

E. Coli, Pseudomonas Species, Aspergillusniger ,Candida Species, And Spectral Studies Of $Th(NO_3)_4$ Complex With 4CABPT Ligand

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ABSTRACT

We report here series of new the Th(IV) complexes with Schiff base having general composition $Th X_{4-n}nL$ ($X=NO_3$, $n=2$) Where L = Schiff base The complexes were characterized on the basis of analytical conductance, molecular weight and spectral studies. The Schiff base behave as neutral monodentate ligand which coordinate to the central metal atom through azomethine nitrogen.

Key words – Schiff base ligand, Th(IV)

a).Preparation of aldehyde:

Preparation of 4-NN-Bis-2'-(cyanoethyl) aminobenzaldehyde was modeled on the procedure given in the literature.

Preparation of 4-NN-Bis-2'-(cyanoethyl) aminobenzaldehyde

NN-Bis-2'-cyanoethylaniline (13.8g., 0.07mol) was slowly added in small portions to the cooled mixture of phosphorous oxychloride (0.05 to 0.07 mol) and dimethylformamide (0.2 to 0.25 mol.), taken in a round bottom flask provided with mechanical stirrer and a reflux condenser, carrying a calcium chloride guard tube. The contents were heated with stirring on a steam bath for three hours. The dark brown liquid was poured over crushed ice and the clear solution was neutralized with sodium acetate. On keeping overnight the solid product separated out. It was collected under suction, washed with little water and recrystallized from ethanol when the aldehyde was obtained as light yellow shining needles. data on m.p. and yield of the aldehydes is given in Table.1

Table 1: Melting points and yields of the aldehydes A and B

S.No.	Aldehyde formed	Melting point (°C)	Yield
1.	4CABPT	110	76.78 %

(b)Preparation of Schiff bases :

General procedure :

The aldehyde (~1m.mol.) and p-touidine (~1.1m.mol.) were taken in a round bottom flask, to which two drops of piperidine were added. The contents were heated for four hours in an oil bath maintained at 100-105°C. The contents first melted and then solidified. The Schiff base was purified by recrystallization from ethanol as yellow solid with sharp melting point.

Mass spectral studies of ligands: Mass spectral studies of the Schiff base ligands chosen and prepared for complex formation, were performed successfully. Molecular weight of the ligands have been confirmed from the parent peaks in their mass spectra. Parent peaks in the mass spectra of the ligand appearing at m/e 309 4CABPT respectively.

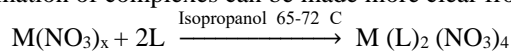
Table 2: Mass spectral peaks for 4-NN-bis-2'-cyanoethylaminobenzalidene p-toluidine 4CABPT]

S.No.	m/e ratio	% Relative Abundance
1	360	05
2	339	05
3	319	10
4	318	35
5	309	100 (Base peak)
6	276	10
7	223	10
8	209	10

9	165	05
10	152	05
11	81	05
12	57	05

SYNTHESIS OF COMPLEXES : For the synthesis of complexes the respective metal salt solutions were treated with the ligands solutions in the required molar concentrations (i.e. 1:2, 1:4, or 1:6). In some cases complexes were isolated immediately in cold while in some cases in hot solutions. In few cases resulting solutions were refluxed for 2-3 hours at ca 65-72 °C. The solvents used were ethanol isopropanol and acetone. Complexes were collected washed with solvent and finally with anhydrous ether and dried in vacuo over anhydrous calcium chloride.

The formation of complexes can be made more clear from the following general reactions.



Heat and reflux

(M = Th)

4CABPT, 2MCABPT

(x = 2)

All the physicochemical studies and analyses were performed as reported earlier .

Complexes	Colour	M.P.	Yield	M.W. found (calcd.)	Analysis			χ (Ohm^{-1} $\text{cm}^2\text{mole}^{-1}$)
					Found	(Calcd.) Analysis		
					C	H	N	
Th(NO ₃) ₄ . 2(4CABPT)	yellow	176	81	1012 (1014)	47.18 (44.11)	4.28 (4.70)	15.24 (15.44)	3.1

INFRARED SPECTRAL STUDIES:

Infra red absorptions of the Schiff bases used in this investigation have been assigned by the comparison of the spectra with those already reported by others along with mono-substituted benzene ring system . The I.R. spectra of new complexes formed by the interaction of the Schiff bases with thorium (IV) recorded in the region 4000-500 cm^{-1} using KBr pallets. On inspecting spectra of ligands and that of the complexes it is inferred that ligands are coordinated through azomethine nitrogen, as an important band appears at 1598-1580 cm^{-1} attributed to $\nu(\text{C}=\text{N})$ (azomethine) mode. In the spectra of all the complexes this band shifts to a lower wave number side and appears in the range 1540-1518 cm^{-1} , indicating the involvement of nitrogen atom of azomethine group in the co-ordination .

