Research Article GU J Sci 36(3): 1022-1032 (2023) DOI: 10.35378/gujs.1089316

Gazi University

Journal of Science

http://dergipark.gov.tr/gujs

# The Study of Interaction Activity of Nickel (ll) Phthalocyanine Complex Bearing Tetra Substituted Phenoxy-3-Methoxybenzoic Acid Groups with DN

Ali ARSLANTAS[1]* Mehmet Salih AGIRTAS[2]

[1] *Department of Biomedical Engineering, The Faculty of Engineering and Architecture, İzmir Bakırçay University, 78050, İzmir, Türkiye*
[2] *Chemistry Department, The Faculty of Science, Van Yüzüncü Yıl University, 65080, Van, Türkiye*

**Highlights**

- Nickel (II) phthalocyanine compound having phenoxy-3-methoxybenzoic acid.
- Studying of DNA interaction with Ni(II) phthalocyanine.
- Potential medicine of phthalocyanine and binding mode of the compound.

**Article Info**

*Received: 17 Mar 2022*
*Accepted: 02 Aug 2022*

**Keywords**

*Calf thymus-DNA, Phthalocyanines, UV/Vis Spectroscopy, Electrophoresis, Melting Temperature*

**Abstract**

Nickel phthalocyanine complex containing 3-methoxybenzoic acid groups was acquired and specified by way of Fourier Transform Infrared, NMR and UV-Visble spectroscopy procedures. Interaction of **PcNi** with the DNA molecule was examined via electronic absorption spectra, fluorescence spectra, melting point, viscosity, and the electrophoresis technics, respectively. The interaction activity of **PcNi** against the DNA was examined by way of absorption spectra titrations and the fluorescence spectra, farther by conducting melting point, viscosity procedures in the buffer of a pH 7.02. The obtained outcomes from these methods demonstrated that **PcNi** indicated substantial binding affinity to the DNA via intercalating by $K_b$ of 1.31 x $10^6$ $m^{-1}$. Further, the interacting activity of **PcNi** on the DNA was analyzed by which electrophoresis technique and this procedure indicated that **PcNi** complex exhibits strong binding affinity on the DNA.

## 1. INTRODUCTION

Phthalocyanines are one of substantial aromatic compounds and they have interesting biological features such as anticancer, enzyme inhibition, and antimicrobic activities. Also, they have a great potential of applications in the different fields for instance sensors, treatment of cancer disease [1-7]. Phthalocyanine compounds are comfirmed to become an accomplished manner of second origin photosensitizer in nowadays because of their marginal toxicity, powerful absorption spectra in the phototdynamic therapy and easy chemical alteration [5]. Predominantly researches are concentered upon their actions versus cancer and many photosensitizers depending phthalocyanine compounds have been permitted for clinical usage. The one of disadvantages of phthalocyanine compounds in the implementations is the insolubility of these compounds in certain solvents and in the presence of grouping because of the ring interactivity [5].

Recently, investigation for the interaction studies of phthalocyanine metal complexes by DNA molecule have gotten great attention during the recent years to develop new anticancer medicine [8-13]. Many studies on DNA binding of phthalocyanine metal compounds were performed in the reported literature for cancer therapy [14]. Most of the scientific studies concentrated targeting cell cycle and DNA interaction mechanism because the DNA molecule is evaluated the target molecule on medicinal compounds. In the reported studies, the interaction properties of DNA molecule for phthalocyanine metal compounds have

* Corresponding author: e-mail: arsoz33@gmail.com

been studied understanding how the tumor hindering activities of the new anticancer medicine are acting [14, 15]. A crucial part of cancer treatment is made up of phthalocyanine compounds that connect to DNA or avoid the DNA resting [16, 17] and therapeutics binding to DNA molecule may modify DNA structure [18]. When drugs connect to DNA, they may generate difference in duplication of DNA molecule and genetic expression [19, 20]. Small compounds are thought to be the main modes of interaction with the DNA molecule, these are intercalative and non-intercalative binding modes [19, 21]. Phthalocyanines may react on DNA by way of intercalative and hydrogen binding mechanisms [22]. In recently, the interaction activities of phthalocyanines on DNA accelerated in the literature due to the hindering of direct or indirect accretion of cancerous tumor [22].

This current study, synthesized Ni(II) phthalocyanine complex **4** (**PcNi**) having phenoxy-3-methoxybenzoic group was characterized by way of Fourier Transform Infrared, NMR and absorption spectroscopy methods. Interaction properties of the complex by Calf Thymus DNA were searched via absorption, fluorescence, melting point, procedure of electrophoresis and viscosity experiment. The findings from these techniques may pave way for further studies about treatment of cancer.

