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Synthesis, structure and antimicrobial activity of Co(II) and Cu(II) complexes with 2-imino, 4-thiobiuret

Shraddha Shukla and A. P. Mishra*

Bio-coordination Research Laboratories, Department of Chemistry, Dr. H.S. Gour University Sagar(M.P), INDIA

ABSTRACT

The coordination complexes of Co (II) and Cu (II) with the Schiff base derived from 4-aminoacetophenone and 1-acetonaphthone with 2-imino, 4-thiobiuret have been synthesized and characterized by micro analytical data, FT-ir and FAB mass spectral studies. Synthesized complexes have been tested for the reactivity and substitution behavior. The Schiff base and metal complexes shows a good activity against the Gram-positive bacteria; Bacillus subtilis, Staphylococcus aureus and Gram-negative bacteria; Escherichia coli, Pseudomonas aeruginosa; Candida albicans, Aspergillus niger, Aspergillus fumigatus and Fusarium oxysporum fungal cultures.

Keywords: Antimicrobial, 2-imino, 4-thiobiuret, 4-aminoacetophenon, 1-acetonaphthone.

INTRODUCTION

The developments and design of new products with the potential for use as biologically active compounds [10,12] has recently became a burgeoning topic within the biological science [19,20], and chemistry in particular [21,22]. The antibacterial activity of various heterocyclic systems is well known, but some heterocyclic cause's toxicity so metal complexes with ligands decreases toxicity. Schiff bases play an important role in inorganic chemistry, as they easily form stable complexes with most transition metal ions. The development of the field of bioinorganic chemistry has increased the interest in Schiff base complexes may serve as models for biologically important species [16,23]. The synthesized chemical compounds, which are used for the treatment of infectious diseases, are known as chemotherapeutic agents.

In this paper, we describe the synthesis, structure and antimicrobial screening of some Schiff base complexes view viz. $[Co(A).(H_2O)_3].Cl_2.2H_2O$; $[Cu(A).(H_2O)_3].Cl_2.2H_2O$ and $[Co(T)(H_2O)_2].Cl_2.3H_2O$; $[Cu(T)(H_2O)].Cl_2.3H_2O$.

FT-IR spectra:

1.$C_{14}H_{14}N_4OS$ **(A):** (KBr, ν, cm^{-1}) 3089 (C-H,Aromatic), 2947 (OH), 1519 (C-O,phenolic), 1612 (C=N), 994 (N-N), 3198 (N-H), 1637 (C=O).

2.$[Co(C_{14}H_{14}N_4OS)(H_2O)_3].Cl_2.2H_2O$ **(1):** 3105 (C-H,Aromatic), 3315 (OH), 1278 (C-O,phenolic), 1610 (C=N), 836 (N-N), 3039 (N-H), 1596 (C=O), 470 (M-N), 422(M-S).

3.$[Cu(C_{14}H_{14}N_4OS)(H_2O)_3].Cl_2.2H_2O$ **(2):** 3100 (C-H,Aromatic), 3321 (OH), 1280 (C-O,phenolic), 1606 (C=N), 735 (N-N), 3050 (N-H), 1598 (C=O), 493 (M-N), 425(M-S).

4.$C_{10}H_{13}N_5OS$ **(T):** (KBr, ν, cm^{-1}) 3199 (C-H,Aromatic), 3011 (OH), 1525 (C-O,phenolic), 1621 (C=N), 960 (N-N), 3190 (N-H), 1686 (C=O).

5.$[Co(C_{10}H_{13}N_5OS)(H_2O)_2].Cl_2.3H_2O$ **(3):** 3176 (C-H,Aromatic), 3037 (OH), 1284 (C-O,phenolic), 1618 (C=N), 896 (N-N), 3040 (N-H), 1612 (C=O), 480 (M-N), 438(M-S).

6.$[Cu(C_{10}H_{13}N_5OS)(H_2O)_2].Cl_2.3H_2O$ **(4):** 3140 (C-H,Aromatic), 3312 (OH), 1292 (C-O,phenolic), 1615 (C=N), 764 (N-N), 3048 (N-H), 1617 (C=O), 498 (M-N), 440 (M-S).

