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Research Article

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A spectroscopic study on Calf thymus DNA binding properties of copper (II) complex with imidazole derivatives of 1,10-phenanthroline ligand

Nasrin Sohrabi*, Alireza Amini Khouzani

Department of Chemistry, Payame Noor University, PO Box 19395-3697, Tehran, Iran

Abstract Employing spectroscopic methods and elemental analysis, copper (II) complex with 1,10-phenanthroline based ligand,$[Cu(FIP)_2](OAC)_2$ (**1**) in which FIP = 2-(Furan-2-yl)-1H-Imidazo[4,5-f][1,10] phenanthroline (FIP) as ligand was synthesized and characterized in this study. The interaction of$[Cu(FIP)_2](OAC)_2$ (**1**) with calf-thymus DNA (ct-DNA) was studied by UV/Vis absorption fluorescence spectroscopy and viscosity measurements in 20 mMTris/HCl buffer solution, pH 7.0 at 25 °C. The$[Cu(FIP)_2](OAC)_2$ (**1**) complex bind to ct-DNA via hydrophobic mode as illustrated by hyperchromism in the UV/Vis absorption band of$[Cu(FIP)_2](OAC)_2$ (**1**), and also the decreasing of ethidium bromide (EtBr)-DNA solution fluorescence. The Copper complex interacts with ct-DNA while having an intrinsic binding constant of 1.65×10^5 M^{-1}. Furthermore, the thermodynamic studies suggested that the interaction processes were endothermic disfavored ($\Delta H > 0$) and entropy favored ($\Delta S > 0$). This was an indication to the fact that the Copper complex might interact with ct-DNA by a non-traditional intercalation mode of binding *via* hydrophobic force. The viscosity-related explorations showed no considerable increasing changes in the viscosity of ct-DNA with the increasing of the concentration of Copper complex.

Keywords Copper complex, Imidazole derivatives, DNA binding.

Introduction

There is a strong tendency toward studying biologically active compounds such as metal complexes which are applied as antitumor drugs in biochemistry and medicine [1-2]. Regarding metal complexes, studying their interaction with nucleic acid is of utmost importance since they have an important role in developing new drugs which have therapeutic effects [3-4]. Generally, metal complexes can interact with DNA via covalent interaction which incorporates the coordination of the nitrogenous base or the phosphate moiety of the nucleic acid to the central metal ion or non-covalent interaction. This latter case includes intercalation, groove binding or external electrostatic binding [5]. DNA repair can be hampered by metal complexes through their interference with enzymes or proteins involved in DNA replication or DNA repair [6]. Among the metal complexes, copper complexes have attracted a lot of attention from researchers due to their interesting binding properties and cleavage reactivity with nucleic acid [7-8]. By the same token, 1,10-phenanthroline and its derivatives are usually served as a very efficient class of ligands, which lead to efficient catalysis in cross coupling reaction of aliphatic alcohols and aryl halides [9], antimicrobial activities [10], efficient DNA-binding and DNA cleavage [11]. Despite the fact that a lot of attention has been paid by researchers to transition metal complexes incorporating imidazole[4,5-f][1,10]phenanthroline derivatives ligands [12-13], there have been few studies on Copper (II) complexes with imidazole derivatives of 1,10-phenanthroline and its interaction with ct-DNA. Recently, we have reported the synthesis and human serum albumin binding of palladium (II) complexes with imidazole derivatives of 1,10-phenanthroline ligand [14], Within this study, we report the interaction between a copper (II) complex with 1,10-phenanthroline-based ligand, [Cu(FIP)2](OAC)2 (**1**) (Scheme 1) and ct-DNA in

order to study the behavior of this class of compounds with ct-DNA. Subsequently, we applied several techniques applied to investigate the interactions between $[Cu(FIP)_2](OAC)_2$ complex and ct-DNA.

