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DNA-Binding Studies of Some Potential Antitumor 2,2'–bipyridine Pt(II)/Pd(II) Complexes of piperidinedithiocarbamate. Their Synthesis, Spectroscopy and Cytotoxicity

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Abstract

In this study two platinum(II) and palladium(II) complexes of the type $[M(bpy)(pip\text{-}dtc)]NO_3$ (where M=Pt(II) or Pd(II), bpy=2,2'-bipyridine, pip-dtc=piperidinedithiocarbamate) were synthesized by reaction between diaquo-2,2'-bipyridine Pt(II)/Pd(II) nitrate and sodium salt of dithiocarbamate. These cationic water soluble complexes were characterized by elemental analysis, molar conductance, IR, electronic and ^{1}H NMR spectroscopic studies. The cyclic dithiocarbamate was found to coordinate as bidentate fasion with Pt(II) or Pd(II) center. Their biological activities were tested against chronic myelogenous leukemia cell line, K562, at micromolar concentration. The obtained cytotoxic concentration (IC_{50}) values were much lower than cisplatin. The interaction of these complexes with highly polymerized calf thymus DNA (ct-DNA) was extensively studied by means of electronic absorption, fluorescence, circular dichroism and other measurements. The experimental results, thermodynamic and binding parameters, suggested that these complexes cooperatively bind to DNA presumably via intercalation. Moreover, the tendency of the Pt(II) complex to interact with DNA was more than that of Pd(II) complex.

Keywords: Platinum(II) and palladium(II) complexes, Cytotoxicity, Dithiocarbamate, Intercalation.

1. Introduction

The reaction between a primary or secondary amines and carbon disulfide in basic media yields dithiocarbamate. This class of compounds has diversified applications in the field of agriculture, industry, analytical and organic chemistry, and biology, as well as their physicochemical properties, which have been summarized in several review and articles.[1–3] Also, it has been reported that dithiocarbamates obtained from cyclic amines are more stable than the aliphatic derivatives.[1] Dithiocarbamates are known to coordinate with transition metal centers as mono or bidentate and their metal complexes present a wide range of applications. The interest to these complexes has been renewed by their utilization as coadjutants in the treatment of AIDS,[4,5] tuberculosis, human cancer or as catalytic applications,[7]and metallocyclation.[9–12] Dithiocarbamato complexes of Pd(II) and Pt(II) exhibit high anti-tumor activity together with a reduced toxicity with respect to cisplatin and analogous compounds.[13] The later may be due to strong bonds of platinum or palladium with dithiocarbamate which prevent or at least limit their reactions with other sulfur-containing renal proteins.[14] Many Pt(II) and Pd(II) complexes with dithiocarbamates are

known to exhibit anti-tumor activities[15,16] and cytotoxic properties against some tumor cells such as lung, ovarian,[17] melanoma, colon, renal, prostate and breast cancer.[18] Furthermore, complexes of dithiocarbamate exhibit anti-tumor activities against leukemic cells,[19] KB tumor cells[20] and *pam.ras* cells.[21] Preparations and studies of several anti-tumor complexes having chelating ligands such as N,N-diamines,[22] N,S-amino-thioether,[23] O,S-donor,[24] diaminoacids,[25,26] dicarboxylic acids[27] and dithiocarbamates[15,28,29] are recent advances of platinum and palladium complexes which have higher activity and reduced toxicity as compared with cisplatin.

The present paper involves synthesis and characterization of two water soluble and structurally related platinum(II) and palladium(II) complexes. Both complexes bear a planar 2,2'-bipyridine ligand. This planar aromatic ligand along with square planar geometry around Pt(II) or Pd(II) centers may make the complexes susceptible to intercalate in DNA. Moreover, we attached a bidentate dithocarbamate to Pt(II) and Pd(II) center which can protect a variety of animal species from renal, gastrointestinal and bone marrow toxicity induced by cisplatin.[15] Thus, keep in mind the following: in mind: (i) the above mentioned structural criteria, (ii) low Ic_{50} values of the above two complexes, (iii) availability of only few literature contributions on the DNA binding studies of such a dithiocarbamate complexes[30–32] and (iv) the fact that the mechanism of action of these complexes with DNA must be quite different from that of cisplatin, an extensive interaction studies of both complexes with calf thymus DNA are presented. In these interaction studies several bindings and thermodynamic parameters as well as evaluation of binding modes have been described. They may throw light on the interaction mechanisms of these types of complexes with DNA of cells and possible side effects of these agents.

2. Experimental

2. 1. Materials

Potassium tetrachloridoplatinate, 2,2'-bipyridine, highly polymerized calf thymus DNA sodium salt and Tris-HCl buffer were purchased from Merck (Germany). Palladium(II) chloride anhydrous was obtained from Fluka (Switzerland). Piperidine, carbon disulfide and ethidium bromide were obtained from Aldrich (England). [Pt(bpy)Cl_2] and [Pd(bpy)Cl_2] were prepared based on what mentioned in the literature.[33] Other used chemicals were of analytical reagent or higher purity grade. Solvents were purified prior to be used by the standard procedures.[34]

2. 2. Physical Measurements

The melting points of the compounds were determined on a Unimelt capillary melting point apparatus. Carbon, hydrogen and nitrogen were analyzed on a Herause CHNO-RAPID elemental analyzer. Infrared spectra (4000–400 cm^{-1}) were determined with KBr disks on a J_{ASCO}*-460 plus* FT-IR spectrophotometer. UV-vis spectra were recorded on a J_{ASCO} *UV/VIS-7850* recording spectrophotometer. ^{1}H NMR spectra were measured on a *Brucker DRX-500* Avance spectrometer at 500 MHz, using TMS as the internal reference in DMSO-d_6. The fluorescence spectra were carried out on a *Hitachi MPF-4* spectrofluorimeter. Circular dichroism spectra were recorded on an *Aviv Spectropolarimeter* model 215. Conductivity measurements of the above platinum and palladium complexes were carried out on a Systronics Conductivity Bridge 305, using a conductivity cell of cell constant 1.0 and doubly distilled water was used as solvent.

2. 3. Preparation of the Ligand and Metal Complexes

2. 3. 1. Pip-dtcNa

This ligand was prepared by a modified literature method:[35] Piperidine (5 mL, 50 mmol) in 30 mL acetone and sodium hydroxide (2 g, 50 mmol) in 20 mL doubly distilled water were mixed and stirred vigorously in an ice bath. Carbon disulfide (10 mL, excess) was added slowly. Stirring continued for one hour in an ice bath and another three hours at room temperature (30 °C). It was then filtered and the volume of the filtrate was reduced to 20 mL on Rota evaporator and was placed in a refrigerator overnight. The product was separated as white needle-like crystals which was filtered off and washed with 15 mL acetone and dried at 40 °C. Yield: 7.5 g (82%) with a melting point of 299.2–299.7 °C. *Anal*. Calc. for $C_6H_{10}NS_2Na$ (183): C, 39.34; H, 5.46; N, 7.65%. Found: C, 39.39; H, 5.50; N, 7.68%.

