Nasrin Sohrabi /J. Pharm. Sci. & Res. Vol. 7(8), 2015, 533-537

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Binding And UV/Vis Spectral Investigation of Interaction of Ni(II) Piroxicam Complex With Calf Thymus Deoxyribonucleic Acid (Ct-DNA) : A Thermodynamic Approach

Nasrin Sohrabi

Assistant Professor, Department of Chemistry, Payame Noor University, PO BOX 19395-3697, Tehran-Iran

Abstract

In this study the interaction of the complex with DNA have been investigated using UV/Vis , and thermal denaturation.The results showed that complex $Ni(Pirox)_2(DMF)_2$ can bind to ct-DNA. The binding constants of the complex with ct-DNA have also been calculated. The thermodynamic parameters for binding of complex with ct-DNA are also determined using van't Hoff equation. Competitive binding with ethidium bromide (EB) indicate that the complex could not intercalate in the DNA.

Keywords: Non-steroidal anti-inflammatory drug (NSAID) , Ni(II) piroxicam complex , Ethidium bromide (EB),Calf thymus deoxyribonucleic acid (ct-DNA), UV/Vis spectroscopy

1. INTRODUCTION

DNA was known as an important cellular target for investigation of interaction of drugs with biological importance such as carcinogens and other classes of them.There are three binding modes of noncovalent interactions of small molecules to DNA:intercalative binding, groove binding and electrostatic interaction[1]Due to sandwich structure of some drugs between the aromatic, heterocycles DNA base pairs the intercalation binding mode is the strongest kind of binding[2]. Piroxicam (Pirox) (4-hydroxy-2-methyl-*N*-2-pridyl-2H-1, 2-benzothiazine-3-carboxadiamide-1,1-dioxide) belongs to the oxicam group a class of enolic acids [3]. Piroxicam (Scheme 1) produces a rapid and effective response in the treatment of many diseases[4]

Ni(II) complexes of these NSAIDs have been shown to have better anti-cancer effects than the bare drugs. It has been shown that the structure of the Ni(II)– piroxicam complex is a bis complex having square geometry and [2].Some of organic dyes are probes for nucleic acids, such as ethidium bromide (EB), bisimidazole, metal complex and photosensitive agent. EB (Scheme2) is a strong mutagen widely used in biochemical research for visualizing nucleic acids[5]. In this work EB was used to compare the binding mode of NiNASID and EB with ct- DNA.

Most of NSAIDs have negative charge at physiological pH It is obvious that this property can affect on interaction with DNA backbone and this interaction is weak.. It has been shown that the presence of metal ion reduces this property and metal complex of NASID can interact with DNA better than NSAID[12,13].When the temperature is increased the double stranded DNA dissociates to single strands, generating hyperchromism effect. The melting temperature (Tm), a valuable parameter to study the metal complex-DNA interactions, is defined as the temperature where half of the total base pairs get unbound[6]. The melting temperature of DNA (T_m) in the presence of a binding molecule or metal can also be used to distinguish between intercalative and external binding modes. Usually, classical intercalation gives rise to higher ΔT_m values than either groove binding or outside stacking [7].

In this study, the interaction of Ni(II)NSAID complex with ct-DNA was investigated by UV/Vis absorption, as well as DNA melting and viscosity measurements. The major binding mode of Ni(II)NSAID complex was estimated using UV/Vis studies. For confirming the binding mode between Ni(II)NSAID and DNA, we used thermal denaturation and the competition of Ni(II)NSAID with EB in their interaction with DNA. This work can provide useful information about the binding mechanism of Ni(II) NSAID with ct-DNA and thus is a helpful tool for anticancer drug design.

