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RESEARCH ARTICLE

CHARACTERIZATION OF THE INTERACTION BETWEEN A COPPER BASED DRUG WITH CALF THYMUS DEOXYRIBONUCLEIC ACID (CT-DNA)

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ABSTRACT

The interaction between copper (II) complex with piroxicam (Pir) as ligand, trans-(Cu $(Pir)_2(DMF)_2$) and calf thymus deoxyribonucleic acid (ct-DNA) using UV/Vis absorption spectroscopy, fluorescence quenching study, thermal melting of ct-DNA and thermodynamic studies was investigated. Spectrophotometric studies showed that the binding constant was $K_b = 3.3104\times10^5$ M^{-1}at 298 K. A competitive binding with ethidium bromide (EB) showed that trans-(Cu $(Pir)_2(DMF)_2$) displace EB from its binding site in ct-DNA. The thermal denaturation experiments show the melting temperature of ct-DNA increases (about 0.5°C) due to binding of trans-(Cu $(Pir)_2(DMF)_2$). The thermodynamic parameters were calculated by van't Hoff equation and indicated that electrostatic and van der Waals play major roles in the interaction. All of the experimental results show that the outside bindingmust be predominant.

Key words: Piroxicam; Non-Steroidal anti-Inflammatory Drug; Calf Thymus DNA; Binding; Thermodynamic Parameters; Fluorescence Quenching.

INTRODUCTION

The investigation of metal complexes with pharmaceutical compounds as ligand is active research area in bioinorganic chemistry because of the synergistic effects from the ligand and the metal that can provide the high activity for drugs (Cavaglioni, 1997; Blaha *et al*., 1997; Roat *et al*., 1997). Non-steroidal anti-inflammatory drugs (NSAIDs) are a well-known class of drugs that are used as antipyretic, analgesic and anti-inflammatory agents. They are used to reduce pain in different arthritis and other post-operative conditions (Duffy *et al*., 1998). Their mode of action is through inhibition of the cyclo-oxygenase-mediated production of prostaglandins (Amin *et al*., 1995). Piroxicam (Scheme 1) belongs to the class of acidic, non -steroidal, anti-inflammatory drugs that form chelate complexes with metals having lower toxicity and higher pharmacological effect (Chiu *et al*., 2004). A variety of recent observations indicated that metal complexes when used in conjunction with anti-inflammatory drugs exhibit synergistic activity (Crouch *et al*., 1985; Weder *et al*., 2002). The biological activity of metal complexes with NSAIDs as ligands is mainly focused on the evaluation of the free radical scavenging activity and the binding properties to biomolecules such as DNA and serum albumins (SAs), which may also serve as their biological targets (Psomas, 2013). Copper, one of the most interesting metals due to its biological role and its potential synergetic activity with drugs has been the subject of a large number of research studies (Crisponi *et al*., 2010; Drewry *et al*., 2011; Sorenson, 1989).

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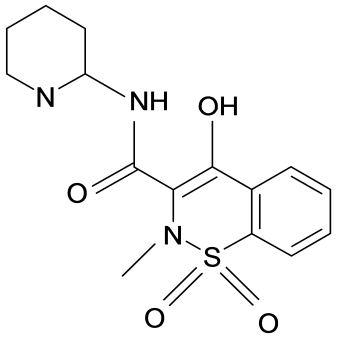
Copper (II) complexes with diverse drugs have shown numerous biological activities such as antitumor (Weder *et al*., 2002; Efthimiadou *et al*., 2007), antioxidant (Dimiza *et al*., 2011), antibacterial (Dimiza *et al*., 2011; Ruiz *et al*., 1998; Hueso-Urena *et al*., 1983) and antifungal (Saha *et al*., 2004). Taking into consideration the significance of the NSAIDs in medicine, we have studied the interaction of Cu (II) with the piroxicam. The interaction of Cu (II) with piroxicam as ligand resulted in the formation of trans-(Cu $(Pir)_2$ $(DMF)_2$) complex. It has been shown that the structure of the Cu (II)–piroxicam complex is a bis complex having octahedral geometry and the crystal of the complex from DMF has monoclinic crystal structure (Sreeja Chakraborty *et al*., 2014). The interaction of trans-(Cu $(Pir)_2$ $(DMF)_2$) with calf-thymus ct-DNA monitored by UV spectroscopy, thermal denaturation and via the ethidium bromide (EB) displacement from the EB-DNA conjugate by fluorescence emission spectroscopy was investigated.

