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Study on DNA Binding Properties of Nickle (II) Phthalocyanine Compound with 2-Isopropyl-5-Methylphenoxy Substituents.

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ABSTRACT

In this study, previously synthesized Ni (II) phthalocyanine compound bearing 2,10,16,24-tetrakis(2-isopropyl-5-methylphenoxy group was chosen for its interaction with calf thymus-DNA. Calf thymus-DNA was used to determine DNA binding properties of Ni (II) phthalocyanine compound. The DNA binding activities of Ni (II) phthalocyanine compound bearing 2-isopropyl-5-methylphenoxy substituent was investigated by using absorption titration, fluorescence emission, cyclic voltammetry, gel elctrophoresis in Tris-HCl buffer at pH 7.0. In addition to above methods, melting point and viscosity experiment were performed to determine the DNA intercation of the compound in Tris-HCl buffer solution. The results showed that Ni (II) phthalocyanine compound binds strongly to calf thymus-DNA via intercalation binding.

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INTRODUCTION

Phthalocyanine compounds show important features such as chemical stability, eminent thermal, dense luminescence, and electron transfering impact [1, 2]. Feasibilities of phthalocyanine compounds have been studied in terms of varied fields, in photodynamic therapy, therapeutical drug and antioxidant properties [3]. Metallophthalocyanine compounds indicate unique electrochemical, physical properties due to their large correlated π regulations that are kept in close affinity with metallic ions [4-6]. The derivatives of phthalocyanine compounds are very suitable tool for photodynamic cancer therapy [7]. For this reason, nowadays, the biological studies of phthalocyanine metal compounds for anticancer treatment have been increased because of their effects in medical treatment [8].

For treatment of genetic diseases such as cancer, DNA molecule is a signifacant target in cancer therapy. Nowadays, transition metal complexes have gained great attatention as anticancer medicine due to their significant effect in medical treatment. Some cancer medicines give an important beginning information about the mechanism of DNA interaction. Transition metal compounds have multiple electrochemical and spectral characteristics which increase their DNA cleavage properties and DNA interaction [9, 10]. DNA molecule has been a substantial target for treatment of genetic disorders such as cancer disease. The interaction probe of phthalocyanine metal compounds with DNA not only ensures an opportunity to find out the intercation methods of some antitumor but also permits to design new DNA targeted phthalocyanine metal compounds [9, 10]. The many studies conducted so far targeting the DNA molecule is a significant intracellular targets of medications interaction of tiny chemical compounds with DNA results in DNA harm, disrupting the division of cell and cell death. As a consequence, the interreactivity of chemical compounds by DNA molecule is an important study area for the developing of novel curing drugs [11].

In this study, we studied the interaction of previously synthesized Ni (II) phthalocyanine compound (**PcNi**) bearing 2-isopropyl-5-methylphenoxy group which is synthesized and characterized according to literature procedure [2], substituents on the peripherial positions by calf thymus-DNA using absorption spectra, fluorescence spectroscopies, cyclic voltammetry, gel

electrophoresis, thermal denaturation profile and viscosity measurement in a Tris-HCI buffer solution (pH 7.0).

METHODS

Chemicals and equipments

In this work, Ni (II) phthalocyanine compound bearing 2-isopropyl-5-methylphenoxy substituent was used. Calf Thymus-DNA (CT-DNA), and Tris-HCl were provided from Sigma Aldrich company. Sodium chloride (NaCl) was purchased from Merck. All chemical compounds were analytical grade and used without further purification. All CT-DNA solutions were prepared by using Milli-Q water. The experiments were carried out in an the medium of Tris-HCl buffer solution at pH 7.0.