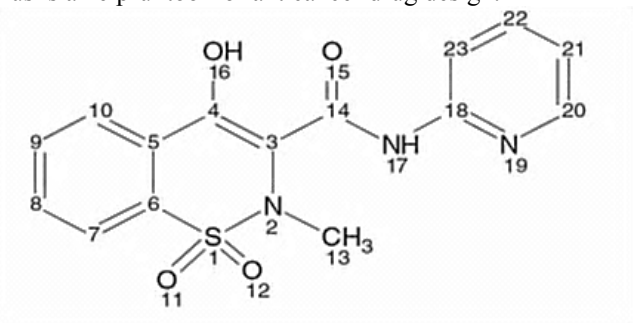

Figure1:Structure of Piroxicam[4].

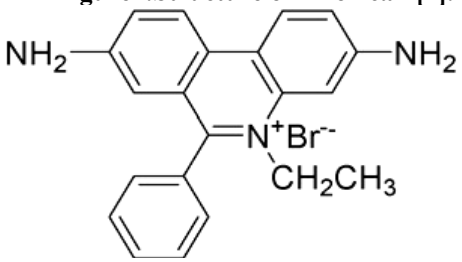

Figure 2.Structure of ethidium bromide(EB)[5]

2. MATERIALS AND METHODS

2.1. Chemicals and Preparations

In this work we use of $Ni(Pirox)_2(DMF)_2$ and Tris-Hcl buffer (pH=7.0). NaCl, EDTA, dimethylformamide(DMF) .All reagents were obtained from Merck Chemical Co and

were in analytical grades and used as received. This ligand $Ni(Pirox)_2(DMF)_2$ was prepared in a similar manner in the literature [7] in order to use in interaction with ct-DNA. Calf thymus DNA (ct-DNA), ethidium bromide (EB) were purchased from Sigma Chemical Co. Stock solution of ct-DNA was prepared by dissolution overnight in 10 mM Tris-Hcl buffer 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 $=1.32\times10^4$ L. mol^{-1}. cm^{-1} at the absorption maximum of 260 nm[8]. Double distilled water was used throughout the experiments.

2.2. Apparatus

The UV/Vis spectrum was measured using a Perkin Elmer Lamba25 UV/Vis spectrophotometer equipped with thermal bath. Tris-Hcl buffer solution was used to control the pH of the media (pH=7.0) and measurements were performed on a Metrahm-82700 pH-meter.

2.3. Procedures

2.3.1. Absorption titration

The absorbance titrations were performed at a fixed concentration of Ni(II)NSAID and varying the concentration of ct-DNA. The binding constant, K_b of Nickel complex to DNA was calculated from Eq (1).

$$\frac{[DNA]}{(\varepsilon_a-\varepsilon_f)}=\frac{[DNA]}{(\varepsilon_b-\varepsilon_f)}+\frac{1}{K_b(\varepsilon_b-\varepsilon_f)} \qquad (1)$$

where [DNA] is the concentration of ct-DNA in base pairs, the apparent absorption coefficients ε_a, ε_f, and ε_b correspond to A_{obsd}/[NiNSAID], the absorbance for free nickel complex, and the absorbance for nickel complex in the fully bounded form, respectively. K_b is the equilibrium binding constant in M^{-1} [9,10].

2.3.2. Thermal Melting of DNA

Melting curves were made using an UV/Vis double beam spectrophotometer Perkin-Elmer Lambda 25Model equipped with a thermal bath. The temperature inside the cuvette was measured with a platinum probe and was increased over the range 25-86 °C at a heating rate 0.5 °C/min. Melting curves of both free DNA and Ni(II)NSAID– DNA complex in Tris–HCl buffer were obtained by measuring the hyperchromicity of DNA absorbance at 260 nm as a function of temperature. The melting temperature, T_m was obtained from the midpoint of the melting curves.

2.3.3. EB binding with DNA

The UV–vis absorption spectra of a fixed concentration of EB without and with various concentrations of DNA are measured, respectively. The scan range is 200–700 nm; the absorption spectra are obtained at room temperature.

2.3.4. Ni(II) piroxicam complex binding with DNA

The UV–vis spectra of Ni(II) piroxicam complex, DNA–EB complex, and the mixture of the Ni(II) piroxicam complex and DNA–EB complex are measured, respectively.