2. 3. 2. [Pt(bpy)(pip-dtc)]NO_3

1 mmol (0.422 g) of [Pt(bpy)Cl_2] was suspended in 160 mL acetone-water (3:1 v/v) mixture and 2 mmol (0.34 g) $AgNO_3$ was added to it. This reaction mixture was stirred in darkness for 7 h at 55 °C and then 12 h at room temperature (30 °C). The AgCl precipitate formed was filtered through Whatman 42 filter paper. The temperature of filtrate was kept at 45–50 °C and then 1 mmol (0.183 g) piperidinedithiocarbamate sodium salt dissolved in 10 mL water was added slowly. Stirring continued for 6 h and then the mixture was evaporated at 35–40 °C to complete dryness. The precipitate obtained was stirred with 30 mL acetonitryl-methanol (3:1 v/v) for 10 min. at 40 °C and filtered. Diffusion of ether into this filtrate gave brownish yellow crystals after 48 h. The crystals were isolated by filtration, washed with 10 mL ether and dried at 40 °C. Yield: 0.333 g (58%) and decomposed at 296.5–297.8 °C. *Anal*. Calc. for $C_{16}H_{18}N_4O_3S_2Pt$(573): C, 33.51; H, 3.14; N, 9.77%. Found: C, 33.56; H, 3.11; N, 9.83%. Absorp-

tion spectrum (H_2O): λ_{max} (ε_M), 366 nm (4080), 321 nm (7908), 309 nm (7252), 285 nm (13964), 209 nm (31800).

2. 3. 3. [Pd(bpy)(pip-dtc)]NO_3

This complex was prepared by converting [Pd(bpy)Cl_2] to [Pd(bpy)$(H_2O)_2$]$(NO_3)_2$ and treating with piperidinedithiocarbamate sodium salt as given for [Pt(bpy)(pip-dtc)]NO_3. Yield: 0.267 g (55%) and decomposed at 278.7–279.2 °C. *Anal*. Calc. for $C_{16}H_{18}N_4O_3S_2Pd$ (484): C, 39.67; H, 3.72; N, 11.57%. Found: C, 39.73; H, 3.70; N, 11.60%. Absorption spectrum (H_2O): λ_{max} (ε_M), 314 nm (12640), 304 nm (12412), 248 nm (35068), 202 nm (33748).

2. 4. Cytotoxicity Assay

The following procedure was similar to that reported earlier[19] except that the 2×10^4 cells/mL were used in place of 1×10^4 cells/mL in Tris-HCl solution of pH 7.0. The means ± S.D. (standard deviation) value of IC_{50} values are from three independent experiments.

2. 5. Biochemical Studies

All experiments involving the interaction of the complexes with ct-DNA have been carried out in Tris-HCl buffer of pH 7.0 medium containing 10 mmol/L sodium chloride.[30, 31] The stock solutions of Pt(II) and Pd(II) complexes (2 mmol/L) were made in this medium by gentle stirring and heating at 35 °C, while that of DNA (4 mg/mL) were at 4 °C until they become homogenous. The metal complex solutions, with and without DNA were incubated at 27 °C and 37 °C. Then, the spectrophotometric readings at λ_{max} (nm) of complexes where DNA has no absorption were measured. Using trial and error method, the incubation time for solutions of DNA-metal complexes at 27 °C and 37 °C were found to be 2 h and 30 min. No further changes were observed in the absorbance reading after longer incubation. The concentration of DNA was determined spectrophotometrically using a molar absorptivity of 6600 M^{-1} cm^{-1} (258 nm).[37] Different techniques to probe the changes on DNA structure induced by complexes have been as follows:

2. 5. 1. Electronic Absorption Titration

Electronic absorption spectroscopy is universally employed to determine the binding parameters (n, K, g) of metal complexes with DNA as reported earlier.[38, 39] Where n is Hill coefficient, g is the number of binding sites per 1000 nucleotides of DNA and K is apparent binding constant. Also, the other thermodynamic binding parameters: molar Gibbs free energy of binding (ΔG°_b), molar enthalpy of binding (ΔH°_b) and molar entropy of binding (ΔS°_b) were determined according to reported method.[25] All measurements were performed separately at 27 °C and 37 °C and were repeated three times for each complex.

2. 5. 2. Denaturation of DNA with Pt(II) and Pd(II) Complexes

The application of UV absorption method to the study of denaturation of DNA in presence of Pt(II) or Pd(II) complexes was similar to that reported earlier.[25] In these studies, the concentration of each metal complex at midpoint of transition, $[L]_{1/2}$, was determined. Also, thermodynamic parameters such as: $\Delta G^\circ_{(H_2O)}$, conformational stability of DNA in the absence of metal complex; $\Delta H^\circ_{(H_2O)}$, the heat needed for DNA denaturation in the absence of metal complex; $\Delta S^\circ_{(H_2O)}$, the entropy of DNA denaturation by metal complex as well as m, measure of the metal complex ability to destabilize DNA were found out using Pace method.[25,40]

2. 5. 3. Fluorescence Studies

Ethidium bromide (EB), one of the most sensitive fluorescence probed, has a planar structure binds with DNA through intercalative mode.[41,42] At first, DNA (60 μM) was added to 2 μM aqueous ethidium bromide solution and maximum quantum yield for ethidium bromide was achieved at 471 nm, so we selected this wavelength as excitation radiation for all of the samples at different temperatures (27 °C and 37 °C) and emission was observed in the range of 540–700 nm. To this solution (containing EB and DNA) different concentrations of the Pd(II) or Pt(II) complex (0.05, 0.1 and 0.15 mM) were added. Addition of any of these metal complexes to DNA-EB system causes obvious reduction in fluorescence intensity. These metal complexes do not exhibit emission in the presence of DNA and there is no influence on the emission intensity of free EB in the absence of DNA. Thus the competitive DNA-binding of metal complexes with EB could provide an evidence of the interaction of the metal complexes between DNA base pairs.

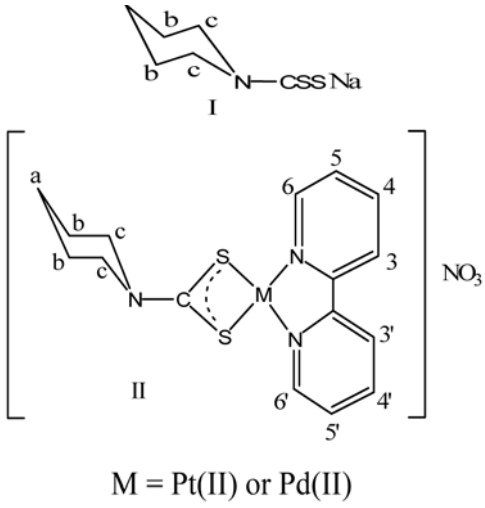
2. 5. 4. Circular Dichroism

This technique is quite sensitive to the changes in the secondary structure of nucleic acids, which allows analyzing any conformational modification of DNA provoked by its interaction with Pt(II) or Pd(II) complexes.[41,42] The DNA concentration in the experiments was 120 μM. Induced CD spectra resulting from the interaction of the Pd(II) or Pt(II) complex with DNA at the two temperatures 27 °C and 37 °C, were obtained by subtracting the CD spectrum of the native DNA and mixture of DNA-Pd(II) or -Pt(II) complex from the CD spectrum of the buffer and spectrum of buffer-Pd(II) or -Pt(II) complex solutions. CD spectrum of each sample was scanned in the range of 200–320 nm using 1 cm path length cells.