Materials and Instruments

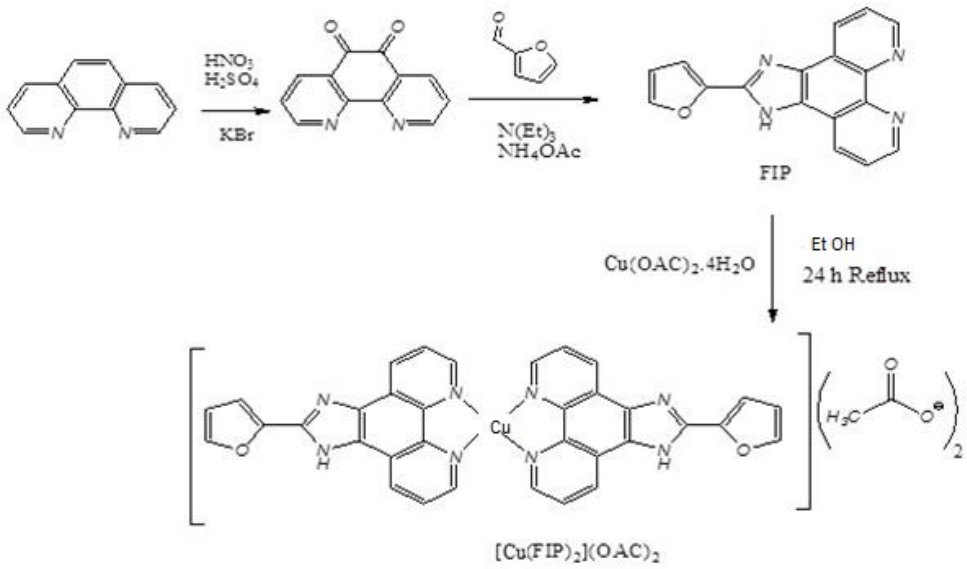
Calf thymus DNA was purchased from Sigma Chemical Co and was used without further purification. All other chemicals were in analytical grades and were purchased from Merck and Sigma. The experiments were run in 20 mMTris/HCl buffer solution at pH 7.0 at 25 °C. Concentrations expressed in moles of base pairs per liter were obtained using $\varepsilon = 1.32 \times 10^4$ $L.mol^{-1}.cm^{-1}$ at the maximum absorption of 260 nm. Infrared spectra were recorded on a FT-IR Nicolet-IR 100 spectrometer using KBr pellets. Elemental analysis (C, H, N) of the Copper complex was performed by CHN Rapid Heraeus elemental analyzer. The electronic absorption spectra were measured on a Perkin Elmer lambda 25 Model recording spectrophotometer. The fluorescence spectra were determined on a Scinco Model spectrofluorimeter.

Synthesis of 2-(Furan-2-yl)-1H-Imidazo [4,5-f][1,10] phenanthroline (FIP)

The same method as the one in the literature [13,14] was used to synthesize the 2-(Furan-2-yl)-1H-Imidazo [4,5-f][1,10] phenanthroline (FIP) as ligand.

Synthesis of [Cu(FIP)2](OAC)2 complex

A ethanolic solution (10 mL) of the $Cu(OAC)_2.4H_2O$ (0.1 mmol) was added a solution of FIP ligand (0.2 mmol) in ethanol while the reaction mixture was refluxed for 2 hours. The mixture was cooled down to room temperature and the resulting brown solid was filtered, then washed with ethanol and recrystallized in ethanol. The product was dried at 60 °C in a vacuum oven. Yield: %58. Analytical Calculated for $CuC_{29}H_{18}N_8O_7$: C, 61.10; H, 4.30; N, 14.24. Analytical found: C, 60.45; H, 4.61; N, 13.87. FT-IR (KBr), υ (cm^{-1}): 3416 (υ_{O-H}), 3046 (υ_{N-H}), 1604, 1574, 1550, 1522 ($\upsilon_{C=N}$), 436 ((υ_{M-L}).