3. Results And Discussion

3.1. DNA-binding study with UV spectroscopy

Complex binding with ct-DNA through intercalation usually result in hypochromism and bathochromism, due to intercalative mode involving a strong stacking interaction between an aromatic chromophore and the base pairs of DNA [11,12]. Electronic absorption spectroscopy is an effective method to examine the binding mode of DNA with metal complexes [13]. If the binding mode is intercalation, the л* orbital of the intercalated ligand can couple with the л orbital of the DNA base pairs, thus, decreasing the л→ л * transition energy and resulting in the bathochromism. On the other hand, the coupling л orbital is partially filled by electrons, thus, decreasing the transition probabilities and concomitantly resulting in hypochromism [14]. It is a general observation that the binding of an intercalative molecule to DNA is accompanied by hypochromism and significant redshift (bathochromism) in the absorption spectra due to strong stacking interaction between the aromatic chromophore of the ligand and DNA base pairs with the extent of hypochromism and red-shift commonly consistent with the strength of intercalative interaction [13]. Thus, in order to provide evidence for the possibility of binding of each complex to ct-DNA, spectroscopic titration of a solution of the complexes with ct-DNA has been performed [15]. The apparent binding constants of [Ni(II)NSAID] and ct-DNA were estimated and used for calculation of Gibbs free energy change of reaction at various temperatures. The spectral features of studied DNA at various [Ni(II)NSAID] concentrations were shown in Figure 3.

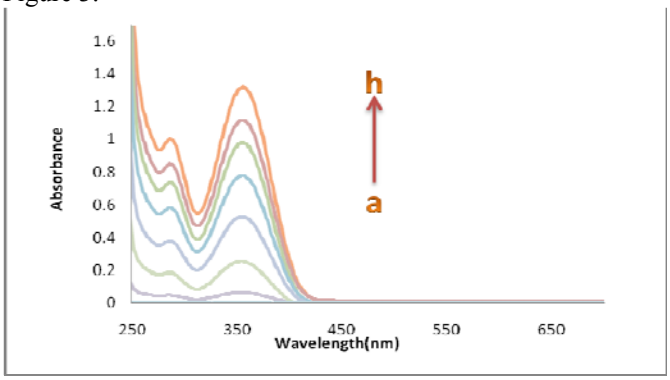

Figure 3. (A). Diffrential Absorption spectra of ct-DNA (13.9 µM) in the absence (a) and presence of [Ni(II)NSAID]; 3.89 µM (b), 7.65 µM (c), 15.29 µM (d), 22.94 µM (e), 30.58 µM (f), 34.33 µM (g), 41.98 µM (h), in 10 mM Tris–HCl buffer, pH 7.0 and at 45 °C.

Determination of thermodynamic parameters 3.1.2.

At range 25-45 ◦C, the binding constant of [Ni(II)NSAID] with DNA is determined, respectively. The results are also represented in Table 1. It is found that the binding constant of [Ni(II)NSAID] increases with the temperature. This suggests that the binding reaction of [Ni(II)NSAID] with ct-DNA is endothermic, and the increasing temperature benefits [Ni(II)NSAID] binding with ct-DNA. This point is further proved by the value of Δ*H*, the enthalpy change of the binding reactions. The equations as follows are used to calculate the thermodynamic parameters.

$$\ln\frac{K_{b1}}{K_{b2}}=\frac{-\Delta H^0_b}{R}\left(\frac{1}{T_2}-\frac{1}{T_1}\right) \qquad (2)$$