FAB-mass spectra:

1. FAB-mass spectra of $[Co(A)(H_2O)_3].Cl_2.2H_2O$*:* The FAB mass spectrum of $[Co(A)(H_2O)_3].Cl_2.2H_2O$ complex has been studied as one of the representative case. The peaks of appreciable intensity have been observed at m/z values obs.(cal.) – 514(509), 492(490), 478(473), 400(401), 363(365), 344(347),289(288) and 107(102) suggesting the fragmentation pattern [14]. The m/z value 514 corresponds to nearest composition $[Co(A)(H_2O)_3]Cl_2.2H_2O$, 363 to $[Co(A)H_2O]$, 344 to [Co(A)], 289 to ligand alone and 102 to Co with chelated ligand moiety.

2. FAB-mass spectra of $[Cu(A)(H_2O)_3].Cl_2.2H_2O$*:* The FAB mass spectrum of $[Cu(A)(H_2O)_3].Cl_2.2H_2O$ complex has been studied as one of the representative case. The peaks of appreciable intensity have been observed at m/z values obs.(cal.) – 513(509), 495(495), 476(475), 405(405), 370(370), 351(350), 290(291) and 107(117) suggesting the fragmentation pattern [14]. The m/z value 513 corresponds to nearest composition $[Cu(A)(H_2O)_3]Cl_2.2H_2O$, 370 to $[Co(A)H_2O]$, 351 to [Co(A)], 290 to ligand alone and 117 to Cu with chelated ligand moiety.

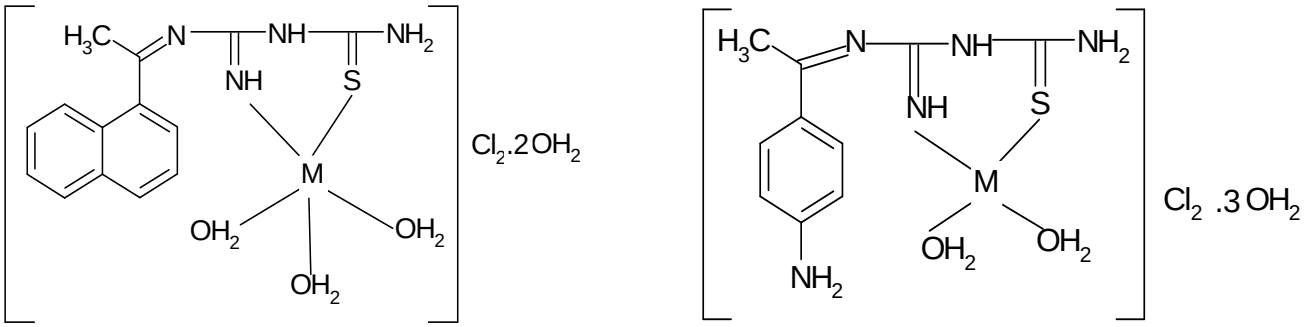
3. FAB-mass spectra of $[Co(T)(H_2O)_2].Cl_2.3H_2O$*:* The FAB mass spectrum of $[Co(T)(H_2O)_2].Cl_2.3H_2O$ complex has been studied as one of the representative case. The peaks of appreciable intensity have been observed at m/z values obs.(cal.) – 476(474), 433(437), 405(401), 376(380), 313(312), 250(253) suggesting the fragmentation pattern.

4. FAB-mass spectra of $[Cu(T)(H_2O)_2].Cl_2.3H_2O$*:* The FAB mass spectrum of $[Cu(T)(H_2O)_2].Cl_2.3H_2O$ complex has been studied as one of the representative case. The peaks of appreciable intensity have been observed at m/z values obs.(cal.) – 475(478), 449(442), 428(424), 396(388), 355(357), 316(310), 257(253) suggesting the fragmentation pattern.

TABLE-I: Analytical and physical data of the metal complexes of ANIT-(A) and AAIT-(T) Schiff base ligands:

Comp./Complexes	Mol. Wt.	Colour	Yield %	Found (calcd.) %				Metal %	Decomposition temp.˚C
				C	H	N	S		
$C_{14}H_{14}N_4OS$-**(A)**	270.37	Magnolia	84	14.79 (14.76)	5.22 (5.21)	20.72 (20.70)	11.86 (11.86)	-	100
$[Co(C_{14}H_{14}N_4OS)(H_2O)_3].Cl_2.2H_2O$**(1)**	508.3	Tobacco green	82	33.06 (33.06)	4.76 (4.75)	11.02 (11.01)	6.31 (6.33)	11.59 (11.57)	180
$[Cu(C_{14}H_{14}N_4OS)(H_2O)_3].Cl_2.2H_2O$**(2)**	440.85	Dirty brown	80	38.14 (38.16)	5.49 (5.46)	12.71 (12.70)	7.27 (7.25)	14.41 (14.41)	150
$C_{10}H_{13}N_5OS$-**(T)**	235.33	Sandalwood	87	51.04 (51.1)	5.57 (5.57)	29.76 (29.79)	13.62 (13.64)	-	120
$[Co(C_{10}H_{13}N_5OS)(H_2O)_2].Cl_2.3H_2O$**(3)**	473.26	Coffee	80	25.38 (25.36)	4.89 (4.85)	14.79 (14.78)	6.78 (6.75)	12.45 (12.48)	210
$[Cu(C_{10}H_{13}N_5OS)(H_2O)_2].Cl_2.3H_2O$**(4)**	405.81	Milliatary dark green	75	29.59 (29.57)	5.71 (5.73)	17.26 (17.23)	7.90 (7.92)	15.66 (15.64)	300

Structure: Suggested structures of the metal complexes of the ligand.



Antimicrobial activity:
All the synthesized compounds/complexes of were tested for their antimicrobial activity.

For antibacterial screening Gram-positive bacteria; *Bacillus subtilis, Staphylococcus aureus* and Gram-negative bacteria; *Escherichia coli, Pseudomonas aeruginosa;* and antifungal screening *Candida albicans, Aspergillus niger, Aspergillus fumigatus* and *Fusarium oxysporum* fungal cultures. All compounds shows good activity against all microbes. In future these complexes will be used as antibiotics.

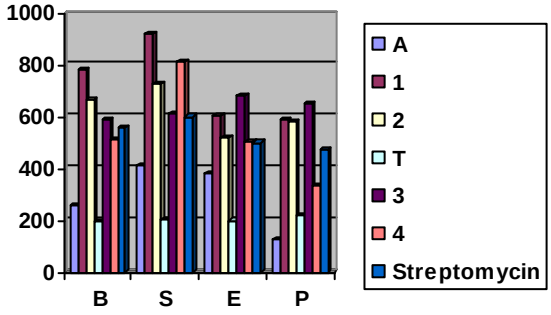
These results exhibited markedly an enhancement in activity on coordination with the metal ions against one or more testing bacterial strains. This enhancement in the activity is rationalized on the basis of the structures of, (A)–(T) by possessing an additional azomethine (C=N) linkage which imports in elucidating the mechanism of transamination and resamination reactions in biological system . It has also been suggested that the ligands with nitrogen and oxygen donor systems might inhibit enzyme production, since the enzymes which require these groups for their activity appear to be especially more susceptible to deactivation by the metal ions upon chelation. Chelation reduces the polarity of the metal ion mainly because of the partial sharing of its positive charge with the donor groups and possibly the π-electron delocalization within the whole chelate ring system thus formed during coordination. This process of chelation thus increases the lipophilic nature of the centralmetal atom, which in turn favors its permeation through the lipoid layer of the membrane. This in turn is responsible for increasing the hydrophobic character and liposolubility of the molecule in crossing cell membrane of the microorganism, and hence enhances the biological utilization ratio and activity of the testing complexes/compound.

A comparative study of the ligand and its complexes indicates that some of the metal chelates exhibit higher antimicrobial activity than the free ligand. The increase in the antimicrobial activity of metal chelates is due to the presence of metal ions in the complexes.

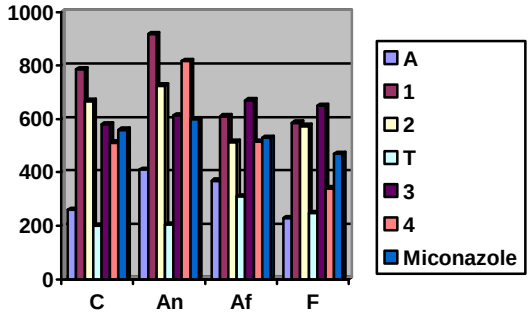
Evaluation of antibacterial screening: All the synthesized compounds/complexes of were tested for their antibacterial activity. For antibacterial screening Gram-positive bacteria; *Bacillus subtilis, Staphylococcus aureus* and Gram-negative bacteria; *Escherichia coli, Pseudomonas aeruginosa* were used. Antibacterial screening was performed by the Well diffusion method using Nutrient Agar as the medium. Two-eight hours old bacterial inoculums containing approximately 10^4-10^6 colony forming units (CFU)/ml were used in these screening. Streptomycin used as a standard drug. Recommended concentration (100µl) of the test sample (1mg/ml in DMSO) was introduced in the respective wells. The M.I.C. values indicate a better activity against the Gram-negative strains except that of P.aeruginosa that usefully is very resistance at antibiotic. The activity of complexes is higher than the ligand. The most

