



## Spectroscopic Evaluation of DNA Binding Activities of Copper (II) Phthalocyanine Complex Consisting of Tetrakis-(4-Tritylphenoxy) Ligand

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### Highlights

- Cu (II) phthalocyanine compound was examined.
- The ability of the Cu(II) phthalocyanine compound to interact with DNA was evaluated.
- The DNA binding mode of the complex was determined.

### Article Info

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### Abstract

The structure and basic properties of Cu(II) phthalocyanine compound possessing tetrakis-(4-tritylphenoxy) group were elucidated in a past study with the help of absorption and infrared spectroscopic equipments. The electronic spectra, emission spectroscopy, gel agarose electrophoresis and thermal melting were employed to reveal the DNA interaction functions of this complex at changing concentrations of CT-DNA. In this experiment, the binding constant for the Cu(II) phthalocyanine compound which contains the tetrakis(4-tritylphenoxy) group was computed to be  $1.53 \times 10^6 \text{ M}^{-1}$ . The data obtained from absorption and fluorescence spectroscopic studies revealed that the CuPc compound reacted with CT-DNA through an intercalating mechanism. Well as the above methods, melting temperature and electrophoresis were also employed to analyse the interaction feature of CuPc with DNA. The interaction of the CuPc compound with DNA was also confirmed by data from melting temperature and electrophoresis experiments. Within the framework of the results obtained, it is predicted that CuPc compound may be a possible cancer therapeutic agent.

## 1. INTRODUCTION

The DNA molecule is a significant targeting molecule which is utilized for treatment of many genetic disorders such as carcinoma. In recent years, it has been observed that there has been a significant increase in biological research due to the significant effects of transition metal-based compounds in cancer treatment [1]. The mechanism by which a drug binds to DNA provides very important preliminary information about the possibility of using the chemical compound as an anticancer drug in cancer treatment [2]. Within this framework, the very broad spectral and electrochemical activities of transition metal-based compounds contribute significantly to the interaction activities of these compounds with DNA [3,4].

Under normal conditions, the presence of too much active oxygen radicals, which arise as a result of metabolic reactions, causes oxidative destruction of biomolecules including DNA. Consequently, it may increase the likelihood of some very critical illnesses as for instance cancer, cardiovascular and neurodegenerative, occurring very quickly. Depending on their chemical structural properties and the root of oxidative stress, metal-based compounds can serve as drugs [5,6].

Phthalocyanine compounds are highly functional molecules, and research on the derivatives of these compounds has been continuing for many years. Due to the thermal and chemical properties of phthalocyanines, they have applications in different fields such as sensors and photodynamic cancer therapy [7]. Additionally, due to the photosensitizing and antioxidant properties of phthalocyanine compounds, research is ongoing for photodynamic therapy in anticancer treatment [8-10]. Because of its capacity to interact with DNA, phthalocyanine complexes have attracted great attention in the last decade for their

application in cancer treatment approaches [11-17]. In studies published in the literature, different complexes of transition metal phthalocyanine compounds were synthesized to be used as chemotherapeutic active substances [18]. The intercalative binding mechanism of manganese (III) and copper (II) phthalocyanine with 3-pyridine-3-propyloxy substituents was previously described by Biyiklioglu et al [19]. The DNA bonding activity and cleavage assay of Cu(II) and Zn(II) phthalocyanines were investigated by Amitha and Vasudevan [20]. The results from these studies show that the compounds bind intercalatively with DNA. However, it is understood that copper phthalocyanine complexes still do not receive the expected importance in this research field. Encouraging the potential anticancer activities of phthalocyanines and their copper complexes, it reveals the importance of investigating the interactions of phthalocyanines and copper complexes with DNA. In the current study, the interaction activities of previously synthesized peripheral tetra-substituted Cu(II) phthalocyanine [21] with CT-DNA (Calf Thymus-Deoxyribonucleic Acid) were investigated using absorption spectroscopy, fluorescence, techniques of melting point and electrophoresis.

## 2. MATERIAL METHOD

### 2.1. Chemicals and Equipment

The some properties and structure of the Cu(II) phthalocyanine complex containing tetrakis-(4-tritylphenoxy) group have been elucidated in a previous study [21]. All the chemicals used as containing NaOH at pH 7.05 and stored at 4 °C. In addition, the stock copper (II) phthalocyanine complex prepared by dissolving DMF, an organic solvent, for use in the study was kept at 25 °C. If necessary, all the solutions used in the study were prepared by diluting the desired sample consumables in this scientific research were procured from Sigma-Aldrich. CT-DNA sample that was utilized as reagent in the research was prepared using Tris-HCl buffer solution volumes using Tris-HCl buffer. Instrumental devices such as UV/Vis, thermal melting point, emission, and electrophoresis were used in this study to determine the interaction activities of the complex. In addition to the above devices, Thermo Scientific Owl Electrophoresis system was preferred in this study to perform gel electrophoresis tests.

