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The interaction between a zinc(II) phthalocyanine compound bearing octakis phenoxyacetamide substituents and calf thymus DNA

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Abstract: In this study, a zinc(II) phthalocyanine compound (**PcZn**) substituted with octakis phenoxyacetamide was synthesized and characterized. Calf thymus DNA (CT-DNA) was used to determine the DNA binding properties of the zinc(II) phthalocyanine compound. This interaction of CT-DNA with **PcZn** was investigated using electronic absorption spectra, emission spectroscopy, a thermal melting temperature study, cyclic voltammetry, and viscosity measurements in a Tris-HCl buffer solution at a pH of 7.2. The findings showed that zinc(II) phthalocyanine interacts with calf thymus DNA via partial intercalative binding mechanisms. In addition to the techniques above, a gel electrophoresis study was also carried out to verify the interaction of CT-DNA with the **PcZn** complex. The results demonstrated that the octakis phenoxyacetamide-substituted zinc(II) phthalocyanine complex binds to CT-DNA via a partial intercalative binding mode.

Key words: DNA binding, absorption spectroscopy, gel electrophoresis, thermal melting, phthalocyanine compound

1. Introduction

Many people suffer from deadly forms of cancer, and many patients worldwide die each day because of this disease.[1] It is known that cancer is one of the most serious health issues across the world and it is anticipated that the disease will continue to kill many people in the coming years.[2] Because of this, cancer therapy and studies on inhibiting cancers have acquired major significance. Therefore, the design of therapeutic cancer medicine is very important to combat this deadly disease.

Metallophthalocyanine compounds have many significant chemical features because of their structures. These compounds are highly useful in different types of medical applications.[2,3] Recently, in the published literature, many scientists have paid particular attention to the cell cycle and the DNA molecule.[3] The interaction of drugs with DNA molecules is a very important approach in the design of cancer medicines.[4] Therapeutic chemical compounds have very significant properties that let them bind to DNA or hinder the resting of DNA molecules.[4] The binding of drugs to DNA molecules can alter the structure of DNA and the duplication or transcription of DNA.[5] The binding of drug agents to DNA can end variations in DNA replication.[6] This interaction is thought to be where the major binding modes of tiny drug molecules to DNA occur through intercalative and nonintercalative binding mechanisms.[5,6] There are several major DNA binding modes of metallophthalocyanine compounds to DNA. These binding mechanisms are intercalation or external binding.

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Intercalation binding mode is the mechanism where small molecules penetrate themselves in between DNA base pairs. External binding, such as electrostatic, dipole-dipole, and hydrogen binding interactions between DNA and intercalators, is a substantial binding mechanism. Hydrogen binding and electrostatic attraction are the major binding forces in groove binding.[2,4]

In the recent literature many studies have been conducted on the interaction of phthalocyanine compounds with DNA. The phthalocyanine compounds bearing different groups interact with DNA via intercalation or nonintercalation binding mechanisms.[7] The chemical reactivities of phthalocyanine compounds can be changed by altering the peripheral ligands and central metal cations.[8] In addition, phenoxyacetamide-substituted zinc(II) phthalocyanine compounds are biologically effective molecules. A zinc(II) phthalocyanine compound bearing octakis phenoxyacetamide ligands can have potential applications, for example in cancer therapy[9] and in antibacterial[10] treatment.

The interaction of phthalocyanine compounds with DNA molecules to prevent the spread of cancerous cells has gained great attention. In the published literature, there are many interesting studies carried out in this area. These kinds of compounds can be very useful for increasing the anticancer therapy research.[2] In the published literature, the binding properties of tetracationic zinc and metal-free phthalocyanine compounds have been studied.[11] The findings of these studies demonstrated that water-soluble quaternized zinc and metal-free phthalocyanine compounds interact with DNA molecules.[11,12] According to another study of a water-soluble morpholine zinc(II) phthalocyanine complex, it was shown to interact with calf thymus DNA (CT-DNA) through intercalation binding mechanisms.[13] The results of another study demonstrated that cationic phthalocyanines can interact with DNA via nonintercalation binding mechanisms.[14] The studies of tetra-substituted zinc(II), manganese(III), and copper(II) phthalocyanine compounds indicated that the compounds bind to DNA through intercalative binding modes.[15] A copper hexamethyleneimino-ethoxy-substituted phthalocyanine compound was studied. The compound showed that it has a strong interaction with DNA.[16] Additionally, the binding properties of a cationic photobleachable cobalt and of palladium phthalocyanines with CT-DNA were also reported. The results of this study showed that the phthalocyanine compounds bind to DNA molecules.[16]