effective antibacterial was manifested against Gram-positive strains except that of S.aureus. The values of minimum inhibitory concentration (M.I.C., μg cm^{-3}) are presented in table-II.

Evaluation of antifungal screening: Antifungal activity of the all synthesized compounds were studied against four fungal cultures; *Candida albicans, Aspergillus niger, Aspergillus fumigatus* and *Fusarium oxysporum*. Potato dextrose agar (PDA) was seeded with 10^5 (CFU)/ml fungal spore suspensions and was transferred to Petri- plates. Disc soaked in 20 ml (10μg/ml in DMSO) of all compounds were placed at different positions on the agar surface. Miconazole used as a standard drug. The M.I.C. values indicate a better activity against the A.niger and C albicans. Complex 1 shows best activity of all fungal cultures. The activity of complexes is higher than the ligand. The most effective antifungal was manifested against the A.niger and C albicans. The values of minimum inhibitory concentration (M.I.C., μg cm^{-3}) are presented in table-III.



Antibacterial activity



Antifungal activity

The MIC values indicate that the metal complexes had a higher antibacterial activity than the free ligand. Such increased activity of the metal complexes can be explained on the basis of the overtone concept [24] and chelation theory [25]. According to the overtone concept of cell permeability, the lipid membrane that surrounds the cell favors the passage of only lipid soluble materials, due to which liposolubility is an important factor controlling the antimicrobial activity. On chelation, the polarity of the metal ion is reduced to a great extent due to the overlap of the ligand orbital and the partial sharing of the positive charge of the metal ion with donor groups. Furthermore, it increases the delocalization of electrons over the whole chelate ring and enhances the lipophilicity of the complex. This increased lipophilicity enhances the penetration of the complex into the lipid membrane and blocks the metal binding sites on the enzymes of the microorganism. However, the antimicrobial activities of the ligand and its metal complexes were lower than those found for the standard antimicrobial drugs.

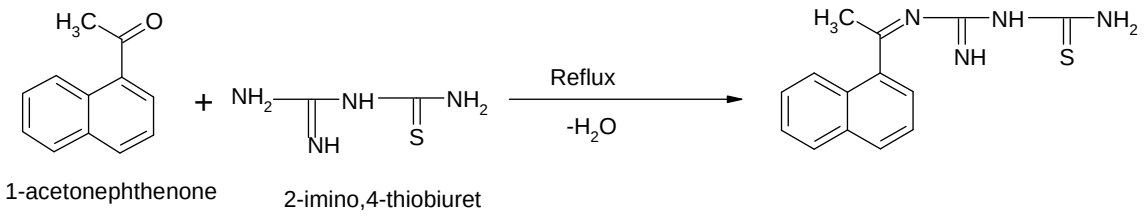
MATERIALS AND METHODS

Apparatus and Reagent: All the used chemicals and solvents were of A.R. grade. Cobalt (II) and Copper (II) chloride were obtained from Aldrich, Fluca, Loba and Merck chemie. Elemental analysis and FAB-mass spectra were recorded at SAIF-CDRI, Lucknow. FT-IR (in KBr), were recorded at SAIF, I.I.T. Dehli.

Synthesis: Schiff bases (ANIT, AAIT) have been synthesized by adding the methanolic solution of 1-acetonephthanone and 4-aminoacetophenone (0.06/0.07mol) with methanolic solution of 2-imino 4-thiobiuret (0.06/0.07mol) in equimolar ratio. The reaction mixture was then refluxed on 10-12 hours. The volume of solvent was reduced until precipitation began, and the mixture was allowed to stand overnight, after which the colored solid was obtained. It was filtered off,

