Inquiries of DNA Interaction of Cobalt (II) Phthalocyanine Compound Bearing 4-tritylphenoxy Groups

[1]Ali Arslantaş* and [2]Mehmet Salih Ağırtaş
[1]*Biomedical Engineering, The School of Engineering and Architecture, İzmir Bakırçay University, 78050, İzmir, Turkey*.
[2]*Chemistry Department, The Faculty of Science, Van Yüzüncü Yıl University, 65080, Van, Turkey*.
ali.arslantas@bakircay.edu.tr*

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Summary: [Tetrakis-(4-tritylphenoxy) phthalocyaninato] Cobalt (II) (PcCo) was previously formed via the reaction of 4-(4-tritylphenoxy)phthalonitrile. The structure of the resulting PcCo was characterized using absorption spectra, infrared and NMR spectroscopies. The deoxyribonucleic acid (DNA) connecting feature for PcCo was inquired in the different concentration of Calf Thymus-deoxyribonucleic acid (CT-DNA) using UV/Vis, fluorescence spectroscopy, gel agarose electrophoresis and thermal denaturation methods. UV/Vis spectrometer and fluorescence spectroscopy verified PcCo bounds to the DNA and the binding constant (K_b) of the PcCo was also calculated. The binding constant K_b is a very important parameter for getting information on the binding mechanism. The K_b of the compound was also calculated as 1.44×10^6 M^{-1}. The value of K_b demonstrated that the complex binds to DNA through an intercalative binding mechanism. Additionally, thermal denaturation and the electrophoresis studies were implemented to analyze the interacting of PcCo via CT-DNA. In the absence of the complex, the melting experiments were conducted for CT-DNA and T_m was found 69.6 ^oC for the DNA and The T_m of PcCo complex was found 76.3 ^oC. Melting point temperature and electrophoresis experiments demonstrated that PcCo binds to CT-DNA through intercalation binding mechanisms. The acquired findings confirmed that PcCo links to the DNA via the intercalating binding manner. Therefore, PcCo may have potential use in cancer treatment. Because of this, further research is needed before this complex can be used in cancer treatment.

Keywords: DNA, Phthalocyanine, Binding Mode, Absorption spectra, Fluorescence spectroscopy

Introduction

Phthalocyanines are an enormous class of compounds in both basic and applied research, possessing a variety of chemical properties that make them interesting chemical reagents for nucleic acid modification. Phthalocyanine compounds are capable of functioning as photosensitizers and enhancing the generation of singlet molecular oxygen due to their intense absorption in the red zone of visible light [1, 2]. Phthalocyanines and the derivatives of these compounds are extremely useful molecules and thereby many search regarding phthalocyanines are continuing for years.

In the development of new reagents for biotechnology and medicine, the binding and reactivity of metal complexes with DNA has long been studied in detail [1]. Metallo phthalocyanine complexes possess various potential implementations in medical sciences, pharmacology, involving biochemical sensors, liquid crystals, films, and photodynamic treatment of cancer [3-8]. Co(II) complexes have been extensively examined in the literature in recently because of their remarkable photophysical and fluorescent characteristics [9]. For instance, metals such as cobalt and copper are considered an elementary essential trace elements with a varied range of biological properties in the human body. Different ligands have exhibited potential anticancer or anti-inflammatory activities when coordinated to transition metal ions. For example, E. Budzisz et al. revealed that metals like Co(II) complexes exhibited significant cytotoxicity against cancer cells [10].

Rosenberg's groundbreaking invention of cisplatin in 1965 opened a new era of metallo-based anticancer research [**11**]. Even today, cisplatin and its analogs are some of the most commonly used effective chemotherapeutic drugs as first-line chemotherapeutic agents in clinical use treatment of testicular and ovarian tumors. Ben-Hur et al. first demonstrated the ability of Pcs to eradicate mammalian cells in 1985 [12].