## 2. EXPERIMENTAL

### 2.1. Material and Method

Chemicals such as $NiCl_2$, $K_2CO_3$, 4-hydroxy-3-methoxybenzoic acid, methanol, acetonitrile, DMF, DMSO, THF were purchased from Merck company (Darmstadt, Germany). Calf Thymus-DNA, NaCl and Tris-HCl had been commercially obtained from Sigma/Aldrich (Darmstadt, Germany). Supplied chemicals had not been purified before usage. In this study, the NMR experiments had been performed with an Agilent spectrometer (Van YYU, Türkiye). For IR measurements, FT-IR were conducted by Thermo Scientific FT-IR spectrophotometer (Van YYU, Turkey). A Hitachi Spectroscopy (Van YYU, Turkey) and Cary 60 UV/Vis spectroscopy (Karabük Uni, Central Laboratory, Turkey) were used for the UV-Vis absorption titration experiments and for the fluorescence titrations were conducted with an Agilent Technologies Cary spectrocopy. Thermo Scientific Electrophoresis device was utilized for the electrophoresis experiments and Ubelohde viscometer was used to carry out viscosity experiments in a Tris-HCl buffer.

### 2.2. The Synthesis of Phenoxy-3-Methoxybenzoic Compound (3)

The synthesis and characterization of phenoxy-3-methoxybenzoic compound (**3**) had been previously reported in the literature [23].

### 2.3. The Synthesis of the Nickle (II) Phthalocyanine Compound (PcNi)

The nickel (II) compound was synthesized with the reaction of the compound (**3**) in medium of $NiCl_2$, which was previously stated in the literature [23]. The yield was 0.017 g (33 %). UV-Vis (THF) λmax (log ε): 672 (5.15) IR spectrum ($cm^{-1}$):3523, 3066, 2914, 1695, 1598, 1587, 1462, 1408, 1263,1217, 1176, 1116, 1091,1058, 1029, 956, 754, 742. $^1$H NMR (400 MHz, DMSO-d6) δ ppm: 7.11, (Ar-H), 3.82($CH_3$), 3.33 (DMSO-$d_6$) 2.48 (DMSO-$d_6$), 1.13 ($CH_3$).

