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Template Synthesis of Macrocyclic Schiff Base Complexes of Transition Metal Ions and their Biological Activity

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The complexes of the type MLX_2 [where M = Zn(II), Co(II), Cu(II) and Ni(II); L is a condensation product of 1,3-dibenzoylbenzene and carbohydrazide/thiocarbohydrazide; X = Cl^-, CH_3COO^-] were synthesized using template method. These complexes were characterized by elemental analysis, spectral analysis (IR, NMR), magnetic moment measurements, molar conductivity, ESR studies and electronic measurements which indicated non electrolytic nature of complexes having distorted octahedral geometry. All the complexes were screened for antimicrobial activity against Gram-positive bacteria (*Bacillus subtilis, Staphylococcus aureus*), Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa*) and phytopathogenic fungi (*Candida albicans, Saccharomyces cerevisiae*). Minimum inhibitory concentration (MIC) of these complexes was also determined and compared with standard antibiotics ciprofloxacin and amphotericin.

Keywords: 1,3-Dibenzoylbenzene, Carbohydrazide, Thiocarbohydrazide, Phytopathogenic fungi, Template method.

INTRODUCTION

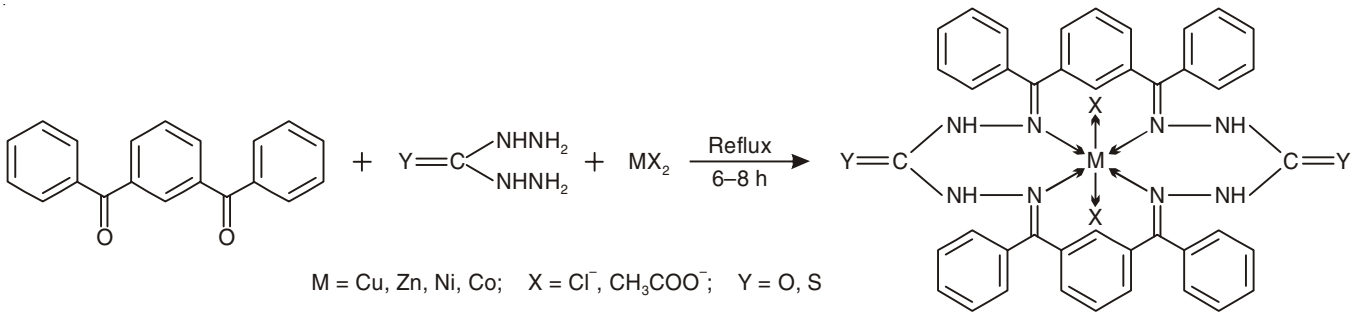
Template reactions have been widely used for the synthesis of macrocyclic complexes, in which transition metal ions are generally used as the template agent [1]. Synthesis of transition metal macrocyclic complexes of Schiff's base ligands had gain a growing interest due to their wide applications in pharmacological field for the use as antimicrobial agents against pathogenic microorganisms [2,3]. New metal based antibacterial and antifungal compounds are in increasing demand these days so coordination chemistry is becoming an emerging area of research. Antimicrobial activities of complexes are due to their lipophilic nature and ability to penetrate through the lipid membrane. Moreover these complexes find their uses in many industries. Macrocyclic complexes are used as catalysts [4] and anticorrosion agents [5]. Macrocyclic complexes are of great importance in many biological processes like oxygen transport and photosynthesis [6]. These complexes are widely used as anticancer [7,8], antifungal [9], anti-inflammatory [10], antidiabetic [11] and antiviral [12] drugs. Because of similarity with biomolecules, the study of macrocyclic Schiff base complexes had reached to a significant era. We have incorporated metal ions onto cyclic/acyclic systems and synthesized biologically active macrocyclic Schiff base complexes of transition metals [13-15]. In continuation of the same work, present complexes have been synthesized and their pharmacological effect has been explored.

