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Research Article

Synthesis, Characterization, Calf Thymus DNA Interaction Studies and Calculation of Thermodynamic Parameters of Platinum Containing N_2S_2 Donor Atoms

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ABSTRACT

Platinum(IV) containing 2-mercaptobenzothiazole disulfide (MBTS) ligand with a disulfide bond was synthesized through the reaction of $[PtAr_2(SMe_2)_2]$ (Ar = Ph) with 2-mercaptobenzothiazole disulfide. Also, the complex containing Ar = *p*-tolyl groups was synthesized and their biochemical properties were compared. In this research, 2-mercaptobenzothiazole (MBT) was constructed during the breaking of the S–S bond in 2-mercaptobenzothiazole disulfide (MBTS) in the reaction, which then act as a sulfur-nitrogen chelating ligand. The synthesized organometallic compounds were identified using various spectroscopy methods such as FT-IR, ^{1}HNMR, elemental analysis, and electronic spectra. Furthermore, the interactions of synthesized compounds and DNA (deoxyribonucleic acid) were studied using different techniques such as electronic and photoluminescence spectroscopy and viscosity investigations. The intrinsic binding constants containing K_b and K_f were calculated for Pt complexes at different temperatures, and the obtained data were compared with each other. The spectra of emission and absorption changed upon the addition of DNA. Moreover, thermodynamic parameters of the interaction between Pt compounds and deoxyribonucleic acid were obtained. The resulting K_b values confirmed the Pt complexes considerably interacted with DNA. The obtained positive changes in enthalpy and entropy confirmed the corresponding hydrophobic interaction. The viscosity of deoxyribonucleic acid was increased during the addition of complexes, indicating the interaction with these compounds. The obtained data indicated the mechanism of intercalative for CT-DNA interactions with Pt complexes. Moreover, comparing the intrinsic binding constants data showed that the interaction of the organoplatinum complex containing phenyl groups with DNA is more than its analogue containing *para*-tolyl groups.

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1. Introduction

Bioorganometallic chemistry is one of the most important fields between two different subjects, containing biochemistry and organometallic compounds with direct metal-carbon bonds. Combination organometallic complexes and biomolecules such as amino acids and DNA, to introduce different new drugs have attracted enormous interest [1-3]. Platinum complexes can be used as therapeutic drugs, and so the medicinal features of these complexes are very significant for their better performance in the treatment of cancer, fungal, viral, and parasitic infections. Platinum is a rare and precious metal ion in organometallic and coordination chemistry, and its compounds have a key role in designing new drugs and catalysts. Platinum complexes can also participate in oxidative addition reactions and increase their oxidation state and coordination number at the same time. Investigations on the biochemical properties of platinum compounds lead to the design and synthesis of many useful compounds for studying their anti-cancer activity [4]. These studies revealed that different factors such as the kind of ligands and metals, coordination number and geometry of the metal centers [5-10] are important for designing new complexes to improve their solubility, anti-cancer activity, and reducing toxicity. Interaction studies of organoplatinum complexes with DNA as an important target for these complexes have attracted a lot of attention. One of the most important platinum complexes in clinical usage is cisplatin. However, its biological uses have some drawbacks and disadvantages, such as resistance against these drug compounds [11, 12]. Interaction of Pt complexes with the other atoms such as sulfur in amino acids and enzymes leads to resistance against the platinum compounds, which can be considered an important side effect. Research works have shown that almost 75–85% of Pt complexes bind proteins and sulfur in biomolecules instead of DNA bases [13]. To prevent binding to natural molecules such as S-donors instead of DNA and reduce drug resistance, new derivatives of platinum complexes have been synthesized and introduced. The formation of adducts with DNA is considered a key step for the anti-cancer activity of these compounds. The N(7) of guanine and adenine in nucleotides are very important in the coordination of platinum complexes with DNA [14-16]. Interaction of Pt complexes with DNA affected conformational and structural changes of double-strand of DNA. According to previous research works, coordination number, geometry, oxidation state, and kind of coordinated ligands are very effective in the interaction of DNA with platinum complexes [17 and 18]. Due to the importance of reducing the side effects of Pt(II) complexes in biochemistry, the design and synthesis of Pt(IV) complexes have attracted much more attention. In this research work, we reported synthesis of di-phenyl Pt(IV) complex **A** containing MBTS. Moreover, interaction of the synthesized complex with CT-DNA was investigated and compared with *para*-tolyl platinum complex **B** analogue which was prepared using similar procedure with a little change from the literature [19].