## 3. RESULTS AND DISCUSSION

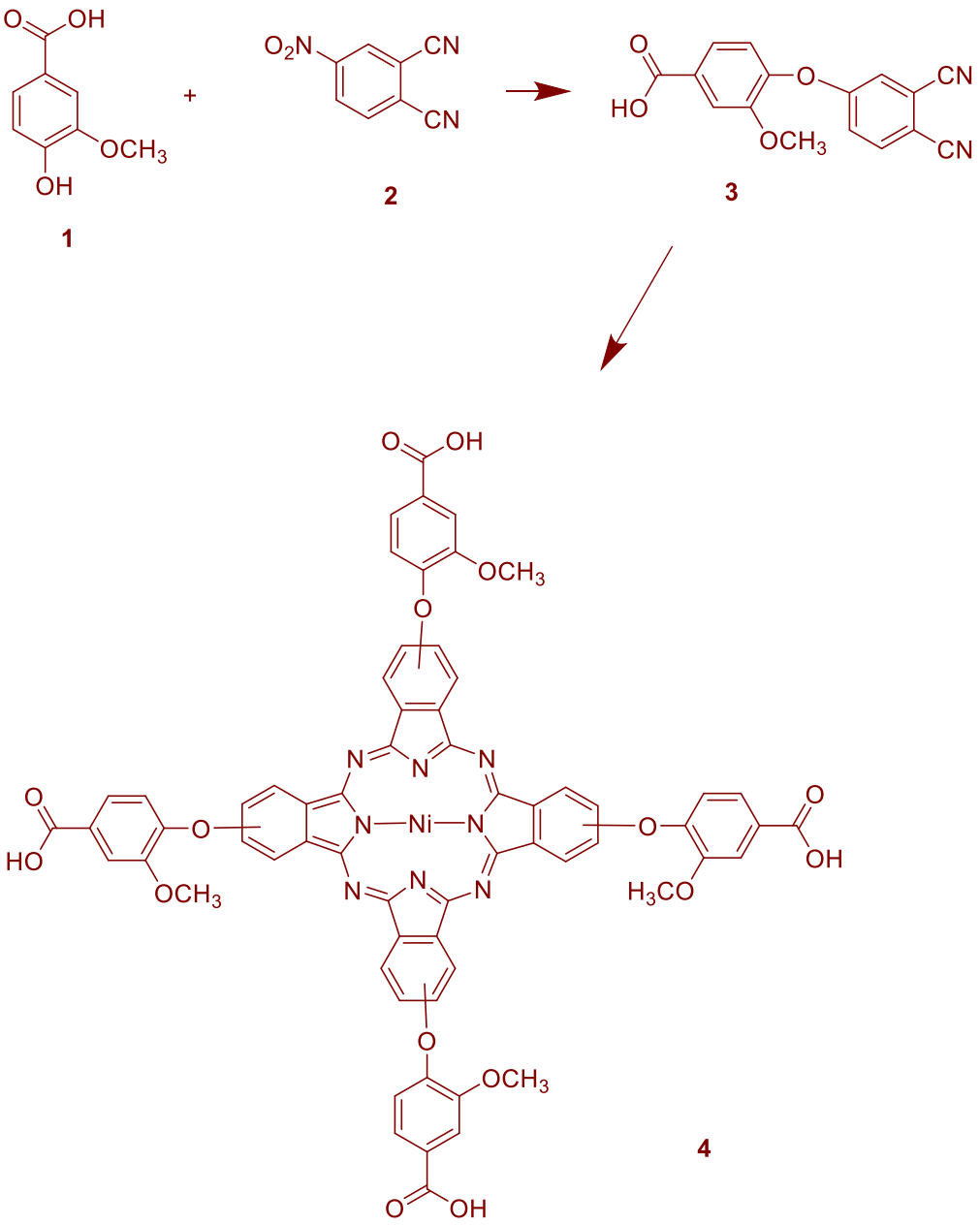


***Figure 1.*** *The synthesis pathway of* ***PcNi*** *phthalocyanine complex*

Synthesized Nickel (II) phthalocyanine complex was produced with cyclotetramerization of the compound (**3**) with $NiCl_2$ at fixed temperature and under $N_2$ gas. The synthesis pathway of Ni(II) phthalocyanine compound is as indicated in Figure 1. **PcNi** was analyzed by spectroscopic procedures like UV-Vis, $^1$H-NMR, IR and the findings had been coherent by anticipated chemical structure. The complex compound of this nickel phthalocyanine dissolves in THF, DMF and DMSO. The FT-IR and $^1$H- NMR spectral data of **PcNi** compound are given in the Figures 2 and 3, respectively.