Scheme 1: The Synthesis procedure and chemical structure of $[Cu(FIP)_2](OAC)_2$ complex

Results and Discussion

DNA Binding: Electronic absorption spectroscopy

The application of electronic absorption spectroscopy is one of the useful techniques in DNA-binding studies [15]. As a result of the intercalative mode involving a strong stacking interaction between an aromatic chromophore and the base pairs of DNA, complex binding with DNA through intercalation usually results in hypochromism and bathochromism. Similarly, hyperchromic effect has been observed that might be ascribed to electrostatic binding [16] or the partial uncoiling of the helix structure of DNA exposing more bases of the DNA

[17]. The absorption spectrum of the[Cu(FIP)2](OAC)2 complex displays an inter-ligand charge transfer bands at 229 nm and the metal to ligand charge transfer band at 284 nm (Fig.1) [14].

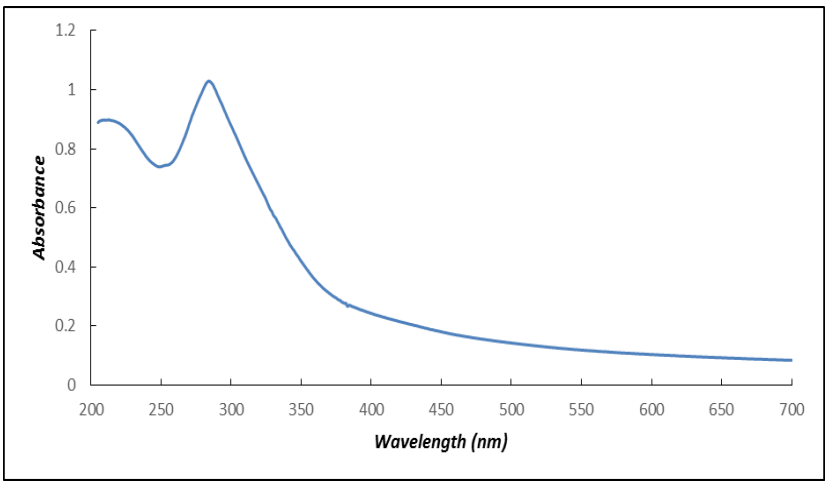


Figure 1: UV/ Vis spectrum of $[Cu(FIP)_2](OAC)_2$ comple

Pursuant to adding DNA, the absorption intensity of the Copper complex in 20 mMTris/HCl buffer solution, pH 7 and at 25 °C increased (hyperchromism), which indicated the interactions between ct-DNA and the Copper complex (Fig.2a),then DNA is saturated with[Cu(FIP)2](OAC)2 complex and absorbance will be constant (Fig. 2b).