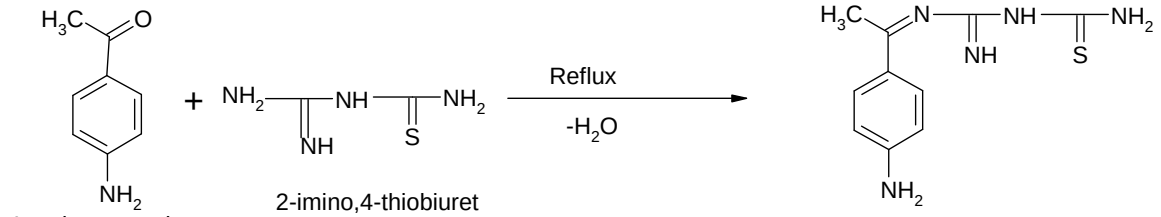
recrystallized thrice with ethanol, finally washed with ether, and dried under reduced pressure over anhydrous $CaCl_2$ in desiccators. The purity of the synthesized compounds was monitored by TLC using silica gel-G. Yield ANIT(A) =84% and AAIT(T) =87%.

All the complexes have been prepared by mixing the methanolic solution of $MCl_2.nH_2O$ (0.005/0.003mol) with the methanolic solution of Schiff bases ANIT-(A)/ AAIT-(T) (.005/.007mole) in 1:1 molar ratio. The resulting mixture was refluxed on water bath for 8-9 hours. The volume of solvent was reduced until precipitation began, and the mixture was allowed to stand overnight, after which the colored solid was obtained. It was filtered off, recrystallized thrice with ethanol, finally washed with ether, and dried under reduced pressure over anhydrous $CaCl_2$ in desiccators. The purity of the synthesized compounds was monitored by TLC using silica gel-G.



1-acetonephthenone [1] 2-imino,4-thiobiuret [2] [3]

Schiff base-ANIT(A)



4-aminoacetophenone [4] 2-imino,4-thiobiuret [2] [5]

Schiff base-AAIT(T)

Biological activity:

Antibacterial bioassay- All the synthesized compounds were screened in vitro for their antibacterial activity against two Gram-positive bacteria; *Bacillus subtilis*, *Staphylococcus aureus* and two Gram-negative bacteria; *Escherichia coli, Pseudomonas aeruginosa* strains using by the Well diffusion method using Nutrient Agar as the medium. Two-eight hours old bacterial inoculums containing approximately 10^4-10^6 colony forming units (CFU)/ml were used in these assays. The wells were dug in the media with the help of a sterile metallic borer with centers at least 24 mm. Recommended concentration (100µl) of the test sample (1mg/ml in DMSO) was introduced in the respective wells. Other wells filled with antibacterial drug *Streptomycin*. The plates were incubated immediately at 37˚c for 24 hours. Activity was determined by measuring the diameter of zones showing complete inhibition (mm). Growth inhibition was compared with the standard drug.

Table-II: The MIC (µg cm^{-3}) of the synthesized compounds for Antibacterial activity:

Compounds	Gram-positive		Gram-negative	
	B.subtilis (B)	***S.aureus (S)***	***E.coli (E)***	***P.aeruginosa (P)***
A	256	410	378	128
1	780	918	604	586
2	664	728	516	578
T	198	206	200	218
3	586	612	680	650
4	512	812	502	332
Streptomycin	560	600	500	470

Antifungal activity- Antifungal activity of the all synthesized compounds were studied against four fungal cultures; *Candida albicans, Aspergillus niger, Aspergillus fumigatus* and *Fusarium oxysporum*. Potato dextrose agar (PDA) was seeded with 10^5 (CFU)/ml fungal spore suspensions and was transferred to Petri- plates. Disc soaked in 20 ml (10µg/ml in DMSO) of all compounds were placed at different positions on the agar surface. The plates were incubated at 32˚c for seven days. The results were recorded as zone of inhibition in mm and were compared with standard drug *Miconazole*.

Table-III: The MIC (µg cm^{-3}) of the synthesized compounds for Antifungal activity:

Compounds	***C.albicans*** **(C)**	***A. niger*** **(An)**	***A.fumigatus*** **(Af)**	***F. oxysporum*** **(F)**
A	260	410	368	228
1	786	918	610	586
2	668	725	514	574
T	200	204	310	248
3	580	614	670	650
4	512	818	516	340
Miconazole	560	600	530	470

RESULT AND DISCUSSION

All the metal complexes are coloured, solid and stable towards air and moisture. They decomposed at high temperature and they are more or less soluble in common organic solvents. The result of elemental analysis (C, H, N and S) with molecular formulae, yield and the melting/decomposition point are presented in table I. The metal complexes exhibit 1:1 (metal:Schiff base ligand) stoichiometry.