3. Results and Discussion

3. 1. Characterization of Compounds

A free ligand, piperidinedithiocarbamate sodium salt (pip-dtcNa) and two complexes [Pt(bpy)(pip-dtc)]NO_3 and [Pd(bpy)(pip-dtc)]NO_3, bpy is 2,2'-bipyridine, were prepared by the reaction of [M(bpy)$(H_2O)_2$] $(NO_3)_2$ with pip-dtcNa in molar ratio of 1:1. The ligand and complexes were characterized by IR, ^{1}H NMR, UV-vis and elemental analysis and conductivity measurements. The molar conductance values of these complexes in water are 110 and 101 $cm^2\,ohm^{-1}\,mol^{-1}$ for Pt(II) complex and Pd(II) complex, respectively. These values suggest that they are 1:1 electrolytes.[43] The chemical analysis and molar conductance data support the formulation of the two complexes (Scheme 1).



Scheme 1. Proposed structures and nmr numbering schemes of (I) pip-dtcNa, (II) [M(bpy)(pip-dtc)]NO_3.

3. 1. 1. Infrared Spectra

In the IR spectra of the ligand and complexes, the two most significant bands are of interest. First, the pip-dtcNa ligand showed a strong absorption at 1470 cm^{-1} which is assigned to N-CSS stretching mode,[44] while the IR spectra of the complexes [Pt(bpy)(pip-dtc)]NO_3 and [Pd(bpy)(pip-dtc)]NO_3, showed absorption at 1549 and 1546 cm^{-1}, respectively. These data suggest that the N-CSS bond order is in between a single bond (ν = 1250–1350 cm^{-1}) and a double bond (ν = 1640–1690 cm^{-1}).[45] As it is clear from from IR spectral data of free dithiocarbamate ligand and the corresponding complexes, the ν(N-CSS) mode has shifted to higher frequencies upon coordination. This indicates that the nitrogen-carbon double bond character has increased due to the electron delocalization towards the palladium or platinum centers. Thus, the above piperidinedithiocarbamate ligand coordinates to Pt(II) or Pd(II) through sulfur atoms. Second, the presence of a single strong band at 967 cm^{-1} for pip-dtcNa, at 1024 cm^{-1} for Pt(II) and at 1019 cm^{-1} for Pd(II) complexes are attributed to ν(SCS) mode.[29] This is a strong indicator of symmetrical bonding of the dithiocarbamate ligand, acting in a bidentate mode in our complexes (Scheme 1). Otherwise a doublet would be expected in the 1000 ± 70 cm^{-1} region which indicates an asymmetrically bonded ligand or a monodentate bound ligand.[45]

3. 1. 2. Electronic Spectra

The electronic absorption maxima of the above Pt(II) and Pd(II) complexes in distilled water with their extinction coefficients are given in the experimental section. Band I at 366 nm for Pt(II) and at 314 nm for Pd(II) complexes are tentatively assigned to metal to ligand charge transfer (MLCT), because these bands are shifted by 16–18 nm on going from dichloromethane to water.[46] Other bands in the spectra of these two complexes may be due to first, second, and higher internal $\pi \rightarrow \pi^*$ transitions of 2,2'-bipyridine ligand.[46] These bands have also overlapping components of $\pi \rightarrow \pi^*$ transitions of dithiocarbamate ligand which may be hidden in the above strong charge transfer transition from Pt(II) or Pd(II) to π^* of 2,2'-bipyridine ligand. The above electronic absorption of data suggests these complexes have square planar configuration[47] and are in agreement with the reported analogous complexes.[15]

3. 1. 3. ^{1}H NMR Spectra

The ^{1}H NMR spectrum of pip-dtcNa ligand shows three multiplet peaks at 1.42, 1.56 and 4.27 ppm which are assigned to H-b, H-a and H-c protons, respectively (see Scheme 1-I). The integrated areas under these peaks correspond to the ratio 4:2:4 and thus support the proposed structure.

The protons of 2,2'-bipyridine moiety in the ^{1}H NMR spectrum of [Pd(bpy)(pip-dtc)]NO_3 appear as a doublet at 8.68 ppm, a triplet at 8.38 ppm, a multiplet at 8.31 ppm and another triplet at 7.77 ppm which are assigned to H-6,6', H-4,4', H-3,3' and H-5,5' protons, respectively (Scheme 1-II).[15] In the [Pd(bpy)(pip-dtc)]NO_3 complex, three multiplet peaks, which were observed at 1.67, 1.74 and 3.85 ppm are assigned to four H-b, two H-a and four H-c protons of dithiocarbamate moiety, respectively (Scheme 1-II). H-b and H-a show downfield shifts of 0.25 and 0.18 ppm while H-c shows upfield shifts of 0.42 ppm in the complex as compared to its value in sodium dithiocarbamate. This suggests the bonding of dithiocarbamate ligand to palladium (II) through sulphur atoms. The integrated areas under the peaks of 2,2'-bipyridine and piperidinedithiocarbamate in the ration 8:10 further support the proposed structure. Similar assignments have been done for protons of 2,2'-bipyridine moiety: 8.72 (doublet, H-6,6'), 8.54 (doublet, H-4,4'), 8.45 (triplet, H-3,3'), 7.76

(triplet, H-5,5') and protons of piperidinedithiocarbamate moiety: 1.71 ppm (multiplet, H-a and H-b), 3.80 ppm (multiplet, H-c) in the analogous complex [Pt(bpy)(pip-dtc)]NO_3. Finally, no changes were observed in the ^{1}H-NMR spectra of the above complexes dissolved in DMSO-d_6 and recorded after 24 h suggesting no dissociation of dithiocarbamate anions. Thus, based on the spectroscopic data, the structures as shown in Scheme 1(II), have been assigned to these two complexes which are also in accord with the observed molar conductance value of 110 and 101 $cm^2ohm^{-1}mol^{-1}$ for [Pt(bpy)(pip-dtc)]NO_3 and [Pd(bpy)(pip-dtc)]NO_3 respectively, as 1:1 electrolytes. Further supports for the proposed structures come from the ratio of the integrated areas under the peaks of 2,2'-bipyridine and dithiocarbamate protons being 8:10 for the Pt(II) and Pd(II) complexes.

3. 2. Cytotoxicity Evaluation

The in vitro anti-tumor properties of Pt(II) and Pd(II) complexes were carried out with human tumor cell line K562.[19] In this experiment, the cell growth was measured after incubation of cells in the presence of compounds (from 0 to 250 μM) to be tested at 37 °C for 24 h. In Figure 1 the cell growth (in %) versus concentration (μM) of above complexes is represented. The 50% cytotoxic concentration (CC_{50}) of each compound was determined 2.5 μM and 10.5 μM for Pt(II) and Pd(II) complexes, respectively. Moreover the IC_{50} value of cisplatin under the same experimental conditions was determined to be 154 μM which is much higher than that of the two prepared complexes. However, the IC_{50} values of these complexes are slightly higher than that of our analogous Palladium(II) dithiocarbamate complexes reported earlier.[19]