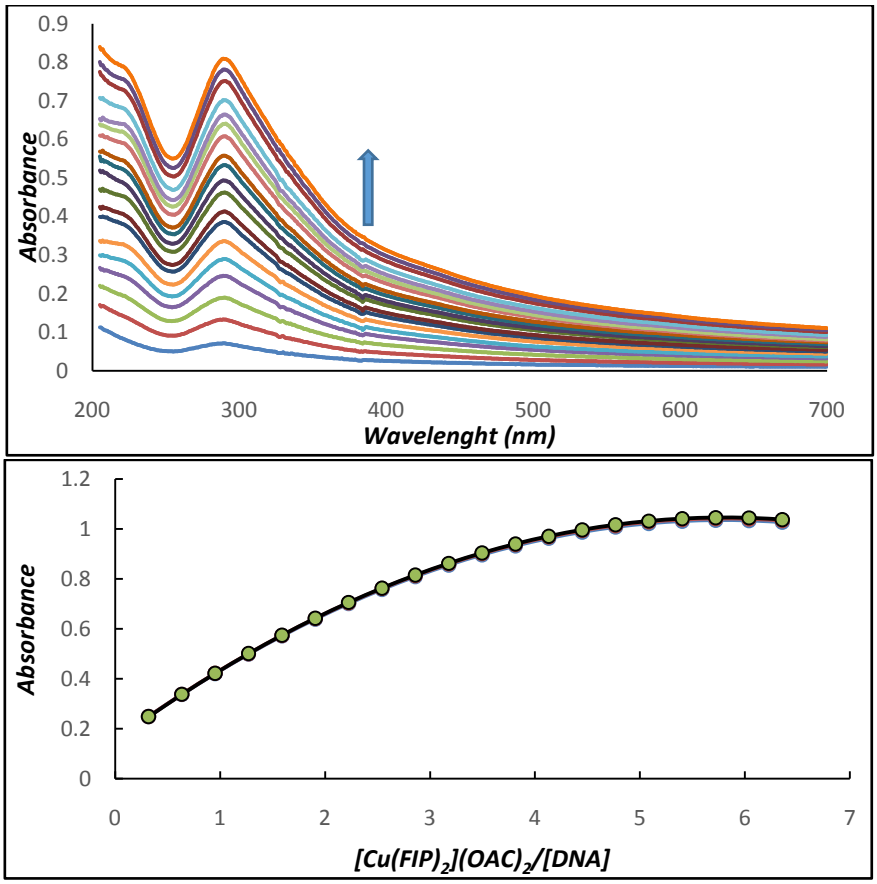


Figure 2.(a)Absorption spectra of $[Cu(FIP)_2](OAC)_2$ (2×10^{-5} M) upon titration with stock solution of ct-DNA in 20 mMTris/HCl buffer solution, pH 7 and at 25 °C.(b) the saturation of DNA with$[Cu(FIP)_2](OAC)_2$ complex.

The Copper complex can bind to double-strand DNA in different binding modes on the basis of the structure and type of the ligand. Due to the fact that DNA double helix possesses many hydrogen binding sites which are accessible in the minor and major grooves, it is likely that the –NH- group of the Copper complex forms hydrogen bonds with DNA, which may contribute to the hyperchromism observed in the absorption spectra [18]. Also, the electrostatic interaction between positively charged Copper complex and the negatively charged phosphate backbone at the periphery of the double helix DNA might have led to the hyperchromic effect [19]. In order to quantitatively compare the binding strength of $[Cu(FIP)_2](OAC)_2$, the intrinsic binding constant was determined according to this equation. (1)[20]:

$$[DNA]/(\varepsilon_a-\varepsilon_f) = [DNA](\varepsilon_b-\varepsilon_f) + 1/K_b\,(\varepsilon_b-\varepsilon_f) \quad (1)$$

in which [DNA] was the concentration of DNA, ε_a, $\varepsilon_f$and $\varepsilon_b$corresponded respectively to the apparent extinction coefficient, the extinction coefficient for the free compound and its fully DNA-bound combination. In the plots of $[DNA]/(\varepsilon_a-\varepsilon_f)$ versus [DNA], K_b was given by the ratio of the slope to intercept (Fig.3). The apparent binding constant of[Cu(FIP)2](OAC)2 complex was calculated to be $(1.65 \pm 0.02) \times 10^5\ M^{-1}$. Moreover, the K_b value obtained was lower than that of classical intercalators [21-23] whose binding constants are on the order of 10^6-10^7.