$$\Delta G^0_b=\Delta H^0_b-T\,\Delta S^0_b \qquad (3)$$

where ΔG, ΔS are the free energy change and the entropy change, respectively. Based on the binding constants at range 25-45 ◦C, the values of ΔH, ΔG and ΔS calculated are listed in Table 1. As we can see that the values of ΔH of [Ni(II)NSAID] is greater than 0, which proves that binding reaction of [Ni(II)NSAID] with ct-DNA is endothermic. Based on the binding constant of [Ni(II)NSAID] with DNA at various temperatures, the binding force of [Ni(II)NSAID] with ct-DNA can be determined. Because ΔH> 0 and ΔS>0, ct-DNA –[Ni(II)NSAID] complex is stabilized mainly by hydrophobic interactions [16]. The acting forces between small molecule and biomacromolecule include hydrogen bonds, van der Waals forces, hydrophobic interaction and electrostatic forces. According to Ross' view, the main binding force can be judged by enthalpy change (ΔH) and entropy change (ΔS). From the viewpoint of thermodynamics, ΔH > 0 and ΔS > 0 imply a hydrophobic interaction; ΔH <0 and ΔS < 0 reflect the van der Waals force and hydrogen bond; ΔH < 0 and ΔS > 0 suggest an electrostatic force. If the temperature was changed by very little, the ΔH can be seen as a constant[17]. The standard enthalpy and entropy change of binding can be estimated from the slope and Y-intercept of the plot of $\Delta G/T$ versus $1/T$, respectively. Such plots for binding of these [Ni(II)NSAID] complexes to ct-DNA in Tris–HCl buffer were shown in Figure 4. .

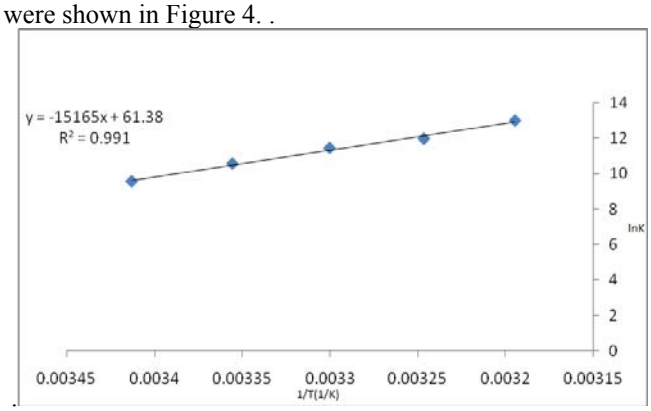

Figure 4. The van't Hoff plots of ct-DNA -Binding of [Ni(II)NSAID]

Table1. Thermodynamic parameters for the interaction between Ni(II)NSAID with ct-DNA

T(K)	ln K_b	ΔH^0_b (Kj/mol)	ΔG^0_b (Kj/mol)	ΔS^0_b(j/mol. K)
293	9.5670	126.0818±11.8	-23.317±25.8	510.31±2.61
298	10.5678	126.0818±11.8	-26.196±25.8	510.31±2.61
303	11.4513	126.0818±11.8	-28.862±25.8	510.31±2.61
308	11.4690	126.0818±11.8	-30.661±25.8	510.31±2.61
313	12.9992	126.0818±11.8	-23.317±25.8	510.31±2.61

3.1.2.Thermal Melting studies

The general method of determining the melting temperatures (Tm) is UV–vis spectroscopy. With the increasing temperature, DNA denatures and the double helixes unfold. Intercalation binding can stabilized double helix structure and T_m increase about 5–8 ◦C, but the non-intercalation binding has no obvious increase in Tm [18]. It is well known that the double-stranded DNA gradually dissociates to single strands and generates a hypochromic effect on the absorption spectra of DNA bases (λmax = 260 nm), when the solution temperature increases. The melting temperature Tm, which is defined as the temperature where half of the total base pairs is unbound, is determined via the thermal denaturation curves of DNA. In general, the melting temperature increases when metal complexes bind to DNA by intercalation, as intercalation of the complexes into DNA base pairs stabilize of base stacking and hence raises the DNA melting temperature[19,20].

