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Spectroscopic and biological studies on OXO-vanadium (IV) complex with dicyandiamide having N-donor sites

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Abstract
The current research work shows a novel oxovanadium (IV) complex ($VC_{12}O_6H_{20}N_4$) by the reaction of $VO(acac)_2$ and DCDA (where acac = acetylacetonate and DCDA = dicyandiamide) as ligand. The synthesized oxovanadium (IV) complex was characterized by different techniques used for elemental analysis, IR, ESR,TG-DTA, XRD, and biological studies. The IR spectra show that the bands associated with [V=O], [V-O], and [V-N] might be assigned to 948 cm^{-1}, 462 cm^{-1}, and 527 cm^{-1}, respectively. The analysis of ESR spectral studies indicates that the eight-line pattern denotes the presence of a single vanadium atom and the monomeric structure of the complex. According to TG curve reveals that the first and second weight loss of the synthesized complex is found at 5.23% and 69.45% due to lattice water molecules and the release of two acetylacetonate molecules and one dicyandiamide molecule. The oxo-vanadium complex's average crystallite size is 76 nm by using Debye Scherer's formula. In biological studies, the complex can interact with CT-DNA through electrostatic binding, causing a small increase in T_m by thermal denaturation. The cyclic voltammetry indicates that the EPA values are shifted to a more negative potential with increasing concentrations of CT-DNA also through electrostatic binding.

Keywords: DCDA (dicyandiamide); CT-DNA (calf thymus); IR (Infra-Red); ESR (Electron Spin Resonance); Thermal denaturation

Introduction
Vanadium is a biometal in the first transition series which is important for animals, plants, and microorganisms. The oxovanadium (IV) complex acts as a catalyst in some biological processes [1] as well as industrial processes [2-4]. The versatile nature of vanadium is due to its possible oxidation states from -1 to +5 which exist in both cationic and anionic forms [5-7] vanadium catalyzed oxygen-transfer reactions haveattracted considerable interest due to their relevance in biological activities, coordination chemistry of oxovanadium (IV) is an interesting area of current research. Diacetyl is a versatile molecule having two reactive carbonyl groups capable of undergoing condensation reaction with a variety of diamine oxovanadium (IV) complexesthat have been found to show insulin memetic activity [8] and potent antidiabetic agent [9]. Vanadium compounds have well-established potential in the oral treatment of both types of diabetes. Vanadium-catalyzedoxygen-transfer reactions have attracted considerable interest dueto their catalytic properties and biological activities, the coordination chemistry of oxovanadium (IV) is an interesting area of current research.
Only a few studies have been reported on the interaction of vanadium complex with DNA, and generally, they have been restricted to cases when vanadium is in its highest oxidation state [10-12]. The geometry, coordination number, and biological efficacy of oxovanadium (IV) are highly ligand-dependent. It is also known that VO^{2+} is less toxic than the vanadate ion (VO_4^{3-}) andwith all these characteristics, the complexes exhibit potential therapeutic applications [13].
The vital role of vanadium in different chemical and biological systems has motivated the development of vanadium chemistry. The coordination chemistry of nitrogen and oxygen donor ligands is an active area of research. It is evident that the -HC≡N, linkage in azomethine derivative is an essential structural requirement for biological activity [14, 15].