Experimental

Materials and Instruments

Calf thymus DNA was purchased from Sigma Chemical Company and was used without further purification. Stock solution of ct-DNA was prepared by dissolution overnight in 10 mMTris-HCl buffer pH=7.0 and was stored below 4 °C in the dark for short periods only. The base-pairs concentration of ct-DNA was determined by its known absorbance measurements using ε 1.32×10^4 L. $mol^{-1}.cm^{-1}$ at the absorption maximum of 260 nm. The concentrations expressed in moles of base pairs per liter were obtained using ε 1.32×10^4 $L.mol^{-1}.cm^{-1}$ at the absorption maximum of 260 nm.



Scheme 1. The molecular structure of Piroxicam {4-Hydroxy-2-methyl-N-(2-pyridyl-2H- 1, 2} benzothiazine-3-carboxamide-1, 1 dioxide}

The complex of trans-(Cu $(Pir)_2(DMF)_2$) was synthesized according to the literature method (20). The electronic absorption spectra were measured by a UV-vis Perkin Elmer Lambda 25 double beam spectrophotometer. The fluorescence spectra were determined by a Hitachi MPF-4 spectrofluorimeter. The Melting experiments were performed using an UV-vis Perkin Elmer Lambda 25 double beam spectrophotometer coupled with a thermo stated cell compartment. The temperature inside the cuvette was determined with a platinum probe and was increased over the range 25-86 °C by a heating rate of 0.5 °C/min (Thermal software). In all of the experiments, we used a potentiometer Metrohm 744-model for measuring pH.

RESULTS AND DISCUSSION

DNA Binding: Electronic absorption spectroscopy

The application of electronic absorption spectroscopy is one of the useful techniques in DNA-binding studies (Barton *et al*., 1984). 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, hyper chromic effect has been observed that might be ascribed to electrostatic binding (Pasternack *et al*., 1983) or the partial uncoiling of the helix structure of DNA exposing more bases of the DNA (23). Pursuant to adding DNA, the absorption intensity of the Cu complex in 20 mMTris/HCl buffer solution, pH 7 and at 25 °C increased (hyperchromism), which indicated the interactions between ct-DNA and the Cu complex (Fig.1).

Then DNA is saturated with-(Cu $(Pir)_2(DMF)_2$) complex and absorbance will be constant (Fig. 2). The Cu 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 Cu complex forms hydrogen bonds with DNA, which may contribute to the hyperchromism observed in the absorption spectra (Shahabadi *et al*., 2012). Also, the electrostatic interaction between positively charged Cu complex and the negatively charged phosphate backbone at the periphery of the double helix DNA might have led to the hyperchromic effect (Xiao *et al*., 2002). In order to quantitatively compare the binding strength of -(Cu $(Pir)_2(DMF)_2$), the intrinsic binding constant was determined according to this Eq. 1(Olmsted, 1977):

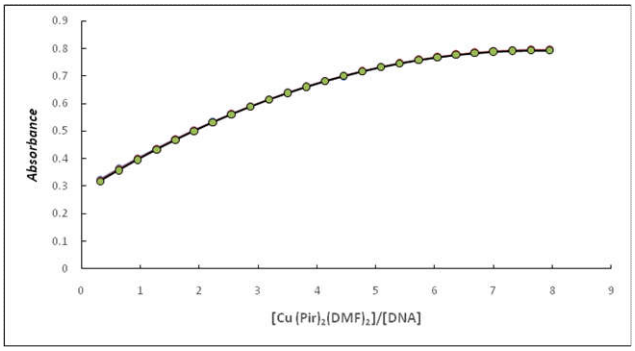


Figure 2. The saturation extent of absorption of ct-DNA with addition of $[Cu(Pir)_2(DMF)_2]$

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

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).

