Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2016, 8(16):138-143
(http://derpharmachemica.com/archive.html)

Synthesis, Equilibrium Studies and Antibacterial activity of Zn(II), Cd(II) and Hg(II) complexes of 4-Formylpyridine N(4)-methylthiosemicarbazone

S. P. Mydhili[a], B. Sireesha[a], Ch. Venkata Ramana Reddy[b]* and P. Acharya Nagarjuna[c]

[a]*Department of Chemistry, Nizam College, OU, Basheerbagh, Hyderabad, 500 001, India*
[b]*Department of Chemistry, Jawaharlal Nehru Technological University Hyderabad, Hyderabad, 500 085, India*
[c]*Department of Microbiology, UCS, OU, 500 007, Hyderabad, India*

ABSTRACT

4-Formylpyridine N(4)-methylthiosemicarbazone (H4FP4MT) was synthesized and characterized by LC-MS, IR, UV-visible, ^{1}H NMR, and ^{13}C NMR spectra. Equilibrium studies of H4FP4MT were carried in 70%v/v DMF-Water medium. Proton dissociation constant of H4FP4MT and the stability constants of Zn(II) and Cd(II) complexes with H4FP4MT were determined pH metrically. Metal complexes, ML_n of H4FP4MT(L) with Zn(II), Cd(II) and Hg(II) were prepared and characterized by various spectro-analytical techniques such as elemental analyses, molar conductance, LC-MS, TGA, IR and ^{1}H-NMR. Elemental analyses, LC-MS and TGA studies reveal the composition of the complexes as ML_2 for all the complexes. Antibacterial activity of H4FP4MT and its metal complexes against gram positive and gram negative bacterial strains were studied and found to be active.

Keywords: Stability constant, bidentate ligand, distorted octahedral geometry, antibacterial.

INTRODUCTION

Thiosemicarbazones are sulphur and nitrogen donor ligands for transition metal ions which possess good biological activity and medicinal properties such as antitumour, antifungal, antibacterial, anticancer etc [1-4]. Presence of an additional potential binding site along with bulky groups at the N(4) position of the thiosemicarbazone moiety significantly enhances biological activity [5,6]. On complexation, lipophilicity that controls the cell permeability of the molecule is modified and complex may be more active with a possible decrease in side effects [7]. In view of the importance of thiosemicarbazone derivatives, the present communication reports the synthesis of 4-formylpyridine N(4)-methyl thiosemicarbazone (H4FP4MT) with modified procedure, determination of proton dissociation constant of H4FP4MT and stability constant of its complexes with Zn(II) and Cd(II) ions in solution. The synthesis, characterization and antibacterial activity of binary metal complexes of H4FP4MT with Zn(II), Cd(II) and Hg(II) also reported.

MATERIALS AND METHODS

All the chemicals used were of AR grade (Sigma-Aldrich). A digital Elico (L1-120) pH meter with a combined glass and calomel electrode was used for equilibrium studies. pH meter was calibrated using 4.0, 7.0 and 9.2 buffers. LC-MS of the ligand and its complexes were recorded on LCMS 2010A, Shimadzu spectrometer, elemental analysis was done on Thermo Finnigan/Eager 300 for EA 11120. Molar conductivity of the complexes ($1x10^{-3}$M) was measured using Digisun model 909 digital conductivity meter. Thermo gravimetric analyses of the complexes were carried on TG balance TA Model: Q/50 in the temperature range of 0°C to 1000°C with a ramp of 20°C per min. IR spectra in KBr were recorded on Schimadzu (Prestige-21) FTIR spectrometer. ^{1}H-NMR (with D_2O exchange) and ^{13}C-NMR were recorded on Varian 400MHz NMR spectrometer and UV spectra in DMSO were recorded on Schimadzu UV 2450 spectrophotometer. Anti bacterial activity was studied following Kirby-Bauer disc diffusion method in sterile nutrient agar medium.

Synthesis of the ligand, L (H4FP4MT)

H4FP4MT(Fig 1) was synthesized by stirring equimolar (0.01M) solutions of N(4)-methyl thiosemicarbazide and 4-formyl pyridine at room temperature for one hour. The progress of the reaction was monitored by TLC. Pale cream coloured product formed was filtered, washed with water, dried and recrystallised from ethanol.

