

Synthesis, Characterization, Antibacterial properties and DNA Binding of a Th(IV) Complex

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Abstract

In this investigation, we have reported the synthesis of the complex $[\text{Th}(5,5'\text{-dmbpy})_3](\text{NO}_3)_4$ (**1**) that 5,5'-dmbpy = 5,5'-dimethyl-2,2'-bipyridine. This complex has been characterized using spectral methods (FT-IR, UV-Vis, ¹H-NMR and luminescence), elemental analysis and the Cyclic Voltammetry (CV) method. The FT-IR data shows that 5,5'-dimethyl-2,2'-bipyridine (5, 5'- dmbpy) ligand adduct to metal centers by nitrogen atoms. Electronic spectra of (**1**) shows ligand field transitions and charge transfer (CT) bands. Electrochemical data for the complex in the DMF solution show reduction and oxidation processes for the Thorium ion and ligand. The interactions of complex (**1**) with FS-DNA have been investigated using UV-Vis and gel electrophoresis. The binding constant (K_b) was calculated using UV-Vis technique ($K_b = 3.5 \times 10^5$). Also, DNA cleavage was studied using agarose gel electrophoresis. The antibacterial properties and fluorescence property of complex (**1**) have also been examined. The in vitro biological screening effects of the complex and ligand were tested against different types of bacterium and $[\text{Th}(5,5'\text{-dmbpy})_3](\text{NO}_3)_4$ (**1**) shows antibacterial properties.

Keywords: Cyclic voltammetry (C.V), Electronic spectra, Thorium ion, gel electrophoresis, antibacterial.

1. Introduction

Compounds with bipyridine derivatives are the most common ligands [1]. The ligand 2,2'-bipyridine has a conjugation system depicted in figure 1. Most recently, great attention has been given to derivative of ligand 2,2'-bipyridine because of their influence in the area of organometallic and coordination chemistry [2, 3]. Thus, compounds which include bipyridine derivative ligands have found applications in various fields such as catalysis, luminescence, biological systems, organic light emitting diodes, and electrochemical studies [4-7].

Actinide ions usually have high coordination numbers and the type of obtainable polyhedron is influenced by the nature of the coordinating ligands [8, 9]. The metal thorium plays an important role in the Indian Nuclear Energy programs. A number of thorium nitrate complexes have been produced and investigated. The compound $[\text{Th}(\text{NO}_3)_4 \cdot (\text{H}_2\text{O})_3]$ was one of the first 11-coordinate compounds to be recognized (Figure 2) [10]. We notified the synthesis of the compound with 5,5'-dimethylbipyridine under formula $[\text{Th}(5,5'\text{-dmbpy})_3](\text{NO}_3)_4$.

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Lanthanide and Actinide ions and their complex are used in bioinorganic chemistry due to their unique properties such as biological, pharmaceutical, magnetic, spectroscopic, luminescent, antibacterial and antitumor activities and DNA-binding affinity [11,12]. The interaction between metal complexes DNA is performed using covalent or non-covalent methods. Covalent interactions comprise the coordination of the nitrogenous base of DNA to the metal ion, whereas non-covalent interactions include three modes: groove binding, intercalation binding, and electrostatic interaction. [13,14].

In this paper, we have reported the interaction of complex **1** with DNA *in vitro*. The DNA binding properties of this complex have been investigated through UV-Vis and gel electrophoresis. The *in vitro* biological screening effects of the synthesized complex (**1**) were tested against different microbial kinds.

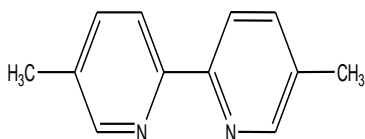


Fig.1. 5,5'-dimethyl-2,2'-bipyridine

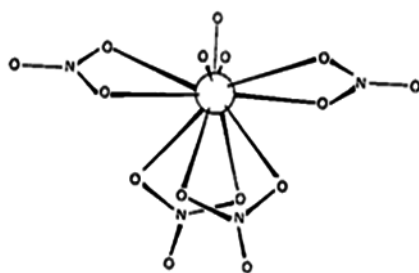


Fig.2. Structure of $\text{Th}(\text{NO}_3)_4(\text{H}_2\text{O})_3$

2. Experimental

2-1. Materials and physical methods

All solvents and chemicals were purchased from Aldrich and Merck. Infrared spectra ($400\text{--}4000\text{cm}^{-1}$) were obtained as 1% dispersions in KBr pellets with a Shimadzu-470 Plus spectrometer. The $^1\text{H-NMR}$ spectrum was recorded at room temperature (25°C) with a Bruker 300 Ultrashield spectrometer in DMSO solution. Cyclic voltammograms were obtained by use of a SAMA500 device. Elemental analysis was

recorded with a Heraeus CHN Rapid analyzer. Melting point was acquired on an electrothermal device type 9100 melting point apparatus. Electronic absorption spectra were obtained on a Cary Bio 300 spectrometer at room temperature and in a DMF solution.