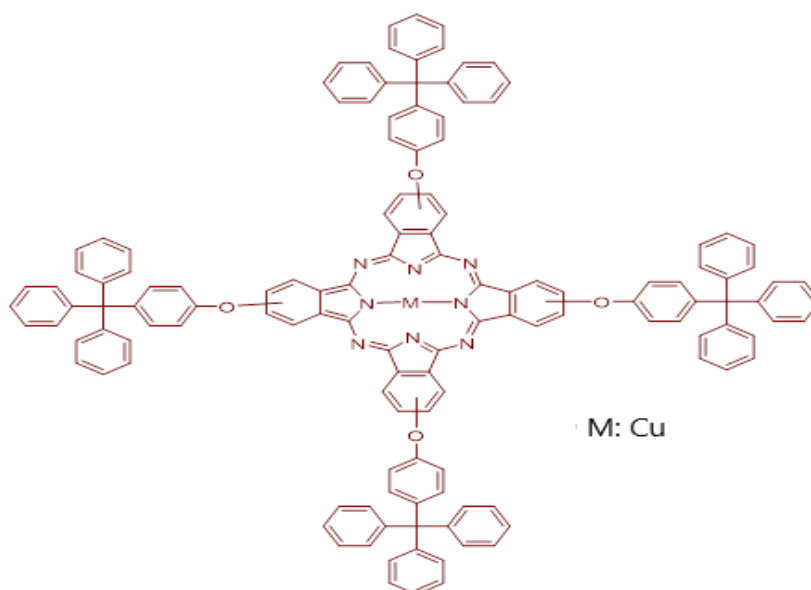
### 2.2. Preparation of Cu(II) Phthalocyanine Carrying 4-(4-Tritylphenoxy)phthalonitrile Group

The compound of 4-(4-tritylphenoxy)phthalonitrile was previously synthesized by our research group to form a phthalocyanine complex with copper (II), a transition metal [21]. The tetrakis-(4-tritylphenoxy)-phthalocyanine copper (II) reported in the existing literature has been isolated, synthesized and characterized corresponding to the earlier study of our research team [21].

## 3. RESULTS AND DISCUSSION

### 3.1. Synthesizing and Analyzing

Figure 1 shows the structure of **CuPc** phthalocyanine complex. The structural analysis of the Cu (II) phthalocyanine complex was carried out using a range of spectroscopic tools including IR and electronic absorption spectroscopy [21]. The evidence gathered from IR and absorption spectra techniques supported the suggested structure of **CuPc**. Copper (II) phthalocyanine was dissolvable in solvents including CHCl<sub>3</sub>, toluene, CH<sub>2</sub>Cl<sub>2</sub>, DMSO, DMF and THF. For **CuPc**, electronic absorption spectra, (THF)  $\lambda_{max}$ , nm (log  $\epsilon$ ) were obtained as 674 (5.21), 608 (4.61), 346 (4.93), accordingly [21]. FT-IR spectra were also acquired for Cu (II) phthalocyanine. IR spectra (cm<sup>-1</sup>) for **CuPc** were observed as 3053(C-H aromatic), 1598(C=C), 1490, 1447, 1442, 1230(Ar-O-Ar), 1085, 1050, 1033, 893, 825, 746, 700, 669, respectively [21]. All of this evidence was consistent with the proposed conformation of the complex.