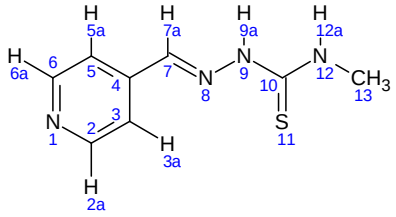


Fig 1. 4-Formylpyridine N(4)-methyl thiosemicarbazone

Yield: 90%; colour: pale cream; m.p. 213°C-215°C; Elemental Anal. Calc. for $C_8H_{10}N_4S$: C 49.42%, H 5.14%, N 28.82%; Found: C 49.65%, H 5.06%, N 28.76%; IR (υ,cm^{-1}; KBr): 3280 υ(N_4-H), 3142 υ(N_3-H), 1597 υ(C=N), 1257 υ(N-CS-N), 985 υ(N-N), 819 υ(C=S); ^{1}H NMR (DMSO, ppm): δ11.76 (s, 1H, N_3-**H**), δ8.7 (d, 1H, N_4-**H**), δ8.58 (d, 2H, Ar-**H**), δ7.75 (d, 2H, Ar-**H**), δ7.97 (1H, s, **H**-C=N), δ3.01(s,3H, N-C**H**$_3$); ^{13}C NMR (DMSO-d_6, δ/ppm): 141.9 (C_2, C_6), 121.5 (C_3, C_5), 139.2 (C_4), 150.4 (C_7), 178.5 (C_{10}), 31.3(C_{13}). UV-visible in DMSO, λ_{max}/nm: 330, 249.5; APCI(+)MS: m/z 195 $[M+1]^+$.

Synthesis of complexes

To the hot methanolic solution of the ligand, L corresponding aqueous metal(II) salt [$Zn(CH_3COO)_2$/$CdCl_2$/ $HgCl_2$] solutions in 1:2 (M:L) molar ratio were added and refluxed for 8-10 hours. pH of the solution was adjusted by addition of few drops of methanolic ammonium hydroxide solution. Solid complexes formed were filtered under hot condition, washed with hot methanol, water and with petroleum ether and finally dried in vacuum [8,9].

[Zn($C_8H_{10}N_4S$)$_2$(H_2O)$_2$](OAc)$_2$ (1)

Colour: yellow; m.p. > 300°C; Elemental Anal. Calc. for $C_{20}H_{30}ZnN_8S_2O_6$: C 39.48%, H 4.93%, N 18.42%; Found: C 42.18%, H 4.51%, N 24.51%; IR (υ_{max},cm^{-1}; in KBr): 3460-3120 υ(N_4-H, N_3-H), 1588 υ(C=N), 1255 υ(N-CS-N), 1008 υ(N-N), 820 υ(C=S); ^{1}H NMR (DMSO, ppm): δ8.70 (d, 1H, N_4-**H**), δ8.58-8.57 (d, 2H, Ar-**H**), δ7.75-7.74 (d, 2H, Ar-**H**), δ7.97 (1H, s, **H**-C=N), δ3.00 (s,3H, N-C**H**$_3$); APCI(-)MS *m/z*: 607 $[M-1]^+$

[Cd($C_8H_9N_4S$)$_2$(H_2O)$_2$] (2)

Colour: yellow; m.p. > 300°C; Elemental Anal. Calc. for $C_{16}H_{22}CdN_8S_2O_2$: C 35.89%, H 4.11%, N 20.93%; Found: C 38.19%, H 4.12%, N 22.41%; IR (υ_{max},cm^{-1}; in KBr): 3329 υ(N_4-H), 1610,1595 υ(C=N), 1010 υ(N-N); ^{1}H NMR (DMSO, ppm): δ8.64 (d, 1H, N_4-**H**), δ8.55-8.51 (d, 2H, Ar-**H**), δ7.79-7.65 (d, 2H, Ar-**H**), δ8.0 (1H, s, **H**-C=N), δ3.01 (s, 3H, N-C**H**$_3$); APCI(+)MS: m/z 536 $[M+1]^+$