UV-Vis spectroscopy studies of binding activities of Ni (II) phthalocyanine compound (**PcNi**) with the DNA were performed in aquartz cuvette using Agilent Technologies Cary 60 UV/Vis spectroscopy (Karabuk, MARGEM, Turkey), fluorescence spectroscopy was conducted using Perkin Elmer LS Fluorescence Spectrometer and Cyclic voltametry (CV) experiments was carried using Ivisumstat Electrochemical Interface electrochemical analyzer. Agarose gel electrophoresis studies were performed with Thermo Owl electrophoresis system. Thermal denaturation profile was performed at 260 nm wavelenght using Agilent Technologies Cary 60 UV-vis spectrocopy and viscosity experiments were carried out using Ubbelohde viscositymeter.

Synthesis of compounds

The synthesis of 4-(2-isopropyl-5-methylphenoxy) phthalonitrile compound and the synthesis of 2,10,16,24-tetrakis(2-isopropyl-5-methylphenoxy phthalocyaninato) nickel(II) (PcNi) is synthesized and characterized according to literature procedure [2].

UV/Vis absorption spectra and fluorescence titration studies

Absorption tittration spectra for DNA binding properties were performed at room temperature at pH 7.0 in a 20 mM Tris-HCl buffer solution containing 20 mM NaCl. The concentration of CT-DNA was calculated by absorbance at 260 nm using a DNA molar extinction coefficient (ε) of 6600 $M^{-1}cm^{-1}$ indicating that the DNA solution was free of proteins [12]. Absorption titration experiments were carried out in the region of 300-800 nm. Absorption titrations of the compound at constant concentration of **PcNi** in Tris-HCl buffer were conducted by adding between 0 to 3.5 μM of CT-DNA. In order to determine the dilution impacts were carried out by control titrations with Tris-HCl buffer solution instead of DNA [13].

Following excitation fluorescence spectroscopy experiments were conducted in the region of 400-750 nm. Fluorescence titration of the compound with the DNA were carried out by increasing concentration of the DNA solution to the compound solution at fixed concentration. In this study, the solutions were allowed to reach to equilibrium for a certain time before measurements were performed [14].

Cyclic Voltammetry studies

Cyclic voltametry experiments at the glass carbon elctrode were carried out by using Ivisumstat Electrochemical Interface electrochemical analyzer at the following setting. Beginning potential was -1.5 V and ultimate potential was 1.5 V and the rate of scan was 10 mV/s. Carbon working electrode, Ag/AgCl reference electrode and plantinnum wire counter electrode were used in this work. A standard single chamber three electrode cell system of 10 mL capacity were used to conducted the all measurements [15, 16]. Cyclic voltametry experiments were performed at ambient temperature in a Tris-HCl buffer.

Melting point Temperature studies

Melting point studies were conducted for the DNA and **PcNi**. The compound and the DNA in Tris-HCl buffer comprising NaCl solution were heated from 20 °C to 95 °C gradually. UV-Vis absorption spectra values were recorded. Thermal denaturation temperature of the DNA was determined in the absence and presence of the compound. Thermal denaturation temperatures were recorded at 260 nm wavelenght [17] by using Agilent Technologies Cary 60 UV/Vis spectroscopy.

Viscosity measurements

In this work, the relative viscosity experiments of **PcNi** with CT-DNA were conducted by using Ubblohde viscometer which was sunmerged in a bathtub maitaining at a fixed temperature (30 °C). The proximate time of flow was achieved after each sample was tested three times. The relative viscosity of the DNA was computed by using $\eta_i=(t_i-t_0)/t_0$, where η_i is the viscosity values of the DNA; t_i is the time of flow of the solution in the absence and presence of the compound; and t_0 is the time of flow of Tris-HCl buffer solution (pH 7.0). The viscosity values were indicated as $(\eta/\eta_0)^{1/3}$ vs [**PcNi**] / [CT-DNA], where η is the relative viscosity of the DNA in the presence of compound and η_0 is the relative viscosity value of the DNA [18].

Agarose gel electrophoresis experiment

For gel electrophoresis study, CT-DNA in 20 mM Tris-HCl buffer at pH 7.0 containing 20 mM NaCl, was allowed to interact with **PcNi**.