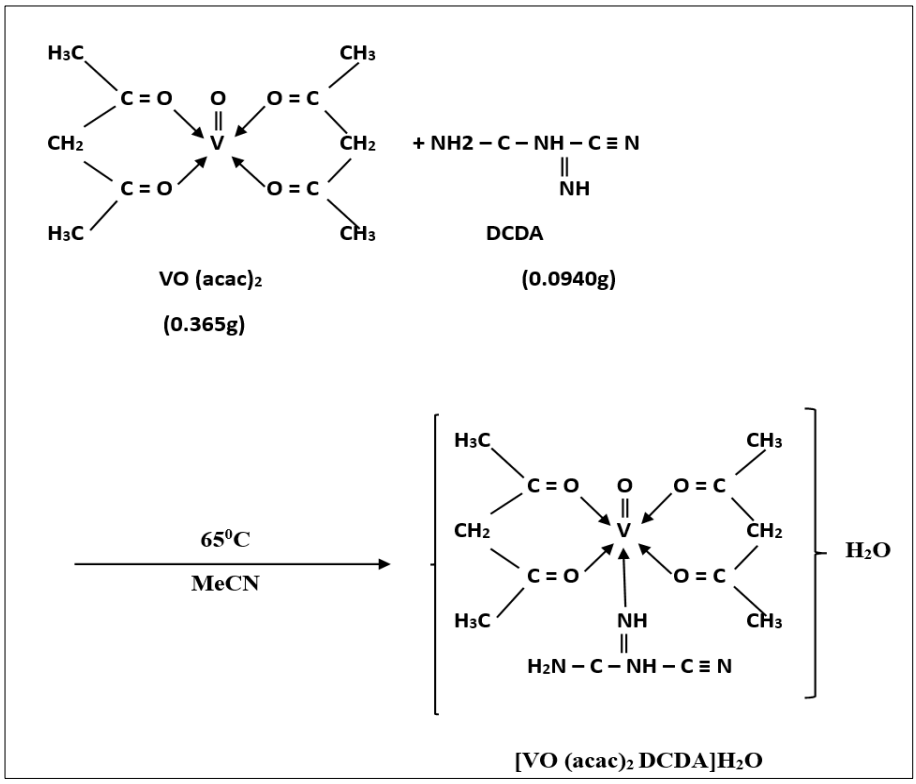
DNA interaction with ions and molecules is an important fundamental issue in life science that is of great importance to understanding the action mechanisms of some anticancer and antibiotic drugs [16, 17]. In order to explore the pharmaceutical importance of vanadyl ion in biological systems, a series of oxovanadium (IV) complexes with ligands derived by the reaction of diacetyl with amino acids such as glycine, alanine, serine, cysteine, and valine are synthesized where VO^{+2} cation appears to act as the kinetic template [18]. Bis (acetylacetonate) oxovanadium (IV) $[VO(acac)_2]$ is also used as a new class of cancer-specific MRI contrast agents that are nontoxic and highly sensitive to cancer metabolism [19]. Considering the importance of vanadium complex and dicyandiamide as well as its DNA interaction. We report here a new oxovanadium (IV) complex $[VO(acac)_2DCDA]H_2O$ using acetylacetonate (acac) and dicyandiamide (DCDA) as ligands. The synthesized complex has been characterized by elemental analysis IR, ESR, TG-DTA, and powder XRD analysis. The interaction of this complex with CT-DNA (calf thymus DNA) was investigated using UV-vis-absorption titration, thermal denaturation, and cyclic voltammetry reported in this paper.

Materials and Methods

All the chemicals and solvents used were of analytical grade reagent (AR) and used without further purification. Bis (acetylacetonate) oxovanadium (IV) $[VO(acac)_2]$ were prepared by standard method. CT-DNA and Tris-HCL molecular biological grades were purchased from Merck (India). Dicyandiamide was directly purchased from the market farm. Deionized sonicated triple distilled H_2O was used throughout the experiment.

Preparation of $[VO(acac)_2$ DCDA$]H_2O$ complex

Dicyandiamide (DCDA) 0.09408g in acetonitrile (30ml) was added dropwise to $[VO(acac)_2]$ 0.365g in acetonitrile (30ml), and the mixture was stirred on a hot plate for 30 hours at roughly 65 ℃. The complex's colour changes from a bluish-green to dark blue. The dark blue solution was filtered and kept at room temperature for slow evaporation. After 2-3 days, a violet crystalline product was obtained. This crystalline product was washed with methanol and dried in the air.