[Hg($C_8H_9N_4S$)$_2$(H_2O)$_2$] (3)

Colour: grey; m.p. > 300°C; Elemental Anal. Calc. for $C_{16}H_{22}HgN_8S_2O_2$: C 30.81%, H 3.53%, N 17.9%; Found: C 32.48%, H 3.31%, N 19.15%; IR (υ_{max},cm^{-1}; in KBr): 3280-3160 υ(N_4-H), 1610,1583 υ(C=N), 991 υ(N-N);^{1}H NMR (DMSO, ppm): δ9.33 (d, 1H, N_4-**H**), δ8.69-8.68 (d, 2H, Ar-**H**), δ7.97-7.96 (d, 2H, Ar-**H**), δ8.11 (1H, s, **H**-C=N), δ3.14 (s,3H, N-C**H**$_3$); APCI(+)MS: m/z 624 $[M+1]^+$

Equilibrium studies

The Zn(II) and Cd(II) metal ion solutions were prepared in double distilled water using corresponding AR grade metal nitrates and were standardized by known methods[10]. pH-metric titrations were carried in 70% v/v DMF-water medium at 303K and ionic strength 0.1MKNO_3. Proton-ligand dissociation constant and formation constants of binary chelates have been determined by Irving Rossotti titration technique [8,11].

Antibacterial studies

Biological activity of the ligand and complexes was tested by Kirby-Bauer disc diffusion method. 0.10mL of test bacteria [gram positive-Staphylococcus aureus, Bacillus subtilis and gram negative -Escherichia coli, Klebsiella pneumonia] was spread over the surface of nutrient agar. Sterile discs of 5mm diameter dipped in DMSO solutions of test samples are placed at equidistance. The potency of all the samples tested was 1000µg/disc. Capacity of the disc is 5µL of the sample. DMSO was taken as control, which has no antibacterial activity. Gentamycin was used as standard. Zone of inhibition was recorded after incubation for 24hrs at 37°C. All these tests were made in triplicate and are averaged.

RESULTS AND DISCUSSION

Equilibrium studies

To understand the chelation ability of H4FP4MT, potentiometric titration of the following sets of solutions (50mL) were carried out by Irving Rossotti titration technique. (i) HNO_3 ($4.0x10^{-3}$M) (ii) HNO_3 ($4.0x10^{-3}$M) + H4FP4MT ($1.0x10^{-3}$M) and (iii) HNO_3 ($4.0x10^{-3}$M) + H4FP4MT ($1.0x10^{-3}$M) + M(II) ion ($2.0x10^{-4}$M) solutions against 0.1M NaOH solution in 70% v/v DMF-Water medium at 303K and 0.1M KNO_3 ionic strength. The titration curves, linear plots are presented in Fig 2.