Melting temperatures were measured in Tris-Hcl buffer solutions pH 7.0 containing 300 µM DNA. The temperature was scanned from 25 to 85ºC. Denaturation curve of ct-DNA represent at various mol ratios of [Ni(II)NSAID] to DNA were investigated and then the melting temperature values (T_m) were estimated by Igor software (Table 2). Then ΔH_m, T_m and other four parameters determined using the following equation and Sigma Plot software (Table3).

$$-\Delta\varepsilon_{260}(T) = \frac{(a_N + b_N T) + (a_D + b_D T)\exp[-\frac{\Delta H_m}{R}(\frac{1}{T} - \frac{1}{T_m})]}{1 + \exp[-\frac{\Delta H_m}{R}(\frac{1}{T} - \frac{1}{T_m})]} \quad \textbf{(4)}$$

ΔG_m determined using the following equation. Then thermodynamic stability curve of ct-DNAand the different molar ratio of Ni(II)NSAID from the drawing changes ΔGm on T produced [21-23].

$$\Delta G = \Delta H_m(1 - \frac{T}{T_m}) - \Delta C_p[(T_m - T) + T\ln\frac{T}{T_m}] \quad \textbf{(5)}$$

Table 2. DNA melting temperature changes upon increasing the molar ratio of Ni(II)NSAID to DNA

[Ni(II)NSAID] / [ct-DNA]	0	0.028	0.055	0.11
T_m (K)	333.171	330.301	330.179	330.118

Table 3. Determination T_m and ΔH_m using equation **(4)** and Sigma Plot software.

[Ni(II)NSAID] / [ct-DNA]	0	0.028	0.055	0.11
T_m (K)	330.628	329.162	330.702	330.708
ΔH (j/mol)	273745.76	382689.74	322336.59	451624.5

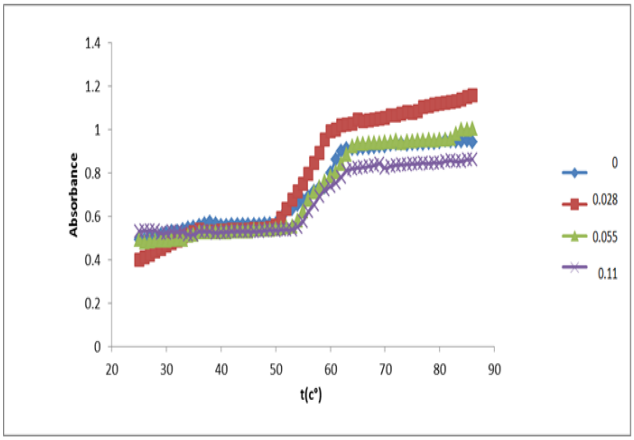

Figure 5. Thermal denaturation graph of ct-DNA and Ni(II)NSAID at different mole ratios of Ni(II)NSAID/[DNA]

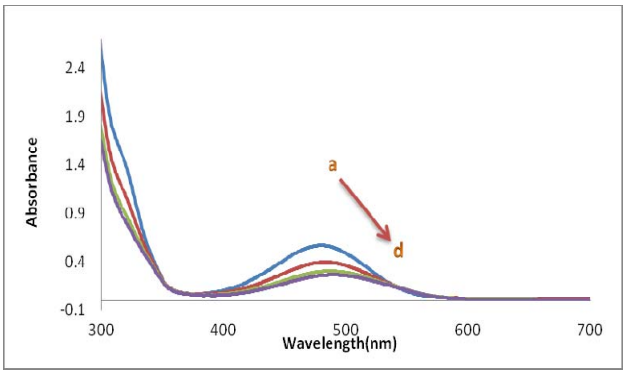

Figure. 6. The effect of DNA on the absorption spectrum (a) of EB. DNA concentrations are 0, 33.92, 67.86 and 101.76 μmol L^{-1} for absorption spectra (a–d), respectively. EB concentration is 31.92 μmol L^{-1}.