The investigation of interactions of two organoplatinum complexes **A** and **B** containing S_2N_2 donor atoms with CT-DNA was performed using different methods such as electronic spectra, fluorescence and viscosity measurements. The binding constants of compounds and DNA (K_b and K_f) were calculated and compared with each other. Furthermore, thermodynamic parameters containing, Gibbs free energy, entropy, and enthalpy changes were calculated for the interaction of deoxyribonucleic acid with the Pt compounds. The nature of the binding between the synthesized

platinum compounds and deoxyribonucleic acid was also suggested using the obtained data.

2. Experimental

2.1. Materials and apparatuses

CT-DNA and the other materials and reagents were prepared by Sigma Aldrich and used without any purification. An argon inert atmosphere was applied for synthesizing the complexes. An FP-6200 spectrofluorometer and a Perkin Elmer Lambda 25 spectrophotometer were applied to record photoluminescence and electronic spectra, respectively. A Bruker Avance DPX 300 MHz was used to obtain proton NMR spectra, and tetramethylsilane was applied as an internal standard. FT-IR spectra were obtained using Perkin-Elmer RXI. The melting points were obtained using a Barnstead Electrothermal 9100. Elemental analyses were acquired by Elementar Vario EL III.

2.2. DNA interactions investigations

To investigate the interaction of Pt compounds with DNA, a Tris buffer solution containing (10 mM HCl/50 mM NaCl, pH=7.4) was prepared. The mixture of deoxyribonucleic acid and compounds was permitted to equilibrate for 10 min before the recording of UV-Vis spectra. The stock solution of calf thymus deoxyribonucleic acid in Tris buffer was prepared and kept at 4 °C. The deoxyribonucleic acid concentration in the stock solution was confirmed through the absorbance in the range of 260-280 nm applying the molar absorption coefficient of 6600 M^{-1} cm^{-1} at 260 nm [20]. The concentration of the complexes was kept constant at 1.0×10^{-6} M during the titration with DNA at different concentrations from 0 to 2.2×10^{-5} M. The emission and absorption spectra were obtained for each injection of DNA solution after 10 minutes.

2.3. Preparation of the Precursors

Cis-[$PtAr_2(SMe_2)_2$] (Ar = Ph, and *p*-tolyl) were prepared through the reaction of [$PtCl_2(Me_2S)_2$] with extra amounts of organolithium in dried ether according to the reported method [21 and 22].

2.4. Synthesis of [$PtPh_2$ MBT], A

A solution of 2-mercaptobenzothiazole disulfide (0.0129 g, 0.0388 mmol) in 8 mL chloroform was used in the reaction with *cis*-[$PtPh_2(Me_2S)_2$] (0.0184 g, 0.0389 mmol) in chloroform (8 mL) and the reaction continued for 48 h in dark and cold place about 10 °C. After filtering the precipitates formed and evaporation of the solvent a pale orange precipitate was acquired and purified using n-hexane several times (Yield: 42%). Pale orange, m.p: 300 ºC (decomp.). Anal. Calc. for [$C_{26}H_{18}N_2PtS_4$] (**A**): (M.W.: 681.77), 45.81, C; 2.66, H; 4.11%., N. Found: 45.57, C; 2.80, H; 4.02%, N. FT-IR (KBr, υ/cm^{-1}): (C-S) 800, (C-H) 724, (C=N) and (C=C) 1632, 1452, 1415, (Pt-N) 503. ^{1}HNMR (300 MHz, $CDCl_3$, δ/ppm): 7.94 (d, $^3J(H^5H^4)$= 8.12 Hz, H^4 of MBT), 7.78 (d, $^3J(H^6H^7)$=8.12 Hz, H^7 of MBT), 7.68 (t, $^3J(H^{4,6}H^5)$=8.12 Hz, H^5 of MBT), 7.61 (m, H^6 of MBT), 7.45 (d, $^3J(H^mH^o)$=8.12 Hz, ($^3J(PtH^o)$=44.52 Hz, H^o of Ph), 7.36 (m, H^m of Ph), 7.26 (m, H^p of Ph).