Violet Colour: The molecular mass of $VC_{12}O_6H_{20}N_4$and F.W. = 367.25 g Yield: 0.2315g (60%). Anal. Calc. for $VC_{12}O_6H_{20}N_4$: V, 13.88; C, 39.24; H, 5.45; N, 15.26. Found: V, 13.47; C, 39.13; H, 5.21; N, 15.14%. Table 1 shows the elemental analytical data of $[VO (acac)_2$ DCDA$]H_2O$

Table 1: Elemental analytical data of $[VO (acac)_2$ DCDA$]H_2O$

Compound		V	C	H	N
$[VO (acac)_2$ DCDA$]H_2O$	Calc	13.88	39.24	5.45	15.26
	Found	13.47	39.13	5.21	15.14

Results and Discussion

The oxovanadium (IV) complex was synthesized and characterized by using various analytical studies such as IR, ESR, TG-DTA, XRD, and biological studies [20] that are reported. Microanalysis shows the C, H, and N present in the complex by using the elemental analyzer model of Perkin-Elmer 2400. The complex is stable in air and soluble in most organic solvents like DMF and DMSO.

Spectroscopic Studies

IR (Infra-Red Spectroscopy)

The IR spectra were carried out on Shimadzu FTIR 8100S using KBr disks in the 450-4000 cm-1 wavenumber range. As a ligand, pure dicyandiamide exhibits a doublet strong nitrile (C≡N) band at 2206 cm^{-1}, 2169 cm^{-1}, and an azomethine (C=N) group band at 1659 cm^{-1} in the IR spectrum as shown in Fig 1.The broad bands in the ranges

3431–3335 cm^{-1} and 3188-3157 cm^{-1} are due to NH_2 asymmetric and symmetric sketches. Fig. 2 shows the IR spectra of the complex of the free nitrile band observed in higher wavenumbers at 2214 cm^{-1} and 2175 cm^{-1} respectively. The complex shows a change in the ligand's (C=N) stretch to a lower wave number at 1662 cm^{-1}, suggesting that the metal has bound the nitrogen in the azomethine. After the complex formed, NH_2 asymmetric and symmetric stretching were detected in the ranges of 3447–3342 cm^{-1} and 3233–3124 cm^{-1}, respectively. The presence of (O-H) in the complex causes the lattice water molecule to be stretched, which results in the band at 3542 cm^{-1}. The band disappears completely from the spectra when the complex is heated to 110 ℃ (see inset in Fig. 1). Additionally, the bands associated with [V=O], [V-O], and [V-N] might be assigned to 948cm^{-1}, 462cm^{-1}, and 527cm^{-1}, respectively. From the IR spectra details of the complex have been shown in Table 2.

Table 2: IR spectral data of the complex

$[VO(acac)_2DCDA]H_2O$	ν (C≡N)	ν(C=N)	ν(V=O)	ν(V-N)	ν(V-O)
Wavenumber	2206 cm^{-1}	1659cm^{-1}	948cm^{-1}	527cm^{-1}	462cm^{-1}

Compound IR spectral band (cm^{-1})

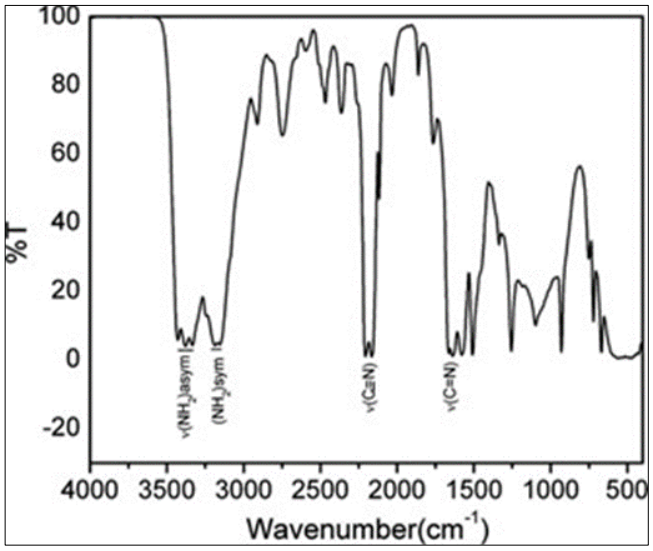


Fig 1: IR spectrum of DCDA.