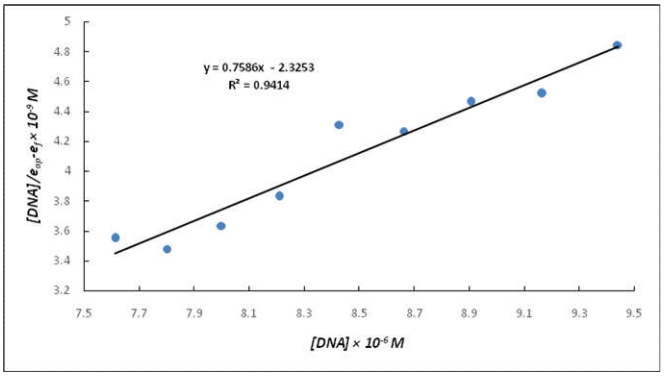


Figure 3. The plot of [ct-DNA]/(|ε_{app} ε_f|) versus [ct-DNA]

The apparent binding constant of-(Cu $(Pir)_2(DMF)_2$) 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 (Ni *et al*., 2006; Eftink *et al*., 1981; Kashanian *et al*., 2010) whose binding constants are on the order of 10^6-10^7. If we compare the intrinsic binding constant of the Cu complex with those of known DNA groove binders, can deduce that this complex binds to ct-DNA via groove binding mode (Ahmad *et al*., 1998; Jayachithra, 2005).

Thermodynamic Studies

In order to better grasp the thermodynamics of the reaction between -(Cu $(Pir)_2(DMF)_2$) 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.2& Eq.3):

$$\text{Ln } K_b = -\Delta H^\circ/RT + \Delta S^\circ/R \quad (2)$$

$\Delta G^o = \Delta H^o - T\Delta S^o = -RT \text{ Ln } K_b$ (3)

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.4).

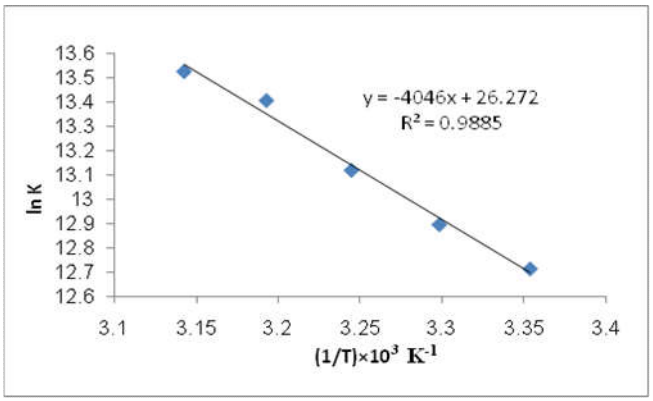


Figure 4. The van't Hoff plot [Ni (FIP)2](OAC)2 binding to ct-DNA

The thermodynamic parameters for the interaction of the Cu 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 Cu 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 Cu 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 Cu complex-DNA interactions.

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 (Pir)2(DMF)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 (Olmsted *et al*., 1977). 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 (Pir)2(DMF)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. 5a which clearly demonstrates a decrease in the fluorescence intensity of the EtBr-DNA solution on adding of the Cu complex is shown in Fig. 6. The binding mode of (Cu (Pir)2(DMF)2) to DNA can be determined according to the classical Stern–Vollmer Eq. 2 (Eftink, 1981)

$F0/F = 1 + KSV\,(Q)$ (4)

Where F_0 and F respectively represent the emission intensity in the absence and presence of quencher, KSV is a linear Stern–Vollmer quenching constant and (Q) is the quencher concentration. KSV was obtained 0.5062 M-1. The Stern–Vollmer quenching plots from the fluorescence titration data are shown in Fig.7.When the Stern–Vollmer plot is linear, it is an indication that only one type of quenching process occurs.