3. 3. DNA Binding Studies

3. 3. 1. Evaluation of Binding Parameters

A fixed amount of each metal complex (25 μL of 2 mmol/L stock) was titrated with increasing concentration of DNA (50–200 μL of 0.2 mmol/L stock) in total volume of 2 mL at 27 °C and 37 °C, separately. In this experiment, change in absorbance, ΔA, was calculated by subtracting the absorbance reading of mixed solutions of each metal complex with various concentrations of DNA, from absorbance reading of free metal complex. The values of ΔA_{max}, change in absorbance when all binding sites on DNA were occupied by metal complex, are given in Table 1 and Fig. 2. In another experiment, a fixed amount of DNA (0.3 ml of 0.2 ml/L stock) was titrated with varying amount of each metal complex (40–170 μl of 0.2 mmol/L stock). The concentration of each metal complex bound to DNA, $[L]_b$, and the concentration of each free metal complex, $[L]_f$, are calculated by using the relationship $[L]_b=\Delta A[L]_f/\Delta A_{max}$. Here $[L]_f = [L]_t - [L]_b$ where $[L]_t$ is the maximum concentration of each metal complex added to saturate all the binding sites of DNA and $\bar{\nu}$ is the ratio of the concentration of bound metal complex to total [DNA]. Using these data ($\bar{\nu}$, $[L]_f$), the Scatchard plots were constructed for the interaction of each metal complex at the two temperatures 27° C and 37 °C. The Scatchard plots are shown in Fig. 3 for [Pt(bpy)(pip-dtc)]NO_3 and the insert for [Pd(bpy)(pip-dtc)]NO_3. These plots are curvilinear concave downwards, suggesting cooperative binding.[39]

To obtain the binding parameters, the above experimental data ($\bar{\nu}$ and $[L]_f$) were substituted in Hill equation, $[\bar{\nu} = g(K[L]_f)^n/(1+(K[L]_f)^n)]$, to get a series of equation with unknown parameters n, K and g. Using Eureka software, the theoretical values of these parameters could be

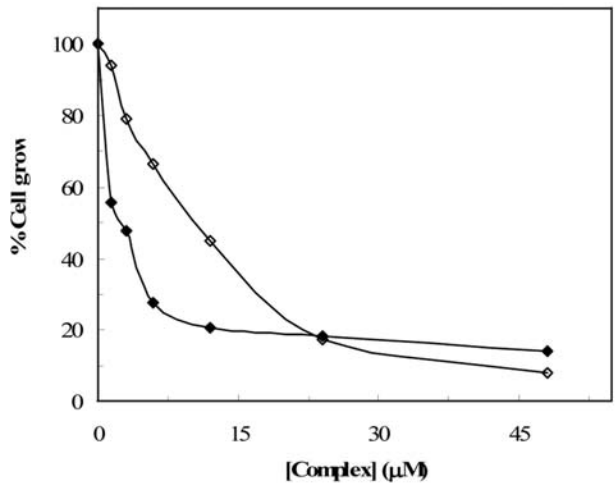


Figure 1. The growth suppression activity of the Pd(II)-complex (◇) and Pt(II) complex (◆) on K562 cell line was assessed using MTT assay as described in material and methods. The tumor cells were incubated with varying concentrations of the complexes for 24 h.

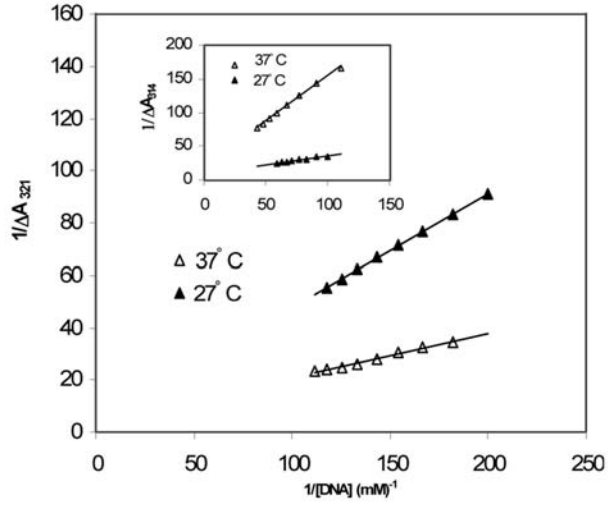


Figure 2. The changes in the absorbance of fixed amount of each metal complex in the interaction with varying amount of DNA at 27 °C and 37 °C. The linear plot of the reciprocal of ΔA versus the reciprocal of [DNA] for [Pt(bpy)(pip-dtc)]NO_3. Insert: for [Pd(bpy)(pip-dtc)]NO_3.

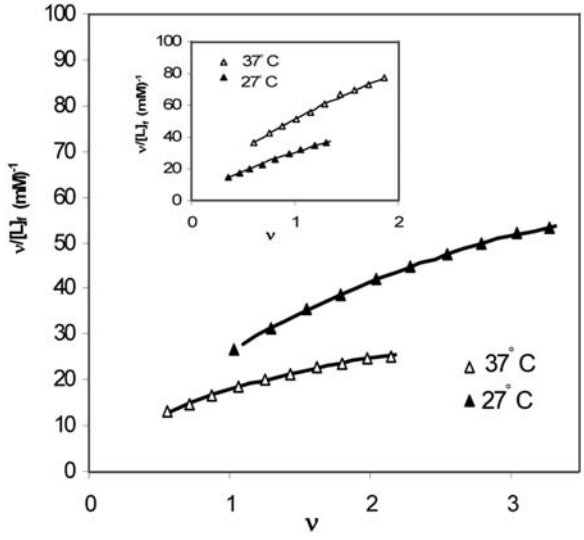


Figure 3. Scatchard plots for binding of [Pt(bpy)(pip-dtc)]NO_3 with DNA. The insert is Scatchard plots for binding of [Pd(bpy)(pip-dtc)]NO_3 with DNA.

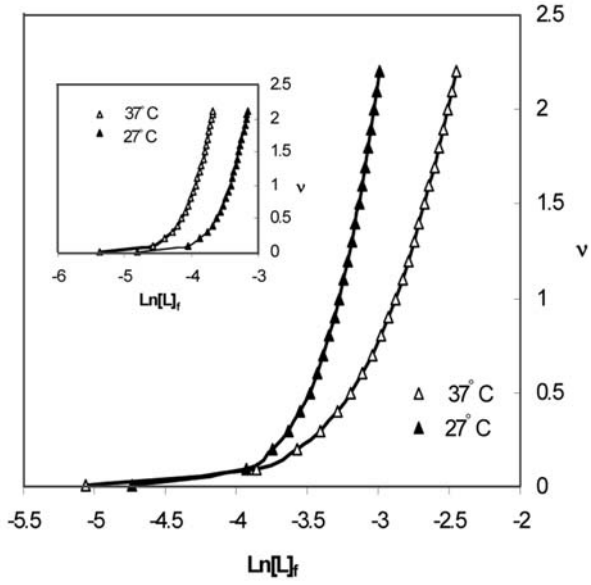


Figure 4. Binding isotherm plots for [Pt(bpy)(pip-dtc)]NO_3 in the interaction with DNA. Insert: for [Pd(bpy)(pip-dtc)]NO_3.

deduced. The results are tabulated in Table 1 which are comparable with those of 2,2'-bipyridine-platinum and -palladium complexes of dithiocarbamate as reported earlier.[15] The maximum errors between experimental and theoretical values of $\bar{\nu}$ are also shown in Table 1 which are quite low. The K, apparent binding constant and n, the Hill coefficient in the interaction of [Pd(bpy)(pip-dtc)]NO_3 with DNA is about higher than that of [Pt(bpy)(pip-dtc)]NO_3 with DNA (see Table 1). This indicates that the cooperativity of Pd(II) complex is more than Pt(II) complex. This is due to this point that palladium complexes are about 10^5 times more labile than their platinum analogs.[48] Similar results were obtained for [Pd(bpy)(ddtc)] NO_3 H_2O,[15] while its Pt(II) analog was highly cooperative.