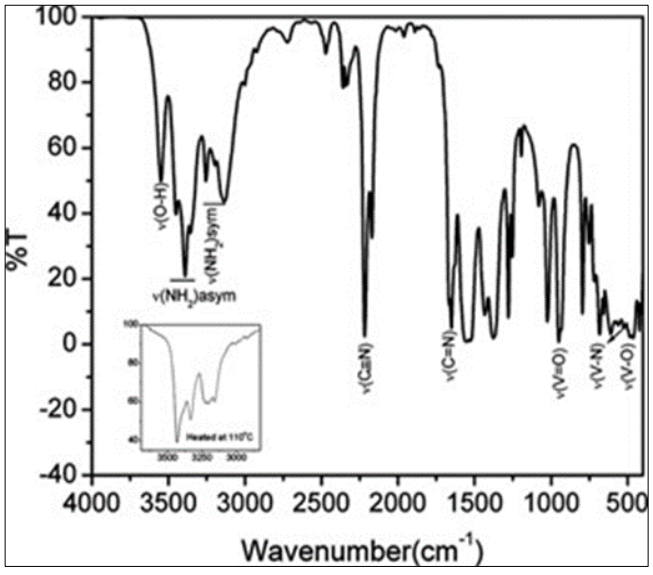


Fig 2: IR spectrum of [VO (acac)$_2$ DCDA]H_2O

ESR (Electron Spin Resonance Spectroscopy)

The ESR spectra of the complex were recorded Varian E-112 spectrometer at the SAIF, IIT Bombay, India. Fig. 3 shows that the complex's X-band ESR spectra were obtained in polycrystalline at room temperature (25 ℃), as well as in a frozen glassy form at -140 ℃. The vanadium (IV) spectrum in DMSO displays an eight-line pattern at room temperature, which denotes the presence of a single vanadium atom and the monomeric structure of the complex. When the DMSO was frozen, the complex showed well-resolved axial anisotropy that was represented by two sets of eight lines. These lines are the consequence of the interaction between the unpaired $3d^1$ electron and the ^{51}V (I=7/2) nucleus's spin and the DMSO molecule. In order to derive the ESR Hamiltonian parameters, the spectrum of the complex in solid at room temperature (25 °C) is too wide and poorly resolved. This is depicted in Fig. 3 in order to get precise ESR values and may be caused by the dipolar contact between the nearby molecules.

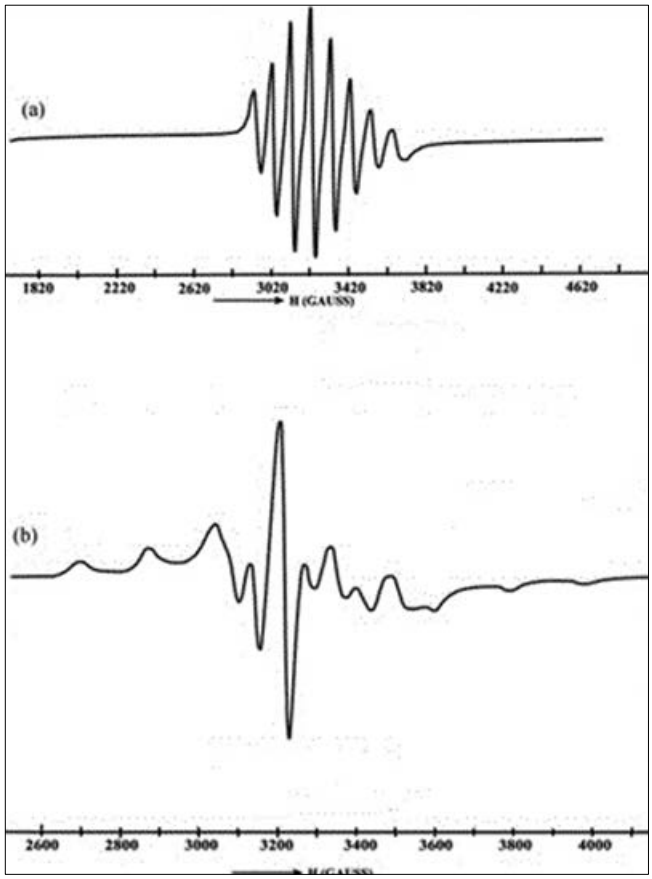


Fig 3: ESR spectra of [VO (acac)$_2$ DCDA]H_2O in solid at (a) 25 ℃ (b) -140 ℃

Thermal Analysis

Thermo Gravimetric Differential Thermal Analysis (TG-DTA)