Furthermore, phthalocyanine compounds are besides recognized about their well conductive

*To whom all correspondence should be addressed.

features [13, 14]. Still, scientists are continuing to study on these compound ds to discover their new properties [15, 16]. The targeting capability and biocompatibility properties of phthalocyanine compounds could easily interact with amphiphilic copolymers for instance polyethylene glycol and polycaprolactone to be used as nanocarrier system to impute phthalocyanine compounds, which may augment the cellular intake capacity **[**17], keeping retainable drug discharge [18], extend their interacting time at the aimed places [19] and develop their intake cleaning [20], which could develop therapeutic effectiveness of photodynamic therapy and decrease toxic effect of phthalocyanine compounds [21].

Deoxyribonucleic acid (DNA) is the main intracellular target of anticancer drugs as it regulates the vast majority of cellular biochemical reactions within the cellular system. The main targets of pharmacological activity are DNA or protein. as many drugs produce their effects by attaching to DNA or protein. Hence, the binding of metal chelates to DNA or protein is the basis for the development of new and more effective metal-based anticancer therapeutics [9].

DNA molecule is an important macromolecule having genetic data and playing a central role in fixing defects, cell reproduction and genetic mutations [22]. DNA is besides one of aims for anticancer medicines [23, 24]. The great affinity of DNA interaction of photosensitizer compounds possesses high potential nuclear aiming capability for effectiveness of photodynamic therapy [25, 26]. McRae et al [27] conducted the binding and photodynamic activities of the metal complex for DNA. They observed that the comlex have a high binding capacity for DNA [27]. Also, Uchiyama et al [28, 29] studied the interaction between a Ga(III) phthalocyanine (Pc) derivative. They discovered that the complex displays binding to DNA. However, the binding activity of different substituted metallo phthalocyanine complexes with DNA have been investigated not so much.

In the current study, PcCo was previously synthesized and the structure of the complex had been characterized using absorption spectra and infrared (IR) spectroscopies [30]. The binding properties between PcCo and calf thymus-deoxyribonucleic acid (CT-DNA) had been studied with ultraviolet visible (UV/Vis), fluorescence spectroscopic, the thermal melting and agarose gel electrophoresis techniques.

Experimental

Materials and methods

The PcCo had been synthesized and characterized the previous study [30]. The chemical reagents such as NaOH, DMF, Tris-HCl, and calf thymus DNA used in this current research were obtained from Sigma-Aldrich and Merck. All of the commercially purchased chemicals were used without any further purification, and all of the chemicals were of analytical grade. CT-DNA was used with no further purification and was solved as a stock solution in double-distilled water. All DNA solutions used were prepared in the buffer containing sodium hydroxide (NaOH) at pH 7.3 and stored in a refrigerator at 4ºC. The stock PcCo was prepared and stored at 25ºC. If needed, the solutions had been diluted to a desirable volume with Tris(hydroxymethyl) aminomethane hydrochloride (Tris-HCl) buffer. UV/Vis absorption data and thermal melting point studies had been gathered using a Agilent Cary 60 UV/Vis spectroscopy and for the emission data were carried out by Perkin Elmer Fluorescence Spectroscopy. Agarose gel electrophoresis experiments had been performed using the Scientific Owl Electrophoresis system at pH 7.3 at 25 ºC.