EXPERIMENTAL

Synthesis of complexes: Template synthesis of metal complexes was carried out by mixing the methanolic solution of 1,3-dibenzoylbenzene, carbohydrazide/thiocarbohydrazide and copper, nickel, cobalt, zinc salts. Carbohydrazide/thiocarbahydrazide (10 mmol) taken in hot methanolic solution, copper, nickel, cobalt, zinc salts (5 mmol) dissolved in same solvent were added to hot diammine solution and refluxed for 0.5 h. 1,3-Dibenzoylbenzene (10 mmol) was added to refluxing mixture. The reaction mixture was refluxed for 6-8 h and then concentrated to half of its volume by evaporation on water bath. Crystalline solids were obtained on cooling the reaction mixture to room temperature (**Scheme-I**). These crystalline solids were filtered and washed with methanol, acetone and diethyl ether and dried *in vacuo*. The complexes were insoluble in water and common organic solvents but soluble in dimethyl sulphoxide and dimethyl formamide. The physico-chemical data of the synthesized complexes are given in Table-1.

Biological activity

Procurement of microbial cultures: Clinical strains of Human pathogenic bacteria such as *Bacillus subtilis* (MTCC121), *Escherichia coli* (MTCC1652), *Pseudomonas aeruginosa* (MTCC741), *Staphylococcus aureus* (MTCC96) and the phytopathogenic fungi *Candida albicans* (MTCC3017) and



Scheme-I: Synthesis of transition metal complexes of carbohydrazide/thiocarbohydrazide and 1,3-dibenzoylbenzene

TABLE-1
PHYSICO-CHEMICAL DATA OF TRANSITION METAL COMPLEXES OF CARBOHYDRAZIDE/THIOCARBOHYDRAZIDE AND 1,3-DIBENZOYLBENZENE

Compd. No.	Formula	Colour	Yield (%)	Molar mass	Elemental analysis (%): Found (calcd.)			
					M	C	H	N
1	$[C_{42}H_{32}N_8O_2Cu(OAc)_2]$	Grey green	68	861.5	7.11 (7.37)	63.76 (64.07)	4.13 (4.41)	12.73 (13.00)
2	$[C_{42}H_{32}N_8O_2NiCl_2]$	Soil	63	809.7	6.91 (7.25)	62.01 (62.24)	3.78 (3.95)	13.51 (13.83)
3	$[C_{42}H_{32}N_8O_2Co(OAc)_2]$	Brown	42	856.9	6.52 (6.87)	64.12 (64.41)	4.08 (4.43)	12.87 (13.07)
4	$[C_{42}H_{32}N_8O_2Zn(OAc)_2]$	Purple	64	863.4	7.24 (7.57)	63.62 (63.93)	4.18 (4.40)	12.61 (12.97)
5	$[C_{42}H_{32}N_8S_2Cu(OAc)_2]$	Black	50	893.5	6.86 (7.10)	61.42 (61.77)	4.01 (4.25)	12.19 (12.53)
6	$[C_{42}H_{32}N_8S_2NiCl_2]$	Green	57	841.7	6.62 (6.97)	59.64 (59.87)	3.42 (3.80)	13.02 (13.30)
7	$[C_{42}H_{32}N_8S_2Co(OAc)_2]$	Black	46	888.9	6.41 (6.62)	61.86 (62.09)	4.02 (4.27)	12.18 (12.59)
8	$[C_{42}H_{32}N_8S_2Zn(OAc)_2]$	White	67	895.4	7.03 (7.30)	61.32 (61.64)	3.92 (4.24)	12.25 (12.51)

Saccharomyces cerevisae (MTCC170), have been procured from Microbial Type Culture Collection IMTECH, Chandigarh. Bacterial strains were sub cultured on nutrient agar (NA) and fungi on malt extract agar (MEA) medium and were incubated aerobically at 37 °C.

Antimicrobial activity: The antimicrobial activity of the complexes have been determined against Gram-positive bacteria (*Bacillus subtilis, Staphylococcus aureus*), Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa*) and phytopathogenic fungi (*Candida albicans, Saccharomyces cerevisiae*) using the agar well diffusion method [16]. Density of all the microbial cultures was adjusted to 0.5 McFarland standards, which were visually comparable to a microbial suspension of approximately 1.5×10^8 cfu/mL. One hundred microlitre (100 µL) inocula of the test microorganisms has been plated out on respective media plates and kept for 15 min for adsorption. Solutions of each complex have been prepared by dissolving the complex in 20 % DMSO. Using sterile cork borer of 8 mm diameter, wells were bored into the seeded agar plates and these were loaded with a 100 µL of the solution of each complex. All the plates are incubated at 37 °C for 24 h. Antimicrobial activity of each complex has been evaluated by measuring the zone of growth inhibition against the test organisms with a zone reader (HiAntibiotic zone scale). Ciprofloxacin and amphotericin were used as a positive control. The experiment was done in triplicate for each organism.