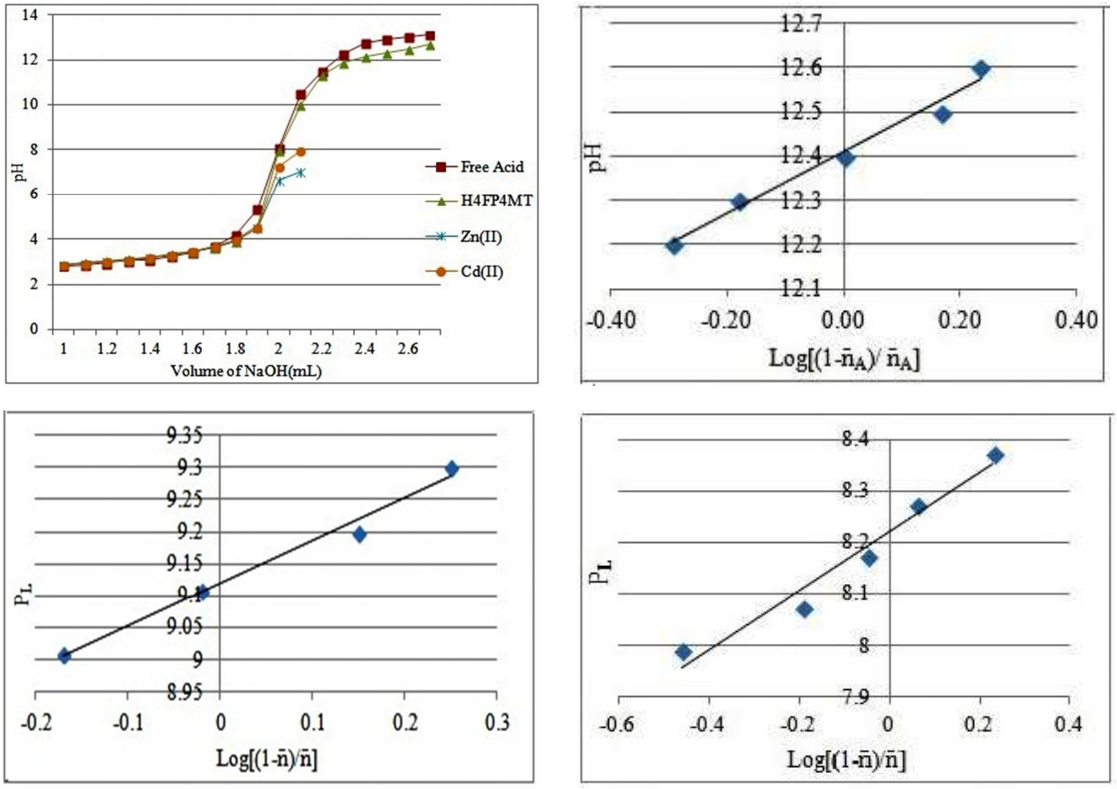


Fig 2. pH titration curves and Linear Plots of Log [(1-$\bar{n}_A$)/ $\bar{n}_A$] vs pH of H4FP4MT, Log [(1-$\bar{n}$)/$\bar{n}$] vs pL of complexes(1,2)

The proton -ligand dissociation constant, pK_a was computed from the linear plots of log [(1-$\bar{n}_A$)/$\bar{n}_A$] vs pH. From the results, it is evident that the ligand has one dissociable proton corresponding to pK_a value 12.41. The ligand can undergo thione-thiol tautomerism and dissociation of proton occurs through thiol-1 form as shown in Fig 3.

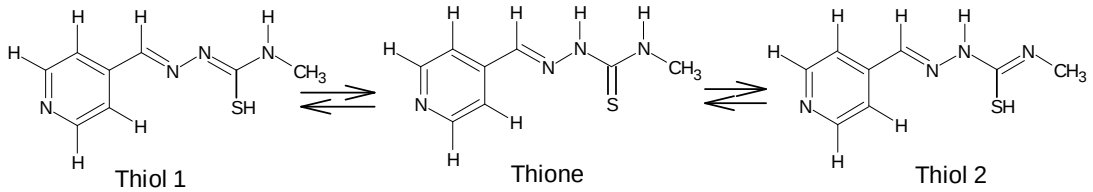


Fig 3. Thione and thiol forms of H4FP4MT

The values of $\bar{n}$ varies from 0.2 to 0.9 indicate formation of 1:1(M:L) complexes in solution. The stability constants (log K) of binary complexes were calculated from linear plots of log [(1-$\bar{n}$)/$\bar{n}$] vs pL. Log K value of Zn(II) complex is 9.12 and that of Cd(II) complex is 8.22 . The greater stability of Zn(II) complex can be attributed to high charge to radius ratio of zinc as compared to cadmium.

Characterization of metal complexes

All the synthesized complexes were coloured, crystalline and stable. Complexes are soluble in DMSO and DMF. Elemental analyses, mass spectral data and TGA confirms the formation of 1:2(M:L) complexes of zinc, cadmium and mercury.

Molar conductivity measurements were recorded in $1x10^{-3}$M DMSO solutions at room temperature. Low molar conductivity values for the complexes (0-20 Scm^2M^{-1}) indicate their nonelectrolytic nature [12]. Presence of chloride ions in complexes(**2,3**) was established from Volhard's test [13].

Liquid chromatograms

Liquid chromatograms of all complexes showed single peaks with retention time in the range of 0.646 to 0.718 min indicating their purity.