The bands assigned to benzene ring vibration compared well with those of mono-substituted derivatives of benzene. Several other modes associated with C-H out of plane deformation appear in the region 785-720 cm^{-1} . In the far IR region, some new medium and weak bands are observed in the range 546-510 cm^{-1} in the complexes where the ligands have no absorptions. These new bands are tentatively assigned to $\nu(\text{M}-\text{N})$ modes observed for several thorium (IV).

Table.3: Infrared absorption frequencies (cm^{-1}) for Thorium (IV) complex of 4-NN-bis-2'-cyanoethylaminobenzylidene-p-toluidine (4CABPT)

Assignments	4CABPT	Th(NO ₃) ₄ . 2(4CABPT)
C = N stretching azomethine	1528.1s	1524.7s
Ring stretching N – Phenyl stretching	1363.7s 1307sh	1367s 1315s
Ring breathing of benzene	1118.5w 969w	1174.5w 969.22sh
Ring breathing and deformation	1528s 1107w	1524.74s 1130m
C – N – C bending	- 812w	920w 820sh
Out of plane ring deformation	747m	727w
Out of plane bending of mono substituted benzene	610w	663w
$\nu(\text{Th}-\text{N})$ Metal - ligand vibration	-	518w

The nitarato complexes : The free nitarate ion (D_{3h} symmetry) has four fundamental vibrations viz. ν_1 , the 'a' type N – O stretching (Raman active) ν_2 the 'a₂' type out of plane, N – O bending (infrared active) ν_3 the 'e' type N – O stretching and ν_4 the 'e' type N – O bending (both IR and Raman active) . On lowering the symmetry from D_{3h} to C_{2v} and C_s , all

the vibration modes split but the number of infrared fundamental vibrations remain the same for C_{2v} and C_s symmetry consequently the two cannot be distinguished in this way.

Because of the fact that various bending modes of nitrate groups to metal ions are possible, the solution becomes even more complicated. For instance, unidentate, symmetrical bidentate and bridging nitrate groups are possible which any possess C_{2v} symmetry. Earlier authors have discussed several means of identifying the mode of coordination of nitrate groups.

For thorium (IV) nitrate complexes the presence of two strong absorptions in region $1550 - 1440 \text{ cm}^{-1}$ and $1270 - 1340 \text{ cm}^{-1}$ is attributed to ν_4 and ν_1 modes of vibrations respectively of covalently bonded nitrate groups, suggest that the groups lie inside the coordination sphere. Other absorptions associated with the covalent nitrate groups are also observed in the spectra of complexes. If the $(\nu_1 - \nu_4)$ difference is taken as an approximate measure to the covalency of nitrate groups, a value of ca 200 cm^{-1} for the complexes studied suggest strong covalency for metal nitrate bonding. The use of high frequency criterion (ν_1) and the magnitude of splitting ν_3 into ν_1 and ν_4 , C_{2v} , for identifying the unidentate or bidentate nature have been questioned.

In some cases Lever separation method, the number and relative energies of nitrate combination frequencies in $1850 - 1650 \text{ cm}^{-1}$ region of the infrared spectrum, has been used as an aid to distinguish the various coordination modes of nitrate group. The weak bands in the region are a combination of the ν_1 (NO) stretching and the ν_4 (NO_2) bending. This combination is allowed by D_{3h} symmetry, although, forbidden by oscillator selection rule. In Lever symmetries the ν_4 mode is expected to splits into two components. Combinations of both of these modes with the ν_1 mode is still allowed by a symmetry and therefore, two combination frequencies may be expected resulting separation corresponding to splitting of ν_4 mode. According to Lever et.al. bidentate coordination involves a greater distortion from D_{3h} symmetry than unidentate coordination. Therefore bidentate complexes are expected to show a large separation of $(\nu_1 + \nu_4)$. By an investigation of the spectra of a series of compounds of known crystal structure, Lever et.al. The separation of monodentate nitrate is appeared to be $5 - 26 \text{ cm}^{-1}$ and that for bidentate nitrate groups $20 - 60 \text{ cm}^{-1}$. This method is tried to the present complexes. A separation of ca $50 - 60 \text{ cm}^{-1}$ in the combination bands in $1500 - 1300 \text{ cm}^{-1}$ region suggests the bidentate nature of coordination for nitrate ions.