Determination of minimum inhibitory concentration (MIC): Minimum inhibitory concentration of the complexes for each test organism has been determined by following the modified agar well diffusion method [17]. A twofold serial dilution of each complex has been prepared. Each complex has been dissolved in 20 % DMSO to achieve a concentration of 100 mg/mL followed by dilution in sterile distilled water (1:1) to achieve a decreasing concentration range of 50 to 0.39 mg/mL. A 100 µL volume of each dilution has introduced into wells (in triplicate) in the agar plates already seeded with 100 µL of standardized inoculum (10^6 cfu/mL) of the test microbial strain. All test plates were incubated aerobically at 37 °C for 24 h and observed for the inhibition zones. Zone of inhibition (> 8 mm) has been observed in each plate. Concentration of the complex that completely inhibited the growth of the microorganism has been taken as minimum inhibitory concentration.

RESULTS AND DISCUSSION

Infrared spectra: Absence of strong absorption peak at 1665 cm^{-1} due to carbonyl group of 1,3-dibenzoylbenzene and medium intensity peaks at 3245/3285 and 3300/3320 cm^{-1} due to NH_2 group of carbohydrazide/thiocarbohydrazide indicate condensation of carbonyl and diamine to form imine (C=N) [18]. A band at 1600 cm^{-1} confirms the presence of C=N group [18,19]. The lower value of this peak may be due to drifting of lone pair electron density of azomethine nitrogen of (C=N) group towards metal atom [20]. A single medium intensity band about 3218 cm^{-1} may be due to ν(N-H) stretching vibrations of carbohydrazide/thiocarbohydrazide. Medium intensity band at about 1714 cm^{-1} may be assigned to the >C=O group of CONH moiety. While ν(C-H) stretching vibrations at 3200 cm^{-1} may be due to aromatic rings of dibenzoylbenzene moiety in the complex. Absence of a band at about 2550 cm^{-1} rules out the possibility of thione-thiol tautomerism. Band near 1300-1000 cm^{-1} may be assigned (C-N) stretching. While the appearance of bands near 800-750 cm^{-1} are assigned to out of plane bending vibrations due to ν(C-H) of aromatic rings [21].

Band near 1640-1550 cm^{-1} and at 1260-1160 cm^{-1} may be assigned to $\nu(COO^-)_{as}$ and $\nu(COO^-)_s$, respectively of acetate ion. The difference between asymmetric and symmetric vibration (about 400 cm^{-1}) shows unidentate coordination of acetate ion with central metal ion [22]. Band at 460 cm^{-1} in far infrared spectra corresponds to ν(M-N) vibrations of metal complexes. This indicates coordination of azomethine nitrogen to the central metal ion.

NMR spectra: Multiplets in the ^1H NMR spectrum of zinc complexes in the region δ 7.5-8.3 may be assigned to protons of aromatic ring of 1,3-dibenzoylbenzene. Broad singlet at about δ 8.3/δ 9.6 may be due to CONH proton of carbohydrazide/thiocarbohydrazide. Absence of broad peak at δ 2.0 due to N-H of carbohydrazide/thiocarbohydrazide confirms the formation of C=N by condensation of carbonyl group of 1,3-dibenzoylbenzene and NH_2 of carbohydrazide/thiocarbohydrazide.

Magnetic measurements and electronic spectral studies

Copper(II) complexes: The effective magnetic moment of copper(II) complexes measured at room temperature is 1.83-1.95 BM. This value agrees well with distorted octahedral geometry of d^9 system. The expected value of one electron octahedral complexes is 1.75 BM. The higher value of magnetic moment of these complexes may be due to spin orbital coupling and spin orbit coupling. Electronic spectra of copper(II) complexes shows a band at 18000-20000 cm^{-1} and a weak low energy shoulder at about 15000-17000 cm^{-1} these bands can be assigned if tetragonal distortion of octahedral symmetry is assumed. Due to tetragonal distortion the *d* orbital splitting should be $x^2\text{-}y^2 > z^2 > xy > xz > yz$. So the electronic transitions involved in the band formation are $^2B_{1g} \rightarrow {}^2B_{2g}$ ($xy \rightarrow x^2\text{-}y^2$) and $^2B_{1g} \rightarrow {}^2E_g$ ($xz, yz \rightarrow x^2\text{-}y^2$) and the low energy shoulder assigned $^2B_{1g} \rightarrow {}^2A_{1g}$ ($z^2 \rightarrow x^2\text{-}y^2$) transitions.