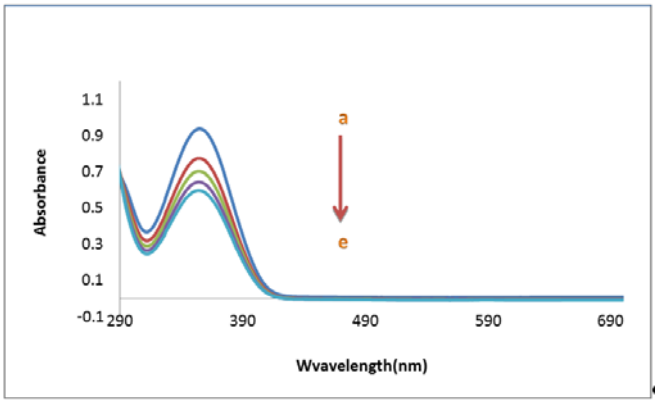

Figure 7. The absorption spectra of Ni(II)NSAID in the absence of DNA (a) and in the presence of DNA (b–e). DNA concentrations are 21.92, 32.14, 42.29 and 52.44 μmol L^{-1} for spectra (b–e), respectively. Ni(II)NSAID is 12.76 μmol L^{-1}.

3.1.3. Competitive studies with EB

The nature of the interaction of EB with nucleic acids was studied by many research reports [23–26]. It is generally recognized that the strong mode of binding of EB to DNA results in the intercalation of the planar phenanthridinium ring between adjacent base pairs on the double helix.

A competitive ethidium bromide (EB) binding study has been undertaken with UV–vis and fluorescence experiments [27] in order to investigate if the complexes could displace EB from its EB-DNA complex.

The UV–vis spectra of EB and DNA–EB complex are presented in Fig. 6. Spectrum (a) in Fig. 6 is the absorption spectrum of 31.92 μmol L^{-1} EB, and the maximum absorption peaks are at 284 and 480 nm. Adding various concentrations of DNA into this EB solution, spectrum (a) changed to spectra (b–d). Obviously, the maximum absorption wavelength shifts from 480 to 498 nm when the concentration of DNA is 101.76 μmol L^{-1} and both the absorbance at 284 and 480 nm decrease progressively with the increasing concentration of DNA. It is well known that bathochromic shift and hypochromism effects are observed in the absorption spectra of small molecules if they intercalate with DNA [28]. The experiment results show that EB intercalates into the DNA bases. Hypochromic effect is suggested to be due to a strong interaction between the electronic states of the intercalating chromophore and that of the DNA bases and the strength of this electronic interaction is expected to decrease as the cube of the distance of separation between the chromophore and DNA bases [28]. The obvious hypochromic effect in this experiment shows that EB gets near to DNA bases.

The result of UV/Vis spectrum analysis about the interaction of DNA with Ni(II)NSAID is shown in Figure 7. Compared with Figure 6, bathochromic shift and hypochromic effect in the absorption are not observed in the absorption spectra of Ni(II)NSAID in Figure 7. From the UV–vis analysis, the conclusion that the binding mode of Ni(II)NSAID with DNA is not the classical intercalation binding can be drawn.

4. CONCLUSIONS

1. Ni(II)NSAID does not show concentration and temperature dependent aggregation over an extended concentration range between 0 to 1.47×10^{-5} M and temperature range between 25-65°C in 5mM phosphate buffer, pH 7.0.
2. Addition of NaCl shows hypochroism along with a short blue shift and no new band appears even in high concentration of salt. This result means that Ni(II)NSAID does not form well defined aggregates even in high concentration of salt.
3. The binding of Ni(II)NSAID complex to ct-DNA, while the wavelength of maximum absorption ,λ_{max} does not show considerable changes the amount of absorption increases. It seems that the driving force is entropy and this interaction is an hydrophobic intraction upon the positive entropy and enthalpy.This result represents the outside-binding and hydrophobic interaction mode.
4. The ct-DNA-binding process is endothermic for Ni(II)NSAID and has the large positive entropy value. These can be represented the predominate role hydrophobe interactions and outside binding mode.
5. The increasing of melting temperature (Tm) of ct-DNA upon addition of Ni(II)NSAID represents the stable interaction between ct-DNA and Ni(II)NSAID . That is also consistent with outside binding mode. Competitive binding with a standard intercalator like ethidium bromide (EB) indicate that the binding mode of Ni(II)NSAID with DNA is not the classical intercalation binding. Very small changes of T_m value confirmed outside binding mode. As observed, the curves all have the same structure and a peak that represents the most stable. The result show that the driving force is entropy and denaturation process was endothermic.