In this study, the DNA binding activities of the previously synthesized octakis phenoxyacetamide-substituted zinc(II) phthalocyanine compound (**PcZn**)[17] with CT-DNA were studied using absorption spectral titration, emission spectroscopy, a thermal melting temperature study, viscosity measurements, cyclic voltammetry, and gel electrophoresis techniques.

2. Results and discussion

2.1. Synthesis and characterization

As shown in Figure 1, an octaphenoxyacetamidephthalocyaninatozinc(II) compound was synthesized and characterized using NMR, FTIR, UV/Vis, and elemental analysis[17], and results showed good agreement with earlier results of studies in the published literature.

2.2. Absorption studies

In general, the interaction of a tiny metal complex with DNA molecules relates to the changes in hyperchromism or hypochromisms and the blue shift or red shift in the wavelengths of the absorption spectra.[6] The binding mechanisms, such as intercalation, usually relate to hypochromisms and red shifts.[6] In general, weak binding shows no change in absorbance levels.[6,8] The intercalative binding mode of a tiny compound leads to

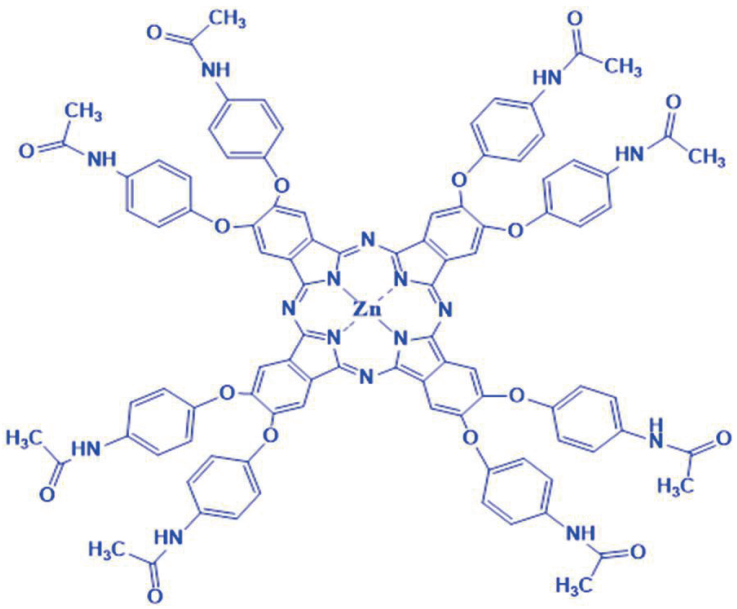

Figure 1. The structure of the zinc(II) phthalocyanine compound (**PcZn**).

hypochromism with blue shifts or red shifts because of a very powerful arrangement of aromatic parts and DNA base pairs.[18,19]. Absorption spectral titration is a very important method in the study of the interaction of metal complexes with DNA molecules.