The binding activities of the compound with the DNA were investigated by 1% agarose gel in Tris-HCl buffer EDTA (ethylenediaminetetraacetic acid) (TBE) solution. 20 µL of samples of DNA + **PcNi** was loaded with loading dye. Agarose gel electrophoresis experiment for DNA + **PcNi** sample was carried out at 80 volt during 3 hours in Tris -HCl buffer ethelendiaminetetraacetic acid (EDTA) (TBE) solution. After gel electrophoresis experiment, the DNA bands were visualized by using Vilber Lourmat UV lamb. For this experiment, Thermo owl gel electrophoresis sytem was used.

RESULTS AND DISCUSSION

Synthesis and characterization

Nickle (II) phthalocyanine compound was obtained by using 4-(2-isopropyl-5-methylphenoxy) phthalonitrile derivative. Ni (II) phthalocyanine compound bearing 2,10,16,24-tetrakis (2-isopropyl-5-methylphenoxy phthalocyaninato) Ni (II) was previously synthesized and characterized according to literature procedure [2]. The chemical structure of nickle (II) phthalocyanine compound bearing 2,10,16,24-tetrakis(2-isopropyl-5-methylphenoxy) group [2] is given in the following Fig. 1. The characterization of previously synthesized Ni (II) phthalocyanine compound was carried out by using NMR, FTIR and UV/Vis absorption spectroscopy analyses and their findings are in reference to literature [2].

The study of DNA-binding

Absorption spectra and fluorescence titration studies

Phthalocyanine metal compounds indicate two types of absorption spectra band in the ground state. One of the these bands, which is renowned Q band, is sighted at about 600-750 nm in the visible area of spectrum because of the $\pi \rightarrow \pi^*$ transition from HOMO (the highest occupied molecular orbital) to LUMO (the lowest unoccupied molecular orbital) of phthalocyanine ring. The other absorption band, which is known as B band, is observed in the ultraviolet region of spectrum at approximately 300-450 nm arising from deeper π levels to LUMO of the ring of phthalocyanine [9, 19]. Due to the intense absorption spectra in the red visible zone and high capacity in generating, metallophthalocyanines are found out to be considerably encouraging as an important photosensitizers.