2.5. Synthesis of [Pt(p-tolyl)$_2$ MBT], B

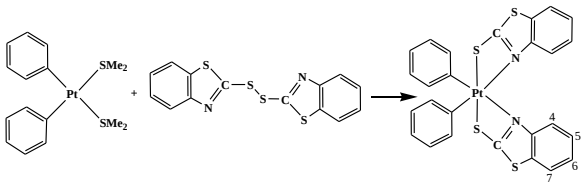
A chloroform solution (8 mL) of 2-mercaptobenzothiazole disulfide (MBTS) (0.0133 g, 0.0400 mmol) was added to the *cis*-[Pt(*p*-tolyl)$_2(Me_2S)_2$] in chloroform (6 mL) (0.0201 g, 0.0400 mmol). After filtering the precipitates formed and evaporation of the solvent a pale orange precipitate was acquired and purified using n-hexane several times (Yield: 44%). Orange, m.p.: 360 ºC (decomp.), Anal. Calc. for [$C_{28}H_{22}N_2PtS_4$] (**B**): (M.W.: 709.82), 47.38, C; 3.12, H; 3.95%, N. Found: 47.12, C; 3.03, H; 3.79%, N. FT-IR (KBr, υ/cm^{-1}): (C-S) 800, (C-H, Me) 3058, (C-H) 724, (C=N) and (C=C) 1583, 1449, 1427, (Pt-N) 433. ^{1}HNMR (300 MHz, $CDCl_3$, δ/ppm): 2.45 (6H, s, CH_3 of *p*-$CH_3C_6H_4$), 7.94 (d, $^3J(H^5H^4)$=7.75 Hz, H^4 of MBT), 7.78 (d, $^3J(H^6H^7)$=7.82 Hz, H^7 of MBT),

7.70(m, H^5 of MBT), 7.60 (t, $^3J(H^{5,7}H^6)$=8.42 Hz, H^6 of MBT), 7.41 (t, 4H, $^3J(H^mH^o)$=8.12 Hz, ($^3J(PtH^o)$=45.42 Hz, H^o of *p*-tolyl), 7.21(t, $^3J(H^oH^m)$=8.12 Hz H^m of *p*-$CH_3C_6H_4$).

3. Results and discussion

3.1. Synthesis, identification and DNA interactions

New Pt(IV) compound **A** including 2-mercaptobenzothiazole disulfide (MBTS) as a ligand was synthesized using the reaction of *cis*-[$PtPh_2(Me_2S)_2$] with 2-mercaptobenzothiazole disulfide (MBTS) in a 1:1 stoichiometry and MBT was constructed during the breaking of the S–S bond in 2-mercaptobenzothiazole disulfide (MBTS), which then act as a sulfur-nitrogen chelating ligand (Scheme 1). Complex **B** was prepared by the reaction of *cis*-[Pt(*p*-tolyl)$_2$(Me_2S)$_2$] with MBTS [19]. Octahedral geometry was suggested for the synthesized Pt(IV) complexes. Six positions of coordination around the platinum center were occupied by two phenyl or two *para*-tolyl groups, and two nitrogen and two sulfur atoms of 2-mercaptobenzothiazole as a chelating ligand and also, aryl groups are in the *cis* position with each other [19 and 23]. Different methods such as ^{1}HNMR, FT-IR, conductometry, and elemental analysis were used to characterize the synthesized complexes. Biological investigations of complex **A**, [$PtPh_2$MBT], indicated remarkable DNA interaction *versus* **B**.



Scheme 1. Synthesis of the Pt(IV) complex with phenyl groups (**A**).

Due to the importance of anticancer properties of Pt complexes, in this article, the interaction with deoxyribonucleic acid was investigated using spectroscopy methods and viscosity measurements to compare its interaction with organoplatinum analogue **B**. The results of this study and the comparison of intrinsic binding constants can lead to introduce of two organoplatinum complexes that can be biochemically important. It can be suggested the synthesized organoplatinum complexes may be potential anticancer agents.

3.2. IR Spectra

Significant peaks in IR spectra related to (C=N) and (C=C) which were appeared in 1632-1415 cm^{-1}. Also, the peak observed at 724 cm^{-1} can be attributed to C-H bending [24]. Moreover, new bands with low intensity can be seen at 430-504 cm^{-1}, which is related to the formation of Pt-S and Pt-N bonds in the complexes [25]. Furthermore, the C-S band appeared at 800 cm^{-1} (Fig. 1).