In the current study, the absorption spectra of the octakis phenoxyacetamide-substituted zinc(II) phthalocyanine compound (**PcZn)** were studied using a UV/Vis spectroscopy technique. The absorption titration spectra of **PcZn** (15 μM) were conducted between wavelengths of 300 and 800 nm in the absence of CT-DNA. The electronic spectra of **PcZn** showed the characteristic absorption spectra in Q bands at 680 nm and 660 nm wavelengths in DMF, as shown in Figure 2. In order to investigate the interaction of **PcZn** with CT-DNA, absorption titrations were carried out in the presence of CT-DNA, and they showed changes in absorbance bands in a Tris-HCl buffer solution at a pH of 7.2. The absorbance bands of **PcZn** dropped in intensity without changing the conformation of bands after increasing the amounts of CT-DNA. The changes in the absorbance bands of **PcZn** during titration with CT-DNA are indicated in Figure 2. The drop in intensities of absorption spectra of **PcZn** demonstrates the existence of intercalative binding modes between CT-DNA and the **PcZn** compound. When the concentrations of CT-DNA were increased from 0.5 to 5 μM, a hypochromism was formed with change in the wavelengths of the absorption spectra. A considerable change in hypochromism demonstrates that **PcZn** interacts with CT-DNA. These results showed that **PcZn** binds to CT-DNA through a partial intercalation binding mode. The binding constant (Kb) of **PcZn** with CT-DNA was calculated using the Wolfe–Shimer equation (Eq. (1)). The Kb constant of **PcZn** was computed to be 1.43×10^6 M^{-1}. The Kb constant demonstrates that **PcZn** interacts with CT-DNA through a partial intercalation binding mechanism.[20]

In addition to the absorption titration experiments, related binding studies were carried out to verify the interaction of **PcZn** with CT-DNA using an ethidium bromide (EB) compound, which is widely used as an intercalator to determine DNA binding mechanisms.[20] In this technique, the chemical compounds bind to the DNA molecule through intercalative binding mechanisms as EB changes sides in the EB and DNA complex, observed by the intensities of the absorption spectra.[21] As indicated in Figure 3, absorption spectra for the

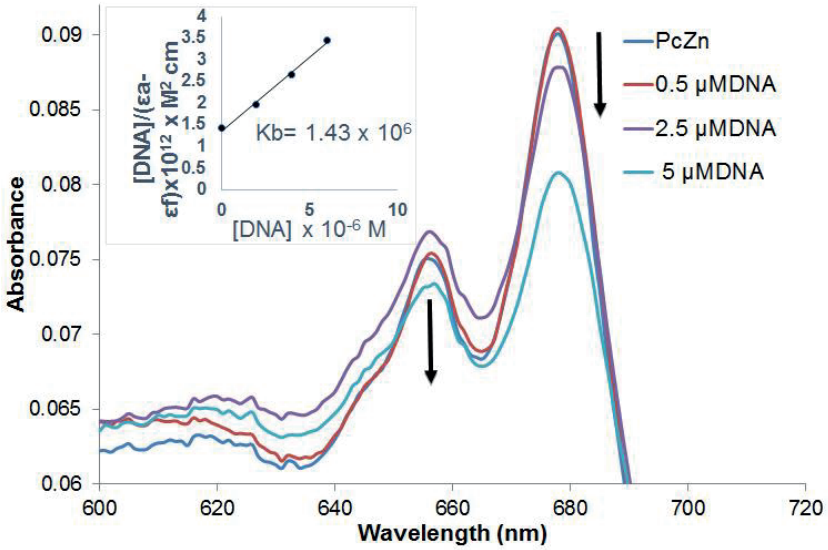

Figure 2. Electronic spectra of **PcZn** in a Tris-HCl buffer at a pH of 7.2 with increasing amounts of CT-DNA. Arrows indicate the absorption spectra changes with increasing amounts of CT-DNA.

related binding experiments were performed with wavelengths of 510 nm to 400 nm. The intensity of absorption spectra for EB around 460 nm dropped and changed with increasing concentrations of CT-DNA. When 1 to 5 μM of **PcZn** was combined with the CT-DNA and EB complex, the intensities of the absorption spectra were increased, as shown in Figure 3. The results demonstrated that **PcZn** changed sides when comparing EB to CT-DNA and EB. As a result, it is shown that **PcZn** binds to CT-DNA via partial intercalative binding mechanisms.