2-2. Synthesis of $[\text{Th}(\text{5,5}'\text{-dmbpy})_3](\text{NO}_3)_4$ (**1**)

A solution of 5,5'-dimethylbipyridine (0.04 g, 0.25 mmol) in 10 ml methanol was added to a solution of $\text{Th}(\text{NO}_3)_4 \cdot 4\text{H}_2\text{O}$ (0.138 g, 0.25 mmol) in 10 ml methanol. The resulting solution was stirred and refluxed at $60\text{--}65^\circ\text{C}$ for 24 h. Brown colored deposits were seen on the walls of the balloon. The product was dissolved in DMF solution. After 10 days, opalescent crystals were isolated.

3. Results and discussion

3.1. IR spectra $[\text{Th}(\text{5,5}'\text{-dmbpy})_3](\text{NO}_3)_4$ (**1**)

The Infrared spectrum of the free ligand 5,5'-dmbpy contains a sharp band at 1615 cm^{-1} related to the stretching frequency of the C=N bond [15]. This frequency shifted to a lower wavenumber in complex (**1**). The IR spectrum of complex $[\text{Th}(\text{5,5}'\text{-dmbpy})_3](\text{NO}_3)_4$ included two sharp bands at $1517\text{--}1300\text{ cm}^{-1}$ associated with $\nu(\text{C}=\text{N})$ and $\nu(\text{C}=\text{C})$ vibrations [16]. This observation indicates the involvement of unsaturated nitrogen atoms of the C=N groups in bonding with the central metal ions [17]. The vibration bands at 3100 cm^{-1} and 2924 cm^{-1} in the complex are assigned to the stretching frequency of the $\nu(\text{C-H})$ bond aromatics and aliphatic, respectively [18]. The bands observed in the range 833 cm^{-1} , 639 cm^{-1} are attributed to $\nu(\text{C}=\text{C}=\text{C})$ and $\nu(\text{C}=\text{C}=\text{N})$ vibrations in the Pyridine rings [19-20]. The band at 1620 cm^{-1} in the complex is associated with the stretching $\nu(\text{N-O})$ in the complex [16]. The band at 512 cm^{-1} was related to Th-N stretching [21].

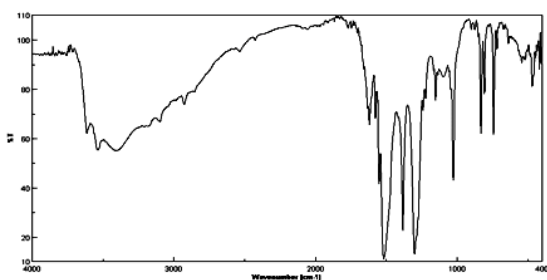


Fig.3. IR spectrum compound $[\text{Th}(5,5\text{-dmbpy})_3](\text{NO}_3)_4$ using disk KBr.

3.2. Electronic excitation study

The UV-visible spectra of $[\text{Th}(5,5'\text{-dmbpy})_3](\text{NO}_3)_4$, was recorded in DMF solution (figure 4). The electronic spectra of complex (1) in DMF solution show several absorption bands in the UV regions. According to evidence affected by the intensity and position from comparison with the spectra of the free ligand 5,5'-dmbpy, the spectra show a very strong band at the range 200-250 nm due to ligand-centered $\pi \rightarrow \pi^*$ transitions [22-24]. The band observed at approximately 313-330 nm is assigned to charge transfer transitions observed in similar complexes [25,26]. Due to the poor solubility of the complex, it was not possible to conduct solvent dependence studies.