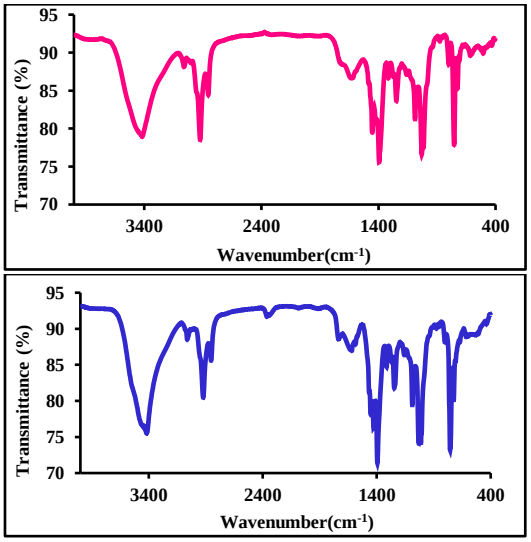


Fig. 1. FT-IR spectra for **A** and **B** complexes.

3.3. Proton NMR Spectra

Proton NMR spectra were applied to better characterize of the compounds **A** and **B**. ^{1}HNMR of compounds **A** and **B** were recorded in $CDCl_3$-d_1. The H^4 of MBT was deshielded by N as an electronegative atom and appeared in down field at higher chemical shifts at 8.12 and 7.94 ppm for **A** and **B** complexes, respectively (Fig. 2). The Pt metal center with I=1/2 and abundance percentage of 34% can couple with the *ortho* hydrogens of the aryl rings to represent a singlet signal and two satellites. Coupling constants were calculated as

^{3}J(PtH^o)=44.52 Hz, H^o of Ph) and ^{3}J(PtH^o)=45.42 Hz, H^o of *p*-tolyl), at 7.45 ppm and 7.41 ppm for **A** and **B** complexes, respectively. Moreover, *ortho* hydrogen can couple with H^m and reveals a doublet with ^{3}J(H^{m}H^o)=8.12 Hz [19 and 26].

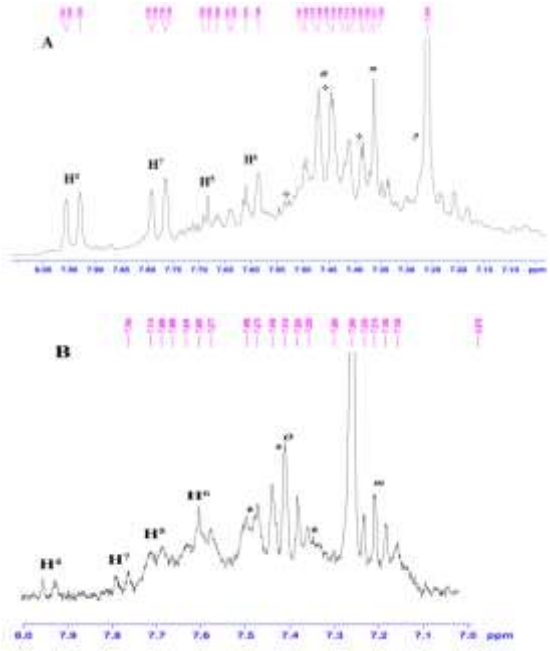


Fig. 2. Expansion of ^{1}HNMR spectra of **A** and **B** complexes in the aromatic region.

3.4. UV/Vis Spectra

Absorption spectra of the Pt compounds were investigated in chloroform (Fig. 3). The peaks at 240 nm and 237 nm with high absorbance were appeared due to the π→π* transitions of the aromatic rings in **A** and **B** compounds, respectively [27]. Furthermore, the peaks at 300 and 299 nm with lower intensities, were attributed to n→π* transitions in complexes, respectively [28 and 29]. MLCT bands cannot be observed in **A** and **B**, due to the higher oxidation state of complexes [30].