Knowing the experimental (dots) and theoretical (lines) values of $\bar{\nu}$ in the Scatchard plots and superimposibility of them on each other, these values of $\bar{\nu}$ were plotted versus the values of $Ln[L]_f$. The results are sigmoidal curves and are shown in Fig. 4 at 27 °C and 37 °C. These plots indicate positive cooperative binding at both temperatures for both of the complexes. Finding the area under

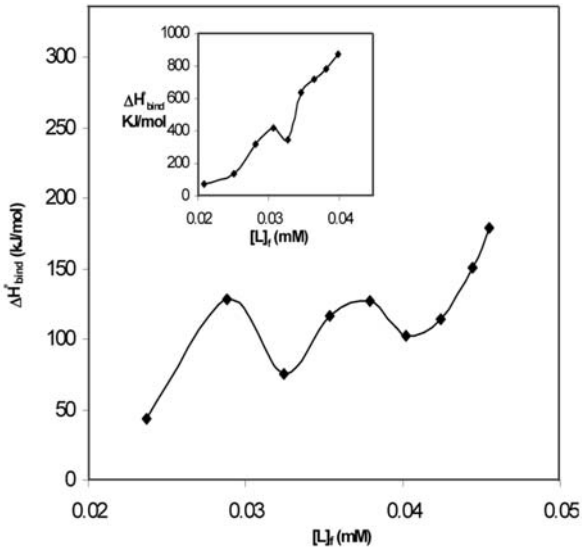


Figure 5. Molar enthalpies of binding in the interaction between DNA and [Pt(bpy)(pip-dtc)]NO_3 (Insert: [Pd(bpy)(pip-dtc)]NO_3) versus free concentrations of complexes at pH 7.0 and 27°C.

Table 1. Values of ΔA_{max} and binding parameters in the Hill equation for interaction between Pt(II) and Pd(II) complexes and DNA in 10 mmol/L Tris-HCl buffer and pH 7.0

	Temperature	[a] ΔA_{max}	[b] g	[c] K (mol/L)$^{-1}$	[d] n	[e] Error
[Pt(bpy)(pip-dtc)]NO_3	27 °C	0.192	6	0.017	3.750	0.015
	37 °C	0.200	6	0.009	2.510	0.030
[Pd(bpy)(pip-dtc)]NO_3	27 °C	0.098	6	0.020	3.910	0.039
	37 °C	0.048	6	0.033	3.800	0.063

[a] change in the absorbance when all the binding sites on DNA were occupied by metal complex [b] the number of binding sites per 1000 nucleotides [c] the apparent binding constant [d] the Hill coefficient (as a criterion of cooperativity) [e] $\bar{\nu}$ maximum error between theoretical and experimental values of

the above plots of binding isotherms and using Wyman-Jons equation,[25] we can calculate the K_{app} and $\Delta G°_b$ at 27 °C and 37 °C for each particular ν and also $\Delta H°_b$. Plots of the values of $\Delta H°_b$ versus the values of $[L]_f$ are shown in Fig. 5 for [Pt(bpy)(pip-dtc)]NO_3 and the insert for [Pd(bpy)(pip-dtc)]NO_3 at 27 °C. Deflections are observed in both plots. These deflections indicate that at particular $[L]_f$, there is a sudden change in enthalpy of binding which may be due to binding of metal complex to DNA or DNA denaturation. Similar observations can be seen in the literature where Pd(II) complexes have been interacted with proteins.[25,26]

3. 3. 2. Thermodynamic Parameters in Denaturation Studies

The maximum unfolding of DNA by interaction with above platinum and palladium complexes occurs when all binding sites are occupied. In this experiment, the sample cell was filled with 1.8 mL DNA (0.1 mmol/L). In this concentration, the absorption of DNA is around 0.8. However, reference cell is filled with 1.8 mL Tris-HCl buffer only. Both cells were set separately at constant temperature of 27 °C or 37 °C and then 25 μL of Pt(II) and 10 μL of Pd(II) complex from stock solutions, were added to each cell. After 3 min., the absorption was recorded at 258 nm for DNA and at 640 nm to eliminate the interference of turbidity. Addition of metal complex to both cells was continued until no further changes in the absorption readings were observed. The profiles of denaturation of DNA by [Pt(bpy)(pip-dtc)]NO_3 and [Pd(bpy)(pip-dtc)]NO_3 are shown in Fig. 6 at two temperatures of 27 °C and 37 °C. The concentration of metal complexes in the midpoint of transition, $[L]_{1/2}$, for Pt(II) complex at 27 °C is 0.207 and at 37 °C is 0.197 mmol/L and for Pd(II) complex at 27 °C is 0.128 and at 37 °C is 0.125 mmol/L. The important observation of this work is the low values of $[L]_{1/2}$ for these complexes[37,41,49] i.e. both complexes (in particular Pd(II) complex) can denature DNA at very low concentrations (~100 μM). Thus, if these complexes will be used as anti-tumor agents, low doses will be needed, which may have fewer side effects.

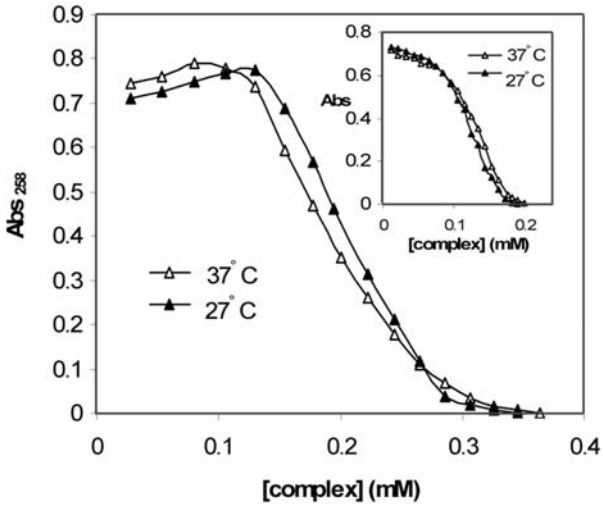


Figure 6. The changes of absorbance of DNA at λ_{max}=258 nm due to increasing the total concentration of [Pt(bpy)(pip-dtc)]NO_3 and the insert, [Pd(bpy)(pip-dtc)]NO_3, $[L]_t$, at constant temperature of 27 °C and 37 °C.

Furthermore, some thermodynamic parameters found in the process of DNA denaturation are discussed here: Using the DNA denaturation plots given in Figs. 6 and the Pace method,[40] the values of K, unfolding equilibrium constant and $\Delta G°$, unfolding free energy of DNA at two temperatures of 27 °C and 37 °C in the presence of [Pt(bpy)(pip-dtc)]NO_3 and [Pd(bpy)(pip-dtc)]NO_3 have been calculated. A straight line is obtained when the values of $\Delta G°$ are plotted versus the concentrations of each metal complex in the transition region at 27 °C and 37 °C. These plots are shown in Fig. 7 for Pt(II) and the insert for Pd(II) systems. The m, slope of these plots (a measure of the metal complex ability to destabilize DNA) and the intercept on ordinate, $\Delta G°_{(H_2O)}$, (conformational stability of DNA in the absence of metal complex) are summarized in Table 2. The values of m for Pt(II) complex are higher than those of Pd(II) complex which indicate the higher ability of Pt(II) to denature DNA. These m values are similar to thoes of Pd(II) complex as well as surfactant reported earlier.[25] As we know, the higher the value of $\Delta G°$, the larger the conformational stability of DNA. However, the values of $\Delta G°$ (see Table 2) decrease by increasing the temperature for both complexes. This is based on expecta-