The spectral bands of $\text{Th}(\text{NO}_3)_4 \cdot n\text{L}$, $\text{L} = \text{Schiff bases}$ studied here were compared with the known bands of $\text{Th}(\text{NO}_3)_4 \cdot 5\text{H}_2\text{O}$ i.e. $1520 (\nu_4)$, $1290 (\nu_1)$, $1030 (\nu_2)$, $808, (\nu_6)$, $745 (\nu_3)$ and $715 \text{ cm}^{-1} (\nu_5)$ in which bidentate character of nitrate group has been established by X-ray diffraction and neutron diffraction studies. It is inferred that the nitrate group in these complexes also behave as bidentate Table(4)

Table.4 : Infrared absorption frequencies (cm^{-1}) of nitrate groups of thorium (IV) and dioxouranium (VI) complexes of Schiff base ligands.

Complexes	ν_4	ν_1	ν_2	ν_6	ν_3	ν_5	Reference
1	2	3	4	5	6	7	8
$\text{Th}(\text{NO}_3)_4 \cdot 2(4\text{CABPT})$	1472	1291sh	1021w	820m	727sh	-	This work

Curtius has reported that the overtone and combination bands of the nitrate group may be used to distinguish monodentate and bidentate nitrate complexes. Many infrared data on uranyl complexes can be found in a work of Bullock, they are consistent with the presence of bidentate nitrate groups.

The fundamental vibration modes of the nitrate complexes studied in the present work are shown in the Table 5.8. It is clear that a weak band at ca $705 - 700 \text{ cm}^{-1}$ may be assigned to ν_5 while ν_3 occurs near $680 - 740 \text{ cm}^{-1}$. The separation of these two frequencies is of order ca $\sim 45 - 35 \text{ cm}^{-1}$ and similar values are reported earlier. Other frequencies, i.e. ν_1 , ν_2 , ν_4 and ν_6 are comparable to those of reported bidentate complexes. Keeping in view it can be said that in the complexes under study, the nitrate group behaves as bidentate ligands.

Electronic Spectral Studies:

The electronic spectral studies of these complexes are of less interest since metal ion does not contain any unpaired electron in its outer most shell. Bands which are around $240 - 220 \text{ nm}$ and bands at $330 - 250 \text{ nm}$ which corresponds to $\pi\pi^*$ transition. Recently, spectral studies of a Series of Th (IV) Chelates has been carried out by Aruna and associates. Sonal Agnihotri and Kishore Arora, has reported spectral studies of Th(IV) Complexes with Schiff base ligands. Synthesis and spectral studies of Th(IV) and dioxouranium (VI) Complexes of some Schiff base ligands have been reported by Mohapatra and associates.

The electronic spectral data of some of the representative complexes along with their corresponding ligands is listed in Table 5.

Table. 5 : Electronic spectral data for some of the Thorium(IV) Complexes of Schiff base ligands.

Compoundsz	$n \rightarrow \pi^*$	$\pi\pi^*$	metal-N absorption
4CABPT	238.8nm	322 nm	-
$\text{Th}(\text{NO}_3)_4 \cdot 2(4\text{CABPT})$	240nm	250 nm	310nm

N.M.R. Spectral Studies :The N.M.R. spectral studies of some of the representative complexes are done and these were compared with the NMR Spectra of corresponding ligands. N.M.R. spectra were recorded on Bruker AC 300 F NMR spectrophotometer at 300 MHz using solvents CD₂Cl₂ and DMSO-*d*₆ and were found to be in good agreement with reported observations. N.M.R. spectra of 4CABPT contains the important signals at 1.42853-1.3354 (pt), 2.73698-2.69401 (t), 3.93494-3.85122, 4.41757-4.30213, 6.65081-6.62248 (d), 6.76639-6.72974 (qt), 7.26142-7.18877 (t), 7.88527-7.81327 (pt), 8.07951-8.05128 (d), 8.33554 (s), and 9.82996 (s), Th(NO₃)₄. 2(4CABPT) has important signals at 143616-1.32871, 2.84503-2.74148, 3.63090 (s) 3.93180-3.88708 (t), 4.00818-3.96411 (t), 4.27329-4.25052 (d), 4.32162-4.29744 (d) 4.41986-4.34856 (qt), 4.54019 (s), 6.71496-6.67900 (qt), 6.83790-6.80841 (d), 6.99191-6.96191 (d), 7.56753-7.53944 (t), 7.81225-7.77971 (qt) 8.14310-8.09046 (t), 8.81487 (s) 9.79035 (s)

Anti-microbial Studies:Anti-microbial namely antifungal, antibacterial studies of complexes as well as that of organic compounds were the field of interest of various workers. Antifungal/Antibacterial activities against various pathogens using Thorium (VI) compound have been reported in the past.