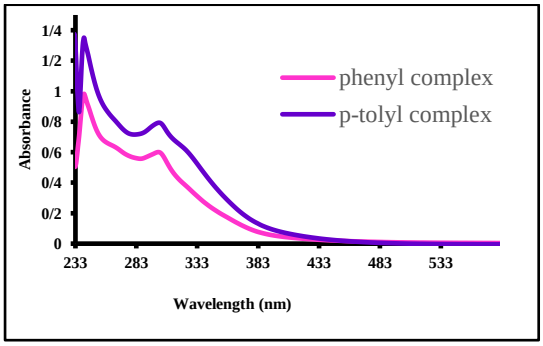


Fig. 3. Electronic spectra of **A** and **B** complexes in chloroform.

3.5. Emission Spectra

Fluorescence spectra of the Pt(IV) compounds were studied in chloroform. Complexes **A** and **B** displayed distinguishable emission bands. The bands appeared at 358 nm and 392 nm with $\lambda_{ex.}$ = 260 and 316 nm are related to the π→π* intraligand fluorescence for **A** and **B** complexes, respectively. Also, complex **A** represented an emission band with higher intensity in comparison to **B**. Moreover, the broad fluorescence band can be considered for different transitions like (ILCT) [31].

4. CT-DNA interaction investigations

4.1 Absorption spectra

To investigate the interaction of Pt compounds with CT-DNA, the UV/Vis spectroscopy technique was used [32]. It was concluded that the intensity of absorption was reduced upon increasing CT-DNA. These changes are depicted in Figs. 4 and 5. The most changes were observed at 305 nm for **A** and **B** complexes, respectively, due to the intraligand π–π* transitions. In this experiment, the concentration of Pt compounds was kept constant (1.0×10^{-6} M), and increasing injection of different values of DNA (0–14 µM and 0-28 µM) showed decreasing absorbance intensity (hypochromic) and a little change of the maximum wavelength (small red shift) for these complexes during the corresponding interaction. This evidence revealed the mechanism of intercalative for CT-DNA interactions with Pt compounds [33]. For calculating the K_b (binding constant) of the compounds and deoxyribonucleic acid, the following equation was used:

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

In this equation, [DNA] showed the concentration of deoxyribonucleic acid in different injections, ε_a is the ratio of A/[complex], ε_b is the extinction coefficient in the fully bound form and ε_f represents the extinction coefficient of the free form of the compound. The K_b was obtained by plotting

[DNA]/($\varepsilon_a - \varepsilon_f$) against [DNA] and calculating from the ratio of slope to intercept [34] (Table 1). The K_b (at 27 °C) for Pt compounds was calculated as 7.13×10^4 and 5.33×10^4 for **A** and **B** complexes, respectively. The obtained data for K_b indicated that Pt complex **A** has stronger binding with CT-DNA compared to the organoplatinum complex **B** due to a little more steric hindrance of aryl groups [35]. The calculated K_b values confirmed that the Pt compounds significantly interacted with deoxyribonucleic acid. Furthermore, the obtained data were comparable with those studied previously for the Pt compounds with intercalation mechanisms [36 and 37].

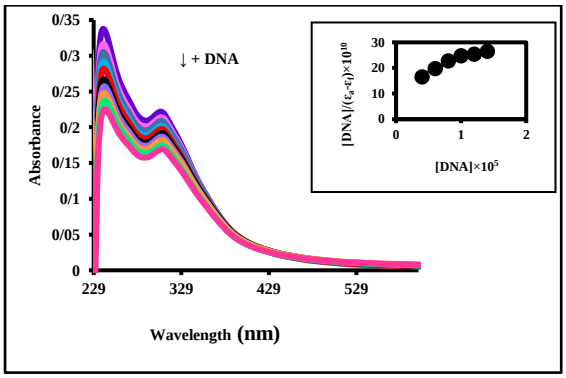


Fig. 4. Variations in the electronic spectra of complex (**A**) during the titration with different concentrations of the deoxyribonucleic acid.

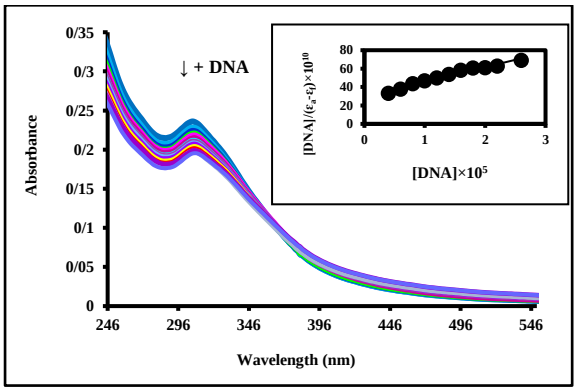


Fig. 5. Variations in the electronic spectra of complex (**B**) during the titration with different concentrations of the deoxyribonucleic acid.