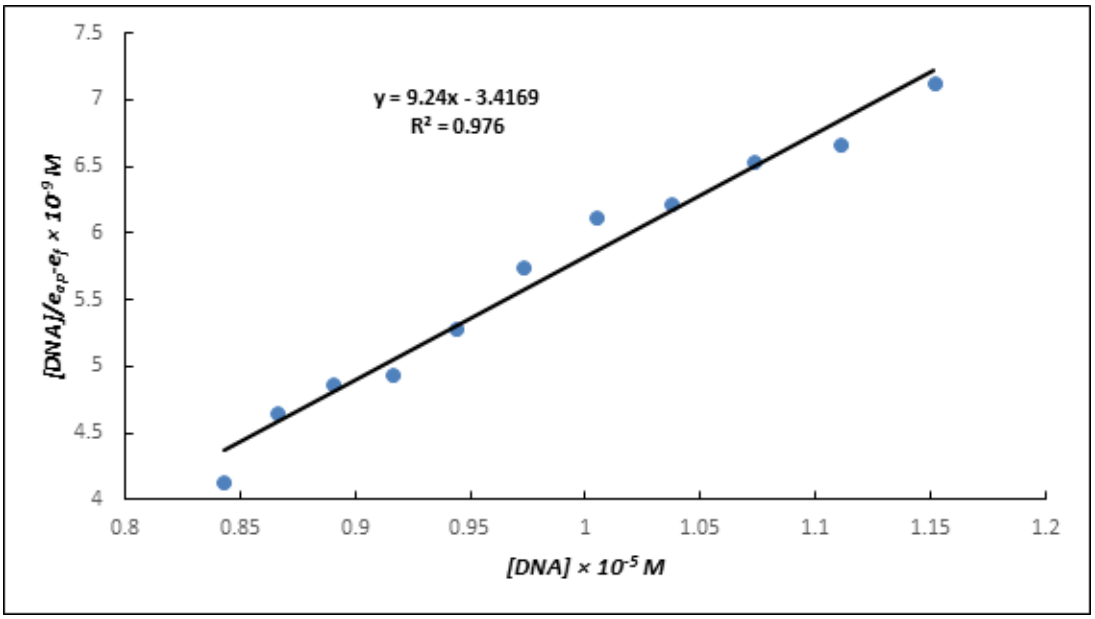


Figure 3: The plot of [ct-DNA]/(|εapp − εf|) versus [ct-DNA]

If we compare the intrinsic binding constant of the Copper complex with those of known DNA groove binders, we can deduce that this complex binds to ct-DNA via groove binding mode [24,25].

Fluorescence spectroscopy

Fluorescence quenching refers to any process which implicates a decrease of the fluorescence intensity from a fluorophore due to a variety of molecular interaction. It is well known that the fluorescence intensity of DNA itself is very weak. Furthermore, no fluorescence was observed in this study for [Cu(FIP)2](OAC)2 complex. Therefore, it is impossible to use its fluorescence emission properties to monitor the interaction of this complex with DNA. Once intercalated into DNA, ethidium bromide (EtBr) displays a dramatic enhancement of DNA fluorescence efficiency [26]. Subsequent to adding a second ligand which competed for the DNA binding sites, fluorescence quenching was observed [27]. Therefore, we carried out competitive binding experiments to investigate the mode of binding between the[Cu(FIP)2](OAC)2 complex and ct-DNA. The fluorescence emission of EtBr (2 µM) bound to DNA (20 µM), while having increasing amounts ct-DNA concentrations, is shown in Fig. 4 which clearly demonstrates a decrease in the fluorescence intensity of the EtBr-DNA solution on adding of the Copper complex is shown in Fig. 5. The competitive binding experiment in the presence of Copper complex and EtBr indicated that the Copper complex could interact as an intercalator. The binding mode of[Cu(FIP)2](OAC)2 to DNA can be determined according to the classical Stern–Vollmer Eq. 2 [28]

$$F_0/F = 1 + K_{SV}\,[Q] \quad (2)$$

Where F_0 and F respectively represent the emission intensity in the absence and presence of quencher, K_{SV} is a linear Stern–Vollmer quenching constant and [Q] is the quencher concentration. K_{SV} was obtained 0.5062 M^{-1}. The Stern–Vollmer quenching plots from the fluorescence titration data are shown in Fig.6.When the Stern–Vollmer plot is linear, it is an indication that only one type of quenching process occurs.