The thermogram was recorded on a Perkin Elmer STA 6000 model. The thermal analysis of the synthesized complex was examined using thermogravimetric analysis. The synthesized oxovanadium complex performed a thermogravimetric examination at temperatures ranging from room temperature (RT) to 900 ℃ with a heating rate of 10 ℃ min^{-1} in the N_2 atmosphere. Fig. 4 shows that the TG-DTA curve of [VO (acac)$_2$ DCDA]H_2O.

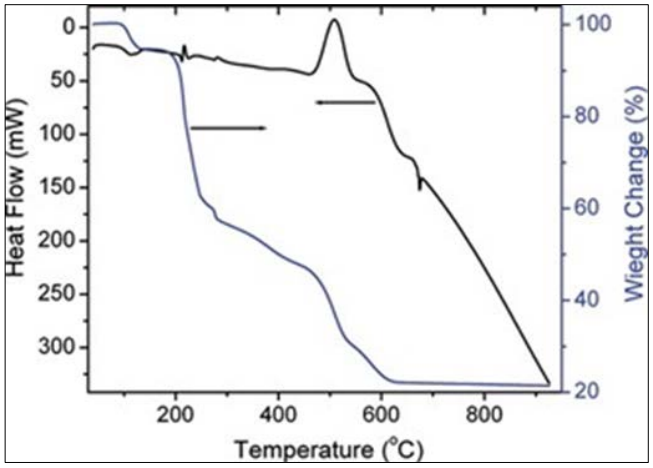


Fig 4: TG-DTA curve of [VO (acac)$_2$ DCDA]H_2O

According to TG curve reveals that the first weight loss of the synthesized complex was found at 5.23%in the range of 104 ℃ to 123 ℃ with a weak endothermic peak at 120 ℃ corresponding to one lattice water molecule. The complex exhibits a second weight loss of 69.45% in the range of 210 °C to 620 °C, with an exothermic peak occurring at 521 °C that is associated with the release of two acetylacetonate molecules and one dicyandiamide molecule.

Structural Analysis

Powder XRD Study

X-ray diffraction patterns were carried out using a PAN analytical powder diffractometer (X pert PRO) using Cu K_x (1.540A^0) radiation fitted with Ni filter. Fig. 5 shows the x-ray powder diffraction pattern of the dicyandiamide complex. The crystallite of the mononuclear oxovanadium complex hasa triclinic structure with unit cell dimension, a = 8.3518 A^0, b = 12.7285 A^0, c = 6.3934 A^0,α = 98.910^{0}β= 101.321^0, λ = 90.808^0 and cell volume = 657.69A^0

The Debye-Scherer formula is used to determine the crystallite size of the synthesis complex as follows:

$$D=\frac{k\lambda}{\beta \cos\theta}=\frac{0.9\,\lambda}{\beta\, cos\,\theta}$$

Where, D = crystallite size, K = constant lies from 0.82 to 1.39 depending on the specific geometry of the scattering object for two-dimensional lattices, the value of K was found to be 0.9 and that of the three-dimensional lattice is 1.39, β = the full width at half maximum of the predominant peak, θ = the diffraction angle andλ = wavelength of x-ray used. The average crystallite size of the oxo-vanadium complex is found to be 76 nm.