4.2 Emission spectra investigations

To further study the binding mode of the Pt complexes and deoxyribonucleic acid interaction, emission spectra were also used. To investigate the corresponding fluorescence spectra, a constant concentration of the Pt complexes was titrated with different concentrations of DNA at 25.0 °C and pH=7.4. Changes in fluorescence spectra within the titration with deoxyribonucleic acid are displayed in Figs. 6 and 7. In these figures, increasing emission intensity can be observed upon the addition of the solution of deoxyribonucleic acid in Tris-HCl buffer. The binding constants of the interaction (K_f) were calculated using the below equation [38].

$\log(F_0\text{-}F)/F = \log K_f + n\log[DNA]$

F_0 and F are the fluorescence intensities of the fluorophore in the presence and absence of various concentrations of deoxyribonucleic acid, respectively. The values of K_f for **A** and **B** complexes were calculated as 5.49×10^4 and 5.52×10^2 at 27 °C, respectively (Table 1).

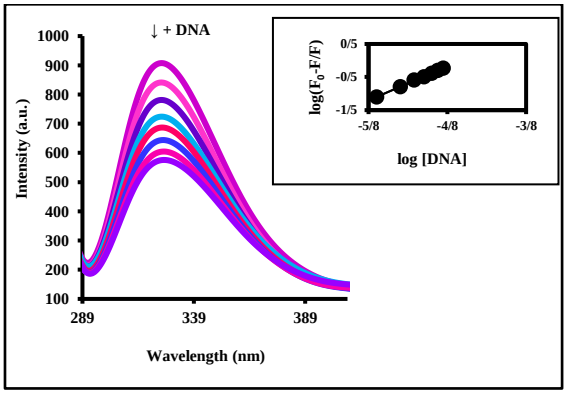


Fig. 6. Variations in the fluorescence spectra of complex (**A**) with increasing concentrations of the deoxyribonucleic acid.

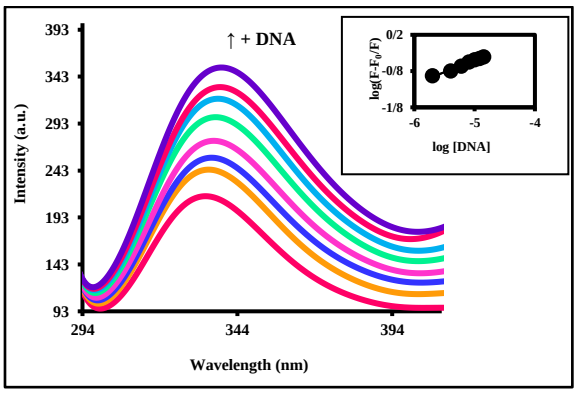


Fig. 7. Variations in the fluorescence spectra of complex (**B**) (λ_{ex}=258) with increasing concentrations of the deoxyribonucleic acid.

4.3 Calculation of thermodynamic parameters

Different kinds of intermolecular forces were reported between the complexes and DNA. These

forces can be divided into four categories: electrostatic interaction, non-covalent interactions, hydrophobic, and van der Waals forces [39]. To determine the intermolecular forces, thermodynamic parameters including ΔH, ΔS, and ΔG were calculated using the obtained binding constants and van 't Hoff equations [40].

$$\ln K = -\Delta H/RT + \Delta S/R$$

$$\Delta G = \Delta H - T\Delta S$$

The intercept and slope of linear graph of lnK *vs* 1/T show ΔS and ΔH, respectively. The graphs of van 't Hoff are displayed in Figs. 8 and 9. The data are indicated in Table 2.

Table 1. Intrinsic binding constants of **A** and **B** complexes at pH=7.4.