Table 2. Thermodynamic parameters of DNA denaturation by platinum(II) and palladium(II) complexes

Compound	Temperature °C	[a] m (kJ/mol)(mmol/L)$^{-1}$	[b] $\Delta G°_{(H_2O)}$ (kJ/mol)	[c] $\Delta S°_{(H_2O)}$ (kJ/mol K)	[d] $\Delta H°_{(H_2O)}$ (kJ/mol)
[Pt(bpy)(pip-dtc)]NO_3	27	155.9	17.49	0.248	91.9
	37	151.9	14.99	0.248	
[Pd(bpy)(pip-dtc)]NO_3	27	80.1	20.33	~ 0	20.5
	37	86.6	19.00	~ 0	

[a] measure of the metal complex ability to destabilize DNA [b] conformational stability of DNA in the absence of metal complex [c] the entropy of DNA denaturation by metal complex [d] the heat needed for DNA denaturation in the absence of metal complex

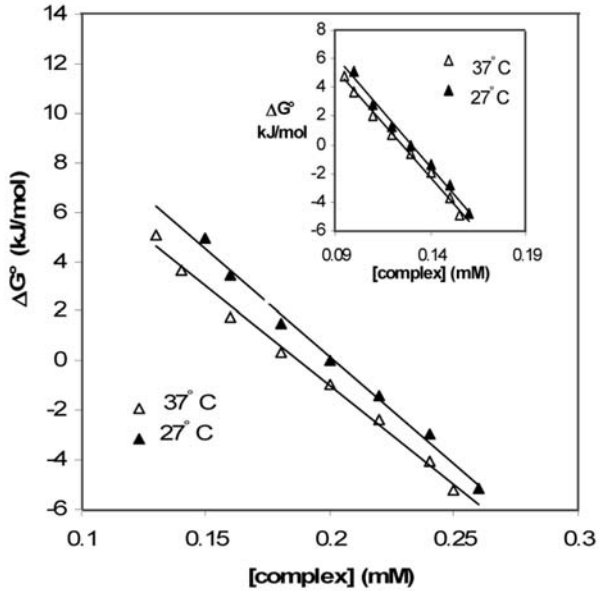


Figure 7. The molar Gibbs free energies plots of unfolding ($\Delta G°$ vs. $[L]_t$) of DNA in the presence of $[Pt(bpy)(pip\text{-}dtc)]NO_3$. Insert: in the presence of $[Pd(bpy)(pip\text{-}dtc)]NO_3$.

tions because in general, most of the macromolecules are less stable at higher temperature.

Another important thermodynamic parameter found is the molar enthalpy of DNA denaturation in absence of metal complexes i.e. $\Delta H°_{(H_2O)}$. For this, we calculated the molar enthalpy of DNA denaturation in presence of each metal complex, $\Delta H°_{conformation}$ or $\Delta H°_{denaturation}$, ($\Delta H°_{con}$ in Fig. 8), in the range of the two temperatures using Gibbs-Helmholtz equation.[50] On plotting the values of these enthalpies versus the concentrations of each metal complex, straight lines will be obtained which are shown in Fig. 8 for $[Pt(bpy)(pip\text{-}dtc)]NO_3$ and the insert for $[Pd(bpy)(pip\text{-}dtc)]NO_3$. Intrapolation of these lines (intercept on ordinate i.e. absence of metal complex) give the values of $\Delta H°_{(H_2O)}$ (see Table 2). These plots show that in the range of 27 °C to 37 °C the changes in the enthalpies in the presence of Pt(II) complex is descending while those of Pd(II) are ascending. These observations indicate that on increasing the concentration of Pt(II) complex, the stability of DNA is decreased while in the case of Pd(II) the opposite trend is observed which may be due to higher tendency of interaction of Pt(II) than Pd(II) complexes with DNA. In addition, the entropy ($\Delta S°_{(H_2O)}$) of DNA unfolding by Pt(II) and Pd(II) complexes have been calculated using equation $\Delta G = \Delta H – T\Delta S$ for each temperature (27 °C or 37 °C) and the data are given in Table 2. These data show that increasing temperature do not change the values of entropies. This might be due to proximity of the temperature range. Also, the metal-DNA complex is more disordered than that of native DNA, because the entropy changes are positive and the extent disorder in Pt(II)-DNA complex is more than Pd(II)-DNA complex (see Table 2).

This again shows that ability of platinum complex in the denaturation of DNA is more than that of the palladium complex. This might be due to that, ligand exchange reaction in Pd(II) complex is 10^5 times faster than that of Pt(II) complex.[48] Moreover, K, apparent binding constant in the interaction of Pt(II) complex with DNA is decreasing with rise in temperature while that of Pd(II) complex increases (Table 1). This indicates that the interaction of Pt(II) complex with DNA is exothermic with higher electrostatic share and Pd(II) complex interact with DNA endothermically with higher hydrophobic share. Similar observations we have already obtained for analogous compounds.[32,51]

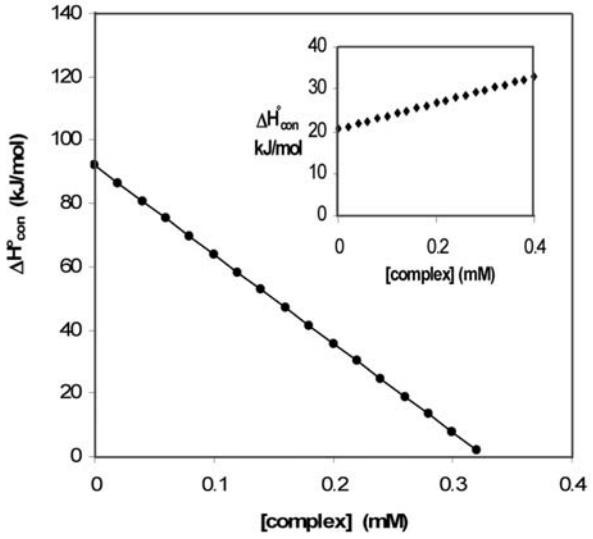


Figure 8. Plots of the molar enthalpies of DNA denaturation ($\Delta H°_{conformation}$ or $\Delta H°_{con}$) in the interaction with $[Pt(bpy)(pip\text{-}dtc)]NO_3$ and the insert with $[Pd(bpy)(pip\text{-}dtc)]NO_3$ complexes in the range of 27 °C to 37 °C

3. 3. 3. Evaluation of Binding Modes

The modes of binding between DNA and the above metal complexes were further investigated by gel filtration and ethanol precipitation experiments.

The solution of each interacted DNA-metal complex (250 μL complex and 50 μL DNA from stock solutions in 2.5 mL buffer) was passed through a Sephadex *G-25* column equilibrated with the same buffer. Elution was done with buffer and each fraction of the column was monitored spectrophotometrically at 321 nm and 258 nm for Pt(II)-DNA system and at 314 nm and 258 nm for Pd(II)-DNA system. The gel chromatograms obtained from these experiments are given in Fig. 9 for $[Pt(bpy)(pip\text{-}dtc)]NO_3$ and the insert for $[Pd(bpy)(pip\text{-}dtc)]NO_3$. These results show that the two peaks obtained at two wavelengths were not clearly resolved which indicate that metal complexes have not separated from DNA and their binding with DNA is strong enough that not readily break.