Nickel(II) complexes: The effective magnetic moment for nickel(II) complexes at room temperature is in the range of 2.92-3.10 BM. This value shows octahedral high spin nature of nickel(II) complexes. The expected value for octahedral two electron system is 2.8 BM. This value is due to spin only moment. Observed higher value of these complexes shows orbital momentum contribution to spin moment of high spin octahedral nickel(II) complexes. Electronic spectra of nickel(II) complexes shows a band at 9500-10000 cm^{-1} with a low energy shoulder at about 11000-12600 cm^{-1}. These bands are formed due to splitting of one band and transitions involved for this band may be $^3B_{1g} \rightarrow {}^3E_g$ and $^3B_{1g} \rightarrow {}^3B_{2g}$ for D_{4h} symmetry [23]. The other two bands are at 17030-17700 cm^{-1} (ν_2) and at 27100-28450 cm^{-1} (ν_3). These bands may be due to $^3A_{2g} \rightarrow {}^3T_{1g}$ (F) and $^3A_{2g} \rightarrow {}^3T_{1g}$ (P) transitions. The $\pi \rightarrow \pi^*$ transition of (C=N) group results in observed high energy band at 35400 cm^{-1}. Effective magnetic moment and electronic spectra confirms high spin nature and distorted octahedral symmetry of nickel(II) complexes.

Cobalt(II) complexes: At room temperature the effective magnetic moment of cobalt(II) complexes lie in the range of 4.45-4.67 BM. This value agrees with three electron octahedral system as the expected value is about 3.75 BM [24]. The higher value of magnetic moment (μ_{eff}) for d^7 complexes may be due to orbital angular momentum contribution and spin orbit coupling. This also confirms high spin nature of cobalt(II) complexes. Electronic spectra of cobalt(II) complexes exhibit three bands at 8200-9300 cm^{-1} (ν_1) 13500-16550 cm^{-1} (ν_2) and 19450-20350 cm^{-1} (ν_3) respectively. These bands also confirm D_{4h} symmetry of high spin cobalt(II) complexes. The transitions assigned to these bands are $^4T_{1g} \rightarrow {}^4T_{2g}$ (F), $^4T_{1g} \rightarrow {}^1A_{2g}$ (F) and $^4T_{1g} \rightarrow {}^4T_{2g}$ (P) transitions.

ESR: The ESR spectrum of copper(II) complexes of 1,3-dibenzoylbenzene and carbohydrazide/thiocarbohydrazide were recorded in solid state on X-band at a frequency of 9.1 GHz under the magnetic field of 2000G. Tetracyanoethylene radical was used as g–marker. The ESR spectrum of complexes of 1,3-dibenzoyl benzene and carbohydrazide at room temperature in solid state exhibit single isotropic peak with g_{iso} value 2.007. The g value of free electron is 2.0023. This shifting of g value is due to interaction between the spin and orbital motion. The value of g_{iso} greater than 2.0023 indicate that the unpaired electron is localized in $d_{x^2-y^2}$ orbital of copper(II) ion. This again shows distortion in the copper(II) complex from O_h symmetry to D_{4h} symmetry. It is known that the transition metal complexes having d-shell more than half filled have g value greater than 2.0023. As g value of these complexes is 2.007 (greater than 2.0023) so this indicate that d-shell in these copper(II) complexes is more than half filled. The ESR spectrum of copper(II) complexes at LNT shows a broad signal with g value 2.119. This g value again confirms distortion in octahedral symmetry.

The ESR spectrum of 1,3-dibenzoylbenzene and thiocarbohydrazide at LNT in solid state show an isotropic peak with g_{iso} value 2.11. Shifting of g value (2.0023) is due to spin orbit coupling. A higher value of g is due to mixing of spin orbital coupling of metal orbitals having unpaired electrons with filled ligand orbitals. Higher value of g also shows distorted octahedral symmetry of these copper(II) complexes.