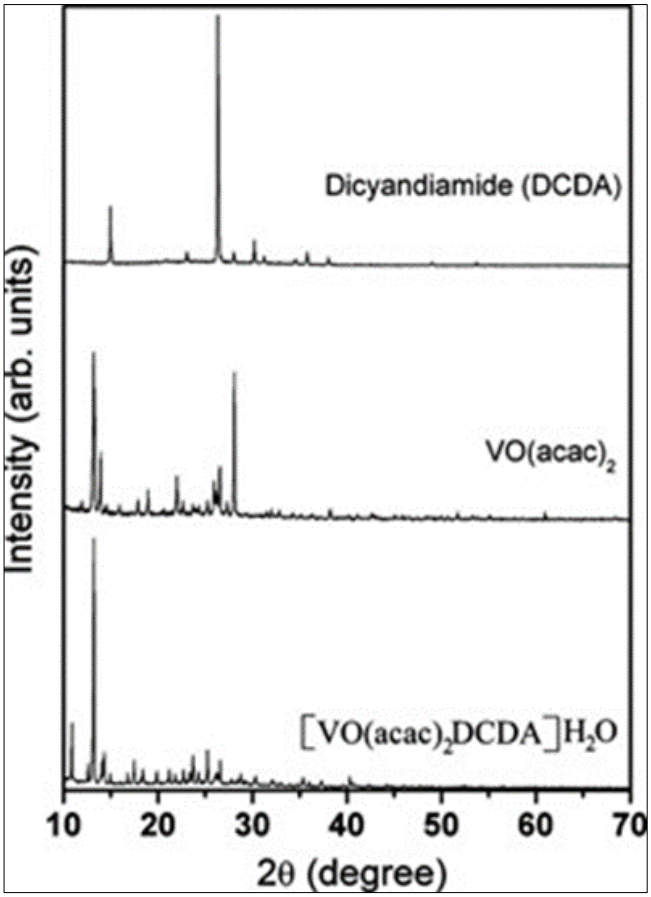


Fig 5: Powder XRD patterns of[VO(acac)$_2$ DCDA]H_2O, VO (acac)$_2$ and Dicyandiamide (DCDA) ligand

Biological study

Thermal denaturation study

Thermal denaturation analyses were carried out using a Peltier temperature-controlling programmer (PTP6) fitted with a Perkin Elmer UV-vis Lamda 35 spectrometer. The melting curves were determined by measuring the absorbance at 265 nm as a function of temperature for the solution of CT-DNA (72μM) in the absence and presence of various concentrations of the oxovanadium complex, as shown in Fig. 6. The temperature was scanned from 25 to 98 °C at a speed of 120 nm per minute.

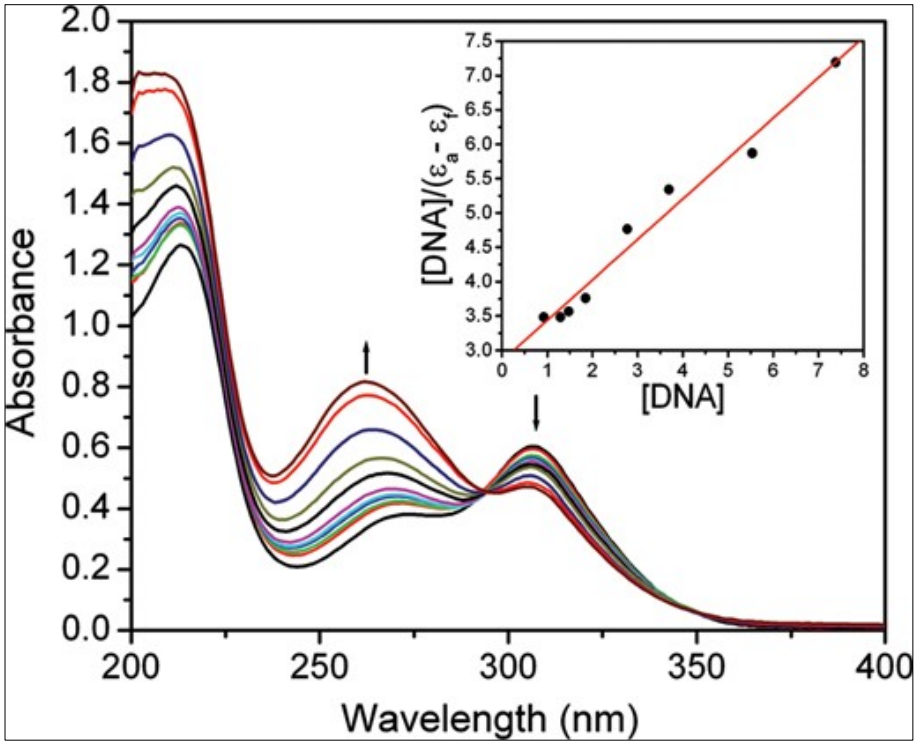


Fig 6: Absorption spectra of $[VO(acac)_2$ DCDA$]H_2O$ complex.