2.3. Emission titration studies

The emission titration technique is widely used in studies of the interaction of DNA molecules with metal complexes. This technique provides more information on the interaction between metal complexes and the

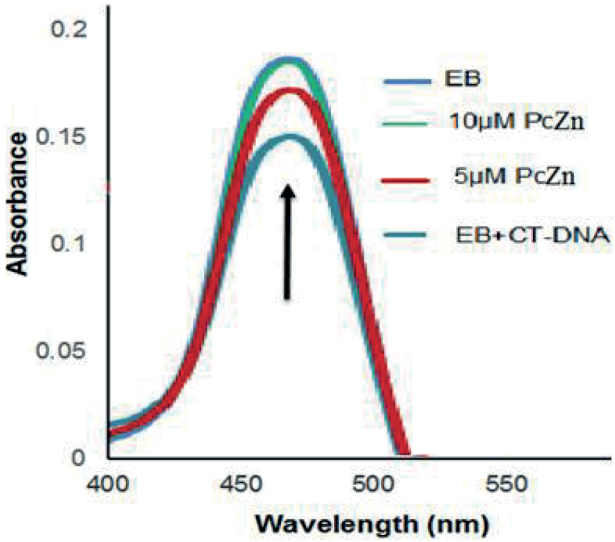

Figure 3. Absorption titration results for free EB and EB bonded to CT-DNA with increasing amounts of **PcZn** in the absence and presence of **PcZn.**

DNA molecule. Emission titration experiments are performed to investigate the binding properties between the DNA molecules and small metal compounds.[22] The emission titration experiments were carried out in the absence and presence of CT-DNA to determine the interaction of CT-DNA with **PcZn**. The zinc(II) phthalocyanine compound produced emission spectra in the absence of CT-DNA in a Tris-HCl buffer at a pH of 7.2 with a peak appearing at a wavelength of approximately 670 nm (black line), as indicated in Figure 4. An increase in intensity of the emission spectra of **PcZn** was monitored in the presence of CT-DNA in Tris-HCl at a pH of 7.2 with respect to the intensity of emission spectra of the original **PcZn**, as shown in Figure 4. For increasing concentrations of CT-DNA (5 μM (purple line), 10 μM (red line), and 15 μM (green line)), **PcZn** produced emission bands at wavelengths of approximately 605, 610, and 645 nm. The emission spectra in the absence and presence of DNA were different. The emission titration spectra experiments were repeated several times and almost the same results were obtained. We speculate that the difference of emission spectra of in the absence and presence of DNA may be due to the type of binding mechanism of CT-DNA with PcZn and the structure of the **PcZn** compound. The intensities ($\boldsymbol{I}$) of fluorescence titration spectra of the **PcZn** compound in a Tris-HCl buffer solution (pH 7.2) at room temperature in the absence and the presence of calf thymus DNA increase, changing upon increasing amounts of CT-DNA.[23] These findings demonstrated that the phthalocyanine compound binds to the CT-DNA molecule.[23]

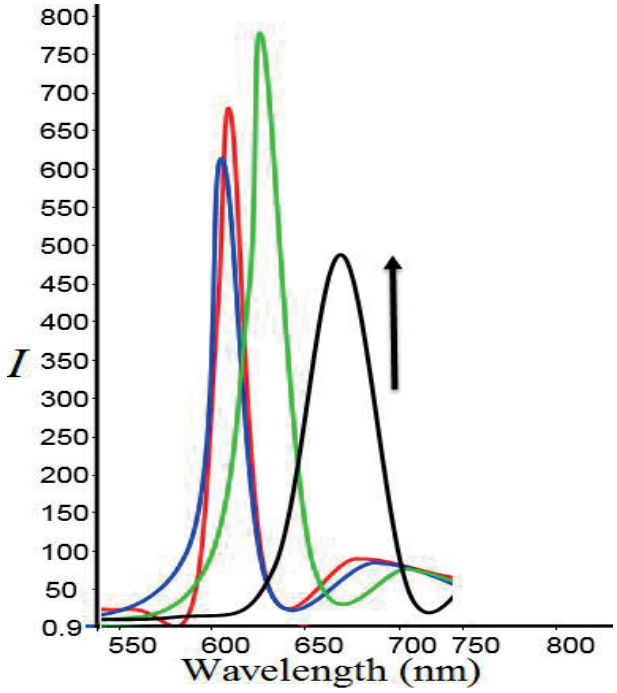

Figure 4. Fluorescence titration spectra of **PcZn** compound (10 μM) (black line) in a Tris-HCl buffer solution (pH 7.2) at room temperature in the absence and the presence of CT-DNA. Arrow indicates the intensity changing upon increasing amount of CT-DNA, where $\boldsymbol{I}$ shows intensity.