MATERIAL AND METHODS:

Antimicrobial activity was determined by disc diffusion method. Filter paper disc measuring 6.0 mm diameter were cut with a punch from Whatman filter paper No.1 sterilized at 160°C in hot air oven for one hour, antimicrobial solutions of desired concentration were dropped on discs and were dried in incubator in 37 °C and stored in freezer. Nutrient agar was poured into plates, keeping depth of the medium 4.0 mm. After the medium solidified, the plates were kept for 30 minutes in an incubator (35 to 55 °C) to remove excess of moisture, 4-5 colonies of pure culture on Nutrient agar /Sabouraud's Dextrose agar were transferred into a culture tube containing peptone water with the help of wire loop. The culture was incubated by lawn culture method. A loop full of 2 mm diameter was used to streak the plates with the test organisms and kept it for 5-10 minutes at room temperature.

The discs were removed with the help of flamed forceps from their respective vials and placed in the plate 15 mm away from the edge, at equal distance and sufficiently separated from each other to avoid overlapping of zone of inhibition, finally pressed them lightly with forceps to make complete contact with surface of medium. The plates were incubated at 35-38°C for 24-30 hours.

CULTURE MEDIA USED

Nutrient agar

Agar 2% was added to prepare nutrient agar for antimicrobial sensitivity test. The pH was maintained between 7.4-7.6.

Composition

- Peptone = 10g
- Sodium Chloride = 05g
- Beef extract = 10g
- Distilled water = 1000ml.

After addition of 2% agar, media was autoclaved at 15-25 minutes and poured in sterilized plates.

Sabouraud's Dextrose agar

Composition

- Peptone = 10.0g
- Dextrose = 40.0g
- Agar = 20.0g
- Distilled water = 1000ml

In the present study new synthesized complex was first dissolved in dimethylsulphoxide or acetone to prepare the drug impregnated disc used in anti-bacterial and anti-fungal by disc diffusion technique. Organism tested were E.coli Pseudomonas, Aspergillus niger, and Candida albicans Diameter of zone of inhibition was measured in millimeter and reported as (+) for intermediate zone (partially sensitive), (++) for sensitive zone, (+++) for resistant zone, (+++++) for significant resistant zone and (-) for no inhibition zone.

TABLE 6

Anti-bacterial activity of Complex

Solubility data - All Solutions were prepared in DMSO

Concentration data -

- Medium - Nutrient agar
- pH range - 7.4 to 7.6
- Period of growth - 24 to 48 hrs.

S.No.	Name of Complexes	E. coli	Pseudomonas
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			<i>species</i>
I	Th(NO ₃) ₄ .2(4CABPT)	-	+

Table 7**Anti-fungal activity of complex**

Solubility data	-	All solutions were prepared in DMSO
Concentration tested	-	
Medium	-	Sabouraud's Dextrose agar
pH range	-	7.5
Period of growth	-	4 days

S.No.	Name of Complexes	<i>Aspergillus niger</i>	<i>Candida Species</i>
I	Th(NO ₃) ₄ .2(4CABPT)	+	-

Zone of inhibition: (+) 0-4 mm (++) 4-8 mm (+++) 8-12 mm (++++) 12-16 mm (-) No inhibition

Results and Discussion:

The analytical data temperature indicate that the complex are non-ionic in nature the complexes are fairly stable at room except. The Iodo complexes which convert in to sticky mass after some time. Four new synthesized Complex was screened for their anti bacterial activity against several species of *E. coli*, and *Pseudomonas* species using agar plate diffusion technique. The testing were carried out in dimethylsulphoxide solution at a concentration of 30 gm/ml. Ofloxacin and tetracycline were used as the standard drugs. Results are assembled in Table 6.

Anti-fungal activity :

antifungal activity against *Aspergillus niger* and *Candida* species at concentration of 30gm/ml Using Sabouraud's Dextrose agar media disc diffusion technique. The testing was carried out in dimethylsulphoxide solution. Amphotericin B discs were used as the standard drugs. Results are assembled in Table 7.

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