Mass spectra

APCI(-) mass spectrum of the complex(**1**) showed molecular ion peak at m/z at 607. 1:2(M:L) can be established from the peak at m/z 453. From APCI (+) mass spectrum of complex(**2**), a peak at m/z 536 $[M+1]^+$ is observed. Peak at m/z 500 indicate loss of coordinated water in the complex and 1:2 (M:L). Analysis of APCI (+) mass spectrum of complex(**3**) indicated molecular ion peak at m/z 624, loss of coordinated water in the complex and 1:2 (M:L) from m/z 588 peak.

Thermogravimetric analysis

Weight loss in complex(**1**) observed in three steps. Loss of two coordinated water molecules can be evidenced from 6% decomposition between 100°C to270°C.This was followed by decomposition of ligand. The residue (59.9%) reveals incomplete decomposition of the complex indicating its thermal stability. Thermogram of complex(**2**) showed decomposition in three major steps. The presence of coordinated water can be evidenced from the curve at 235°C-250°C (5%) in the first step. Slow weight loss from 250°C to 940°C in corresponds to the decomposition of the ligand followed by cadmium metal and indicates 1:2 (M:L) ratio. In complex(**3**) loss of two coordinated water molecules can be evidenced from 5.5% decomposition between 180°C to 220°C. Weight loss (90.4%) from 220°C to 791°C indicates decomposition of the complex Thermograms of the complexes are shown in Fig 4.

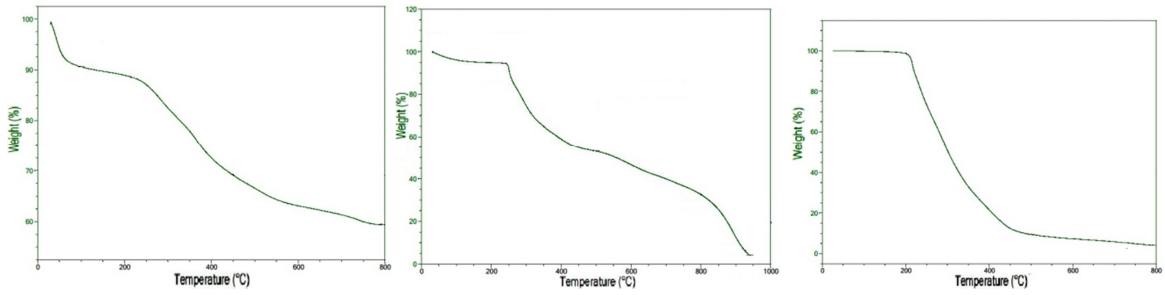


Fig 4. Thermograms of complexes(1,2,3)

IR spectral data

Table 1. IR Spectral data (KBr, cm^{-1})

Compound	υ(C=N)	υ (N_3-H)	υ (N-N)	υ (N-CS-N)
H4FP4MT(**L**)	1597	3142	985	1257
Complex(**1**)	1588	3120	1008	1255
Complex(**2**)	1610,1595	----	1010	----
Complex(**3**)	1610,1583	----	991	----

In IR spectra of the complexes (Table 1, Fig 5), υC=N shifted towards lower frequency suggesting the coordination of azomethine nitrogen with metal ions [14,15]. $υN_4$-H at 3280cm^{-1} of the ligand showed a shift (3460-3120 cm^{-1}, broad peak) in complex(**1**). This infers coordination of thioamide nitrogen with the metal ion. υ(N-CS-N) is found missing in complex(**2,3**) suggesting the coordination of thiolate sulphur [16,17]. From the far IR region of the spectra, there is evidence for the presence of (M-N), (M-S), (M-Cl), and (M-OH_2) bands in the complexes. Analysis of IR spectral data shows that the ligand is bidentate with azomethine and thioamide nitrogen atoms in complex(**1**) and in complexes(**2,3**) azomethine nitrogen and thiolate sulphur are potential donor sites forming five membered chelate with the metals.