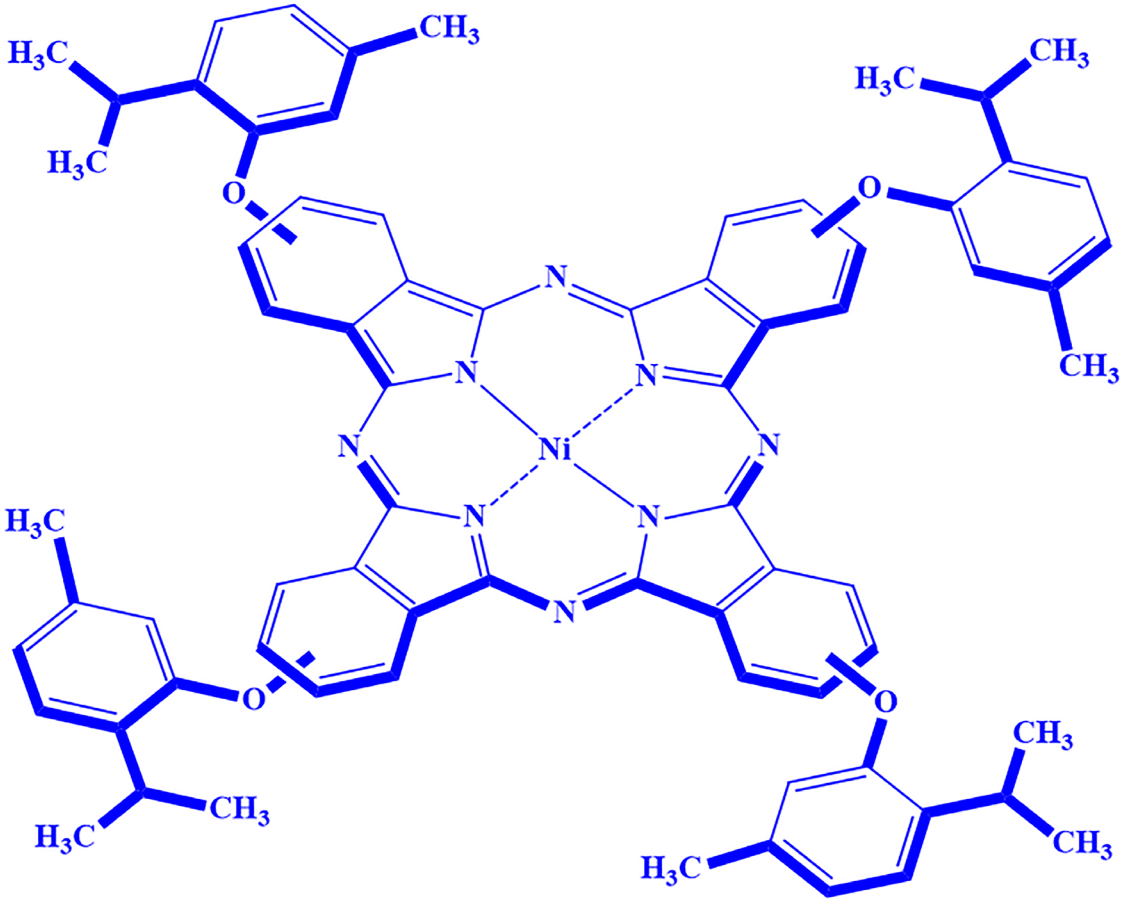

Fig. 1. Chemical structure of nickle (II) phthalocyanine compound (**PcNi**).

Phthalocyanine metal compounds can bind to the DNA via intercalation and non-intercalative binding modes. In general, intercalation interaction of a tiny chemical compound with DNA causes changes in absorbance (hyperchromism or hypochromism) and a red or blue change in wavelength [19] of compound because of π-packing interaction between aromatic groups [20] in comperison with DNA non-intercalation binding mode causes small changes in absorbances and wavelengths in absorption spectra [9, 21]. Generally, an interaction such as intercalation binding mode is involved a hypochromism and a red shift [19].

Absorption titrations of **PcNi** in the absence and presence of the DNA were carried out to investigate binding activities of nickle (II) phthalocyanine compound with the DNA. In order to study the DNA binding properties of nickle (II) phthalocyanine compound, UV-Vis absorption titration was carried out between the range of 300 and 800 nm wavelengths. The absorption titration of **PcNi** in

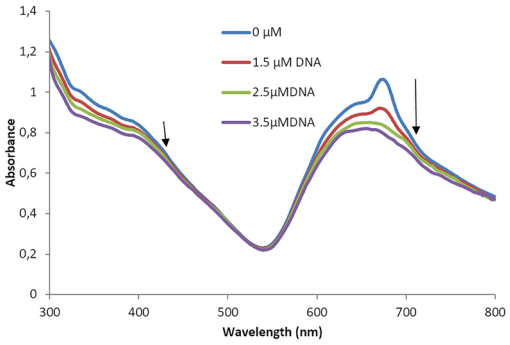

Fig. 2. UV/Vis absorption spectra of **PcNi** in Tris-HCI buffer solution at pH 7.0 on increasing amount of the DNA. The arrows indicate changes in absorbance on increasing amount of CT-DNA.

DMF was observed at about 678 for Q-band absorption and around 390 nm for B-band absorption in the absence of the DNA. Absorption titration of the compound showed hypochromism and red shifts in the presence of the DNA as shown in Fig. 2. As the amount of CT-DNA was increased from 0 to 3.5 µM, a strong hyprochromic change was observed with a small wavelength changing. The significant hypochromic change suggests that a strong interaction occures between nickle (II) phthalocyanine compound and the DNA molecule. These findings indicate that **PcNi** binds to the DNA via intercalative binding mode.