2.4. Thermal melting temperature study

In this study, thermal melting temperature (T_m) experiments were conducted on CT-DNA in the absence and presence of **PcZn** to investigate the binding activities of the **PcZn** complex with CT-DNA. The melting

temperature of the DNA provides significant knowledge regarding the properties of the DNA double-stranded helix with changes in temperature at a 260 nm wavelength.[21] The intercalative binding mechanisms of metal complexes with the DNA molecule increase the melting temperatures because of the efficiency of binding, whereas nonintercalative binding modes for metal complexes with DNA drop or stay the same.[22] In the absence and presence of **PcZn**, the thermal melting temperatures of CT-DNA were determined using UV/Vis spectroscopy. The results of the thermal melting temperature study are shown in the Table. In the absence of the compound, the thermal melting temperature of CT-DNA was found to be 74.83 °C, as shown in Figure 5. The melting temperature in the presence of **PcZn** was found to be 82.76 °C, as in Figure 5. These findings showed that the **PcZn** compound interacts with CT-DNA via partial intercalation binding mechanisms because of an increase in thermal melting temperature values.

Table. The thermal melting temperatures (T_m) of CT-DNA in the absence and presence of **PcZn** complex.

	Thermal melting temperature (T_m)
CT-DNA	74.83 °C
CT-DNA + **PcZn**	82.76 °C

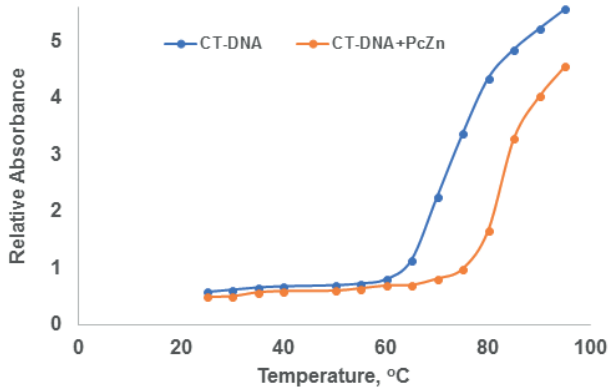

Figure 5. Thermal melting temperature profile of CT-DNA in the absence of **PcZn** (blue line) and in the presence of **PcZn** (orange line).

2.5. Cyclic voltammetry study

Cyclic voltammetry is one of the techniques commonly applied to investigate the interaction of metal complexes with DNA molecules. The technique provides significant information for the initial assessment of the study of electronic absorption spectra.[23] When metal compounds interact with the DNA molecule, the peak potential and peak current of the complexes shift in the presence of the DNA molecule.[24]

In this study, a cyclic voltammetry experiment was performed to investigate the interaction of **PcZn** with CT-DNA in the absence and presence of CT-DNA in a Tris-HCl buffer at a pH of 7.2. The findings are shown in Figure 6. In the absence of CT-DNA (blue line), the **PcZn** compound produced a couple of peaks related to **PcZn** that indicated the cathodic (EPc) peak potential and anodic peak potential (EPa). The EPc and EPa were found to be approximately –0.41 V (EPc) and –0.39 V (EPa) for **PcZn**, as indicated in Figure 6.

The cyclic voltammetric peak current decreased considerably in the presence of CT-DNA. The reduction of peak current upon the addition of CT-DNA is attributed to the small diffusion[25,26] of the **PcZn** compound

binding to the DNA molecule. The EPc and EPa were observed to be –0.45 V (EPc) and 0.15 V and 0.80 V (EPa) for **PcZn**, as indicated in Figure 6 (red line) at a fixed concentration of **PcZn**. This showed a successive drop in peak current. The peak potential and the peak current of the compound showed that CT-DNA binds to the **PcZn** compound via a partial intercalation binding mode.