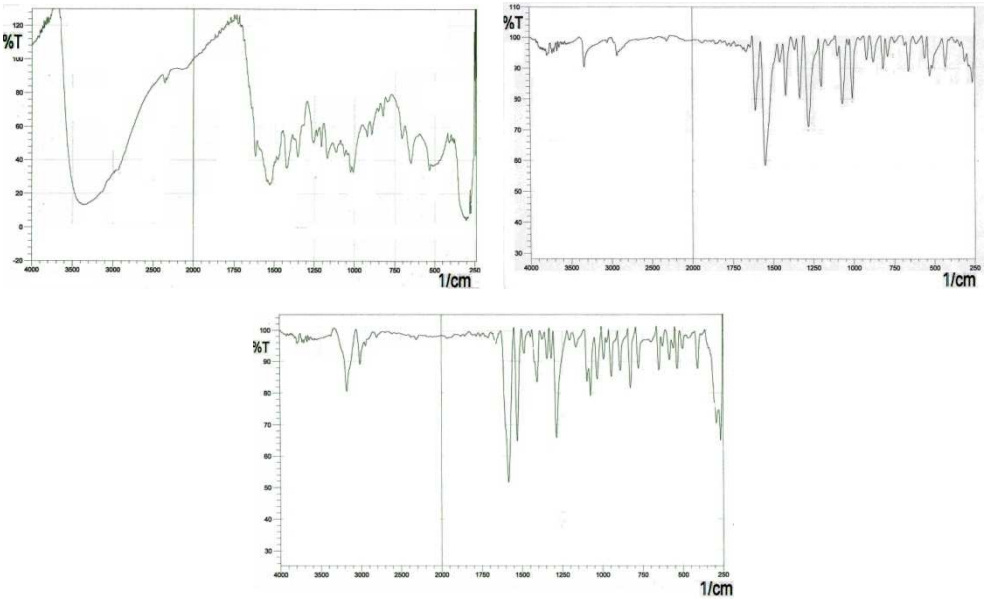
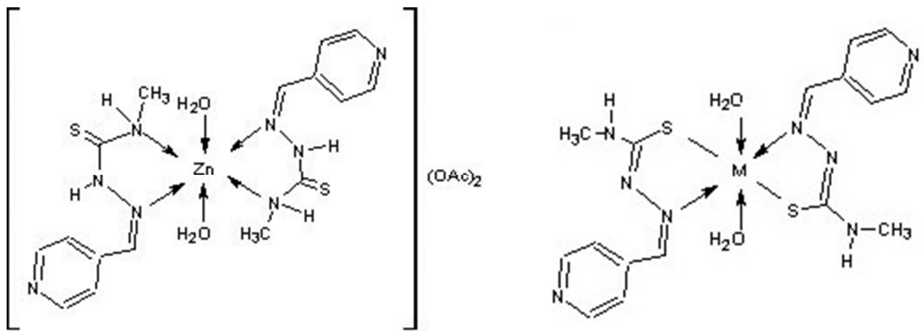


Fig 5. IR Spectra of complexes(1,2,3)

^{1}H-NMR

In the ^{1}H-NMR spectra of complexes (**2,3**), peak at δ11.76ppm which belongs to hydrazine proton in the spectrum of ligand is absent. The characteristic peak of thiol proton at δ 4ppm is also absent. This indicates the coordination of thiolate sulphur to metal ion [2]. The proton of N_4-H appears as a doublet in the ligand and in complexes due to interaction with methyl protons [17].

From the equilibrium studies and various spectro analytical data of complexes (**1-3**), the proposed structures of the complexes are as shown in Fig 6.



M=Cd(II), Hg(II)

Fig 6. Proposed structures of complexes(1,2,3)

Antibacterial studies

Antibacterial activity of H4FP4MT and its complexes have been tested on both gram positive and gram negative bacteria (Table 2). The ligand and complex(**1**) can inhibit the growth of only gram positive bacteria, while complexes(**2,3**) are found to inhibit the growth both gram positive and gram negative bacteria under study. It is observed that the complexes(**2,3**) exhibit more activity than ligand. This can be attributed to the chelating ability of the metal to ligand. Metal atom partially shares its positive charge with the donor atoms of the ligand leading to delocalization of π electron cloud over the chelating ring. Due to this the lipophilic character of the metal gets enhanced and favours its permeability into bacterial cell membranes and inhibits the growth of the bacteria [18].