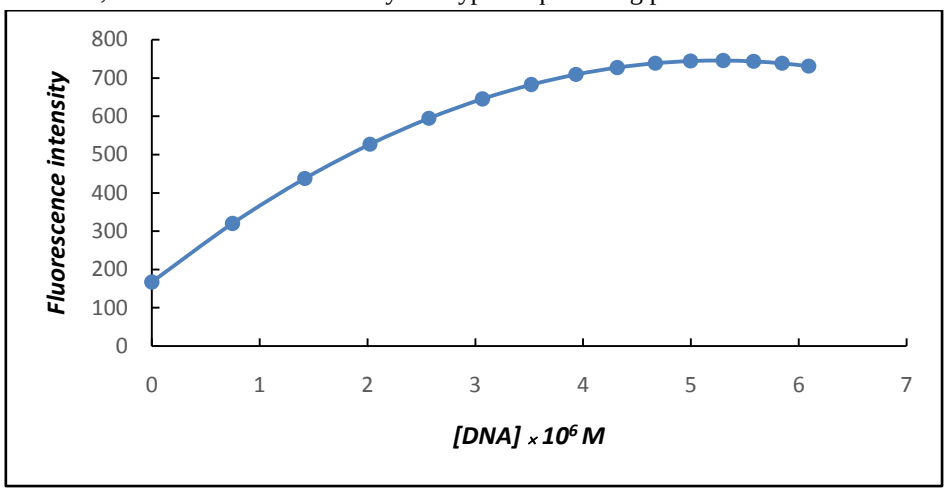


Figure 4: Emission spectra of the EtBr-ctDNA complex in 20 mMTris/HCl buffer solution, pH 7.0 and at 25 °C.

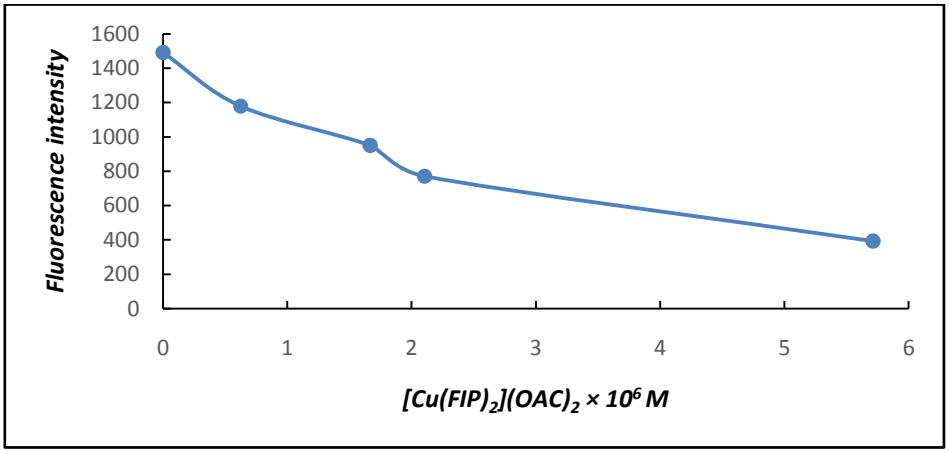


Figure 5: Emission spectra of the EtBr-ctDNA complex in the presence of the increasing amounts of [Cu(FIP)2](OAC)2 complex in DNA in 20 mMTris/HCl buffer solution, pH 7.0 and at 25 °C.

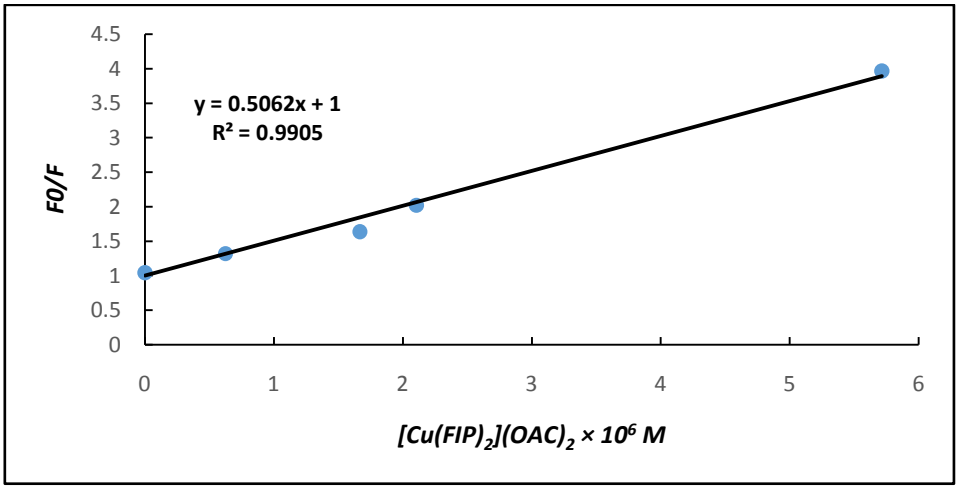


Figure 6: Plots of F0/F versus the concentration of $[Cu(FIP)_2](OAC)_2$ complex for the binding of $[Cu(FIP)_2](OAC)_2$ with ct-DNA at room temperature.