Thermal denaturation measurement shows that when the temperature is raised, the double-stranded DNA gradually dissociates into single strands. The melting curves of the CT-DNA in the absence and presence of complexes are illustrated in Fig. 7. The T_m (melting temperature) of CT-DNA in the absence of the complex is 75 °C and it reaches ca. 76% in the presence of the complex. The small increase in Tm (ΔTm ~ 1^0C) in the presence of the complex suggests that the complex possibly interacts with DNA by electrostatic binding. Table 3 shows that the absorbance value of thermal denaturation curves of CT-DNA

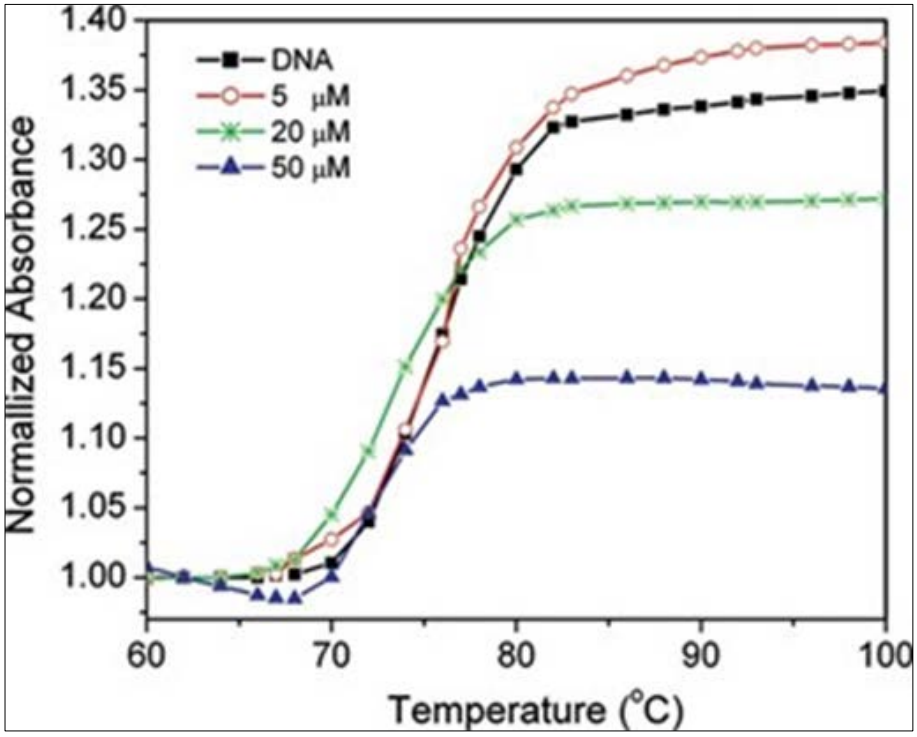


Fig 7: Thermal denaturation curves of CT-DNA (72μM) alone and in the presence of varying amounts (5μM,20μM,50μM) of $[VO(acac)_2DCDA]H_2O$

Table 3: Thermal denaturation curves of CT-DNA

Concⁿ	Temp °C	Normalized absorbance	
		Absent	Present
5µM	70		1.025
	75		1.10
	80		1.32
	85		1.35
	90		1.37
	95		1.38
20µM	70		1.05
	75		1.15
	80		1.25
	85		1.26
	90		1.26
	95		1.26
50µM	70		1.00
	75		1.13
	80		1.14
	85		1.14
	90		1.14
	95		1.14
(alone) DNA 72µM	70	1.00	1.00
	75	1.12	1.12
	80	1.30	1.30
	85	1.34	1.34
	90	1.35	1.35
	95	1.36	1.36

The melting curves of the CT-DNA in the absence and presence of complex are shown by thermal denaturation studies. The Tm of T-DNA in the absence of the complex is 75 ℃ and it reaches 76 ℃ in the presence of the complex. A small increase in Tm in the presence of the complex is suggested.