Table 2. Anti Bacterial Activity of ligand and complexes

Compound	*S.aureus*	*B.subtilis*	*E. coli*	*K.pneumoniae*
H4FP4MT(**L**)	6mm	6mm	-	-
Complex(**1**)	6mm	6mm	-	-
Complex(**2**)	15mm	18mm	6mm	8mm
Complex(**3**)	20 mm	25 mm	17 mm	18 mm

CONCLUSION

Equilibrium studies reveal the monobasic nature of 4-Formylpyridine N(4)-methyl thiosemicarbazone and form stable 1:1 complexes with Zn(II) and Cd(II) ions in solution. In solids, H4FP4MT acts as a bidentate ligand forming distorted octahedral complexes ML_2 with metal ions. All the metal complexes show good antibacterial activity.

Acknowledgements
The authors are thankful to Osmania University, Hyderabad and Loyola Academy Degree & PG College, Secunderabad for providing necessary facilities to carry out this work.

REFERENCES

[1] E. N. Nfor, S. N. Esemu, G. A. Ayimele, E. A. Eno, G. E. Iniama, O. E. Offiong, *B. Chem. Soc. Ethiopia*, **2011,** 25, 361-370.
[2] S. A. Elsayed, A. M. El-Hendaway, S. I. Mostafa, I. S. Butler, *Inorg. Chim. Acta*, **2010**, 363, 2526-2532.
[3] J. Wiecek, D. Kovala-Demertzi, Z. Ciunik, J. Wietrzyk, M. Zervou, M. A. Demertzis, *Bioinorg. Chem. Appl*, **2010**, Article ID 718606, 7pages.
[4] S. Chandra, S. Raizada, M. Tyagi, P. K. Sharma, *Spectrochim. Acta A*, **2008,** 69, 816-821.
[5] S. K. Jain, B. S. Garg, Y. K. Bhoon, *Spectrochim. Acta A*, **1986**, 42, 959-968.
[6] M. E. Hossain, M. N. Alam, J. Begum, M. A. Ali, M. Nazimuddin, F. E. Smith, R. C. Hynes, *Inorg. Chim. Acta*, **1996,** 249, 207-213.
[7] D. Kovala-Demertzi, P. N. Yadav, J. Wiecek, S. Skoulika, T. Varadinova, M. A. Demertzis, *J. Inorg. Biochem*, **2006,** 100, 1558-1567.
[8] B. Sireesha, G. Bhargavi, C. Sita, Ch. Sarala Devi, *Bulletin of pure and applied sciences*, **2006**, 25C, 1-6.
[9] B. Sireesha, S. P. Mydhili, G. Sravan, Ch. VenkataRamana Reddy, *IOSR-Journal of Applied Chemistry*, **2016,** 49-57.
[10] G. H. Jeffery, J. Basset, J. Mendham, R. C. Denny, Vogel's Text Book of Quantitative chemical Analysis, ELBS, London, **1997,** 5th Edition.
[11] H. M. Irving, H. S. Rossotti, *J. Chem. Soc*, **1954,** 2904-2910.
[12] R. Aman, G. Matela, C. Sharma, S. Chaudhary, *Arabian J. Chem*, **2015,** 8, 698-705.
[13] A. I. Vogel; A Text Book of Qualitative Inorganic Analysis, Longman, London, **1968,** 3rd Edition.
[14] D. X. West, H. Beraldo, A. A. Nassar, *Transit. Metal. Chem*, **1999**, 24, 25-28.
[15] G. Kanagaraj, G. N. Rao, *Polyhedron*, **1993,** 12, 383-387.
[16] R. L. de Lima, L. R. d Souza Teixeira, T. M. G. Carneiro, H. Beraldo, *J. Braz. Chem. Soc*, **1999,** 10, 184-188.
[17] H. Beraldo, R. Lima, L. R. Teixeira, A. A. Moura, D. X. West, *J Mol.Struct*, **2001**, 559, 99-106.
[18] S. Chandra, S. Parmar, Y. Kumar, *Bioinorg. Chem. Appl*, **2009**, Article ID 851316, 6 pages.