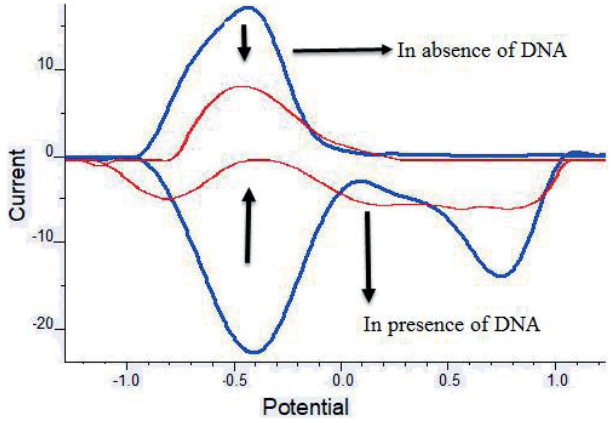

Figure 6. Cyclic voltammetry diagram for **PcZn** in the absence of CT-DNA (blue line) and the presence of CT-DNA (red line). Arrows indicate drop in cathodic peak potential (EPc) in the presence of CT-DNA.

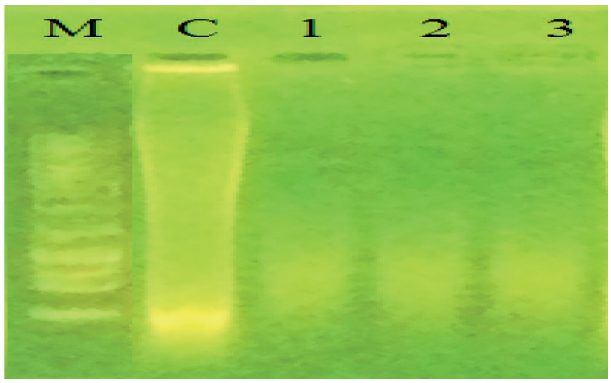

Figure 7. The gel electrophoresis diagram for CT-DNA in a Tris-HCl buffer solution at a pH of 7.2 in the absence and presence of **PcZn**. Lanes 1 to 3 belong to CT-DNA (15 μM) and **PcZn** (5, 10, 20 μM), respectively. C is the control CT-DNA and M is the DNA marker.

2.6. Gel electrophoresis study

In this part of the study a gel electrophoresis technique[27,28] was used to study the interaction of the zinc(II) phthalocyanine compound with CT-DNA molecules at room temperature in the absence and presence of **PcZn**. The binding activities of CT-DNA with **PcZn** were investigated to confirm the impact of variable amounts of **PcZn** on the DNA. It is very clear that the intensities of the DNA bands were considerably decreased for increasing concentrations of **PcZn**. The bands of CT-DNA were visualized under UV light, as indicated in Figure 7. The drop in the intensities of bands of CT-DNA after the binding of **PcZn** to CT-DNA was due to the degeneracy of the DNA double-stranded helix.[7,29] The findings of the gel electrophoresis method demonstrated that the Zn(II) phthalocyanine compound binds to the CT-DNA molecules. The changes in the bands of the DNA were observed in lanes 1 to 3 when these bands were compared to the control DNA (**C**), as indicated in Figure 7. Lane C represents the control CT-DNA and lanes 1, 2, and 3 belong to the CT-DNA and **PcZn** complex**.** Lane M shows the DNA marker. When the interactive ability of **PcZn** with CT-DNA was compared to lane C, it indicated that the **PcZn** compound shows an effective binding activity with CT-DNA. It was seen that the C band did not show any considerable change. As a result, the findings of the gel electrophoresis experiments explicitly demonstrated that **PcZn** binds to CT-DNA.

2.7. Viscosity measurements

Viscosity studies are predominantly used to investigate the interaction of DNA molecules with metal complexes via the increase and decrease in viscosity of DNA after adding the metal complexes. Increasing viscosity demonstrates that metal complexes interact with DNA molecules through intercalative binding mechanisms that lead to the degeneracy and extension of the DNA molecule. In contrast, a decrease in viscosity signifies