ABBREVIATIONS

NSAID non-steroidal anti-inflammatory drug
Pirox Piroxicam
ct-DNACalf thymus deoxyribonucleic acid
EB ethidium bromide

ACKNOWLEDGEMENTS

We are extremely grateful to Payame-Noor University for their financial supports.

REFERENCES

[1] Zhang G.,Hu X., Pan J. "Spectroscopic studies of the interaction between piricarb and calf thymus DNA"spectrochimica Acta part A 78(2011)687-694.

[2] Strekowski L., Wilson B., Mutant. Res. 623(2007)3-13.

[3] He X. Q., Lin Q. Y.,Hu R. D., Lu X. H. spectrochim. Acta A 68(2007)184-190.

[4] Roy S., Banerjee R., Sarkar M.," Direct binding of Cu(II)-complexes of oxicam NSAIDs with DNA backbone", *J. Inorg. Biochem.***100** (2006) 1320–1331.

[5] Shuyun Bi, Hanqi Zhang, Chunyu Qiao,Ying Sun, Chunming Liu," Studies of interaction of emodin and DNA in the presence of ethidium bromide by spectroscopic method",*Spectrochimica Acta Part A* **69** (2008) 123–129.

[6] Noor-ul H. Khan, Nirali Pandya, Nabin Ch. Maity, Manoj Kumar, Rajesh M. Patel, Rukhsana I. Kureshy, Sayed H.R. Abdi, Sandhya Mishra, Satyabrata Das , Hari C. Bajaj," Influence of chirality of V(V) Schiff base complexes on DNA, BSA binding and cleavage activity", *Eur. J. Med. Chem.***46** (2011) 5074-5085 .

[7] Burcu Turanlı-Yıldız, Tugba Sezgin, Z. Petek Cakar, , Canan Uslan, B. Sebnem Sesalan, Ahmet Gul, " The use of novel photobleachable phthalocyanines to image DNA"*, Synth. Met.***161** (2011) 1720– 1724.

[8] Dezhampanah. H, Bordbar. A. K, Tangestaninejad. Sh, "Thermodynamic investigation of a new water- soluble porphyrin with calf thymus DNA", *Eur. J. Biochem.* **1** (2010) 307-311.

[9] Dezhampanah. H, Bordbar. A. K, Tangestaninejad. Sh, "Thermodynamic investigation of a new water- soluble porphyrin with calf thymus DNA", *Eur. J. Biochem.* **1** (2010) 307-311.

[10] M.T. Carter, M. Rodriguez, A. Bard, Voltammetric studies of the interaction of metal chelates with DNA. 2. Tris-chelated complexes of cobalt(III) andiron(II) with 1,10-phenanthroline and 2,2'-bipyridineJ. Am. Chem. Soc. 111 (1989) 8901–8911.

[11] B. Peng, H. Chao, B. Sun, H. Li, F. Gao, L.-N. Ji, Structural, magnetic, electrochemical, catalytic, DNA binding and cleavage studies of new macrocyclic binuclear copper(II) complexes J. Inorg. Biochem. 100 (2006) 1487–1494.

[12] A.M. Pyle, J.P. Rehmann, R. Meshoyrer, C.V. Kumar, N.J. Turro, J.K. Barton, Nickel–quinolones interaction Part 3 — Nickel(II) complexes of the antibacterial drug flumequine J. Am. Chem. Soc. 111 (1989) 3053–3063.

[13] H. Chao, W.-J. Mei, Q.-W. Huang, L.-N. Ji, The Interaction of Sheep Genomic DNA with a Cobalt(II) Complex Containing *p*-Nitrobenzoate and N,N′-Diethylnicotinamide Ligands J. Inorg. Biochem. 92 (2002) 165–170.