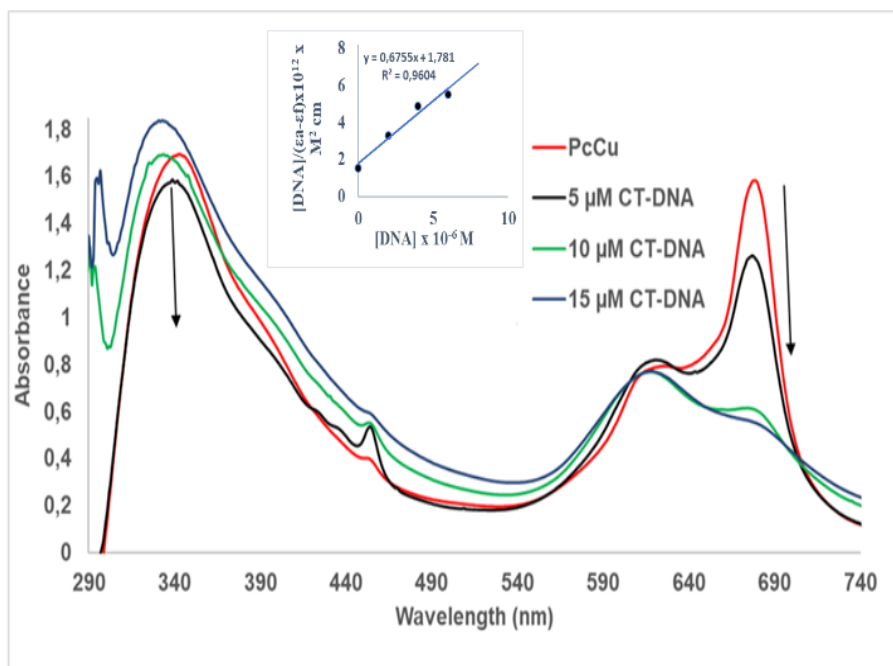
**Figure 1.** Chemical formula of **CuPc** complex

### 3.2. DNA Binding Assays

#### 3.2.1. Electronic Absorption Titration Study for CuPc

The binding of **CuPc** to the DNA has been explored by means of electronic absorption assays in order to comprehend their binding nature to the DNA. The samples of DNA had been made up in the buffer solution for this experiment. Electronic absorption spectra analysis of the stock solution of DNA at pH 7.05 had been read and the data proved that the DNA sample was found to have no protein. The amount of DNA was quantified from the absorbance spectra at 260 nm [20]. The DNA samples were preserved refrigerated at low temperatures and were utilized within a few days [20,22]. The stock specimen of **CuPc** complex was generated in DMF. Electronic absorbance was scanned between 300–850 nm at a pH of 7.05. Absorbance spectra titration tests were conducted for a fixed quantity of **CuPc** (20  $\mu\text{M}$ ) and variable quantities of DNA (15  $\mu\text{M}$ ). After each addition, the absorbances were followed, and the results were registered. The absorption data were used to obtain the binding coefficient ( $K_b$ ) for **CuPc** to DNA using Equation (1) [23]

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

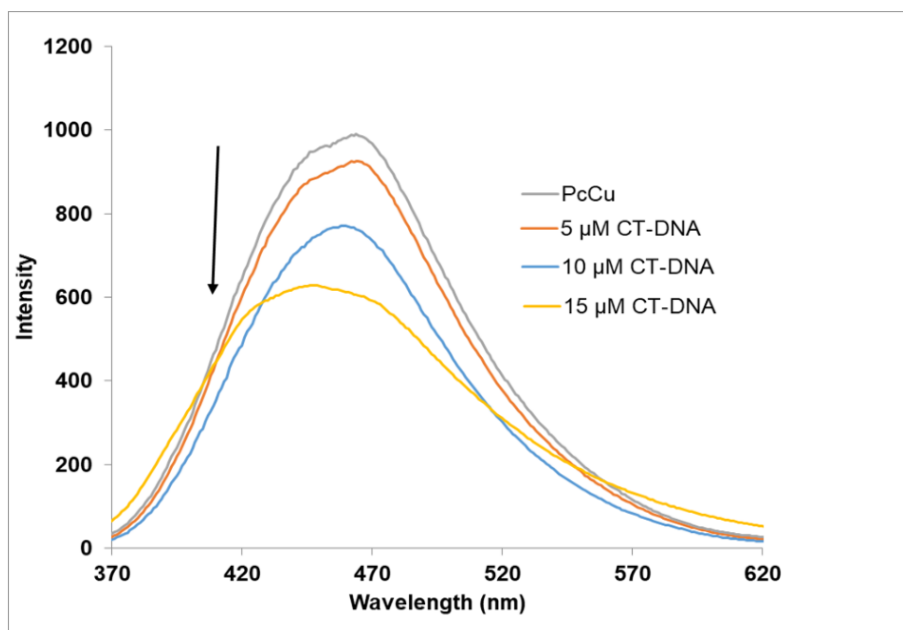


**Figure 2.** Spectra of electronic titration of **CuPc** (20  $\mu\text{M}$ ) in enhancement amounts of CT-DNA (0, 5, 10 and 15  $\mu\text{M}$ ) at a pH of 7.05 in Tris-HCl buffer

The absorbance titration of CuPc with CT-DNA is displayed in Figure 2. For **CuPc** complex,  $K_b$  value was obtained and it had been computed as  $1.53 \times 10^6 \text{ M}^{-1}$  by applying Equation (1) [23]. As the quantity of DNA increased, **CuPc** produced hypochromism with absorbance bands at around 674 and 346 nm as presented in Figure 2. The  $K_b$  values of intercalative agents like idarubicin were calculated in published paper [24,25]. The tendency of the absorbance to alter with the addition of DNA is therefore related to the  $K_b$  value of CuPc. It can be assumed that **CuPc** binds to DNA via an intercalative route.