Synthesis of 4 -(4-tritylphenoxy) phthalonitrile and phthalocyanine complex

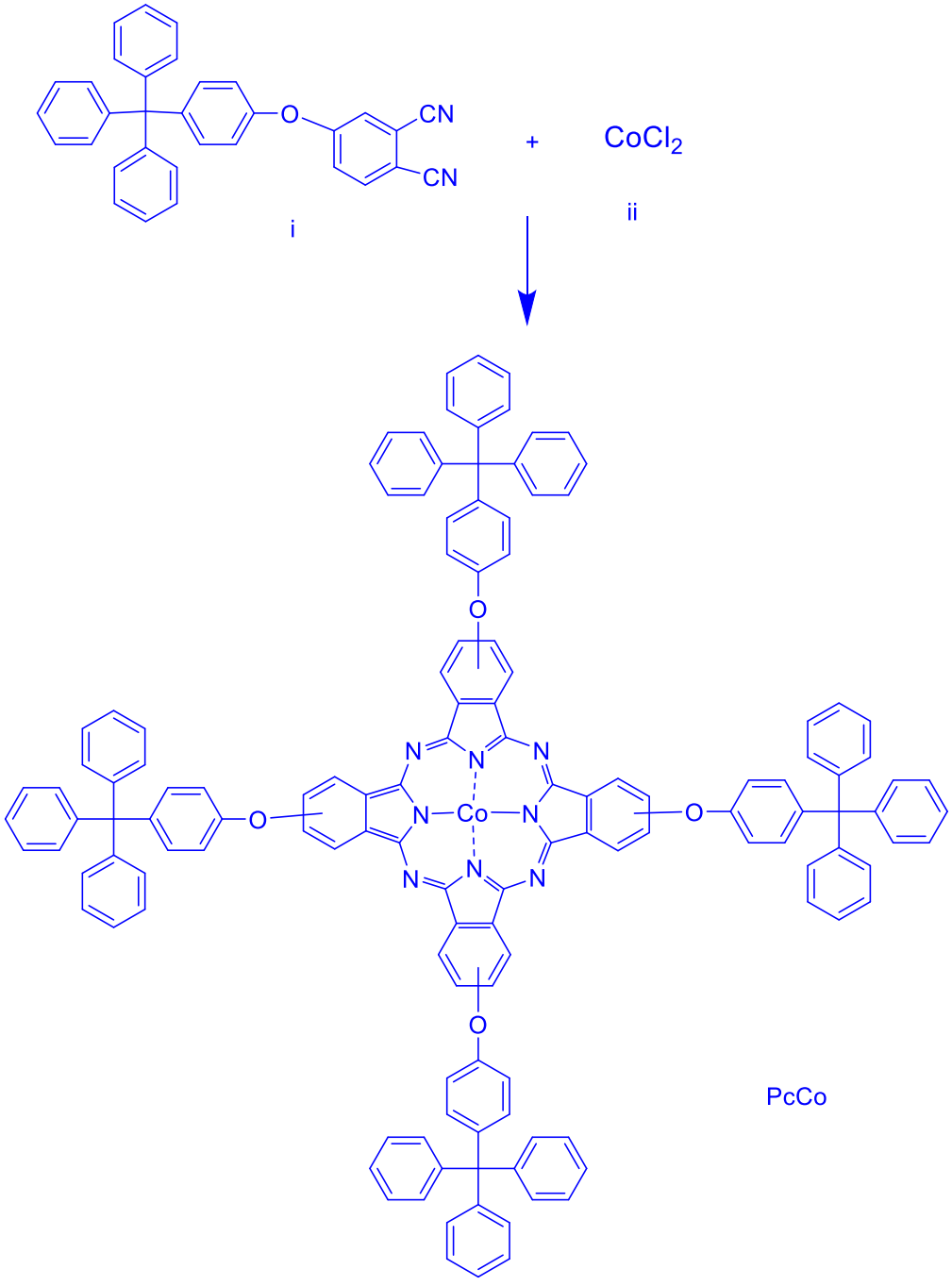
The 4 -(4-tritylphenoxy) phthalonitrile compound had been prepared and synthesized according to the study of our research group [30]. PcCo reported in literature was synthesized and characterized according to our previous study [30]. This compound is soluble in dichloromethane (CH_2Cl_2), trichloromethane ($CHCl_3$), tetrahydrofuran (THF). toluene, dimethylformamide (DMF), dimethyl sulfoxide (DMSO). Yield: 0.024 g. UV/Vis) (THF) λmax, nm (log ε): 662 (5.24), 600 (4.72), 328 (5.14). IR spectrum (cm^{-1}): 3055(C–H aromatic), 1598(C=C), 1490, 1467, 1232(Ar – O – Ar), 1166, 1093, 1056, 956, 893, 746, 700.

