afterward adding of the DNA sample. In this study, also the binding constant ($K_b$) was computed by using Wolfe-Schimer equation [24]. With increases in amount of the DNA, the absorbance values of Pc4 gradually dropped. The dropping of absorbance values demonstrated that the complex Pc4 interacts with CT-DNA and also three main absorbance bands were observed with hypochromism, and these bands were located at around 362, 625 and 682 nm related to the red shift as illustrated in Figure 2. The complex showed hypochromism, which reducing in absorption spectra is called as a hypochromism and incrementing in absorbance values is defined as a hyperchromism. Hypochromicity is related to a mild bathochromic shifting generally originated from the intercalation binding mechanism, comprising a packing interaction among a chromophore compound and DNA base pairs [13]. The Pc4 complex is quite planar central part and it can likely interact with the DNA by an intercalating binding mechanisms. The obtained results from absorption titration method showed that Pc4 complex binds to the DNA molecule through an intercalative mechanism, and the $K_b$ value for Pc4 was obtained as 2. 17 x 10 6 $M^{-1}$ as indicated in Figure 2. The calculated Kb value for the complex also demonstrated that Pc4 binds to the DNA with the intercalation mechanisms.

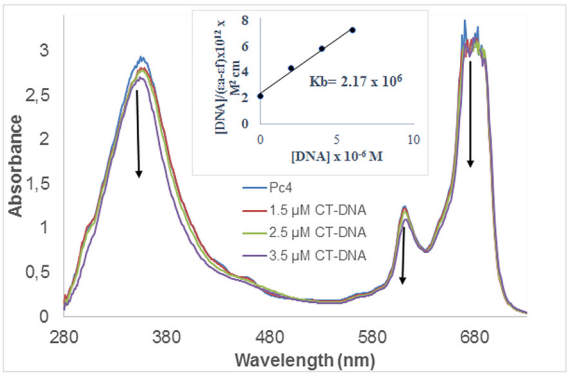


**Figure 2.** Electronic titrations of Pc4 in the buffer system on addition of calf thymus DNA. [Pc4] = 20µM and [CT-DNA] = 0–3.5µM. The arrows represent the dropping in absorption intensities on increasing the calf thymus DNA amount.

## The emission titration studies

The emission study is frequently used to determine the investigating of DNA-drug binding activity because this technique is a very sensitive to explain DNA interaction probes and it can provide further information about the intercalation of molecular compounds [25]. In this present study, the DNA binding activity of Pc4 to the DNA was investigated using emission titration spectra. When the complex Pc4 was interacted with the DNA, it was seen that the intensities of emission spectra were dropped gradually as indicated in Figure 3. The dropping in emission intensity demonstrated that the complex binds to the DNA using hypochromic mechanism. It is seen in Figure 3 that the complex Pc4 gave a strong emission spectra in the absence of CT-DNA at pH 7.03 at around 457 nm. The strong fluorescence spectra could be originated from the ligand [25]. On the adding of the DNA, the decreases in intensity of emission spectra for Pc4 were illustrated in Figure 3. The findings from this technique demonstrated that Pc4 interacts with the DNA via the

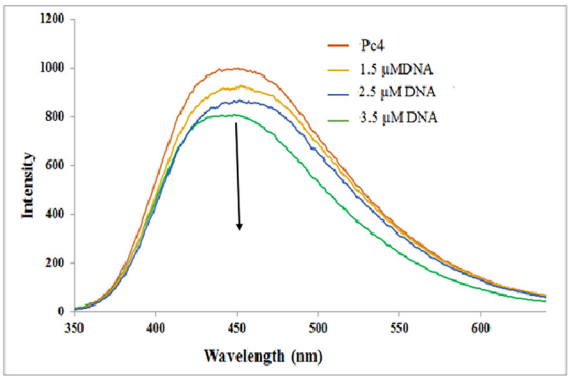


**Figure 3.** Fluorescence titrations of Pc4 in the buffer solution in the absence and presence of the DNA. [Pc4] = 20µM and [CT-DNA] = 0 – 3.5µM. The arrow displays the intensity change on mounting the DNA concentration.

mechanism of intercalative binding.

## The viscosity studies for DNA Binding

In addition, the above methods, the viscosity technique was also applied to search DNA binding activity of Pc4. The viscosity method could supply more information about DNA interaction mechanisms that is very precise to change in length of DNA molecule. Generally, when a chemical complex inserts into the base pairs of DNA, DNA molecule elongates because the base pairs of DNA are decomposed to adapt the attached ligand, which causes to increases in the DNA viscosity [25]. On the other hand, chemical compounds react with DNA by non-intercalative binding mode may decrease the length of DNA by twisting the DNA [26] but, non-intercalative and electrostatic binding mechanisms cause to very a little impact upon DNA viscosity.

In the present study, the changing in the DNA viscosity in the presence of Pc4 was monitored. When the complex reacts with the DNA molecule in an intercalative binding mechanisms, it has an impact upon the DNA viscosity. It is indicated in Figure 4 that on the adding of Pc4 to the DNA, the surge in the DNA relative viscosity was observed. The increasing in CT-DNA viscosity that could relate to the reacting of Pc4 with the DNA molecule. The obtained findings proved that Pc4 attaches to the DNA via intercalative binding mechanisms with a strong affinity.