Thermodynamic Studies

In order to better grasp the thermodynamics of the reaction between [Cu(FIP)2](OAC)2 complex and ct-DNA, it is useful to determine the contributions of thermodynamic parameters. From the viewpoint of thermodynamics, $\Delta H > 0$ and $\Delta S > 0$ reflect hydrophobic interaction; $\Delta H < 0$ and $\Delta S > 0$ imply an electrostatic force; $\Delta H < 0$ and $\Delta S < 0$ suggest the van der Waals force and hydrogen bond. The enthalpy change can be regarded as a constant in case the temperature does not vary significantly. Based on the binding constant at different temperatures, the free energy change can be estimated by the following equations (Eq.3 & Eq.4):

$$\text{Ln } K_b = -\Delta H^o/RT + \Delta S^o/R \quad (3)$$

$$\Delta G^o = \Delta H^o - T\Delta S^o = -RT \text{ Ln } K_b \quad (4)$$

in which K_b is the binding constant at the corresponding temperature and R is the gas constant. The plot of $\ln K_b$ versus 1/T allows the determination of ΔH^o and ΔS^o (Fig.7). The thermodynamic parameters for the interaction of the Copper complex to ct-DNA are summarized in Table.1. Accordingly, the spontaneity of the interaction process can be seen through the negative sign for ΔG^o values. By applying this analysis to the binding system of the Copper complex and ct-DNA, we obtained these results: $\Delta H > 0$ and $\Delta S > 0$. Therefore, hydrophobic interactions are the main forces acting during the binding of Copper complex to ct-DNA while the mode of binding is hydrophobic. Consequently, the release of water molecules or counter ions results in positive enthalpy and entropy values in Copper complex-DNA interactions.

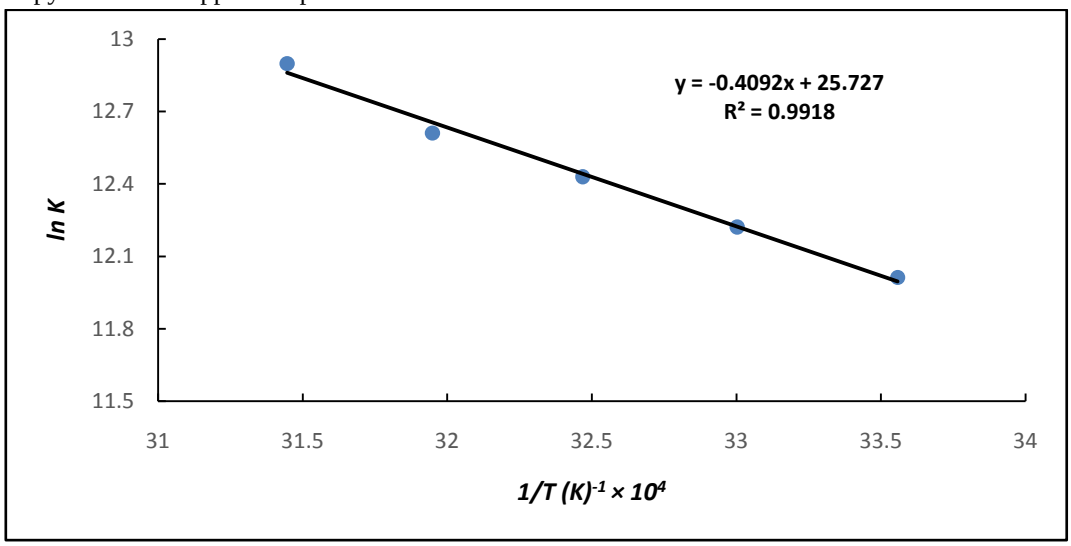


Figure 7: The van't Hoff plot [Cu(FIP)2](OAC)2 binding to ct-DNA.