Results and discussion

The assay of synthesis and characterization

The synthetic pathways of [Tetrakis-(4-tritylphenoxy) phthalocyaninato] cobalt (II) (PcCo) and 4-(4-tritylphenoxy) phthalonitrile were indicated in Scheme 1. The structure of of PcCo was analyzed by spectroscopic procedures like UV/Vis and Fourier Transform Infrared (FT-IR) methods [30]. Data obtained from UV/Vis and FT-IR techniques confirmed the proposed structure of PcCo compound. PcCo was soluble in $CHCl_3$, toluene, CH_2Cl_2, DMSO,

DMF, and THF. UV/Vis) (THF) Λ_{max}, nm (log Ɛ) for the complex PcCo was measured as 662 (5.24), 600 (4.72), 328 and (5.14) [30]. In the FT-IR spectrum of PcCo, aromatic vibration peak was observed at 3055 cm^{-1}, C=C vibrations were observed at 1598 cm^{-1}, and C-O-C vibrations were observed at 1232 cm^{-1}, in accordance with the data in the literature [30] as shown in Fig. 1. This situation supports the structure. Since this complex compound is paramagnetic, its ^1H NMR spectrum is not given. The CN groups in phthalonitrile disappear after being converted to phthalocyanine. This situation is considered as a sign of phthalocyanine formation.



Scheme-1: The synthesis scheme of PcCo compound: PcCo compound is obtained by heating 4-(4-tritylphenoxy) phthalonitrile (i) with $CoCl_2$(ii) at 270 °C for 7 min.

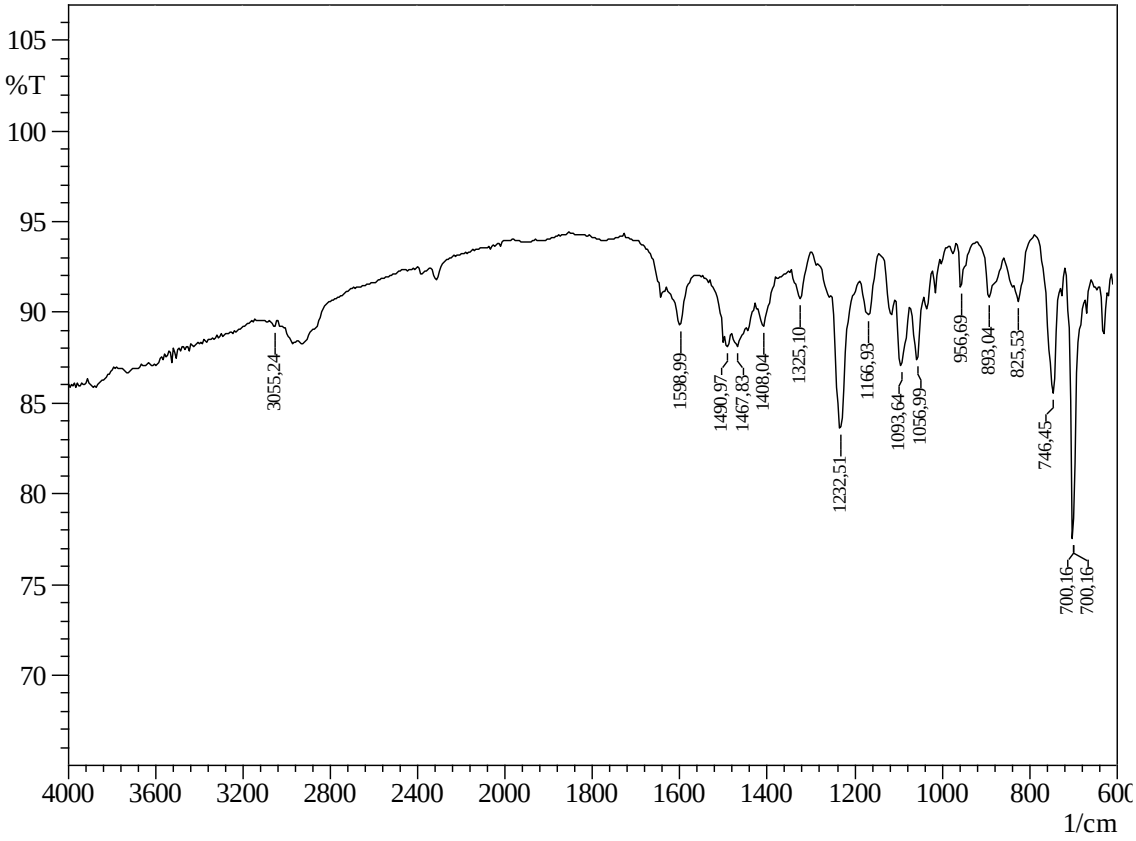


Fig. 1: FT-IR spectrum of PcCo complex.

