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SYNTHESIS, SPECTRAL, THERMAL AND ANTIBACTERIAL INVESTIGATIONS OF MIXED LIGAND COMPLEXES OF THORIUM(IV) DERIVED FROM 8-HYDROXYQUINOLINE AND SOME AMINO ACIDS

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Abstract: Mixed ligand Th(IV) complexes of the type $[M(Q)(L)(NO_3)_2] \times 2\ H_2O$ have been synthesized using 8-hydroxyquinoline (HQ) as a primary ligand and N- and/or O- donor amino acids (HL) such as L-lysine, L-aspartic acid and L-cysteine as secondary ligands. The metal complexes have been characterized on the basis of elemental analysis, electrical conductance, room temperature magnetic susceptibility measurements, spectral and thermal studies. The electrical conductance studies of the complexes in DMF in 10^{-3} M concentration indicate their non-electrolytic nature. Room temperature magnetic susceptibility measurements revealed diamagnetic nature of the complexes. Electronic absorption spectra of the complexes show intra-ligand and charge transfer transitions, respectively. Bonding of the metal ion through N- and O- donor atoms of the ligands revealed by IR studies and the chemical environment of the protons is also confirmed by NMR studies. The thermal analysis data of the complexes indicate the presence of crystallized water molecules. The agar cup and tube dilution method have been used to study the antibacterial activity of the complexes against the pathogenic bacteria *S. aureus*, *C. diphtheriae*, *S. typhi* and *E. coli*.

Keywords: thorium complexes; synthesis, spectral, antibacterial investigations

Most of the metal complexes with 8-hydroxyquinoline possess biological activities (1). The use of 8-hydroxyquinoline as an *in vivo* agent in microbiological system has been reviewed (2). Numerous reviews on the relationship of metal complexes to biological response have been reported (3).

Thorium forms complexes with high coordination number due to its high charge and effective ionic size (4, 5). The antibacterial and cytotoxic activity of Th(IV) with 8-hydroxyquinoline has been reported (6).

The present paper reports the synthesis and characterization of mixed ligand Th(IV) complexes. The complexes are prepared using 8-hydroxyquinoline as a primary ligand and L-lysine, L-aspartic acid and L-cysteine as secondary ligands.

EXPERIMENTAL

Materials

Analytical grade thorium(IV) nitrate pentahydrate was used without further purification. L-lysine, L-aspartic acid and L-cysteine and 8-hydroxyquinoline were obtained from S.D. Fine Chemicals, Mumbai. Solvents like ethanol and dimethylformamide and laboratory grade chemicals whenever used were distilled and purified according to standard procedures (7, 8).

Preparation of mixed ligand complexes

Mixed ligand thorium (IV) complexes were prepared from thorium nitrate pentahydrate, 8-hydroxyquinoline (HQ) as a primary ligand and different amino acids such as L-lysine, L-aspartic acid and L-cysteine as secondary ligands.

To an aqueous solution (10 cm^3) of thorium nitrate pentahydrate (570 mg, 1 mmol), ethanolic solution (10 cm^3) of 8-hydroxyquinoline (145 mg, 1 mmol) was added. The mixture was stirred and kept in a boiling water bath for 10 min. To this hot solution an aqueous solution (10 cm^3) of amino acid (1 mmol) was added with constant stirring. The mixture (1:1:1 molar proportion) was again heated in a water bath for 10 min till the temperature reached

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50°C. The complexes were precipitated by raising the pH of the reaction mixture by adding diluted ammonia solution. The mixture was cooled and the solid complex obtained was filtered, washed with water followed by ethanol. The complexes thus prepared were dried under vacuum and were used for further studies.

Instrumentation

The complexes were analyzed for C, H and N contents on Thermo Finnigan Elemental Analyzer, Model No. FLASH EA 1112 Series at Department of Chemistry, I.I.T., Mumbai. Metal content was estimated gravimetrically by standard procedure (9). The molar conductance values were measured in DMF (10^{-3} M) on an Equiptronics Autoranging Conductivity Meter Model No. EQ-667. Room temperature magnetic susceptibilities were measured by a Guoy method using $Hg[Co(SCN)_4]$ as a calibrant at Department of Chemistry, I.I.T., Mumbai. The electronic absorption spectra of all the complexes in DMF solution (10^{-3} M) in the ultraviolet and visible region were recorded on Shimadzu UV/VIS-160 Spectrophotometer. FT-IR spectra were recorded in KBr discs on a Perkin-Elmer FT-IR spectrophotometer Model 1600 at Department of Chemistry, I.I.T., Mumbai. NMR spectra were recorded on JEOL-300 MHz instrument using TMS as an internal standard at The Institute of Science, Mumbai. Thermal Analysis (TG and DTA) were carried out in controlled nitrogen atmosphere on a Perkin-Elmer Diamond TG-DTA instrument at Department of Chemistry, I.I.T., Mumbai by recording the change in weight of the complexes on increasing temperature up to 900°C at the heating rate of 10°C/min.