Fluorescence spectroscopy technique is also performed to determine binding activities between metal compounds and DNA [18]. Owing to fluorescence titration method is a prevalent and precision technique in the studies of DNA-binding, this method gives an important information about the binding activities between chemical compound and DNA molecules.

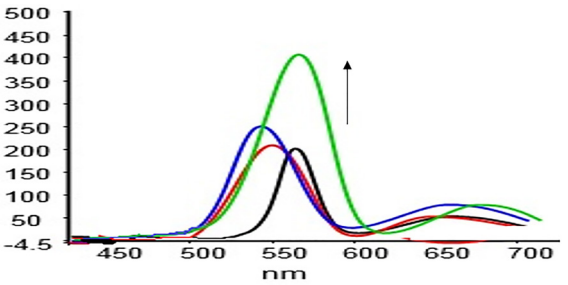

Fig. 3. Fluorescence emission titration of 1.5 µM **PcNi** (black curve) in the absence of CT-DNA in the buffer solution at pH 7.0. Tthe arrow indicates the intensity shift on increasing the DNA concentration (1.5 µM (red curve), 2.5 µM (blue curve) and 3.5 µM (green curve)), where **I** shows intensity.

As shown in Fig. 3, **PcNi** gives intense emission in the absence of DNA in a Tris-HCl buffer solution at ambient temperature with peak displaying at about 563 nm. On the addition of CT-DNA, a clear change in intensities of emission of the compound was observed comparing to the original for **PcNi** as indicated in Fig. 3. The compound gives emission at about 578 nm in the presence of CT-DNA. These results indicate that Ni (II) phthalocyanine compound interacts with CT-DNA in the buffer solution at 7.0.

Cyclic Voltammetry studies

Cyclic voltametry technique is largely operated to investigate binding activities between DNA and chemical compounds and it procures a substantial supplementary information to preliminary evaluated spectral studies [22]. This method is very practical for metal compounds because of their oxidation states. If metal compounds interact with DNA, peak potential and peak current of metal compound change in the presence of DNA molecule [23].

Cyclic voltametry measurements were performed to find out the binding activities between CT-DNA and **PcNi** in the buffer solution at pH 7.0 and the results were shown in Fig. 4. In this study, in the absence and presence of the DNA cyclic voltametric measurements were conducted. The compound has a couple of waves pertains to **PcNi** with the cathodic (EPc) and anodic peak potential (EPa) in the absence of CT-DNA. Anodic peak potential (EPa) and cathodic (EPc) potential were determined to be - 0.11 V (EPc) and - 0.52 V and 0.05 V (EPa) for the compound as shown in Fig. 4a.