The inquire of DNA binding

DNA interacting features with PcCo were monitored by UV-Vis to explain its binding activities. The pattern of DNA was produced in the buffer and then the adsorption titrations of stock pattern of CT-DNA in a Tris-HCl solution at pH 7.3 was measured at 250 and 300 nm. The DNA solution was found to contain no protein. The amounts of DNA for each nucleotide phosphate group had been estimated from absorption titration at 260 nm accounting its value of extinction coefficient [31]. The DNA pattern was stored at 4 ^{o}C and used for several time [31, 32]. The stock sample of PcCo was produced in DMF and diluted with the buffer solution. Electronic absorption titrations were conducted using a quartz sample holder and scanned between the range of 250-900 nm at 25 ^{o}C. Absorption spectra assays were performed for holding fixed amounts of PcCo (20 µM) and changeable amounts of the DNA (0 to 15 µM) by an increasing of 5 µM. UV/Vis absorptions were monitored and recorded after every addition. The sample mixtures of the complex and CT-DNA were permitted to incubate over several minutes for very run and shifts in the absorbance spectra were observed. Also, the binding constant (K_b) for PcCo to the DNA was calculated the using of UV/Vis data and Wolfe-Schimer Equation (1) [33].

$$\frac{[DNA]}{\varepsilon a-\varepsilon f} = \frac{[DNA]}{\varepsilon b-\varepsilon f} + \frac{1}{Kb(\varepsilon b-\varepsilon f)} \qquad (1)$$

Where [DNA] stands for the amount of the DNA, and ε_a, ε_b and ε_f represent the constants of the free complex. The absorption titration of CT-DNA of PcCo is shown in Fig. 2. The intrinsic binding coefficient value for the PcCo complex was calculated and by applying equation (1), the K_b value for the complex was obtained as 1.44 x 10^6 M^{-1}. On rising the amount of CT-DNA, PcCo complex produced hypochromic with a red shift band to the absorbance band about at 672 nm as indicated in Fig. 2. The K_b value of recognized intercalative anticancer medicine like doxorubicin and idarubicin are calculated [34, 35]. For this reason, by comparing the K_b value of PcCo and the tendency of absorbance changing by the adding of the DNA, it can be expected that PcCo reacts with the DNA via intercalative binding mechanism.