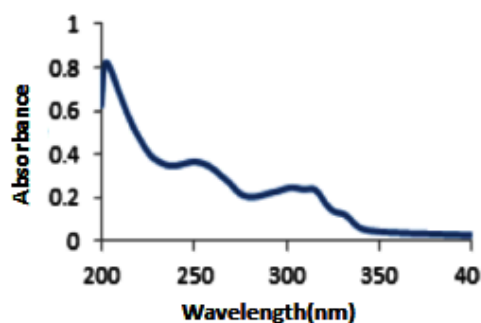


Fig.4. UV absorption spectra of $[\text{Th}(5,5'\text{-dmbpy})_3](\text{NO}_3)_4$

3.3. Electrochemical Studies $[\text{Th}(5,5'\text{-dmbpy})_3](\text{NO}_3)_4$ (1)

Cyclic voltammogram of the complex was recorded at -2.0 to 2.5 V vs. Ag/AgCl using different switching potentials at varying scan rates. The cyclic voltammogram of the complex was obtained at 25°C in DMF solution containing 0.1M TBAH as supporting electrolyte with scan speed of 500 mvs⁻¹ (Figure.5). The voltammogram of the complex shows a quasi-reversible

wave in the potential $E_c = -1.98\text{V}$ related to the reduction of the ligand 5,5'-dmbpy and the quasi-reversible wave at potential $E_c = -1.4\text{V}$ assigned to the reduction of thorium ion [27-29].

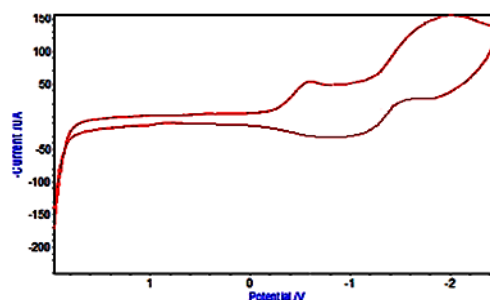


Fig.5. Cyclic voltammograms for compound $[\text{Th}(5,5'\text{-dmbpy})_3](\text{NO}_3)_4$ in DMF at 25 °C.

3.4. ¹H-NMR spectrum of $\text{Th}[(5,5'\text{-dmbpy})_3](\text{NO}_3)_4$

The hydrogen atoms of the aromatic rings (H_c , H_d), (H_b) and methyl groups (H_a) were seen at approximately 7-9 ppm, 3.34 ppm and 2.46 ppm respectively. Figure.6 [30].

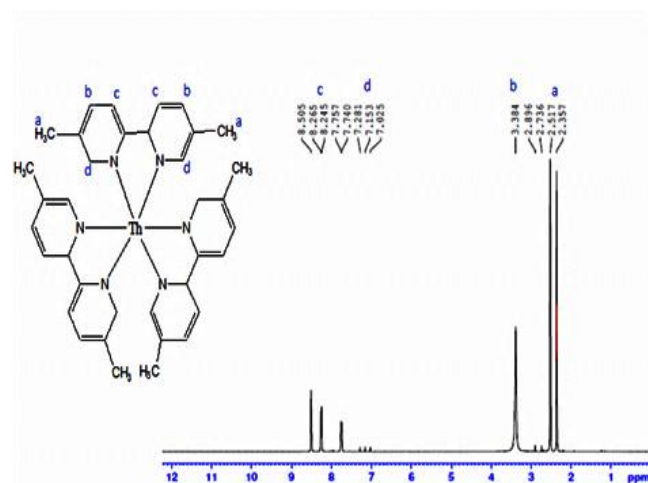


Fig.6. ¹H NMR spectrum of $\text{Th}[(5,5'\text{-dmbpy})_3](\text{NO}_3)_4$ in DMSO

3.5. Elemental analysis of compound

The proposed structures for the compounds were approved with elemental analysis. The results of the elemental analysis in the Table 1 are in good agreement with those calculated for the proposed molecular formula.

Table 1. Elemental analysis (CHN)

Element	C	H	N
Compound	Th	Experi	Th
Experimental	Exp.	Calc.	Experi
	ental		ental

	47.				13.	
1	3	47.2	4.16	4.12	8	13.7

3.6. Luminescent property

In recent years, luminescent metal compounds have been studied for important applications in the fields of biological probes and solar energy sensors and optoelectronic tools [31-33]. The free 5,5'-dimethyl-2,2'-bipyridine ligand displays a maximum emission peak at 347 nm and two shoulders emission peaks at 307 and 425 nm which can be attributed to the $\pi \rightarrow \pi^*$ transitions of the ligand [34]. The thorium complex is emissive at room temperature. Figure 7, shows an intra-ligand fluorescence related to the $\pi \rightarrow \pi^*$ transitions of the ligand 5,5'-dmbpy with maxima occurring at 364 nm [35]. Many aromatic ligands, such as 5, 5'-dimethyl-2,2'-bipyridine, possess some degree of fluorescence, which usually increases when coordinated to metal [36]. The thorium complex shows increased intensity of luminescence, compared with the free ligand 5,5'-dmbpy. Increased property and intensity of luminescence after complexation are of high interest due to the photochemical application of the corresponding compounds [37] (figure 7).