Pharmacological effect of complexes: All the complexes have been tested for antimicrobial activity against Gram-positive bacteria, Gram-negative bacteria and phytopathogenic fungi. Most of the complexes are active against these microorganisms (Table-2). The MIC of most of the complexes is comparable to the standard drugs ciprofloxacin and amphotericin-B. Complex **5**, $[C_{42}H_{32}S_2N_8Cu(OAc)_2]$, completely inhibited the growth of *Pseudomonas aeruginosa* at 3.125 mg/mL while the inhibition of growth for the same bacteria due to complex **4**, $[C_{42}H_{32}O_2N_8Co(OAc)_2]$; **7**, $[C_{42}H_{32}S_2N_8Zn(OAc)_2]$ and **8**, $[C_{42}H_{32}\ S_2N_8Co(OAc)_2]$ is at 6.25 mg/mL (Table-3). This complex can be used against mentioned microorganisms after testing their toxicity on human body.

Conclusion

Synthesized macrocyclic Schiff base metal complexes may be assigned distorted octahedral geometry as revealed by magnetic susceptibilities, electronic spectra, conductance measurements, IR and NMR studies. These complexes are found soluble in DMSO and DMF but insoluble in common organic solvents. Conductance measurements show their non-electrolytic nature. These complexes show biological activity against tested microorganisms. More research can be continued on these complexes to explore their other effects on biological systems. Moreover these complexes can be used in pharma-

TABLE-2
in vitro ANTIMICROBIAL ACTIVITY OF COMPLEXES THROUGH AGAR WELL DIFFUSION METHOD

Compd. No.	Complex	Diameter of growth of inhibition zone (mm)					
		Gram-positive bacteria		Gram-negative bacteria		Fungi	
		Bacillus subtilis	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Candida albicans*	*Saccharomyces cerevisiae*
1	$[C_{42}H_{32}N_8O_2Cu(OAc)_2]$	15	NA	15	24	NA	NA
2	$[C_{42}H_{32}N_8O_2NiCl_2]$	NA	NA	13	21	NA	NA
3	$[C_{42}H_{32}N_8O_2Co(OAc)_2]$	NA	NA	21	25	NA	NA
4	$[C_{42}H_{32}N_8O_2Zn(OAc)_2]$	NA	NA	10	30	NA	NA
5	$[C_{42}H_{32}N_8S_2Cu(OAc)_2]$	20	15	15	32	24	26
6	$[C_{42}H_{32}N_8S_2NiCl_2]$	15	NA	NA	20	NA	NA
7	$[C_{42}H_{32}N_8S_2Co(OAc)_2]$	15	NA	20	26	NA	NA
8	$[C_{42}H_{32}N_8S_2Zn(OAc)_2]$	NA	NA	10	26	NA	NA
	Ciprofloxacin	24	26.6	25.0	22	NA	NA
	Amphotericin-B	NA	NA	NA	NA	16.6	19.3

NA = No activity

TABLE-3
MINIMUM INHIBITORY CONCENTRATION (MIC) OF COMPLEXES

Compd. No.	Complex	Minimum inhibitory concentration (mg/mL)					
		Gram-positive bacteria		Gram-negative bacteria		Fungi	
		Bacillus subtilis	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Candida albicans*	*Saccharomyces cerevisiae*
1	$[C_{42}H_{32}N_8O_2Cu(OAc)_2]$	50	Nt	Nt	12.5	Nt	Nt
2	$[C_{42}H_{32}N_8O_2NiCl_2]$	Nt	Nt	Nt	50	Nt	Nt
3	$[C_{42}H_{32}N_8O_2Co(OAc)_2]$	Nt	Nt	12.5	12.5	Nt	Nt
4	$[C_{42}H_{32}N_8O_2Zn(OAc)_2]$	Nt	Nt	Nt	6.25	Nt	Nt
5	$[C_{42}H_{32}N_8S_2Cu(OAc)_2]$	25	Nt	Nt	3.125	12.5	6.25
6	$[C_{42}H_{32}N_8S_2NiCl_2]$	50	Nt	Nt	25	Nt	Nt
7	$[C_{42}H_{32}N_8S_2Co(OAc)_2]$	50	Nt	25	6.25	Nt	Nt
8	$[C_{42}H_{32}N_8S_2Zn(OAc)_2]$	Nt	Nt	Nt	6.25	Nt	Nt
	Ciprofloxacin	6.25	6.25	6.25	12.5	–	–
	Amphotericin-B	–	–	–	–	12.5	12.5

Nt = Not tested

ceutical field after investigating their harmful effects. If found safe these type of complexes can be used to replace existing drugs which becomes resistant to bacterial strains.