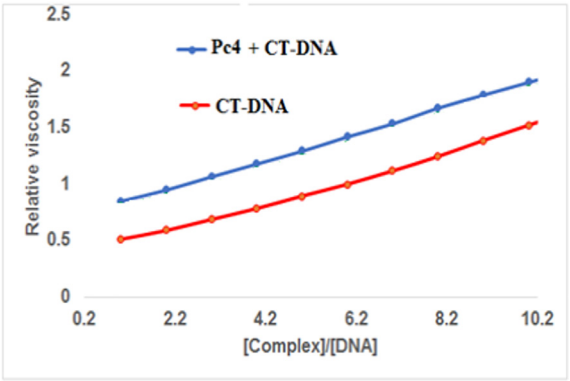


**Figure 4.** The viscosity study of Pc4 indicating the impact of surging amounts of the complex upon the DNA viscosity.

### Thermal denaturation studies for DNA binding

The solution of CT-DNA + Pc4 was incubated at the certain time for each 5 °C and the values of absorption titration were recorded. The recorded absorbance values versus temperature chart were plotted as illustrated in Figure 5. It is observed in Figure 5 that thermal melting temperature of CT-DNA was recorded as approximately 70.40 °C, and the Tm value of CT-DNA + Pc4 was observed as 78.63 °C. Mostly, if the thermal melting variation of the DNA sample and CT-DNA + Pc4 is great, the DNA binding activity is believed to be an intercalation. If this value is not great, the DNA binding mechanism is considered as a non-intercalation. The obtained result from the viscosity measurement demonstrated that Pc4 reacts with the DNA by an intercalative binding mechanism.

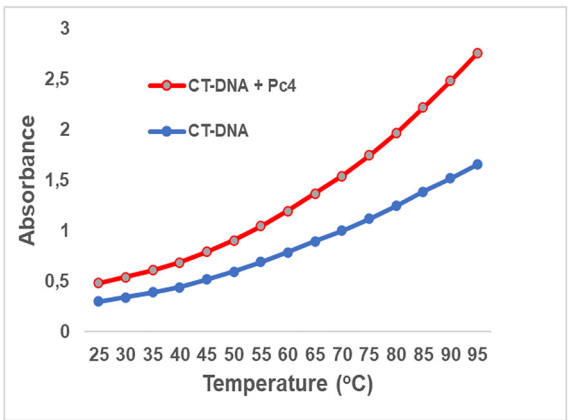


**Figure 5.** $T_m$ study of the DNA showing impact of the compound on melting temperature of the DNA. The Tm measurements of the DNA (blue line) and DNA + Pc4 (red line).

### The agarose gel electrophoresis study for DNA binding

In the literature, the binding activities of the compounds to CT-DNA were studied using agarose gel electrophoresis technique analyzing of the impact of different amounts of the Pd(II) compounds on CT- DNA. In this study, results showed that the intensities of CT-DNA bands obtained for the compounds after interacting with CT-DNA were dropped, as compared with control CT-DNA band. The drop in the intensities of the DNA bands observed after interacting of the compounds with the DNA is thought to be damage deformation of CT-DNA [27].

In addition to above studies, DNA binding activity for Pc4 complex was studied using agarose gel electrophoresis technique. First of all, the migrating of CT-DNA + Pc4 was recorded afterwards GelRed staining as shown in Figure 6. The lane M refers the ladder of DNA, and the lanes 1, 2 and 3 refer the compound Pc4, respectively with varied amounts of the DNA. The amount of DNA surged from the lanes 1 to 3. The concentration of Pc4 was hold fixed at 25 μM, whereas the amounts of DNA was changed from 15 to 25 μM. Then, the intensity of the DNA band was recorded in the absence of Pc4 and also the band intensity of the DNA was monitored in the presence of Pc4. It is clearly seen that in Figure 6, the DNA bands intensities were dropped and the migrating of CT-DNA bands were slightly vanished because of the DNA neutralization. The results demonstrated that Pc4 interacts by the DNA.

## CONCLUSION

In the present study, the objective of the study was to explain the binding activity of the metal complex to CT-DNA for potential use of an anticancer medicine. First of all, the zinc (II) phthalocyanine compound was synthesized and analyzed with electronic spectra, FT-IR and NMR instruments according to the reported procedure in literature. The DNA binding activity of the complex was evaluated using with various methods such as absorption spectra, fluorescence titrations, melting point, viscosity

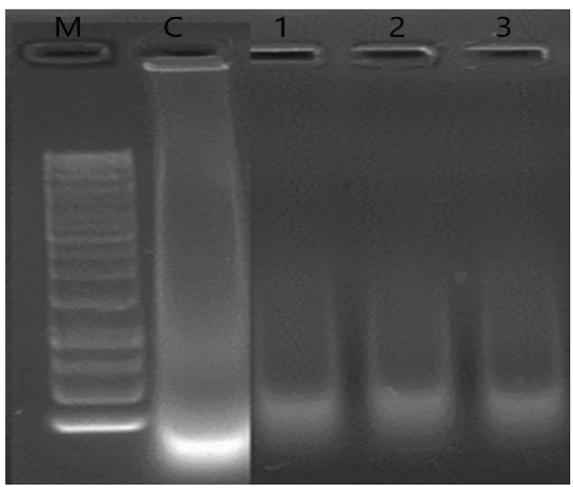


**Figure 6.** Agarose gel electrophoresis studies for Pc4 complex at pH 7.03 on surging the amount of CT-DNA. Lane M: DNA ladder, Lane C: control CT-DNA, Lanes 1 to 3: (Pc4 (20 μM) + CT-DNA (15, 20, 25 μM), respectively.

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