Antibacterial screening

Agar cup method

In the agar cup method, a single compound can be tested against a number of organisms or a given organism against different concentrations of the same compound. The method was found suitable for semisolid or liquid samples and was used in the present work. In the agar cup method, a plate of sterile nutrient agar with the desired test strain was poured to a height of about 5 mm, allowed to solidify and a single cup of about 8 mm diameter was cut from the center of the plate with a sterile cork borer. Thereafter, the cup was filled with the sample solution of known concentration and the plate was incubated at 37°C for 24 h. The extent of inhibition of growth from the edge of the cup was considered as a measure of the activity of the given compound. By using several plates simultaneously, the activities of several samples were quantitatively studied.

Tube dilution method

The test compound (10 mg) was dissolved in suitable solvent (10 mL) such as dimethyl sulfoxide or distilled water to prepare a stock solution of concentration 1000 µg/mL. From this stock solution, aliquots of 5 to 250 µg/mL were obtained in test broth.

The test compounds were subjected to *in vitro* screening against *Staphylococcus aureus*, *Corynebacterium diphtheriae*, *Salmonella typhi* and *Escherichia coli* using Mueller Hinton broth as the culture medium.

Bacterial inoculums were prepared in sterilized Mueller Hinton broth and incubated for 4 h at 37°C. This was dispersed (5 mL) in each borosilicate test tube (150 × 20 mm). The test sample solution was added in order to attain a final concentration as 5 to 250 µg/mL. The bacterial inoculums 0.1 mL of the desired bacterial strain (*S. aureus*, *C. diphtheriae*, *S. typhi* and *E. coli*) containing 10^6 bacteria/mL were inoculated in the tubes. The tubes were incubated at 37°C for 24 h and then examined for the presence or absence of the growth of the test organisms.

The lowest concentration which showed no visible growth was noted as minimum inhibitory concentration (MIC).

RESULTS AND DISCUSSION

Characterization of metal complexes

The synthesis of mixed ligand Th(IV) complexes may be represented as follows:

$$Th(NO_3)_4 \times 5\ H_2O + HQ + HL \rightarrow$$
$$[Th(Q)(L)(NO_3)_2] \times 2\ H_2O + 2\ HNO_3 + 3\ H_2O$$

where, HQ is 8-hydroxyquinoline and HL is an amino acid.

All the complexes are colored, non-hygroscopic, thermally stable solids (Table 1), indicating a strong metal-ligand bond. The complexes are insoluble in common organic solvents such as ethyl alcohol, acetone, chloroform etc., but are fairly soluble in DMF.

The elemental analysis data (Table 2) of metal complexes are consistent with their general formulation as 1:1:1, mixed ligand of the type $[M(Q)(L)(NO_3)_2] \times 2\ H_2O$. The molar conductance values (Table 3) of the complexes in DMF at 10^{-3} M concentration are very low (< 1) indicating their non-electrolytic nature (10).

Table 1. Color, decomposition temperature and pH of the thorium complexes.

No.	Complex	Color	Decomposition temperature (°C)	pH
1.	$[Th(Q)(Lys)(NO_3)_2] \times 2\ H_2O$	Green	230	7.00
2.	$[Th(Q)(Asp)(NO_3)_2] \times 2\ H_2O$	Yellow	220	7.00
3.	$[Th(Q)(Cys)(NO_3)_2] \times 2\ H_2O$	Green	220	7.00

Q represents the deprotonated primary ligand – 8-hydroxyquinoline, whereas Lys, Asp and Cys represent deprotonated secondary ligands L-lysine, L-aspartic acid and L-cysteine, respectively.

Table 2. Empirical formula, molecular weight and elemental analysis data of thorium complexes.