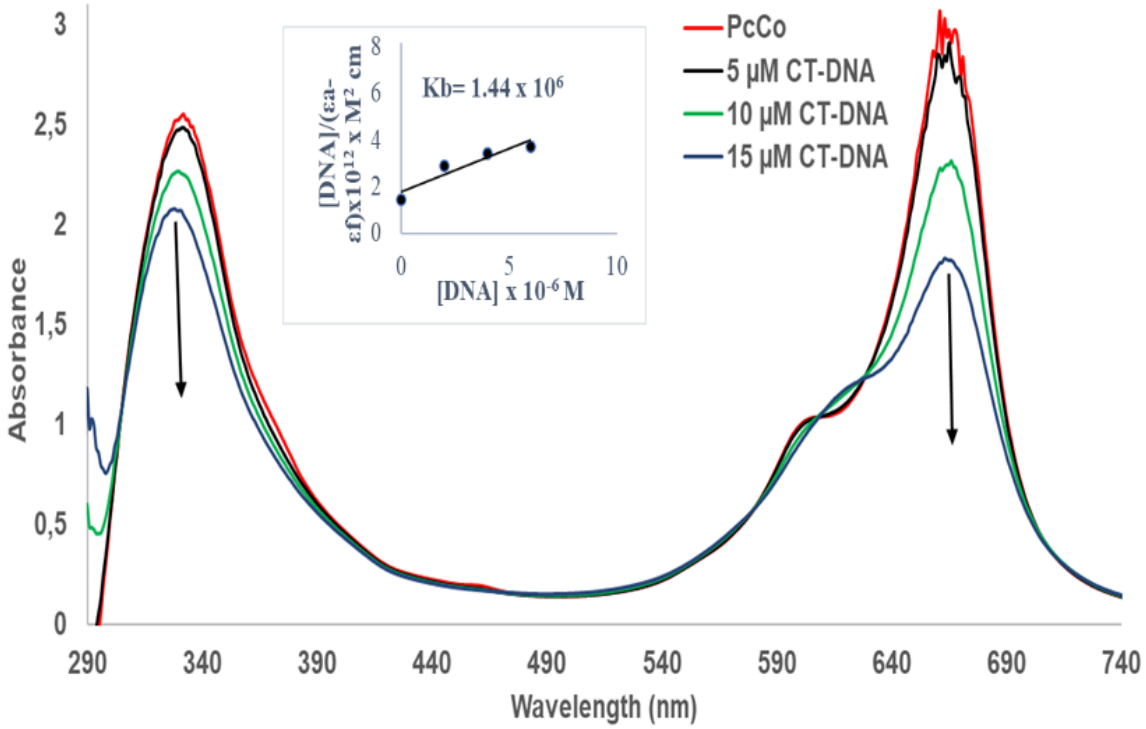


Fig. 2: The spectra of UV/Vis titrations of PcCo (20 µM) upon increasing concentration of CT-DNA (0–15 µM) at pH 7.3.

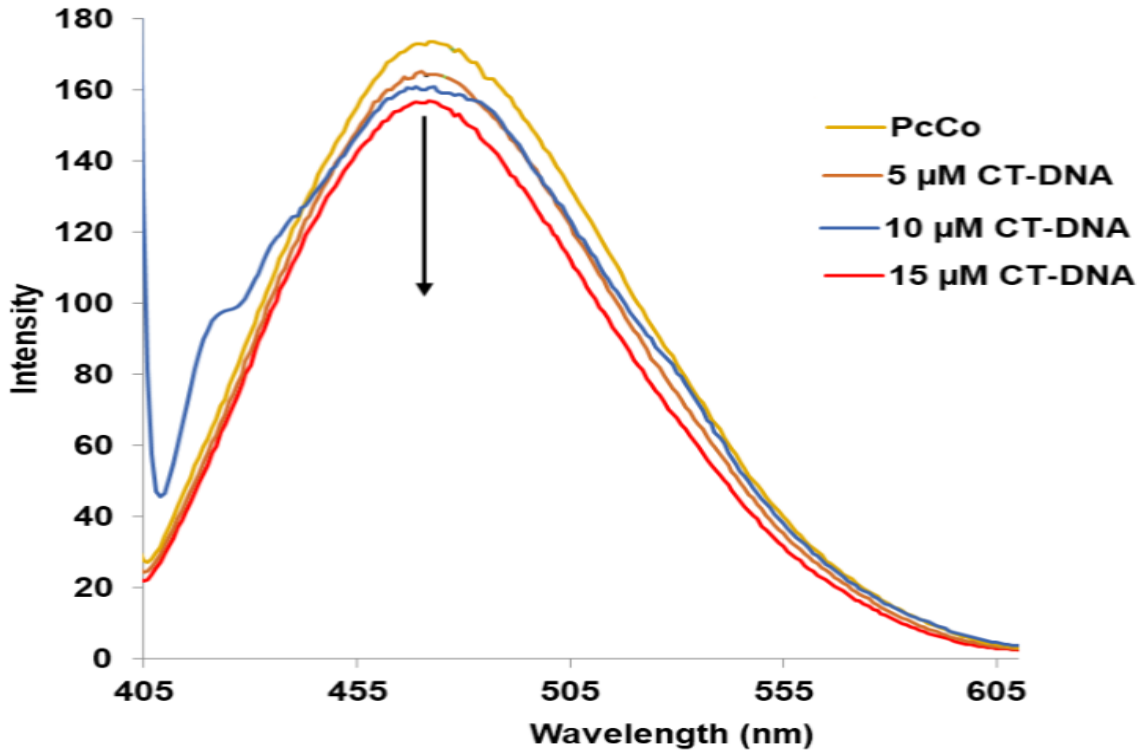


Fig. 3: The spectra of emission of the PcCo complex in the buffer in the absence and way with DNA. The arrow represents changes in intensities of the emission spectra on rising amount of CT-DNA.