The above mode of binding between DNA and the metal complexes was further studied by precipitating of

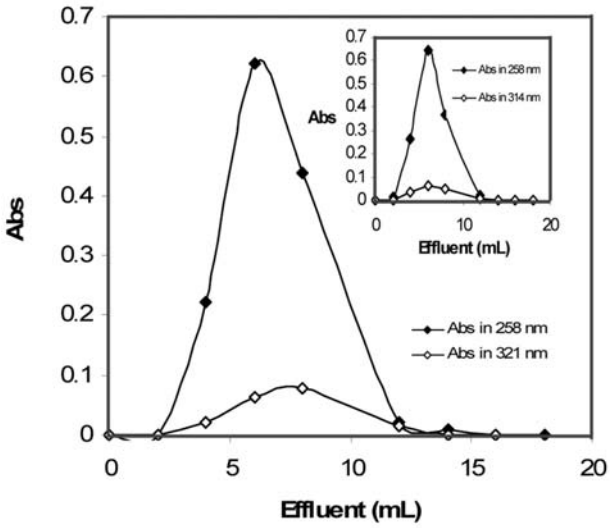


Figure 9. Gel chromatogram of [Pt(bpy)(pip-dtc)]NO_3-DNA complex, obtained on Sephadex G-25 column and the insert for [Pd(bpy)(pip-dtc)]NO_3-DNA complex.

DNA from interacted DNA-metal complexes with absolute ethanol. In this experiment the precipitated DNA was separated out and washed with alcohol. This precipitate was redissolved in Tris-HCl buffer and the solution was monitored spectrophotometrically for DNA at 258 nm, Pt(II) complex at 321 nm and Pd(II) complex at 314 nm. The presence of DNA-metal complex in this solution as observed by spectral method suggests that the above non-covalent interactions could be involved in the bonding. Similar results were obtained for another series of Pt(II) and Pd(II) complexes of 2,2'-bipyridine and amino acids.[52]

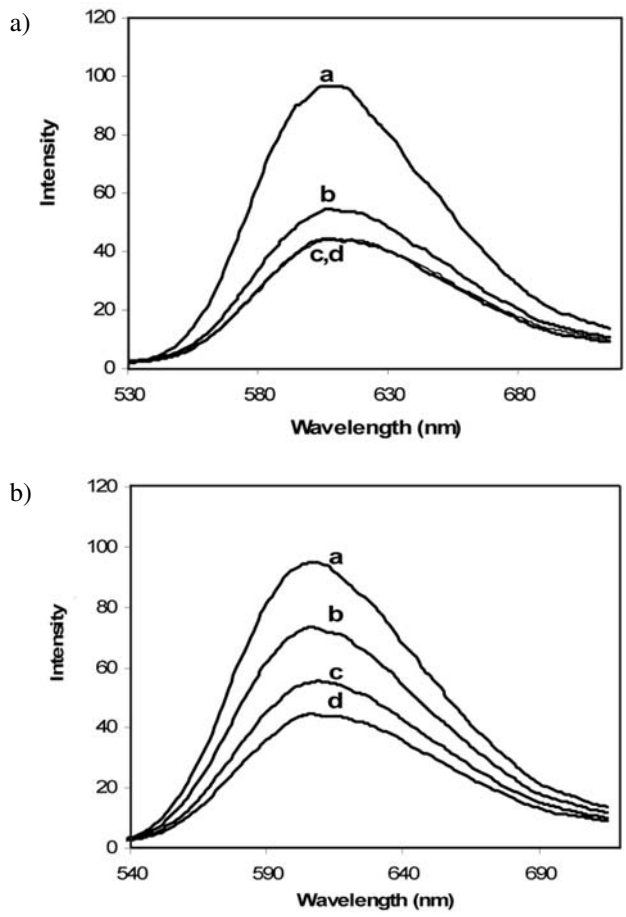


Figure 10. Fluorescence emission spectra of interacted EB-DNA in the absence (a) and presence of different concentrations of Pt(II) complex (A) and Pd(II) complex (B): 0.05 mM (b); 0.1 mM (c); 0.15 mM (d) at 27 °C.

3. 3. 4. Fluorescence Titration Studies

The fluorescence of ethidium bromide (EB) increases after intercalating in DNA. If the complex intercalates into DNA, it leads to a decrease in the binding sites of DNA available for EB-DNA system.[41,42,53] Figs. 10 show fluorescence emission spectra of intercalated EB in DNA, with increasing concentrations of Pd(II) and Pt(II) complexes at 27 °C. Figs. 10 also show a significantly reduction of the ethidium intensity by adding the different concentrations of Pd(II) or Pt(II) complex. (Similar observations were made at 37 °C). These results suggest that the above metal complexes presumably intercalate in DNA. As indicated in Figs. 10, the fluorescence intensity of DNA intercalated ethidium bromide is quenched far better in the presence of Pt(II) complex (Fig. 10(A)) rather than that of Pd(II) complex (Fig. 10(B)). This is probably due to the point that the square planar geometry around Pt(II) complex is less distorted than Pd(II) complex.

Furthermore, involvement of the intercalate bonding in the metal-DNA complex was supported using fluorescence Scatchard analysis.[54] Saturation curves of the fluorescence intensity for a series of DNA-Pt(II)/Pd(II) complexes, at increasing concentrations of each complex (0.05, 0.1 and 0.15 mM) are obtained by adding increasing concentrations of EB (2,4, ... 20 μM). The binding isotherms for the interaction of [Pd(bpy)(pip-dtc)]$^+$ and [Pt(bpy)(pip-dtc)]$^+$ complexes are represented as fluorescence Scatchard plots and are given in Figs. 11(A) and 11(B) respectively. The complexes show competitive inhibition of EB binding (Type-A behavior), in which the slope that is K_{app} (association constant) decreases in the presence of increasing amounts of metal complexes, with no change in the intercept on the abscissa that is n (the number of binding sites per nucleotide).[55] Table 3 lists the values of K and n. The number of binding sites thus remains the same as obtained for DNA-EB complex that is 0.5. This implies that both complexes are intercalating in DNA and thereby competing for intercalation sites occupied by EB. For other planar aromatic compounds similar modes of binding have been seen.[15,55,56]

Table 3. Binding parameters for the effect of platinum and palladium complexes on the fluorescence of EB in the presence of DNA.

	r_f [a]			
complex	**0.00**	**0.83**	**1.66**	**2.5**
[Pt(bpy)(pip-dtc)]NO_3	2.49 [b](0.47)[c]	1.258(0.47)	0.686(0.47)	0.434(0.47)
[Pd(bpy)(pip-dtc)]NO_3	1.862 (0.47)	0.863(0.47)	0.376(0.47)	0.237(0.47)

[a] formal ratio of metal complex to nucleotide concentration. [b] Association constant [c] Number of binding sites (n) per nucleotide