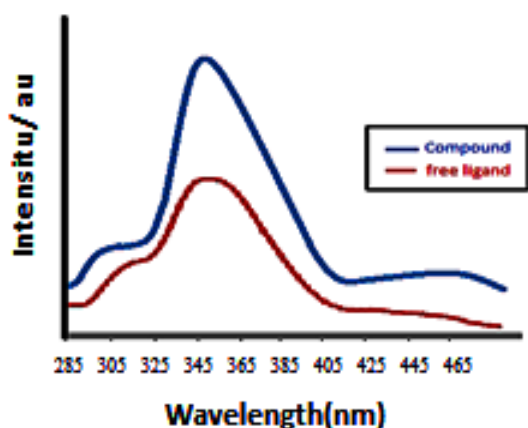


Fig.7. Fluorescence spectra of complex thorium and free ligand 5,5'-dmbpy in the solvent methanol at room temperature

3.7. Electronic absorption spectra

UV-Vis spectroscopy was employed to determine the binding strength and binding modes of small molecules to DNA. The absorption spectra of the fixed

concentration of complex **1** ($1.2 \times 10^{-5} \text{ M}$) in the absence and presence of DNA ($1.4 \times 10^{-4} \text{ M}$, 0-20 μl) can be seen in Figure 8. According to this figure, increased concentrations of FS-DNA led to decrease in the absorption band. We recorded the UV-Vis spectra between complex (**1**) and FS-DNA (Figure 8). The experiment confirms that there is an interaction between complex (**1**) and FS-DNA.

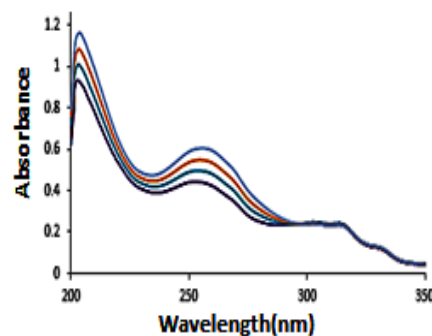


Fig.8. Electronic absorption spectra of complex (**1**) in the absence and presence of different concentrations of DNA.

The binding constant (K_b) can be determined using the curve of $[\text{DNA}]/(\epsilon_a - \epsilon_f) \times 10^{12}$ versus $[\text{DNA}] \times 10^6$. The binding constant is obtained from the ratio of the slope to the intercept (figure 9). The value of K_b finally related to UV-Vis (3.5×10^5) is lower than that K_b related to a classical intercalator such as Ethidium Bromide (EB) (1.4×10^6) [42-44]. The EB is a classical intercalator with a completely flat structure. Ethidium Bromide easily interacts with DNA and can change the DNA structure. Finally, it can be estimated that the binding mode of DNA-compound **1** was not intercalation. Also, there is hyperchromic shift which indicates that there is no intercalation interaction [38-41]. As a result, binding mode between complex **1** with FS-DNA is groove binding, therefore the hydrogen bonding has an important role in the interaction of DNA with complex **1**.

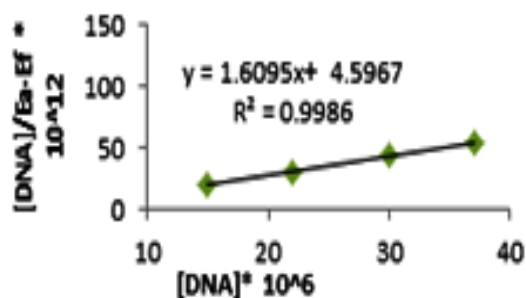


Fig.9. The curve of $[DNA]/(\epsilon a-\epsilon f)\times 10^{12}$ versus $[DNA]\times 10^6$