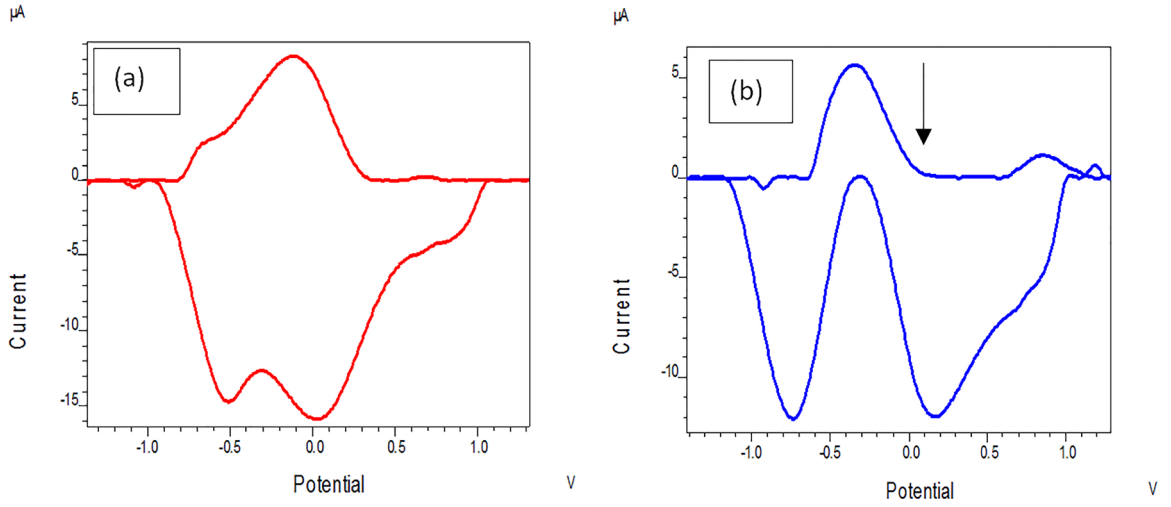

Fig. 4. Cyclic voltammogram of **PcNi** in the a) absence (red line) and b) presence (blue line) CT-DNA of increasing concentration of the DNA. The arrow shows decrease in cathodic peak on increasing amount of the DNA.

On the addition of CT-DNA to the compound, the cyclic voltametric peak currents in the presence of the DNA decreased substantialy. Cathodic peak potential (EPc) and anodic peak potential (EPa) were recorded to be – 0.34 V (EPc) and – 0.72 V and 0.18 V (EPa) for **PcNi** as indicated in Fig. 4b. These results show that the DNA interacts with the compound [24, 25]. In the presence of the DNA, the dropping of the voltametric peak current is referred to low diffusion of **PcNi** binding to CT-DNA. All these results demonstrate that Ni (II) phthalocyanine compound binds to CT-DNA.

Melting point temperature studies

Melting point temperature values for DNA can provide substantial information regarding DNA double helix stability along temperature at 260 nm. The intercalation binding mode of compounds with DNA molecule can cause to increase in melting temperature due to strength of binding mode. In process of non-intercalative interaction of compounds with DNA molecule can decrease melting point temperature [26, 27]. In this study, melting point experiments were conducted to investigate the interaction of compound **PcNi** with CT-DNA in the absence and presence of **PcNi** were indicated in Table 1. In the absence of the compound, melting point of the DNA was observed 73.58 °C, and in the presence of the compound, melting point of the DNA was found to 82.82 °C. The findings show that **PcNi** binds strongly to the DNA through intercalation binding owing to increase in melting point temperature.

Viscosity measurements

In general, binding modes between DNA and molecular compounds are determined by increasing and dropping of relative viscosity of DNA molecule after adding of compounds. An incerase in relative viscosity shows that

Table.1 In the absence and presence of Ni (II) phthalocyanine compound, melting point temperatures (T_m) of the DNA.

Sample	*Melting point temperature (Tm)*
CT- DNA	*73.58 °C*
CT-DNA + PcNi	*80.82 °C*

compounds interact with DNA via intercalative binding mode and DNA base pairs which induces disintegration and elongation of DNA molecule and decreasing in the relative viscosity shows that compounds have non-intercalation interaction between base pairs of DNA. This disintergation of DNA molecule arises from the packing of the ligands between DNA base pairs. The packing of compounds between the base pairs causes a significant change in the structure of DNA molecule [13, 28]. In this work, viscosity measurements were performed in the absence and presence of **PcNi** to investigate binding mode of the compound. Fig. 6 indicates that there is incease in CT-DNA relative viscosity after the inceasing in the amount of **PcNi** because of its successive addition. The findings demonstrate that the compound interacts with the DNA through intercalation binding mode.