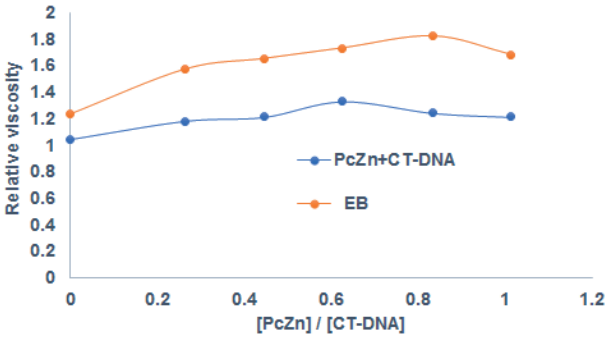

Figure 8. The relative viscosity of CT-DNA with increasing concentrations of **PcZn** and EB.

that metal complexes bind to DNA molecules via nonintercalative binding mechanisms. The packing of the ligands of metal compounds between the base pairs of DNA leads to a considerable shift in the DNA's molecular structure.[30,31] Viscosity experiments were performed to investigate the interaction of **PcZn** with CT-DNA, as shown in Figure 8. In the absence of CT-DNA and **PcZn**, the relative viscosity of EB was measured. Later, in the presence of EB, the relative viscosity of CT-DNA and **PcZn** was monitored. As shown in Figure 8, the viscosity of CT-DNA increased on successive additions of the **PcZn** complex. This result showed that the **PcZn** complex interacts with CT-DNA via partial intercalative binding mechanisms.

2.8. Conclusions

In this study, the interaction of a **PcZn** complex with CT-DNA was investigated using electronic absorption spectroscopy, emission titration, a thermal melting temperature study, viscosity measurements, cyclic voltammetry, and gel electrophoresis methods. Electronic absorption spectra, emission titration, and thermal melting temperature studies demonstrated that the zinc(II) phthalocyanine complex interacts with CT-DNA through a partial intercalative binding mechanism. The decrease in absorbance intensities of CT-DNA and **PcZn** supports the partial intercalative binding mechanism. The results of cyclic voltammogram studies demonstrated successive decreases in peak current and the change in peak potential of the **PcZn** complex indicated that CT-DNA interacts with the zinc(II) phthalocyanine complex. Similarly, the findings of the gel electrophoresis study showed that **PcZn** interacts with CT-DNA. It is concluded that **PcZn** shows DNA binding activities in a Tris-HCl buffer. The results demonstrated that the zinc(II) phthalocyanine complex could be a potential candidate in treatment agents for cancer due to its DNA interaction.

3. Experimental

3.1. Chemicals and equipment

In this study, octaphenoxyacetamidephthalocyaninatozinc(II)[17] was used to investigate its interaction with CT-DNA. Tris-HCl and CT-DNA samples were provided from Sigma-Aldrich and sodium chloride was purchased from Merck. All the used chemical reagents and solvents were of biochemical grade. Therefore, they were used without purification. The solutions of CT-DNA were prepared using Milli-Q water, which was obtained from a commercial company.

Electronic absorption spectra for the binding activities of CT-DNA with the zinc(II) phthalocyanine complex (**PcZn**) were performed using Agilent Technologies Cary 60 UV/Vis spectroscopy. Emission spectroscopy

studies were carried out using PerkinElmer LS fluorescence spectroscopy. An Ivisumstat electrochemical analyzer was used for cyclic voltammetric (CV) studies. The Thermo Scientific Owl Electrophoresis System was used for the gel electrophoresis method. Thermal melting temperature was studied at 260 nm using UV/Vis spectroscopy. An Ubbelohde viscometer was used for viscosity measurements. All methods were performed in a Tris-HCl buffer solution at a pH of 7.2 at room temperature.

3.2. Synthesis of compounds

3.2.1. The preparation of 1, 2-bis(phenoxyacetamide)-4,5-dicyanobenzene compound

The 1,2-bis(phenoxyacetamide)-4,5-dicyanobenzene compound was prepared and characterized according to the literature procedure.[17]

3.3. Electronic absorption spectra and emission spectroscopy studies

Electronic absorption titrations were performed between 300 and 800 nm. Absorption titration spectra for the Zn(II) phthalocyanine complex at a fixed concentration of **PcZn** in a Tris-HCl buffer at a pH of 7.2 were obtained by increasing the concentration of CT-DNA from 0.5 to 5 μM. For determining dilution affects, the results were compared to those of control titrations with Tris-HCl buffer solution instead of the DNA.[32] For the phthalocyanine complex, the binding constant (K_b) was obtained from the Wolfe–Shimmer equation:[33]

$$[DNA]/(\varepsilon a - \varepsilon f) = [DNA]/(\varepsilon b - \varepsilon f) + 1/(Kb(\varepsilon b - \varepsilon f)) \quad (1)$$

Here, εa, εf, and εb are the absorption coefficients. Kb is calculated from the ratio of slope to intercept. The plot is obtained from $\frac{[DNA]}{(\varepsilon b \varepsilon f)}$ versus $[DNA]$.