CONCLUSION

The coordination complexes of Co (II) and Cu (II) with the Schiff base derived from 4-aminoacetophenone and 1-acetonaphthone with 2-imino, 4-thiobiuret have been synthesized and characterized. The synthesized compound shows a good activity against the Gram-positive bacteria; *B. subtilis, S.aureus* and Gram-negative bacteria; *E.coli, P.aeruginosa* and fungal cultures; *C. albicans, A. niger, A. fumigatus* and *F. oxysporum*. It may be suggested that the chelated complexes deactivate various cellular enzymes, which play a vital role in various metabolic pathways of these microorganisms. It has also been proposed that the ultimate action of the toxicant is the denaturation of one or more protein of the cell, which as a result, impairs normal cellular process [16, 17, 18].

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REFERENCES

[1] H.K.D.H. Bhadeshia, university of Cambridge.
[2] M.M. Omar, G.G. Mohamed and A.A. Ibrahim, *Spectrochim. Acta Part A*, **2009**, 73, 358.
[3] Y.F. Wang, J. Liu, H. Xian and G. Zhao, *Molecules*, **2009**, 14.
[4] S. Sarkar, Y. Aydogdu, Dagdelen F, Bhaumik BB and Dey K, *Mate. Chem. and Phys.*, **2004**, 88, 357.
[5] N.H. Al-Sha'alan, *Molecules*, **2007**, 12, 1080.
[6] N. Yanagihara, Y. Yumoto, K. Arai, K. Kawabe and Y. Kojima, *Polyh.*, **2000,** 19, 1311.
[7] F.A.Cotton, G.Wilkinson, C.A.Murillo and M. Bochmann, Adv. Inorg. Chem., **2003**, 6th Edition, Wiley, New York.
[8] A.P.Mishra, M.Khare and S.K.Goutam, *Synth. React. Inorg. Met.Org.Chem.*, **2002,** 32, 1485.
[9] A.P.Mishra and L.R.Pandey, *Ind. J. Chem.*, **2005**, 44A, 94.
[10] A.J.P.Willian, Bioinorg. Chem., Ame. chem. Soci.: **1971,** Washington, DC.
[11] L.I.U.Jian-ning, W.U.Bo-wan, ZHANG Bing, L.I.U. Yongchumn, *Turk. J. Chem.*, 2006, 32, 41.
[12] A.Mastrolorenzo and C.T.Supuran, *Met. Based Drugs* **2001**, 7, 99.
[13] E.Hamed, M.S.Attia and K.Bassiouny, *Bioinorg. Chem. and Appl.*, **2009**, 979680.
[14] Z.H. Chouhan, Asifa Munawar and T. Claudia Supuran , *Met. Based Drugs*, **2001**, 8, 3.
[15] H.Nora, Al-Sha´alan, *Molecules.*, **2007**, 12, 1080.
[16] Z.H.Chouhan, S.K.A. Sheazi, *Syn.Rea.Inorg.Met-Org.Chem*. **1999**, 29, 105.
[17] M.A.Ali, M.H.Kabir, M.Nazimuddin, S.M.H.Majumdar, M.T.H.Tarafder, M.Akhair, *Ind. J.Chem*. **1988**, 27A, 1064.

[18] H.William, V.Stephen, Theo. and Appl. of Microbio. Assays, **1989,** Academic press, San Diego.
[19] A,Scozzafavac, L.Menabuoni, F.Mincione, G.Mincione and C.T.Supuran, *Bioorg.Med.Chem.Lett*., **2001**, 11, 575.
[20] M.J.Seven and L.A.Johnson, Metal Binding in Medic, 4th edn., **1960**, Lippincott Co: Philadelphia, PA.
[21] C.Walsh, *Scien*. **2001**, 409, 226.
[22] McCleverty *J. Prog. Inorg. Chem*. **1968**, 10, 49.
[23] C.H.Colins, P.M.Lyne; University Park Press, **1970,** Baltimore.
[24] Y. Anjaneyulu, R. P. Rao, *Syn. Rea. Inorg. Met. Org. Chem*. **1986,** 16, 257.
[25] L. Mishra, V. K. Singh, *Ind J. Chem*. **1993 ,** 32A, 446.