Viscosity Study

Optical photophysical probes provide necessary but not sufficient support for a binding model. Measurements of DNA viscosity provide a critical test of binding in solution in the absence of crystallographic structural data. The elongation of the double helix DNA through facilitating the separation of DNA base pairs are the outcomes of intercalating agents which subsequently lead to an increase in DNA viscosity. On the other hand, molecules that bind in the DNA grooves by partial and/ or non-classical intercalation, cause less pronounced or no change in the viscosity of DNA solution[29,30]. To further explore the binding mode of[Cu(FIP)2](OAC)2 complex, viscosity measurements were conducted on ct-DNA by varying the concentration of the Copper complex. The values of relative specific viscosity (η/η_0)vs. R (R =[Cu(FIP)2](OAC)2 [ct-DNA]) (where η_0 and η are the specific viscosities of ct-DNA in the absence and in the presence of the[Cu(FIP)2](OAC)2 complex, respectively) were plotted (Fig.8). As the concentration of the complex increases (**1**), the relative viscosity of DNA increases steadily which is consistent with DNA groove binding and/or outside binding. This process is known to enhance DNA viscosity [31].

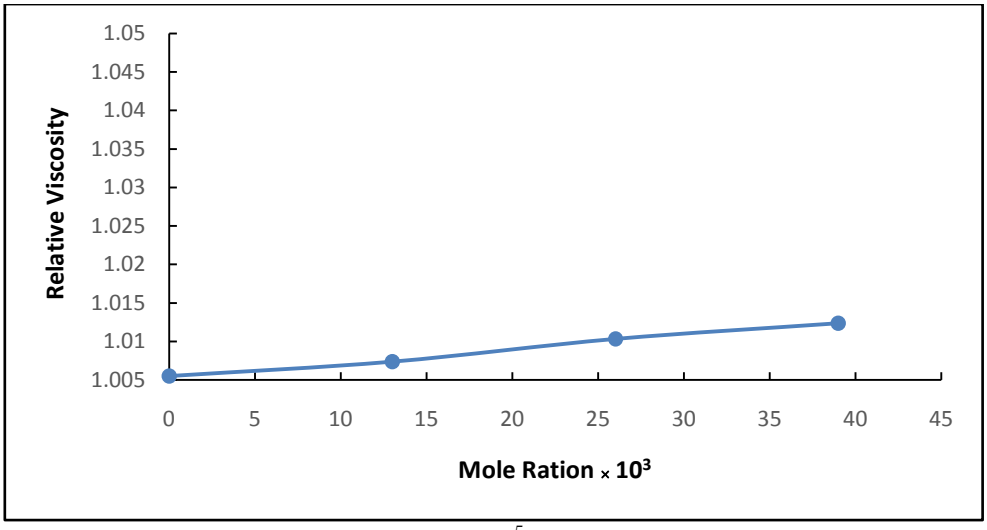


Figure 8: Relative viscosity of ct-DNA (1.35×10^{-5} M) in the presence of increasing amounts of $[Cu(FIP)_2](OAC)_2$ at stoichiometric ratios R= $[Cu(FIP)_2](OAC)_2$/[ct-DNA] =0.0-0.039, plotted as (η/ηo) vs. R. Measurements were done in 20 mMTris/HCl buffer, pH 7.0 and at 25 °C.

Conclusion

In summary, we investigated the binding of ct-DNA with a Copper (II) complex,$[Cu(FIP)_2](OAC)_2$ which contained imidazole derivatives of 1,10-phenanthroline ligand .According to the results of UV/Vis and fluorescence spectroscopies, there is a Copper complex bind to ct-DNA via hydrophobic mode. The thermodynamic parameters ($\Delta H^o > 0$ and $\Delta S^o > 0$) showed that the hydrophobic interaction leads to the increasing entropy which is brought about by interaction with the complex. The negative ΔG^o values for interaction of ct-DNA with the Copper complex indicate the spontaneity of the complexation. The results showed that the relative viscosity of DNA increases steadily which is consistent with DNA groove binding.

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