The fluorescence studies of DNA binding of PcCo complex

Fluorescence spectroscopy is a technique generally used to analyze the bindings between tiny compounds. The benefits of this technique on other methods are very precision, large concentration range and selectiveness [36]. The pathways of interacting of therapeutic chemicals with DNA may be determined using emission spectroscopy. Fluorescence study provides more data about medicines and their binding mechanisms to the DNA molecule [37, 38].

Additionally, the emission binding studies were conducted to make clear the interacting mechanism of PcCo with CT-DNA because this method is very sensitive technique in the interaction studies of medicine-DNA and it may procure further help to the intercalative binding mechanisms of medicinal compounds. As indicated as in Fig. 3, in the absence of the DNA, PcCo produces emission peak around at 470 in the buffer at 25 ^{o}C. In the way of CT-DNA, on the adding of the DNA, the intensities of PcCo drops step by step. The decreases in the intensities proved that PcCo interacts with the DNA molecule via an intercalating mechanism. These findings obviously showed that the [tetrakis-(4-tritylphenoxy) substituted Co(II) phthalocyanine interacts with DNA molecule via an intercalating mechanism, which was coherent to the results of adsorption titration result.

The assay of gel electrophoresis

The interacting features of PcCo with DNA were studied using the electrophoresis technique by use of analyzing of the impact of various amounts of PcCo complex over DNA. The obtained findings were showed in Fig. 4. It is clearly seen that band intensities observed for PcCo after interaction with the DNA were dropped the comparing to the bands of the control CT-DNA (C). Dropping in band intensities recorded after interaction of phthalocyanine compounds with the DNA is linked to become owing to breakdown of the double helix of DNA molecule. The previously reported studies suggested that DNA damage can have happened through backbone split because of a nucleophilic raid of the residues [39]. In the literature, it was stated that the band intensities in agarose gel electrophoresis studies visualized with the emission spectra of ethidium bromide binds to the DNA base pairs via intercalative binding mechanism is subject to not only number of molecular compounds, but also the length of DNA molecule [40]. For this reason, the decrease in the electrophoretic band intensities of CT-DNA on binding by PcCo compound may be owing to overlapping of metal complex on binding between the bases inside the helix of the DNA or surface interaction at the reactive sites of nucleophile upon the double double helix of DNA.

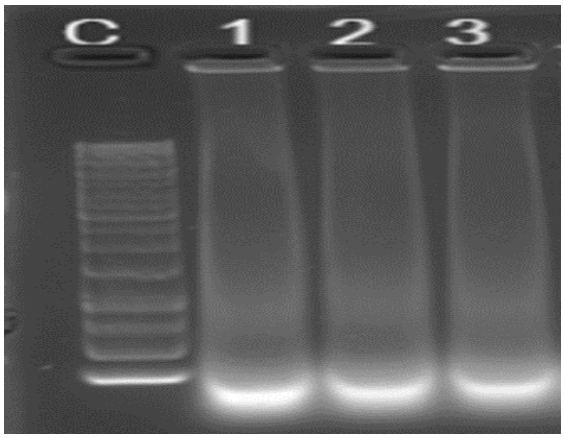


Fig. 4: The gel electrophoresis of the DNA patterns indicating the binding activities between the PcCo compound and the DNA in the buffer solution. Lane C: control CT-DNA. Lanes 1–3: 15 µM CT-DNA + (10, 15 and 20 µM) PcCo, respectively.

Thermal denaturation experiments

In this study, the outcomes of adduct yield forming upon the firmness of DNA double helix were analyzed by monitoring the melting temperatures (T_m) of CT-DNA melting temperatures (T_m). Melting temperature behavior of CT-DNA in the presence of PcCo may provide comprehention within their conformational structure variance when temperature is increased, and ensure knowledge regarding the connecting activities of chemical compounds to DNA molecule. In the literature, reported studies diclosed that when compounds bind to DNA, the stability of DNA improves. Generally, the T_m of DNA rises when metal compounds interact with DNA molecule via intercalative binding mechanism, as an intercalative binding of PcCo between the base pairs DNA leads to the stabilization of base pairs stacking of DNA and therefore increases the T_m of DNA. Mostly, electrostatic interaction throughout DNA phosphate backbone produces merely a little changing in melting temperature, while an intercalative binding mechanism causes to a remarkable increase in melting point of DNA owing to the stabilization of base paired duplex of DNA [41, 42]