[14] P.D. Ross, S. Sabramanian, Thermodynamics of protein association reactions: forces contributing to stability *Biochemistry* 20 (1981) 3096.

[15] W.C. Lin, X.T. Wei, H.W. Xue, M. Kelimu, R. Tao, Y.S. Song, Z.C. Zhou,___MUTATION RESEARCH-FUNDAMENTAL AND MOLECULAR MECHANISMS OF MUTAGENESIS Mutat. Res. 466 (2000) 187–195.

[16] C.V. Kumar, R.S. Turner, E.H. Asuncion, New asymmetric monomethine cyanine dyes for nucleic-acid labelling: absorption and fluorescence spectral characteristics New asymmetric monomethine cyanine dyes for nucleic-acid labelling:absorption and fluorescence spectral characteristics J. Photochem. Photobiol. A: Chem. 74 (1993) 231.

[17] G.A. Neyhart, N. Grover, S.R. Smith, W. Kalsbeck, T.A. Fairley, M. Cory, H.H. Thorp,Binding and kinetics studies of oxooxidations of DNA by oxoruthenium(IV) J. Am. Chem. Soc. 115 (1993) 4423.

[18] B. Selvakumar, V. Rajendiran, P.U. Maheswari, H. Stoeckli-Evans, M. Palaniandavar, Structures, spectra, and DNA-binding properties of mixed ligand copper(II) complexes of iminodiacetic acid: The novel role of diimine co-ligands on DNA conformation and hydrolytic and oxidative double strand DNA cleavageJ. Inorg. Biochem. 100 (2006) 316.

[19] Ahmad. F, Rishi. V, Anjum. F, Pfeil. W, " Role of non-compatible osmolytes in the stabilization of proteins during heat stress" *J. Biochem.* **329** (1998) 137–143.

[20] Pace. C. N, Hebert. E. J, Shaw. K. L, Schell. D, Both. V, Krajcikova. D, Sevcik. J, Wilson. K.S, Dauter. Z, Hartley. R. W, Grimsley. G. R, " Conformational stability and thermodynamics of folding of ribonucleases Sa, Sa2 and Sa3", *J. Mol. Biol.* **279** (1998) 271–286.

[21] W. Fuller, M.J. Warning, Ber. Bunsenges.Mechanism of ethidium bromide inhibition of RNA polymerase" Phys. Chem. 68 (1964) 805.

[22]. C G. Reinhardt, T.R. Krugh, Biochemistry 17 (1978) 4845.

[23] J.B. Lepecq, C.C. Paoletli, Anal. Biochem. 17 (1996) 344.

[24] A. Dimitrakopoulou, C. Dendrinou-Samara, A.A. Pantazaki, M. Alexiou, E. Nordlander, D.P. Kessissoglou, structural characterization, antibacterial and antiproliferative activities of [(n-Bu)2Sn(H-1L)] (LH = H-Aib-L-Leu-OH, H-Aib-L-Ala-OH)", J. Inorg. Biochem. 102 (2008) 618–628.

[25] K. Jiao, Q.X. Wang, W. Sun, F.F. Jian, Synthesis, characterization and DNA-binding properties of a new cobalt(II) complex: Co(bbt)2Cl2 J. Inorg. Biochem. 99 (2005) 1369–1375.

[26] A.M. Pyle, J.P. Rehmann, R. Meshoyrer, C.V. Kumar, N.J. Turro, J.K. Barton,Photochemical conversions and storage of solar energy J. Am. Chem. Soc. 111 (1989) 3051.

[27] E.C. Long, J.K. Barton, Methods of studying Nucleic acids/Drug interactions Chem. Res. 23 (1990) 271.

[28] C. Cantor, P.R. Schimmel, in:W.H. Freeman (Ed.), Biophysical Chemistry, Part 2, W.H. Freeman, San Francisco, 1980, p. 398.