No.	Complex	Empirical formula	Mol. weight	Elemental analysis found (calculated)				
				%M	%C	%H	%N	%S
1.	$[Th(Q)(Lys)(NO_3)_2] \times 2\ H_2O$	$ThC_{15}H_{23}N_5O_{11}$	681.37	34.03 (34.05)	26.40 (26.42)	3.35 (3.38)	10.29 (10.27)	–
2.	$[Th(Q)(Asp)(NO_3)_2] \times 2\ H_2O$	$ThC_{13}H_{16}N_4O_{13}$	668.29	34.70 (34.72)	23.30 (23.34)	2.35 (2.39)	8.35 (8.38)	–
3.	$[Th(Q)(Cys)(NO_3)_2] \times 2\ H_2O$	$ThC_{12}H_{14}N_4O_{11}S$	656.34	35.30 (35.35)	21.92 (21.94)	2.10 (2.13)	8.51 (8.53)	4.86 (4.88)

Table 3. Molar conductance data of thorium complexes.

No.	Complex	Molar conductance $Mhos.cm^2mol^{-1}$
1.	$[Th(Q)(Lys)(NO_3)_2] \times 2\ H_2O$	0.0002
2.	$[Th(Q)(Asp)(NO_3)_2] \times 2\ H_2O$	0.0001
3.	$[Th(Q)(Cys)(NO_3)_2] \times 2\ H_2O$	0.0001

Magnetic studies

The magnetic moments of the complexes were calculated from the measured magnetic susceptibilities after employing diamagnetic corrections and revealed their diamagnetic nature (11).

Electronic absorption spectra

The electronic spectra of the metal complexes in DMF were recorded in the UV-visible region. The spectra shows three transitions in the range 36765–36900, 29762–29851 and 26316–26954 cm^{-1} ascribed to $\pi \rightarrow \pi^*$, $n \rightarrow \pi^*$ and the charge transfer transitions from the ligands to the metal, respectively.

Infra-red spectra

The FT-IR spectra of the metal complexes were recorded for KBr discs over the range 4000–400 cm^{-1}. On the basis of the reported infrared spectra of amino acids, 8-hydroxyquinoline and their metal complexes (12–14), some of the important bands have been assigned.

A broad band observed in the region between 3436–3413 cm^{-1} due to asymmetric and symmetric O–H stretching modes and a band in the range 1602–1601 cm^{-1} due to H–O–H bending vibrations indicating presence of a crystallized water molecules further confirmed thermal studies.

The ν(CO) band is observed at ~1120 cm^{-1}. The position of this band undergoes variation depending on metal complex under study (15). A strong ν(CO) band observed at ~1104 cm^{-1} indicates the presence of oxine moiety in the complexes coordinated through its nitrogen and oxygen atoms as uninegative bidentate ligand (16). The ν(C=N) mode observed at 1580 cm^{-1} in the spectra of free HQ ligand is found to be shifted to lower wave number, in the range 1498–1496 cm^{-1} in the spectra of complexes. A negative shift in this vibrational mode on complexation indicates the coordination through ternary nitrogen donor of HQ. The in plane and out of plane ring deformation modes observed at ~554 and ~790 cm^{-1}, respectively, confirm coordination through nitrogen atom of HQ with the metal.

Broad bands at 3040 and 2960 cm^{-1} due to N-H (asymmetric) and N-H (symmetric) vibrations of free amino acid moiety are shifted to higher wave numbers i.e., in the range 3150–3100 and 3068–3050 cm^{-1}, respectively, in the spectra of metal

complexes, suggesting coordination of the amino group through nitrogen with the metal ion.

The $\nu_{asymmetric}(COO^-)$ band of the free amino acid, i.e., 1610–1590 cm^{-1} is shifted to lower wave number, in the range 1572–1569 cm^{-1} and the $\nu_{symmetric}(COO^-)$ mode observed at ~1400 cm^{-1} in the spectra of free amino acids is found to be shifted to lower wave number i.e., 1384–1383 cm^{-1}, in the spectra of complexes indicating the coordination of the carboxylic acid group *via* oxygen with the metal ion (12). The C-N symmetrical stretching frequency observed at ~950 cm^{-1} in the spectra of amino acids was shifted to lower wave numbers, in the range 900–894 cm^{-1} in the spectra of the complexes, confirming coordination through the amino group of the amino acids.

An important feature of infrared spectra of the metal complexes with HQ is the absence of band ~3440 cm^{-1} due to the O-H stretching vibration of the free O-H group of HQ (14). This observation leads to the conclusion that complex formation takes place by deprotonation of the hydroxyl group of HQ moiety.

The FT-IR spectra of the metal complexes show no absorption bands near 1352 cm^{-1} where ionic nitrate is known to absorb (17), indicating

Table 4. Thermal data of thorium complexes.