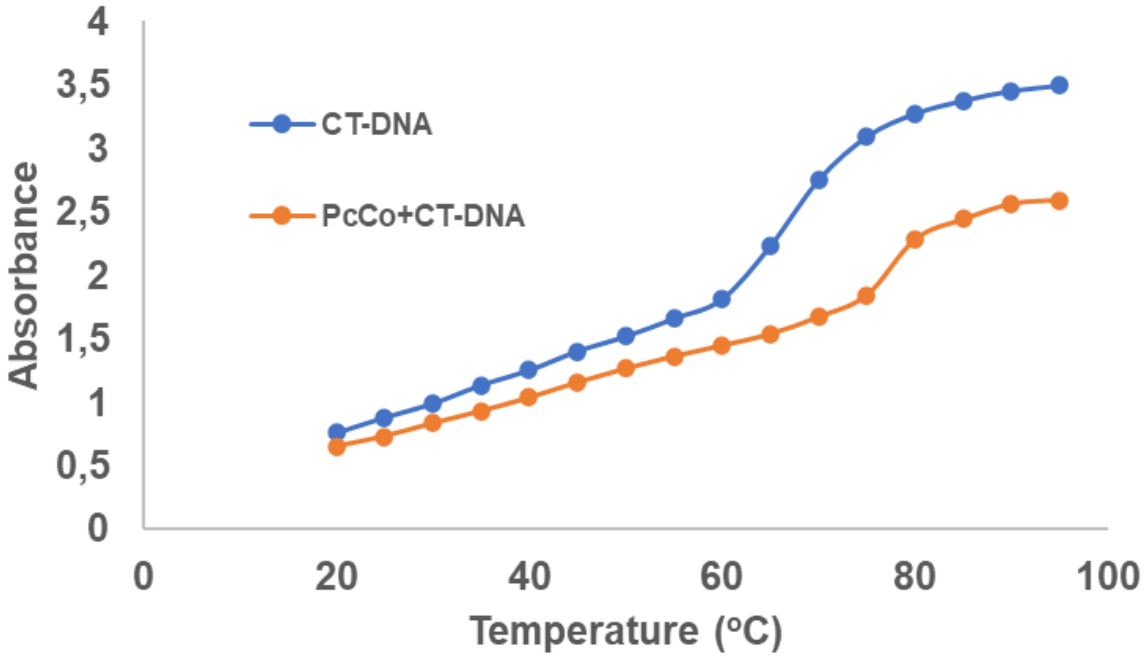


Fig. 5: Tm curves of the DNA in the absence and presence of PcCo, indicating the rising in melting temperature.

The melting temperature studies of CT-DNA for the present PcCo complex indicates an acceptable positive change in T_m of almost 5 °C for PcCo complex. The T_m curve of the DNA in the absence and way of PcCo was as indicated in Fig. 5. In the absence of PcCo, the melting temperature experiments were performed for CT-DNA and recorded a T_m of 69.6 °C at a pH of 7.3. In the way of PcCo complex, the T_m rised around 76.3 °C. The major rise in melting temperature of DNA along with PcCo compound is comparable with that recorded for standard intercalator agents [43-45].

Conclusions

The [Tetrakis -(4-tritylphenoxy) phthalocyaninato] cobalt (II) (PcCo) was previously characterized with UV/Vis and FT-IR techniques. The interaction activity of PcCo with CT-DNA was examined with absorption titrations, emission spectra, gel elctrophoresis and melting temperature experiments. The K_b constant was procured by UV/Vis absorption experiments verified that the complex interacts with the DNA an intercalative binding mechanism. The results produced from emission titrations demonstrated that PcCo binds to the DNA via an intercalating mode. All the obtained findings proposed that the interaction mode of the complex with CT-DNA was an intercalative binding. Additional above studies, the binding of PcCo was also analyzed with electrophoresis and melting temperature experiment upon the DNA. The findings obtained from these techniques demonstrated that PcCo binds to the DNA molecule via intercalation. The compound has potential to use as therapeutic material owing to DNA binding.

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