Table 1.Thermodynamic parameters of the equilibrium of interaction of [Ni(FIP)2](OAC)2 with ct- DNA in 20 mMTris/HCl buffer solution,, pH 7.0 at various temperatures

ln K_b	H_b (cal/mol)	G_b (cal/mol)	S_b(cal/mol. K)
12.71±0.04	80.40×10^2±802.61	-75.34×10^2±25.82	52.23±2.61
12.89±0.04	80.40×10^2±802.61	-77.70×10^2±25.82	52.15±2.61
13.12±0.04	80.40×10^2±802.61	-80.35×10^2±25.82	52.16±2.61
13.40±0.04	80.40×10^2±802.61	-83.44×10^2±25.82	52.32±2.61
13.52±0.04	80.40×10^2±802.61	-85.52×10^2±25.82	52.15±2.61

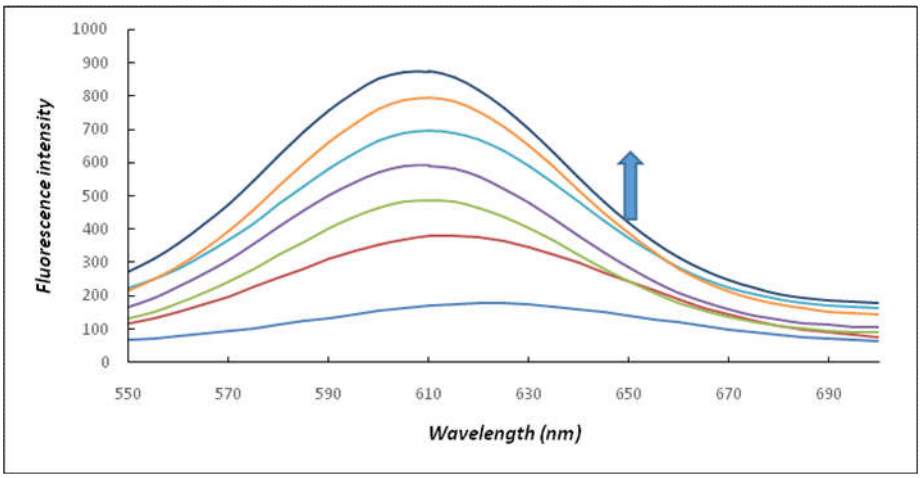


Figure 5a.Emission spectra of the EtBr complex in the presence of the increasing amounts of DNA in 20 mMTris/HCl buffer solution, pH 7 and at 25 °C

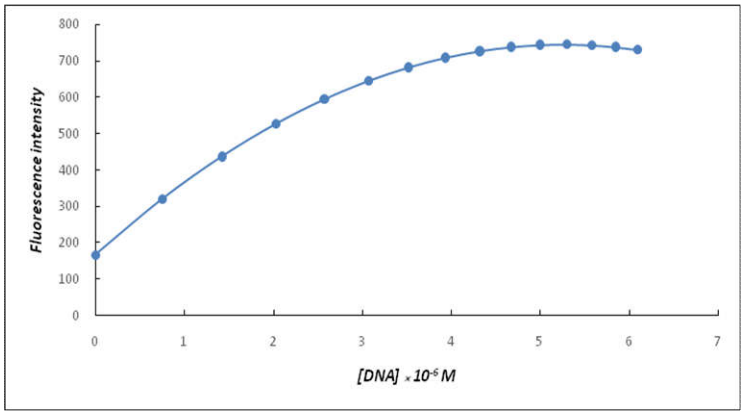


Figure 5b.Saturated emission spectra at of the EtBr complex in the presence of the increasing amounts of DNA in 20 mMTris/HCl buffer solution, pH 7 and at 25 °C

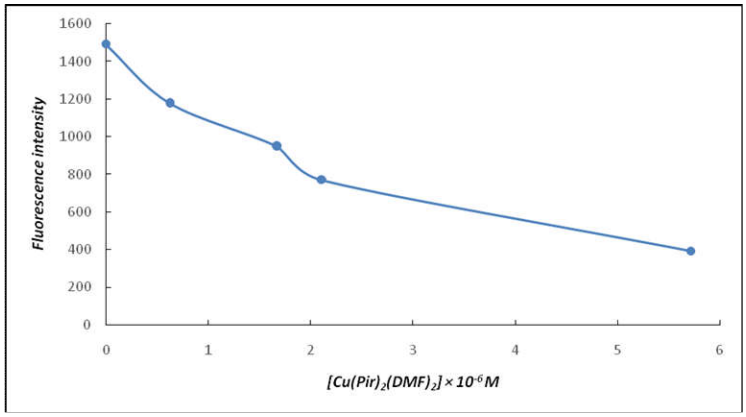


Figure 6. Decrease of emission spectra of the EtBr-ct-DNA complex in the presence of the increasing amounts of [Cu (Pir)2(DMF)2] complex in DNA in 20 mMTris/HCl buffer solution, pH 7 and at 25 °C

