:**

The magnetic moment values of the cobalt (II) complexes (3.80-4.02 BM) indicate the paramagnetic nature of the complexes corresponding to three unpaired electrons. This is also in good agreement with the tetrahedral<sup>[19]</sup> environment around the metal ion.

**Conductance values:**

The molar conductance were measured for different complexes in N, N' di methyl formamide. The conductance values (λ<sub>m</sub>, in ohm<sup>-1</sup> cm<sup>2</sup> mol<sup>-1</sup>) are C<sub>1</sub> (21.54), C<sub>2</sub> (21.67), C<sub>3</sub> (20.98) and C<sub>4</sub> (21.34) respectively.

The conductance values indicate that the complexes are non electrolytic in nature.

**Antimicrobial activities:**

*In vitro* antifungal activity of metal complexes against *F. oxysporum* f. sp. *lycopersici* and *A. solani* were studied. Radial growth of fungal hyphae were measured in mm. Results are shown in the following Table 1, and Table 2. The results showed that all the complexes have antifungal activity against the fungus *F. oxysporum* f. sp. *lycopersici* and *A. solani*. The data showed that out of four complexes C<sub>4</sub> complex was most effective against the radial growth of the pathogen at all concentrations. C<sub>3</sub> may be placed at second position. C<sub>1</sub> at third position followed by C<sub>2</sub>.

**Table 1. In vitro antifungal activity of metal complex against Fusarium oxysporum f. sp. lycopersici.**

S. No.	Complex	Doses (ppm)					Control
		100	500	1000	2000	3000	
1	C <sub>1</sub>	46.7	45.5	39.5	32.0	25.5	47.0
2	C <sub>2</sub>	50.0	46.5	39.5	35.2	28.5	47.0
3	C <sub>3</sub>	41.5	39.5	38.2	30.5	27.2	47.0
4	C <sub>4</sub>	31.0	26.5	20.5	15.5	10.5	47.0

**Table 2. In vitro antifungal activity of metal complex against Alternaria solani.**

S. No.	Complex	Doses (ppm)					Control
		100	500	1000	2000	3000	
1	C <sub>1</sub>	45.5	44.5	40.5	34.5	28.5	47.0
2	C <sub>2</sub>	48.0	45.5	40.5	37.5	29.4	47.0
3	C <sub>3</sub>	43.5	38.5	35.5	33.6	27.2	47.0
4	C <sub>4</sub>	29.0	26.2	18.5	14.5	10.2	47.0

The results of *in vitro* studies revealed that at 3000 ppm concentration all the four complexes showed the maximum inhibition in the radial growth of the fungi *Fusarium oxysporum* f. sp. *lycopersici* and *Alternaria solani*. Out of these four complexes the radial growth was minimum in the case of 4- picoline complex (C<sub>4</sub>). This clearly indicates that C<sub>4</sub> complex may be considered as the best to control the fungal growth out of the four complexes. It binds the protein to grow, resulting the inhibition of growth of pathogen. The antifungal activity of this complex was compared with known drugs Kanamycin and Fluconazol. The literature reveals that these two drugs are very effective against the radial growth of *Fusarium oxysporum* f. sp. *lycopersici*<sup>[20]</sup> and *Alternaria solani*. It was found that at 3000 ppm concentration the radial growth of these two drugs Kanamycin and Fluconazol as 10.8 mm and 10.6 mm respectively. Whereas the radial growth of *F. oxysporum* f. sp. *lycopersici* and *A. solani* reported in this study were 10.5 and 10.2 mm respectively by using C<sub>4</sub> at 3000 ppm concentration. This shows the better inhibition than the drugs Kanamycin and Fluconazol.

**Conclusion**

The work described in this paper involved the synthesis and spectroscopic characterization of cobalt (II) complexes with di basic oxalic acid and heterocyclic amine ligands. These complexes were characterized by using different physico-chemical techniques. The IR spectra revealed that oxalate ion behaves as bidentate chelating ligand co-ordinated to the metal ion through oxygen atom of -COOH group and heterocyclic amine ligands co-ordinated through nitrogen atom of

- NH<sub>2</sub> group. The magnetic moment and electronic spectra confirm the presence of tetrahedral geometry of the complexes. The *in vitro* antifungal activity on the radial growth of the fungus refers that the complexes have significant inhibition efficiency against *F. oxysporum* f. sp. *lycopersici* and *A. solani*. Transition metal complexes with bioligands represent a novel group of antimicrobial agents with potential application for the control of fungal infections and used to treat the drug resistant fungal pathogen.

The metal complexes have been screened for their fungitoxicity by employing on *F. oxysporum* f. sp. *lycopersici* and *A. solani*. Metal complexes are invariably found to be more toxic to the fungal species than the free ligand. The increased activity of the metal complexes against fungal species screened can be explained on the basis of chelation theory<sup>[21]</sup>.

Chelation reduces considerably the polarity of the metal ion mainly because of partial sharing of its positive charge with donor groups and possible delocalization of electron over the entire chelate ring. This aspect increases liophilic character of the neutral chelate favouring its diffusion through the lipid layers of the fungal membranes. Further the presence of un co-ordinated hetero atoms such as O and N in the chelate molecule will involve in the formation of hydrogen bonds with the active centers of the cell constituents resulting in the interference of the normal cell processes. Thus the presence of polar groups like C=O, C-N, C-O etc. in the chelate molecules are expected to enhance their fungitoxicity<sup>[22]</sup>. The molecules tested have greater chance of interaction with the nucleotide bases present in the cells of fungal species. Further the co-ordinatively unsaturated metal centers present in the complexes may bind with some of the functional groups to achieve higher co-ordination number. This leads to increased uptake of the complex by the fungal species resulting in higher degree of growth inhibition.

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