Cyclic Voltammetry Study

The Cyclic Voltammetry was recorded at the CH602C electrochemical analyzer. Fig. 8 shows the cyclic voltammograms of the complex at different concentrations of CT-DNA studied in tris buffer with increasing concentrations of the calf-thymus DNA. The peak current of both the iPc (cathodic scan reduction peak) and iPc values for 1.7 µM, 3.4 µM, 5.2µM and 6.9 µM concentrations of DNA are 0.080 mA, 0.075 mA, 0.074 mA, and 0.071 mA, respectively, while iPa (anodic scan oxidation peak) values are 0.110 mA, 0.089 mA, 0.085 mA, and 0.082 mA, respectively. The EPa values were shifted to a more negative potential with increasing concentrations of CT-DNA, as shown in Fig. This phenomenon implied the formation of a new association complex. These results clearly suggest that the oxovanadium complex binds to CT-DNA through electrostatic binding. Table 4 presents the measurement of the voltammogram in different concentrations of CT-DNA.

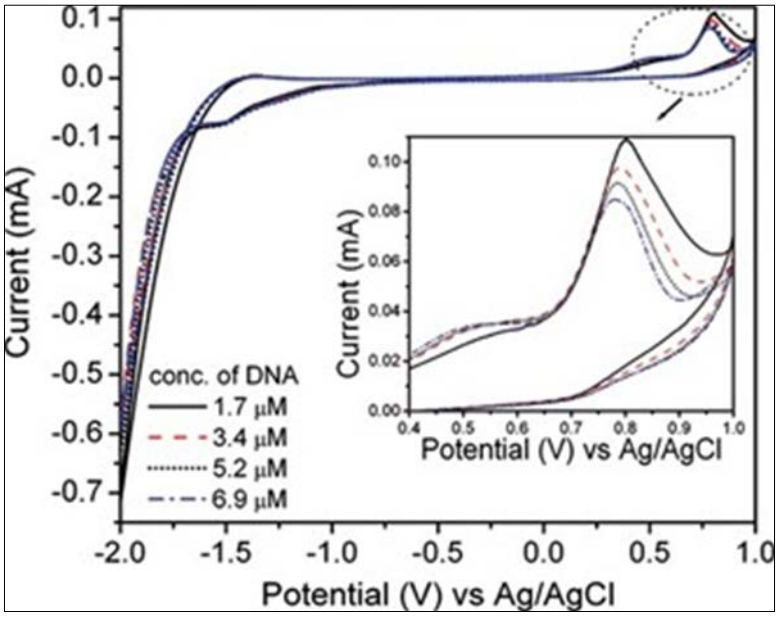


Fig 8: Cyclic voltammogram of 1 x 10^{-3}M [VO(acac)$_2$DCDA]H_2O in tris-HCl, NaCl buffer solution(p^H= 7.4) withincremential addition of CT-DNA;Inset shows the enlarged voltammogram.

Table 4: Measurement of voltammogram in different concentrations of CT-DNA

Concⁿ. of CT-DNA	Current in ampere (mA)		Potential (v) vs Ag/Agcl
	ip_c	ip_a	
1.7µM	0.080	0.110	0.82
3.4µM	0.075	0.089	0.79
5.2µM	0.074	0.085	0.78
6.9µM	0.071	0.082	0.78

Conclusion

The oxovanadium (IV) complex was synthesized and characterized by different techniques. The IR spectrum suggested that the ligand dicyandiamide is bound to the complex through azomethine nitrogen. The crystallite of the mononuclear oxovanadium complex has a triclinic structure. The spectra of the oxovanadium (IV) in DMSO reveal an eight-line pattern that represents the monomeric structure and well-resolved axial anisotropy of the complex. The interaction of the synthesized complex with CT-DNA indicates a weak binding propensity to CT-DNA with a binding constant of 2.063 x 10^2 M^{-1} through non-interactive mode. The result reveals that the synthesized complex is applicable to study in biological applications.

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