No.	Complex	Decomposition temperature (°C)	Temperature range (°C)	% Weight loss		Decomposition product
				found	calculated	
1.	$[Th(Q)(Lys)(NO_3)_2] \times 2\ H_2O$	230	90–100	5.20	5.28	$[Th(Q)(Lys)(NO_3)_2]$
			400–410	21.20	21.28	$[Th(Q)(NO_3)_2]$
			530–550	34.50	34.64	$[ThO_2]$
2.	$[Th(Q)(Asp)(NO_3)_2] \times 2\ H_2O$	220	90–100	5.35	5.39	$[Th(Q)(Asp)(NO_3)_2]$
			400–450	19.72	19.75	$[Th(Q)(NO_3)_2]$
			500–550	35.25	35.31	$[ThO_2]$
3.	$[Th(Q)(Cys)(NO_3)_2] \times 2\ H_2O$	220	90–100	5.40	5.48	$[Th(Q)(Cys)(NO_3)_2]$
			400–450	18.20	18.28	$[Th(Q)(NO_3)_2]$
			500–550	35.90	35.96	$[ThO_2]$

Table 5. Antibacterial activity (mm) of thorium complexes by agar cup method.

No.	Complex	Test			
		S. aureus	*C. diphtheriae*	*S. typhi*	*E. coli*
1.	$[Th(Q)(Lys)(NO_3)_2] \times 2\ H_2O$	21	22	12	12
2.	$[Th(Q)(Asp)(NO_3)_2] \times 2\ H_2O$	28	24	14	12
3.	$[Th(Q)(Cys)(NO_3)_2] \times 2\ H_2O$	20	22	12	13
4.	Tetracycline	30	25	26	26

Table 6. MIC data of thorium complexes.

No.	Complex	MIC (µg/mL)			
		S. aureus	*C. diphtheriae*	*S. typhi*	*E. coli*
1.	$[Th(Q)(Lys)(NO_3)_2] \times 2\ H_2O$	20	25	70	80
2.	$[Th(Q)(Asp)(NO_3)_2] \times 2\ H_2O$	20	25	75	80
3.	$[Th(Q)(Cys)(NO_3)_2] \times 2\ H_2O$	20	20	70	80
4.	$Th(NO_3)_4 \times 5\ H_2O$	100	100	220	200
5.	Tetracycline	1.5	2.0	1.5	2.5