### 3.2.2. Fluorescence Analysis of CuPc Complex for DNA Binding

Emission spectrometry is a procedure frequently utilized to probe the bonds between the small molecular complex and DNA molecule. The benefits of fluorescence procedure over other approaches are its high level of precision, broad concentration spectrum and specificity. This method is frequently conducted to examine drug-DNA binding, as it is one of the most widespread and accurate instrument in DNA binding studies and can provide greater evidence to support the intercalative binding mechanism of metal compounds [25,26]. The route of interaction of medicinal agents with DNA can be elucidated utilizing emission spectrometry, and other procedures based on emission spectra can also offer further valuable insight. Emission experimentation yields detailed information on the localization of agents and the attachment mechanisms to DNA [27,28]. Arrow marks changes in intensities with increasing amount of DNA.

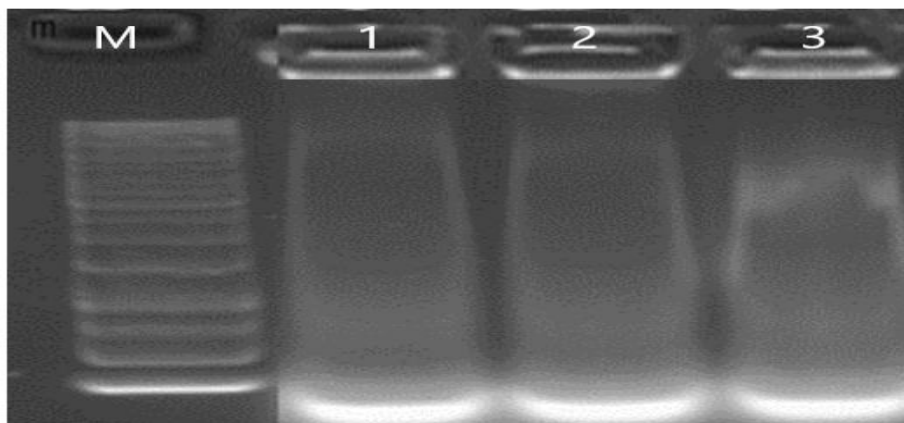


**Figure 3.** The spectrums of the fluorescence titration of CuPc with DNA in the buffer

In addition, for the elucidation of the interaction process of CuPc with CT-DNA, the emission binding examinations were made to clarify the binding of medicinal drugs and DNA, by the fact that this procedure is a tender instrumentation in the interaction examinations of medicinal drugs-DNA and the device can yield further forward aid to an intercalating bonding process of compounds. In absence of DNA, **CuPc** complex produces an emission spectrum as illustrated in Figure 3, with an emission peak at around 465 nm. When CT-DNA is present, the intensities of **CuPc** complex decline successively as the DNA is added. The decreasing intensities suggested that **CuPc** binds to the DNA molecule via intercalative binding pathway. These results apparently demonstrated that CuPc interacted with the DNA by an intercalation pathway, consistent with the adsorption titration data.