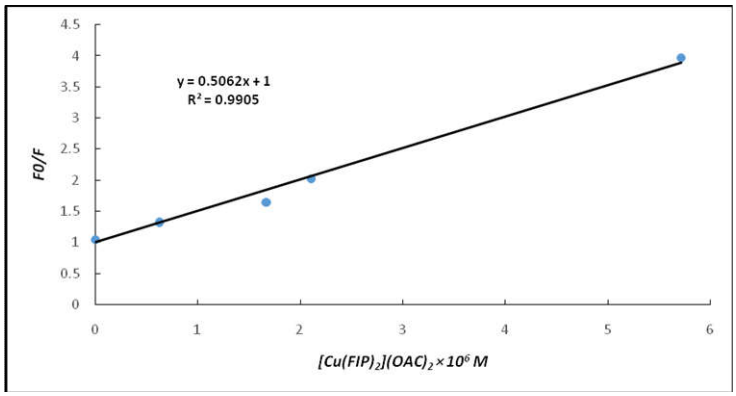


Figure 7.Plots of F0/F versus the concentration of [Cu (Pir) 2(DMF)2]complex for the binding of [Cu (Pir)2(DMF)2] with ct-DNA at room temperature.

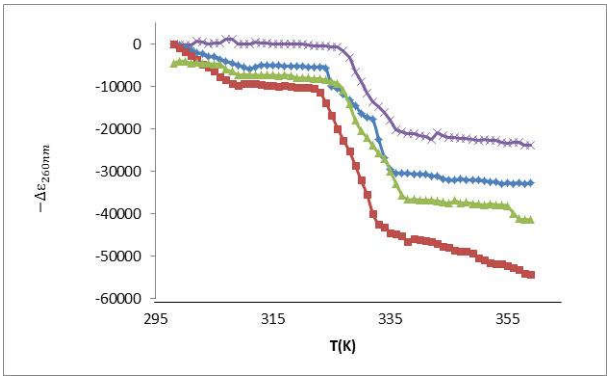


Figure 8. Molar Absorbance coefficient change in (λmax = 281 nm) with temperature for different mole ratios of Cu complex to ct-DNA (▲)0.0, ( )0.014,( )0.056,(—)0.127

Table 3. Maximum stability temperatures and experimental melting for ct-DNA at different mole ratios of Cu complex toct-DNA

$\frac{[Cu(Pirox)_2(DMF)_2]}{[ct\ DNA]}$	0	0.028	0.055	0.11
T_s	330	329	330	330
T_m (K) (exp)	333.171	330.301	330.179	330.118
T_m (K)(theory)	330.628	329.162	330.702	330.708

Table 4. Six Fitting parameters in Eq. 20 that were determined by using sigmaplot software for different mole ratios of Cu complex to ct-DNA

$\frac{[Cu(Pirox)_2(DMF)_2]}{[ct\ DNA]}$	0	0.028	0.055	0.11
$(a_N\times10^{-3})M^{-1}\ cm^{-1}$	-3.566±0.374	-8.871±0.176	-6.055±0.308	-1.476±3.766
$(b_N\times10^{8})M^{-1}\ cm^{-1}\ K^{-1}$	2.114±0.513	16.92±0.261	6.953±0.2073	5.640×10^{5}±12.126
$(a_D\times10^{-4})M^{-1}\ cm^{-1}$	-4.036±2.436	5.163±0.955	1.0982±1.895	1.633±0.5782
$(b_D\times10^{-2})M^{-1}\ cm^{-1}\ K^{-1}$	0.221±0.693	-2.907±0.274	-1.414±0.541	-1.112±0.166
T_m (K)(theory)	330.628	329.162	330.702	330.708
H (kJ/mol)	273.745	382.689	322.336	451.624