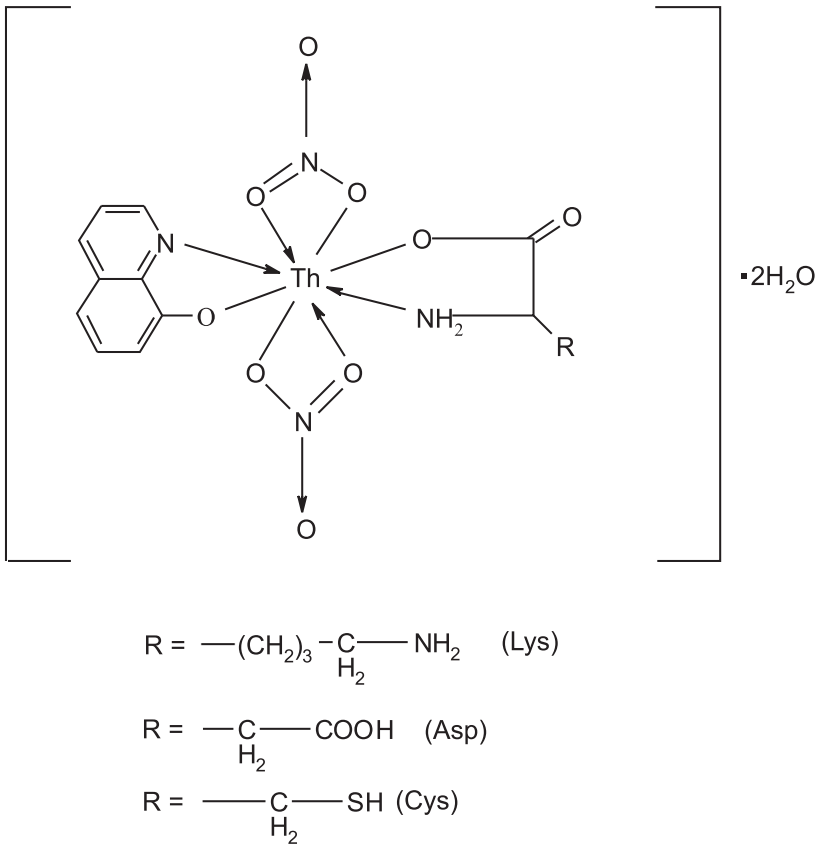

Figure 1. Proposed structures and bonding for the complexes

absence of ionic nitrate. Other bands observed at ~1466, ~1275, ~1035 and 731 cm^{-1} corresponding to ν_1, ν_4, ν_2 and ν_3 vibrations agree with frequencies reported for bidentate nitrate group (18, 19). The weak band observed at 2581 cm^{-1} in the spectra of the complex with L-cysteine may be ascribed to the S-H vibration.

Some new bands of weak intensity observed in the regions around 606–605 cm^{-1} and 485–484 cm^{-1} may be ascribed to the M-O and M-N vibrations, respectively (20). It may be noted that these vibrational bands are absent in the infra-red spectra of HQ as well as amino acids.

NMR spectra

^{1}H NMR spectra of complexes in DMSO exhibit a singlet at δ 2.4 ppm due to amino group protons and broad multiplet in the region δ 6.6–8.2 ppm due to the aromatic ring protons. The presence of water molecules in the complexes is confirmed by the appearance of a new signal around δ 3.4 ppm, attributed to H_2O protons (21).

The complex with L-lysine shows multiplet at δ 1.29–1.33 ppm for two protons of methylene group, another multiplet at δ 1.50–1.58 ppm for four protons of two methylene groups, two triplets at δ 2.63 and 2.88 ppm for two protons of methylene group and one proton of –CH group, respectively, and a broad singlet at δ 5.23 ppm for two protons of other amino group which was D_2O exchangeable.

The complex with L-aspartic acid shows doublet at δ 2.60 ppm for two protons of methylene, triplet at δ 2.88 ppm for one proton of –CH and singlet at δ 11.15 ppm for one proton of carboxylic acid group.

The complex with L-cysteine shows doublet at δ 2.50 ppm for two protons of methylene group, triplet at δ 2.92 ppm for one proton of –CH group and singlet at δ 2.34 ppm for one proton of another –SH group.

Thermal studies

The TG and DTA studies of the complexes have been recorded in the nitrogen atmosphere at the constant heating rate of 10^{o}C/min.

The TG of the complexes show that they are thermally quite stable to varying degree. The complexes show gradual loss in weight due to decomposition by fragmentation with increasing temperature as presented in Table 4. All the complexes show similar behavior in TG and DTA studies. The thermogram of these complexes shows the loss in weight corresponding to two water molecules in the temperature range 90–100°C, followed by weight loss due to amino acid moiety in the range 400–450°C. The final step of the decomposition observed in the range 500–550°C corresponds to the weight loss of nitrate as well as HQ moieties present in the complexes.

The DTA of the complexes display an endothermic peak in the range 90–100°C which indicates the presence of crystallized water molecules. As the temperature is raised, the DTA curve shows a small exotherm in the range 400–450°C and a broad exotherm in the range 500–550°C attributed to decomposition of amino acid moiety and nitrate along with 8-hydroxyquinoline moieties present in the complexes, respectively. The formation of a broad exotherm is possibly due to simultaneous decomposition of ligand moieties and their subsequent oxidation to gaseous products like CO_2, H_2O and NO_2 etc. (11).

Like most of the metal organic complexes, these complexes also decompose to a fine powder of metal oxide i.e., ThO_2. The constant weight plateau in TG after 600°C indicates completion of the reaction. The ThO_2 form was confirmed by X-ray diffraction pattern of the decomposed product (11).

On the basis of the physico-chemical studies, the bonding and structure for the metal complexes may be represented as shown in Figure 1.

Antibacterial studies

All the metal complexes were screened against *Staphylococcus aureus*, *Corynebacterium diphtheriae*, *Salmonella typhi* and *Escherichia coli*.

The studies based on agar cup method revealed that the complexes are sensitive against *S. aureus* and *C. diphtheriae* and less sensitive against *S. typhi* and *E. coli* (Table 5).

The minimum inhibitory concentration (MIC) of ligand and the metal salts ranges between 50–220 µg/mL while that of metal complexes ranges between 20–80 µg/mL (Table 6). The complexes are found to be more active against *S. aureus* and *C. diphtheriae* as compared to *S. typhi* and *E. coli*. As compared to standard antibacterial compound – tetracycline, the complexes show moderate activity against selected strains of microorganisms (22).