3.8. Agarose gel electrophoresis

Agarose gel electrophoresis method of FS-DNA display the interaction metal complex **1** with FS-DNA in tris buffer solution. The DNA cleavage by complex (**1**) was performed to test the ability of complex (**1**) to decrease the movement of FS-DNA in the agarose gel, using incubation of several samples containing an amount of DNA ($1.4\times 10^{-3}M$), and various concentrations of complex **1** in the Tris-buffer solution. All samples of different concentrations of complex (**1**) were mixed with 4 μL of loading buffer Methylene blue+ 5 μL FS-DNA and shaken in order to mix. Next, the solution was loaded onto an agarose gel, and the samples were electrophoresed for 15 min at a voltage of 100 v. After putting under the lights UV, the strips appear. Figure 10 proves the interaction complex **1** with the DNA. Agarose gel electrophoresis of Cleavage of DNA ($1.4\times 10^{-3}M$) with several concentrations of the Th(IV) compound at room temperature: lane (DNA): DNA control; lane 2: Th(IV) compound ($1.2\times 10^{-5} M$) + FS-DNA, lane 3: complex ($2.9\times 10^{-5} M$) + DNA, lane 4: complex ($6.5\times 10^{-5} M$) +FS-DNA, lane 5: complex ($9.8\times 10^{-5} M$) + FS-DNA.

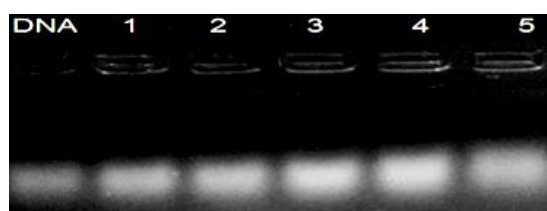


Fig.10. Gel electrophoresis of Cleavage of FS- DNA by several concentrations of complex(**1**) at 25 °C: one lane having: DNA control; lane 1-5: complex (**1**)+ DNA.

3.9. In vitro antibacterial activity

The antibacterial activities of the ligand and complex(**1**) were tested using the disc diffusion method against the species Staphylococcus aureus ATCC 25923 (gram positive bacteria) and Escherichia coli ATCC 25922 (gram negative bacteria). The MIC of complex(**1**) was tested against bacterial strains via a broth dilution method.

The minimum inhibitory concentrations (MIC) of the studied metal complex(**1**) and free ligand are summarized in Table 2. A comparative investigation of the ligand and the complex shows that complex(**1**) exhibits higher antibacterial activity than the free ligand. The increased activity of complex(**1**) can be explained on the basis of Tweedy's chelation theory and Overtone's concept [45,46]. After chelation, there is a decrease in the polarity of the Th(IV) atom, because of the partial sharing of its positive charge with the donor groups and also due to π -electron delocalization on the whole chelate ring. Complex(**1**) prefers interactions with lipids which are important constituents of the cell wall. Also, the decreasing polarity led to increasing the lipophilic character of the chelates. An interaction between the lipid and the complex is successful, which may lead to further breakdown of the cell [47]. The results revealed that complex(**1**) is most effective against Staphylococcus aureus and Escherichia coli, while the free ligand has no activity versus them since the complex has less polarity related to the aromatic ring of free ligand. The increased power of its lipophilic and final complex has more antibacterial activity than ligand. Complex(**1**) is more toxic towards the Gram-positive strain (Staphylococcus aureus) than the Gram-negative strain (Escherichia coli), Due to differences in the structure of the cell wall.

Table 2. The MIC and MBC and inhibition diameter zone values (mm), of the free ligand and its corresponding Th(IV) compound.

Compound	MIC(mg/ml)	MBC(mg/ml)	Inhibition zone(mm)
E-coli	S.aureus	E-coli	S.aureus
Complex(1)	0.003-0.006	0.006-0.012	22-18

5,5-dmbipy0.0125-0.0125 0.025-0.0256-6

4. Conclusions

In summary, in this article we reported the synthesis of the thorium (IV) complex with 5,5'-dmbpy as the ligand. This complex has been characterized using FT-IR, UV-Vis, ¹H-NMR spectroscopies, elemental analysis and cyclic voltammetry (CV) techniques. The luminescence properties of 5,5'-dmbpy ligand and complex (1) were investigated. The intensity of luminescence properties increased after complexation. The interaction of complex (1) with FS-DNA was carried out using UV-Vis spectroscopy and agarose gel electrophoresis. The experimental results indicate that complex (1) interacts with DNA at quite low concentrations. The results indicate that the interaction mode of complex (1) and FS-DNA are groove binding. Agarose gel electrophoresis analysis shows that the destruction of DNA grows during the increasing process of the concentration of complex (1) added to DNA. This has been attributed to interactions between complex (1) and DNA. The biological activity of complex (1) showed that the Th(IV) complex displays antibacterial activity.

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