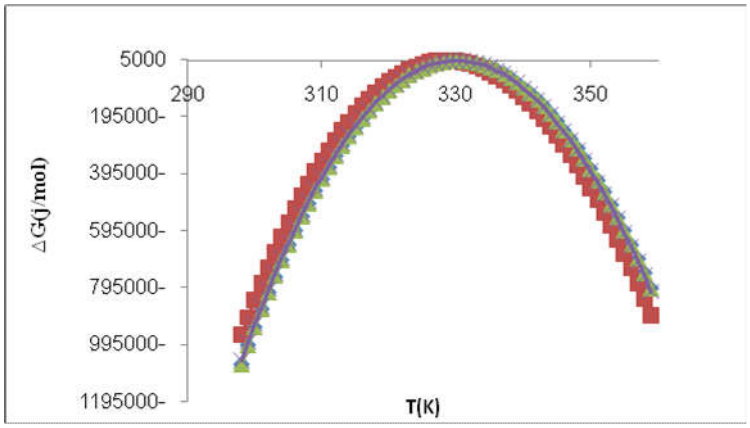


***Figure 9.*Denaturation Gibbs Free Energy changes with temperature for different mole ratios of Cu complex to ct-DNA (▲)0.0, ( )0.014,( )0.056,(—)0.127**

Thermal Denaturation of ct- DNA

Melting experiments were made in phosphate buffer solutions pH 7.0 containing 97 µM ct-DNA. The temperature was scanned from 25 to 93ºC. The melting temperature (Tm) was taken for the free ct-DNA in the absence of ligand, and for the different molar ratios of Cu Complex to ct-DNA. Results of such studies for Cu Complex-ct-DNA complex are shown in Fig.8. By using Igor software and determining $\delta\Delta\varepsilon/\delta\Delta T$, T_m was determined graphically and was listed in Table 3. The evaluation of thermodynamic parameters, obtained from spectroscopic techniques, is based on the equilibrium constant K for a transition between the native state and the denaturatedstate (Drewry, 2011). $\Delta\varepsilon_{281}$ is an Absorbance coefficient change in any temperature with temperature 25˚C in λ_{max} = 260nm for ct-DNA (Ahmad *et al*., 1998) in the absence and presence of Cu Complex.

$$\Delta\varepsilon_{281}=\frac{\Delta\varepsilon_N+\Delta\varepsilon_D\exp\left[\frac{-\Delta H_m}{R}\left(\frac{1}{T}-\frac{1}{T_m}\right)-\Delta Cp\left[\frac{T_m}{T}-1+\ln\left(\frac{T}{T_m}\right)\right]\right]}{1+\exp\left[\frac{\Delta H_m}{R}\left(\frac{1}{T}-\frac{1}{T_m}\right)\right]-\Delta Cp\left[\frac{T_m}{T}-1+\ln\left(\frac{T}{T_m}\right)\right]} \quad (13)$$

There are six parameters in Eq.13 that were determined by fitting, using sigmaplot software. These parameters are summarized in Table 4.Using these parameters, we can calculate the $\Delta G_D(T)$ at 25 to 93ºC by Eq. 14.The result is represented in Fig.8. The result shows more stable state about Ts=341.15 to 348.15 K at different mole ratios of Cu Complex to ct-DNA. The accurate values are brought in Table 5.

The stability of a globular protein is usually quantified in the Gibbs free energy values since ΔG_D is the work required for disruption of the native protein structure. For that reason, the difference in Gibbs energy at a given temperature can be expressed by the Gibbs–Helmholtz eq.(6) (Jayachithra *et al*., 2005).

$$\Delta G_D(T)=\Delta H_m\left(1-\frac{T}{T_m}\right)-\Delta Cp\left[(T_m-T)+T\ln\frac{T}{T_m}\right] \quad (14)$$

Conclusion

In summary, we investigated the binding of ct-DNA with a Copper (II) complex-(Cu $(Pir)_2(DMF)_2$).According to the results of UV/Vis and fluorescence spectroscopies, there is a Cu complex bind to ct-DNA via outside mode. The thermodynamic parameters ($\Delta H^o > 0$ and $\Delta S^o > 0$) showed that the electrostatic 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 Cu complex indicate the spontaneity of the complexation. Due to melting results, there is no obvious change in melting temperatures ,Tm, in different mole ratio of Cu complex to ct-DNA. Therefore, the dominant force is entropy and the mode of this interaction is outside binding and electrostatic van der Waals play major roles in the interaction (Ross, 1981).

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