The results show that, as compared to the activity of metal salts and free ligand, the metal complexes show higher activity. The activity of metal complexes is enhanced due to chelation. The chelation reduces considerably the polarity of the metal ions in the complexes, which in turn increases the hydrophobic character of the chelate and thus enables its permeation through the lipid layer of microorganisms (23).

CONCLUSIONS

Based on the above results the following conclusions may be drawn.

The higher decomposition temperatures of the complexes indicate a strong metal-ligand bond and electrical conductance studies show non-electrolytic nature of the complexes, respectively. Magnetic studies indicate diamagnetic nature of the complexes. Electronic absorption spectra of the complexes show intra-ligand and charge transfer transitions, respectively. IR spectra show bonding of the metal ion through N- and O- donor atoms of the two ligands. ^{1}H NMR study reveals the chemical environment of protons and the presence of water molecules in the complexes. Thermal analysis confirms the presence of crystallized water molecules.

On the basis of above results, coordination number eight is proposed for thorium complexes.

The antibacterial study shows that complexes are found to be more active against *S. aureus* and *C. diphtheriae* as compared to *S. typhi* and *E. coli*. Compared to standard antibacterial compound, tetracycline, the complexes show moderate activity against the selected strains of microorganisms.

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REFERENCES

1. Howard-Lock H.E., Lock C.J.L.: in Comprehensive Coordination Chemistry, Wilkinson G., Gillard R.D., McCleverty J.A., Eds., vol. 6, p. 755, Pergamon Press, Oxford 1987.
2. Shulman A., Dwyer F.P.: in Chelating Agents and Metal Chelates, Dwyer F.P., Mellor D.P. Eds., p. 383, Academic Press, New York 1964.

3. Chenouwert M.B.: Pharmacol Rev. 8, 57 (1956).
4. Bagnall K.W.: in Comprehensive Coordination Chemistry, Wilkinson G., Gillard R.D., McCleverty J.A.: Eds., Vol. 3, p. 1129, Pergamon Press, Oxford 1987.
5. Mutterties E.I., Roesky H., Wright C.M.: J. Am. Chem. Soc. 88, 4856 (1966).
6. Thakur G.A., Athlekar S.V., Dharwadkar S.R., Shaikh M.M.: Acta Pol. Pharm. Drug Res. 64, 9 (2007).
7. Perrin D.D., Perrin D.R., Armarego W.L.F.: Purification of Laboratory Chemicals, 2nd edn., Pergamon Press, Oxford 1980.
8. Vogel A.I.: Textbook of Practical Organic Chemistry, 5th edn., Longmans Green and Co. Ltd., London 1989.
9. Vogel A.I.: Quantitative Inorganic Analysis, 4th edn. ELBS, London 1965.
10. Geary W.J.: Coord. Chem. Rev. 7, 81 (1971).
11. Thakur G.A., Shaikh M.M.: Acta Pol. Pharm. Drug Res. 63, 95 (2006).
12. Islam M.S., Ahmed M.S., Pal S.C., Reza Y., Jesmine S.: Indian J. Chem., 34A, 816 (1995).
13. Bhagwat V., Sharma V., Poonia N.S.: Indian J. Chem. 15A, 46 (1977).
14. Nakamoto K., Morimoto Y., Martell A.E.: J. Am. Chem. Soc. 83, 4528 (1961).
15. Charles R.C., Freiser H., Friedel R., Hillard L.E., Johnson W.D.: Spectrochim. Acta 8, 1 (1956).
16. Panda S., Mishra R., Panda A.K., Satpathy K.C.: J. Indian Chem. Soc. 66, 472 (1989).
17. Balkrishnan P.V., Patil S.K., Sharma H.D., Venkatsetly H.V.: Can. J. Chem. 43, 4052 (1965).
18. Chowdary M.C.: Polyhedron 6, 285 (1987).
19. Addison C.C., Simpson W.B.: J. Chem. Soc. 598, (1965).
20. Nakamoto K.: Infrared and Raman Spectra of Inorganic. and Coordination Compounds, 4th edn., p. 233, John Wiley and Sons, New York 1986.
21. Hosny W.M.: Synth. React. Inorg. Met.-Org. Chem. 28, 1029 (1998).
22. Prasad R.V., Thakkar N.V.: J. Mol. Cat. 92, 9 (1994).
23. Chohan Z.H., Misbahul A.K., Moazzam M.: Indian J. Chem. 27A, 1102 (1988).

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