	T (K)	K_b	K_f
Complex **A**	290	4.02×10^4	5.49×10^4
	300	7.13×10^4	
	310	9.65×10^4	
Complex **B**	290	2.67×10^4	5.52×10^2
	300	5.33×10^4	
	310	7.20×10^4	

Table 2. Thermodynamic data for binding of the deoxyribonucleic acid and **A** and **B.**

Complex	T (K)	ΔG (kJ mol^{-1})	ΔH (kJ mol^{-1})	ΔS (J $mol^{-1}K^{-1}$)
B	290	-24.63	37.41	213.96
	300	-26.77		
	310	-28.91		
A	290	-25.62	32.84	201.61
	300	-27.64		
	310	-29.65		

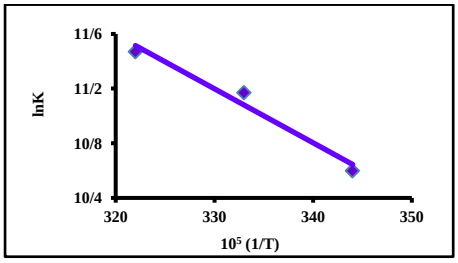


Fig. 8. The van't Hoff plot for binding of deoxyribonucleic acid A.

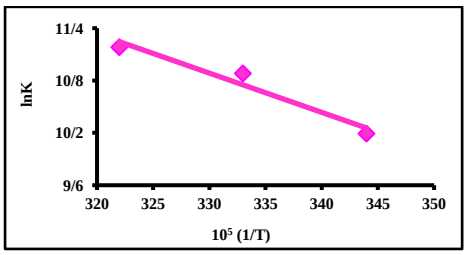


Fig. 9. The van't Hoff plot for binding of deoxyribonucleic acid B.

Three different types of interaction forces based on the obtained signals for thermodynamic parameters are proposed: positive entropy and enthalpy changes show hydrophobic forces; 2) negative entropy and enthalpy changes show hydrogen bonds and van der Waals interactions and 3) negative enthalpy changes and positive entropy changes indicate electrostatic interactions [41]. The data of thermodynamic parameters are indicated in Table 2. The positive obtained values of ΔS and ΔH for platinum complexes **A** and **B** indicated hydrophobic forces between the Pt compounds and deoxyribonucleic acid. Also, with increasing temperature ΔG become more negative (Table 2).

4.4 Viscosity investigations

Viscosity studies were applied to study the length change of DNA during interaction with compounds as a simple technique. Intercalative mode makes elongation of the double helix of DNA and increases its viscosity because of increasing in the separation of purine and pyrimidine bases to embed the complex between the bases [42,43]. Viscosity changes of the deoxyribonucleic acid solution in the

presence of organoplatinum are depicted in Fig. 10. The figure showed the increased viscosity of deoxyribonucleic acid because of the interaction with the complexes. The obtained data confirmed the intercalation mechanism and was consistent with the spectroscopic data.

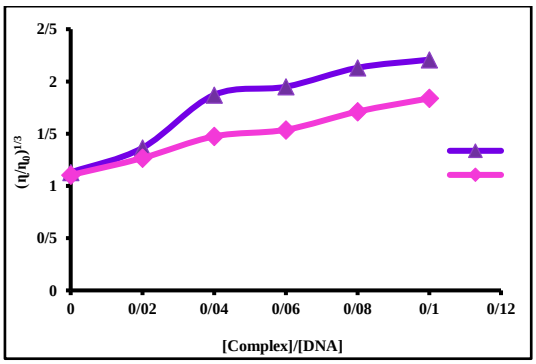


Fig. 10. Viscosity changes for the interaction of DNA and **A** and **B** complexes.

5. Conclusions

Di-phenyl Pt(IV) complex containing N_2S_2 donor atoms was synthesized through the cleavage of S–S bond of MBTS ligand. During this reaction, coordination number and oxidation state of platinum were increased. Also, *p*-tolyl Pt(IV) complex was synthesized in order to compare the interactions of complexes with DNA. Investigations of interactions with DNA were performed using different methods such as electronic and photoluminescence spectroscopies and viscosity method. The intrinsic binding constants were calculated at three different temperatures containing 17, 27 and 37 °C. The results of these studies suggested an intercalation mechanism for the interaction of deoxyribonucleic acid and Pt complexes. Moreover, thermodynamic data were obtained through the van't Hoff equation. The resulting positive amounts of ΔS and ΔH displayed hydrophobic forces for the interaction between the synthesized Pt complexes and deoxyribonucleic acid. Comparing the binding interaction constants data (K_b and K_f) showed the interaction of **A** containing diphenyl groups with DNA is more than **B** due to a little steric of phenyl groups in compare to *p*-tolyl.

Acknowledgments

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Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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