REFERENCES

1. N.E. Borisova, M.D. Reshetova and Y.A. Ustynyuk, *Chem. Rev.*, **107**, 46 (2007).
2. M. Salavati-Niasari, M. Bazarganipour, M.R. Ganjali and P. Norouzi, *Transition Met. Chem.*, **32**, 9 (2007).
3. T. Aboul-Fadl, F.A. Mohammed and E.A. Hassan, *Arch. Pharm. Res.*, **26**, 778 (2003).
4. K.C. Gupta and A.K. Sutar, *J. Coord. Rev.*, **252**, 1420 (2008).
5. R. Ahamad, R. Prasad and M.A. Quraishi, *Corros. Sci.*, **52**, 933 (2010).
6. S. Chandra and K. Gupta, *Transition Met. Chem.*, **27**, 196 (2002).
7. S.M.M. Ali, M.A.K. Azad, M. Jesmin, S. Ahsan, M.M. Rahman, J.A. Khanam, M.N. Islam and S.M.S. Shahriar, *Asian Pacific J. Trop. Biomed.*, **2**, 438 (2012).
8. K. Chaubey and S.N. Pandeya, *Int. J. Pharm. Technol. Res.*, **4**, 590 (2012).
9. G. Kumar, D. Kumar, S. Devi, R. Johari and C.P. Singh, *Eur. J. Med. Chem.*, **45**, 3056 (2010).
10. S.M. Sondhi, N. Singh, A. Kumar, O. Lozach and L. Meijer, *Bioorg. Med. Chem.*, **14**, 3758 (2006).
11. V.C. da Silveira, J.S. Luz, C.C. Oliveira, I. Graziani, M.R. Ciriolo and A.M.C. Ferreira, *J. Inorg. Biochem.*, **102**, 1090 (2008).
12. V.E. Kuz'min, A.G. Artemenko, V.P. Lozitsky, E.N. Muratov, A.S. Fedtchouk, N.S. Dyachenko, L.N. Nosach, T.L. Gridina, L.I. Shitikova, L.M. Mudrik, A.K. Mescheriakov, V.A. Chelombitko, A.I. Zheltvay and J.-J. Vanden Eynde, *Acta Biochim. Pol.*, **49**, 157 (2002).
13. R. Malhotra, S. Kumar, Jyoti, H.R. Singal and K.S. Dhindsa, *Indian J. Chem.*, **39A**, 421 (2000).
14. S. Kumar, R. Malhotra and K.S. Dhindsa, *Polyhedron*, **11**, 1383 (1992).
15. S. Singh, R. Malhotra, A. Hooda and K.S. Dhindsa, *Bull. Soc. Chim. Belg.*, **105**, 108 (1996).
16. K.R. Aneja, C. Sharma and R. Joshi, *Jundishapur J. Microbiol.*, **4**, 175 (2011).
17. A. Nostro, A. Germano, V. D'Angelo, A. Marino and M.A. Cannatelli, *Lett. Appl. Microbiol.*, **30**, 379 (2000).
18. Q. Zeng, J. Sun, S. Gou, K. Zhou, J. Fang and H. Chen, *Transition Met. Chem.*, **23**, 371 (1998).
19. L.K. Gupta and S. Chandra, *Transition Met. Chem*, **31**, 368 (2006).
20. C. Lodeiro, R. Bastida, E. Bertolo, A. Macias and A. Rodriguez, *Transition Met. Chem.*, **28**, 388 (2003).
21. S. Chandra and L.K. Gupta, *J. Indian Chem. Soc.*, **82**, 454 (2005).
22. K. Nakamoto, Infrared and Raman spectra of inorganic and coordination compounds, *Wiley Interscience Publication*, New York (1978).
23. V.B. Rana, P. Singh, D.P. Singh and M.P. Teotia, *Transition Met. Chem.*, **6**, 36 (1981).
24. V.B. Rana, P. Singh, D.P. Singh and M.P. Teotia, *Polyhedron*, **1**, 377 (1982).