### 3.2.3. Gel Electrophoresis Analysis of CuPc/DNA Interaction

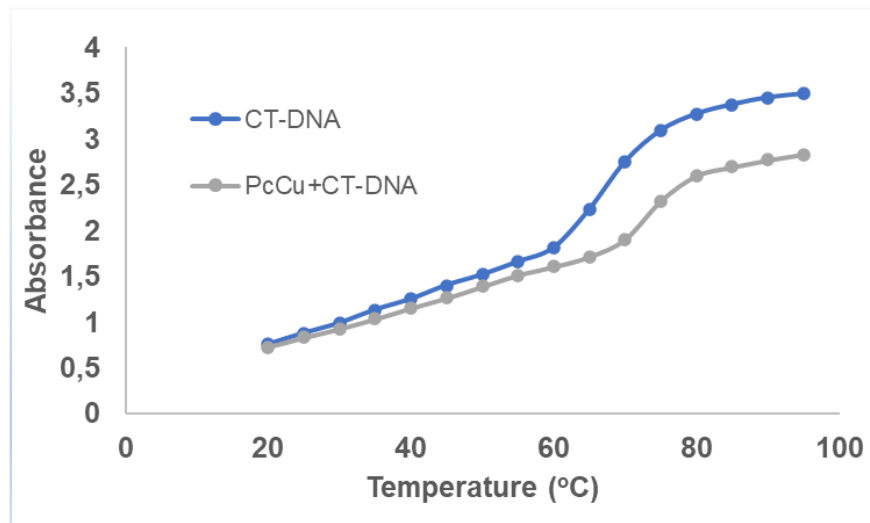
The interaction abilities of earlier synthesized **CuPc** to the DNA were assessed using gel electrophoresis by analyzing the influence of varying amounts of **CuPc** complex CT-DNA and its consequences obtained were illustrated in Figure 4. When comparison with the bands of standard DNA, there was a significant reduction in the band intensities detected for the CuPc after interacting with the DNA. The decline in band intensity detected after the interaction of CuPc with DNA is attributed to the distortion of the DNA double helix. Previously, research reports had proposed that damaging the DNA may have been caused by the splitting of the backbone due to a nucleophilic attack of the residues [28]. In the literature, work on agarose gel electrophoresis has suggested that band intensities can be governed not only by the number of molecular bonds, but also by the length of the DNA molecule [29]. In conclusion, overlapping of the metal complex during binding between bases in the DNA helix or surface interaction at the reactive sites of the nucleophile in the DNA double helix may explain the decrease in the electrophoretic band intensities of CT-DNA after binding by the CuPc phthalocyanine complex.



**Figure 4.** The binding activity studies between **CuPc** complex and the DNA in the buffer solution are shown by gel electrophoresis. Lane M represents standard DNA ladder. Lanes (1-3) indicate (5, 10 and 15  $\mu\text{M}$  CT-DNA) + 20  $\mu\text{M}$  **CuPc** complex, respectively

### 3.2.4. Experiments on the Thermal Denaturation

In this report, by analyzing the CT-DNA melting temperatures ( $T_m$ ), the influence of the amount of adduct formation on the stabilisation of DNA supercoil has been studied.  $T_m$  response of CT-DNA in the presence of a metal compound can unveil the conformational structure variance with rising temperature, and assure insight into binding ability of tiny metal compounds to DNA molecule. Literature reports suggest that when metal compounds bind to the DNA double helix, molecular stability is promoted. The  $T_m$  of DNA is often elevated when metal compounds react with DNA by intercalative binding, because the intercalative binding of metal compounds between base pairs of DNA produces a stabilisation of the stacking of base pairs of DNA and thus enhances the  $T_m$  of DNA. In most cases, electrostatic forces across the DNA phosphate backbone generate only a minor modification of the melting temperature, whereas an intercalative binding process induces a substantial rise DNA melting point due to stabilisation of DNA molecule [30,31].



**Figure 5.** The melting temperature ( $T_m$ ) graphs of DNA in lack and in the existence of **CuPc**, displaying rise of the melting temperatures

Analysing the melting point of DNA for current **CuPc** reveals an allowable favourable shift in the  $T_m$  of approximately 5 °C for **CuPc**. Figure 5 presents  $T_m$  graphs of DNA in the absence and presence of **CuPc**. In the absence of **CuPc**,  $T_m$  tests were done for DNA and  $T_m$  was detected as 69.6 °C in the buffer at pH 7.05, and for the **CuPc** complex,  $T_m$  of around 75.7 °C was determined. The substantial rise in the  $T_m$  of the DNA along with **CuPc** complex is very similar to that reported for the standard intercalating substances [32-34].

#### 4. CONCLUSION

The tetra-(4-tritylphenoxy)-substituted copper (II) phthalocyanine complex had been earlier produced by synthesis and characterised by UV/Vis and FTIR methodologies. Emission, spectral absorption, electrophoresis and melting point assays were employed to examine the reaction behaviour of **CuPc** with CT-DNA.  $K_b$  values generated by electronic spectroscopy proved that **CuPc** interferes by DNA via intercalating intercation. Data derived from emission analysis for the **CuPc** complex have revealed that CuPc connects to DNA via intercalative manner of interaction. The nature of CuPc interacting with CT-DNA was proposed to be intercalating bonding by all the data generated. In addition to the above examinations, the binding abilities of the **CuPc** complex were also tested on calf thymus DNA utilizing agarose gel electrophoresis and melting temperature experimentation. The data generated by these procedures disclosed that **CuPc** intercalates with DNA molecule. The complex has the potential to be used for therapeutic medicine due to its bonding to DNA.

#### CONFLICTS OF INTEREST

No conflict of interest was declared by the authors.

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