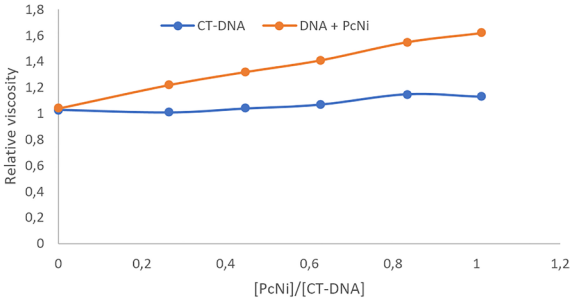

Fig. 6. In the absence and presence of Ni (II) phthalocyanine compound, relative viscosity of the DNA.

Gel Electrophoresis Studies

In this study, agarose gel electrophoresis experiments [27, 29] was conducted to investigate interaction of compound **PcNi** with CT-DNA at room temperature in the absence and presence of the previously synthesized the compound. The interaction of **PcNi** with the DNA was conducted to investigate the effects of varied concentration of the compound on the DNA using gel electrophoresis. The results are indicated in Fig. 7. As compared to band of control DNA (C), the results show that the intensity of the DNA bands were dropped after binding of the compound to DNA. The dropped in intensity of the DNA bands after interaction **PcNi** with the DNA is thought to be deformation of DNA double stranded [30].

The gel electrophoresis experiments clearly showed that Ni (II) phthalocyanine compound interacted with the DNA as there was shift in lane 1-3 bands as compared to CT-DNA control (C) as shown in Fig. 7. The interaction of the compound as compared with that of C is because of its efficient the DNA binding ability. As indicated in Fig. 7, it

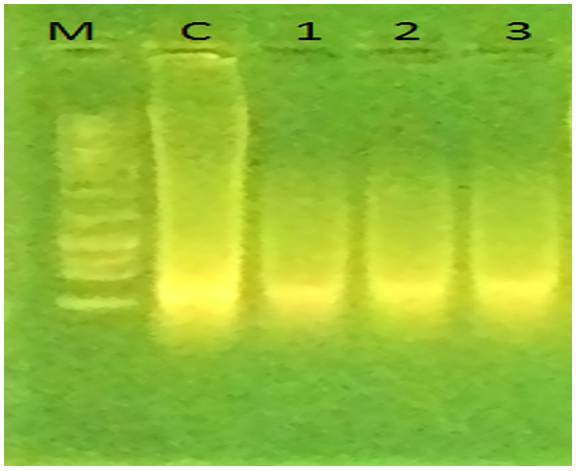

Fig. 7. Agarose gel electrophoresis of CT-DNA in the absence and presence of **PcNi** in a Tris-HCl buffer at pH 7.0. Lane M: DNA Marker; lane C: control DNA; Lanes 1-3: -DNA + **PcNi** (20 μM), respectively.

was found that DNA control (Lane C) band did not indicate any remarkable change of the band of lane C. Lane 1, 2 and 3 belong to **PcNi**. It was clearly seen that the compound interacted with the DNA as compared with the control DNA (C). In addition to this, the interaction of the compound with the DNA caused partial DNA neutralization [31]. The exsiting of smearing in gel diagram showed some cleavage as seen in Fig.7 and the cleavage effect of **PcNi** is comparable to that of C band is due to its effective interaction ability with DNA. As a result, these results indicate that compound Ni (II) phthalocyanine compound can strongly interact with CT-DNA.

CONCLUSION

The binding activities of Ni (II) phthalocyanine compound with the DNA were investigated using UV/Vis absorption spectra and fluoresence titration and the findings showed that the compound interacts strongly with the DNA molecule. The big change in melting point of DNA after binding of the compound also confirms intercalation binding. Cyclic voltametric experiment values indicate that the clear negative peak potentials were recorded on the addition of the DNA also support the intercalation binding of the compound to DNA. Gel electrophoresis experiments also showed that the compound interacts strongly with DNA. The results obtained from viscosity measurements also supports intercalation binding mode between the DNA and Ni (II) phthalocyanine compound. As a result, the compound indicates intense binding activities with the DNA. These results show that nickle (II) phthalocyanine compound could be a candidate compound in cancer treatment due to its DNA binding activities.

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