In addition to the above study, further verifying the interaction of CT-DNA with octaphenoxyacetamide-phthalocyaninatozinc(II), competitive DNA binding studies of **PcZn** with EB were performed using electronic absorption spectra. Initially, the EB and CT-DNA complex was produced by adding EB to a CT-DNA sample. Later, **PcZn** was added to the EB + CT-DNA complex. Then the changes in absorbance of EB and CT-DNA were monitored.[30]

The emission titration studies were conducted between 500 and 750 nm after excitation. Emission titration spectra experiments were performed with increasing amounts of CT-DNA added to a **PcZn** sample at a fixed concentration. After adding DNA to **PcZn**, the solution was allowed to reach chemical equilibrium for a certain period of time before the measurements were recorded.[34,35]

3.4. Cyclic voltammetry study

For this experiment, for cyclic voltammetric studies, an Ivisumstat Electrochemical Interface Electrochemical Analyzer was used at a glassy carbon. An Ag/AgCl reference electrode, a glassy carbon working electrode, and a platinum wire counter electrode were used in this part of study. A single-compartment three-electrode cell system of 10 mL capacity was used to perform the experiments.[35] In this technique, all CV measurements were recorded in a Tris-HCl buffer solution at a pH of 7.2.

3.5. Thermal melting temperature study

The study of thermal melting temperature (T_m) of CT-DNA is a measurement of stability of double-stranded DNA with temperature.[13] In the absence and presence of the **PcZn** complex, the study of T_m was recorded

for CT-DNA. CT-DNA and **PcZn** sample in buffer at a pH of 7.2 consisting of NaCl solution were heated from 25 °C to 95 °C progressively every 5 min. The values of electronic absorption titration were recorded after each heating of the sample. The T_m measurements were performed at 260 nm[36–39] using Agilent Technologies Cary 60 UV/Vis spectroscopy.

3.6. Viscosity measurement studies

In this technique, the viscosity measurements of **PcZn** with CT-DNA were recorded using an Ubbelohde viscometer that was put in a water bath at a constant temperature around 30 °C. CT-DNA concentration was kept constant while the concentrations of **PcZn** and EB were increased gradually to investigate the DNA binding activity of **PcZn** with CT-DNA. The approximate flow time was measured by testing the sample three times. In the absence and presence of the compound, the solution viscosity was calculated with $\eta_i = (t_i - t_0)/t_0$, where η_i is the viscosity value of CT-DNA, t_i is the flow time of the **PcZn** and CT-DNA solution, and t_0 is the flow time of the buffer solution at a pH of 7.2. The values of findings were indicated as $(\eta/\eta_0)^{1/3}$ vs. [**PcZn**]/[DNA], where η is the viscosity of CT-DNA in the presence of the compound and η_0 is the viscosity of the DNA.[40]

3.7. Gel electrophoresis experiment

In this method, gel electrophoresis experiments were carried out in 20 mM of Tris-HCl buffer system at a pH of 7.2 consisting of 20 m NaCl. The binding of **PcZn** to CT-DNA was studied using agarose gel in Tris-HCl buffer EDTA (TBE) solution. The CT-DNA (15 μM) and **PcZn** (5 to 20 μM) samples were loaded with the dye in the buffer solution. The gel electrophoresis measurements were performed at 80 V for 3 h in TBE solution. Then, in gel electrophoresis experiments, CT-DNA bands were visualized using a UV lamp. In this work, the Thermo Scientific Owl Gel Electrophoresis system and a 1-kb DNA standard marker were used for the experiments.[41–45]

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