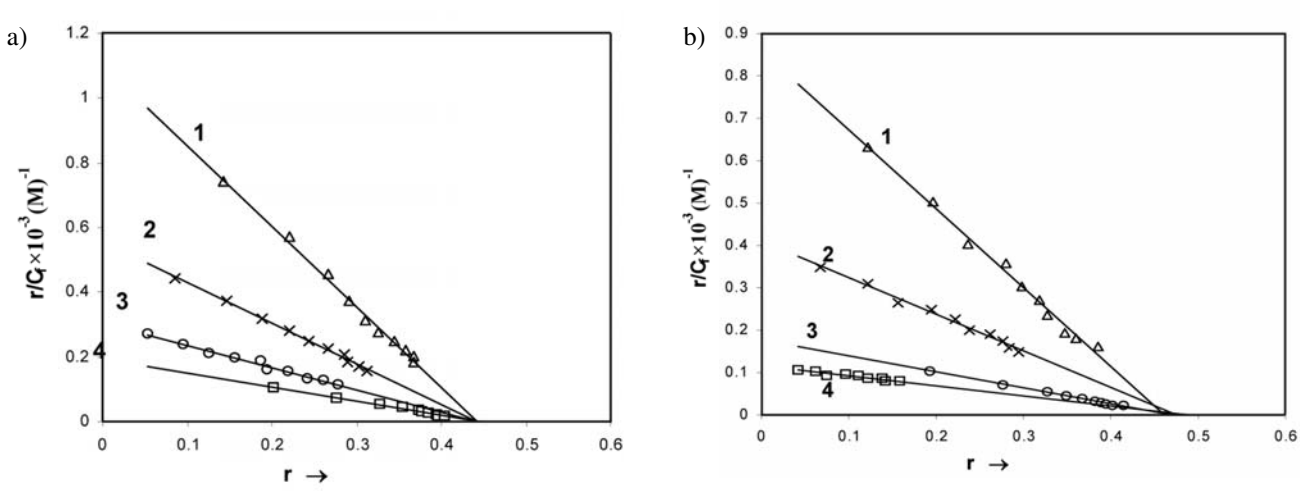


Figure 11. Competition between [Pd(bpy)(pip-dtc)]NO_3 (A) and [Pt(bpy)(pip-dtc)]NO_3 (B) with ethidium bromide for the binding sites of DNA (Scatchard plot).
In curve no. 1, Scatchard's plot was obtained with calf thymus DNA alone. Its concentration was 60 μM. In curves nos. 2, 3 and 4 respectively, 50, 100 and 150 μm metal complex, were added, corresponding to molar ratio [complex]/[DNA] of 0.83, 1.66 and 2.5. Solutions were in 10 mM NaCl, 10 mM Tris-HCl (pH 7.0). Experiments were done at room temperature.

3. 3. 5. Circular Dichroism Studies

In the CD studies, when the complexes produce variations in the molar ellipticity Δθ, this means that some modifications are also produced on the bases stacking and on the magnitude of the winding angle between adjacent base pair, that is on the bending and winding of DNA helix. Thus CD spectra of calf thymus DNA incubated with platinum or palladium complexes recorded at several ratios.[36] The observed CD spectrum of DNA consists of a positive band at 265 nm due to base stacking and a negative band at 247 nm due to helicity, which are characteristics of DNA in the right-handed B-form. The complexes have no CD spectrum when are free in the solution but have an induced CD spectrum when interact with DNA. When our compounds were incubated with DNA, the CD

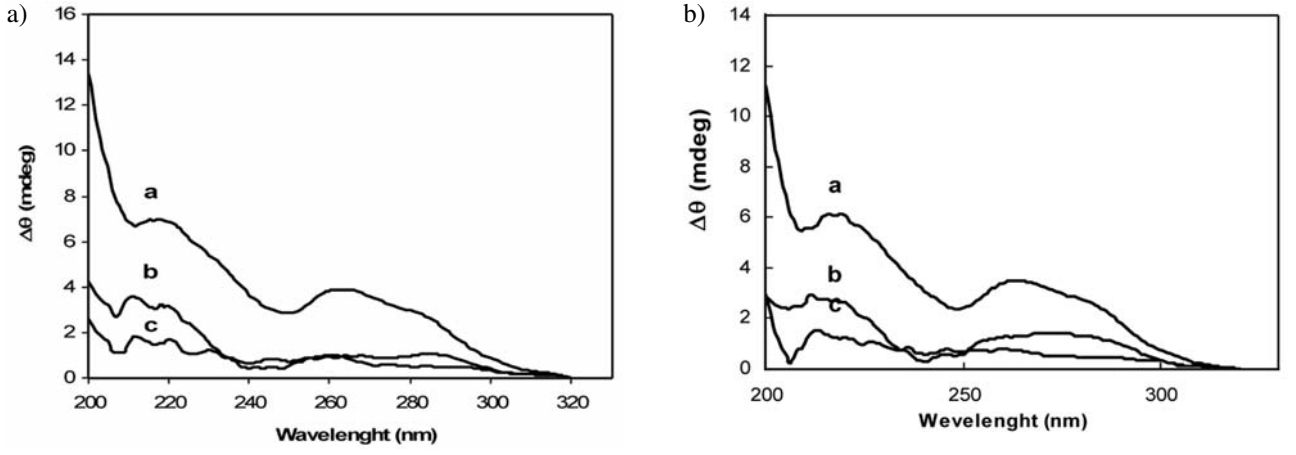


Figure 12. CD spectra of interacted ct-DNA with the Pt(II) complex (A) and Pd(II) complex (B) at 27 °C: a, free ct-DNA (120 μM); b, in the presence of 100 μM complex and c, in the presence of 200 μM complex.

spectra displayed changes of both positive and negative bands (Fig. 12 for [Pt(bpy)(pip-dtc)]NO_3 and the insert for [Pd(bpy)(pip-dtc)]NO_3). Moreover, at concentrations lower than $[L]_{1/2}$, the complexes may induce certain conformational changes in DNA as indicated by significant decrease in the intensities of positive and negative bands (Fig. 12,b). However, at concentrations higher than $[L]_{1/2}$, the precipitate formed and spectra collapsed completely (Fig. 12,c). Similar observations have been seen in the interaction of crocin and crocetin with calf thymus DNA.[57]

4. Conclusion

Two water soluble Pt(II) and Pd(II) complexes of formula [M(bpy)(pip-dtc)]NO_3 have been prepared and characterized by spectroscopic methods. They have been found to be better cytotoxic agents than cisplatin against chronic myelogenous leukemia cell line, K562. They cooperatively bind to calf thymus DNA through intercalation. Both complexes can denature DNA at very low concentrations. Several binding and thermodynamic parameters are also presented.

5. Acknowledgements

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Povzetek

Sintetizirali smo dva kompleksa tipa [M(bpy)(pip-dtc)]NO_3, kjer pomeni M=Pt(II) ali Pd(II), bpy=2,2'-bipiridine, pip-dtc=piperidin ditiocarbamat, ki smo jih okarakterizirali z elementno analizo, uporabo IR, elektronske in ^{1}H NMR spektroskopije ter meritvami električne prevodnosti. Ugotovili smo, da je ciklični ditiokarbamat koordiniran bidentatno k Pt(II) ali Pd(II) centru. Biološko aktivnost kompleksov smo v mikromolarnih koncentracijah testirali napram mielogenskim levkemijskim celicam, K562. Ugotovili smo, da je njihova citotoksičnost (Ic_{50}) nižja od vrednosti za cisplatin. Interkacije proučevanih kompleksov z visoko polimerno obliko DNA iz telečjega timusa (ct-DNA) smo raziskovali z elektronsko absorpcijo, fluorescenco, cirkularnim dihroizmom in ostalimi metodami. Eksperimentalno dobljeni termodinamski parametri in parametri vezanja kažejo, da se kompleksi kooperativno vežejo na DNA verjetno pretežno s interkalacijo. Ugotovili smo, da je tendenca interakcije Pt(II) kompleksa z DNA močnejša kot pa pri Pd(II) kompleksu.