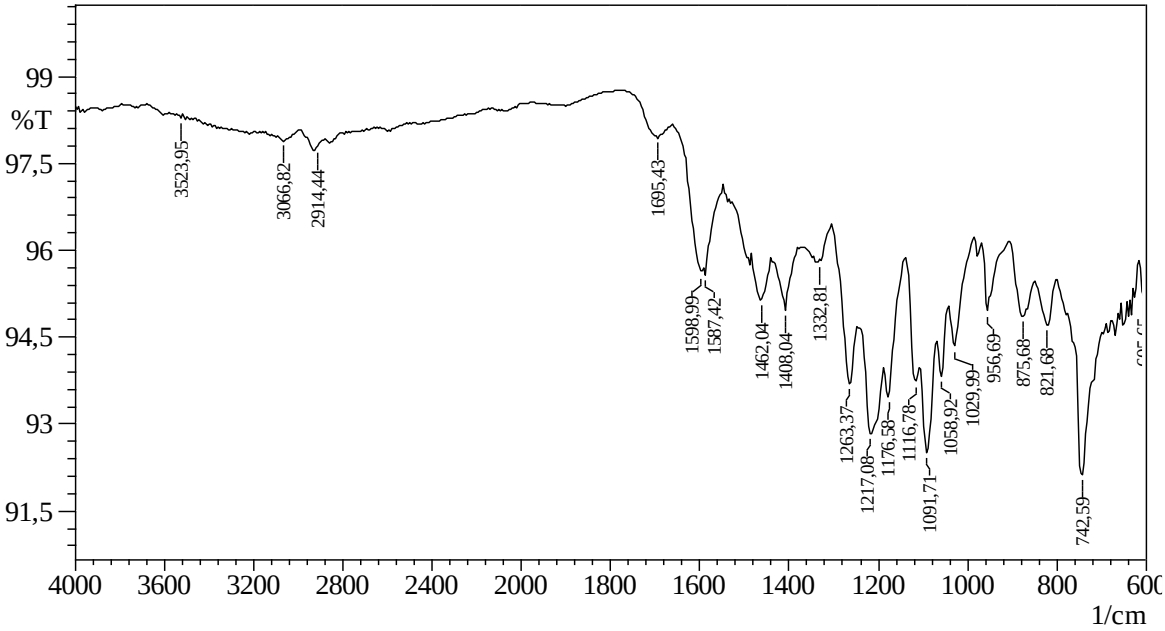


***Figure 2.*** *The FT-IR spectra of* ***PcNi*** *complex*

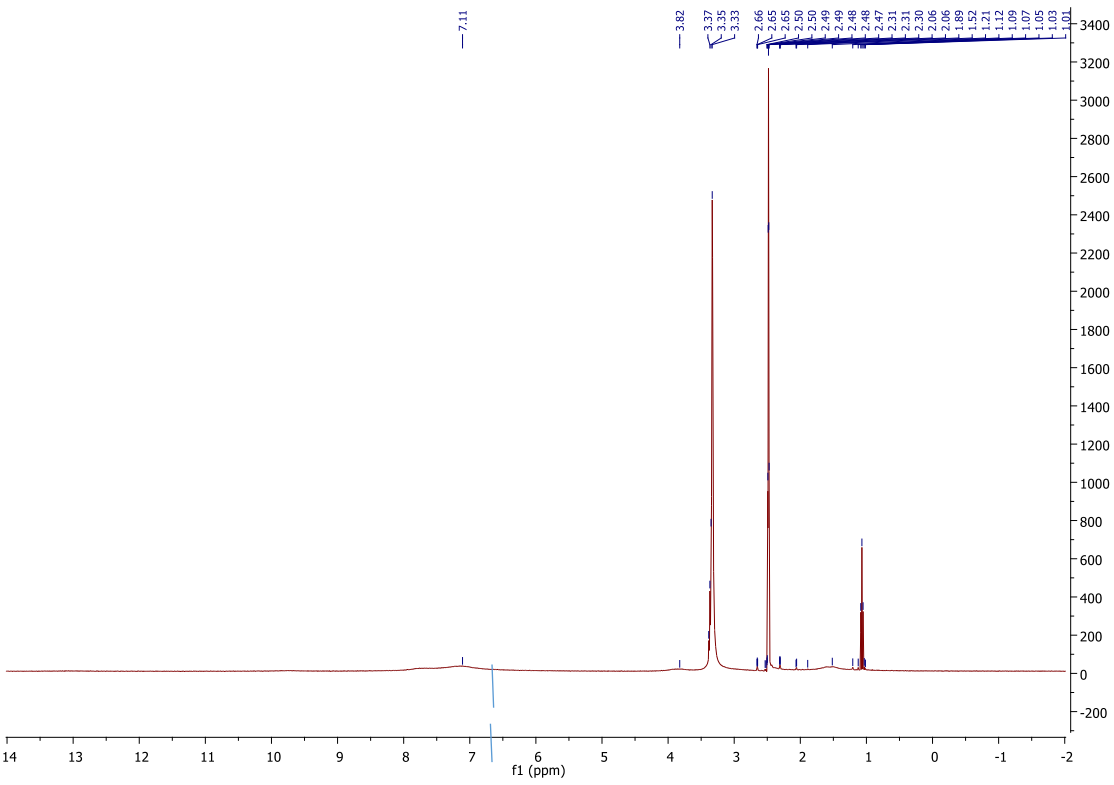


***Figure 3.*** *$^{1}$ H NMR spectra of PcNi complex*

### 3.1. DNA Binding Investigations

The binding of **PcNi** to DNA were examined with absorption titration measurements to determine its binding activities to the DNA [24]. The DNA sample was solved in a Tris-HCl. All the chemical solutions were produced in dimethylformamide (DMF) and then subtilized in the buffer solution at pH 7.02. UV-Vis absorption titrations were conducted in the range of 260–850 nm at 25ºC as illustrated in Figure 4. absorption measurements were implemented by holding amount of **PcNi** fixed (20 µM) and changeable the amounts of CT-DNA (0 to 3.5 µM) with a rising of amounts of the DNA. UV-Vis absorption spectra were measured following each of addition of the DNA. The **PcNi** + DNA solution was permitted to incubate for 5 min for each running and changing in absorption spectra were performed at 25 ºC. The rises in concentration of CT-DNA, absorbances of the complex slowly declined. Interaction activity of the compound to the DNA were observed via hypochromism of two main absorbance bands, which were located at around 350, 686 nm connected to the red shift as represented in Figure 4. From obtained UV-Vis spectra parameters, the binding constant ($K_b$) of **PcNi** by the DNA was specified with Wolfe-Schimer equation [25]. The $K_b$ values for **PcNi** was determined as 1.31 x $10^6$ $m^{-1}$, using Wolfe-Schimer equation. In case of mounting the amount of the DNA, the complex **PcNi** deduced hypochromism by a red shift. As a result, by checking the binding constant value and the tendency of absorption spectra changing by the DNA addition, it can be expected that **PcNi** interacts by DNA in an intercalative binding.

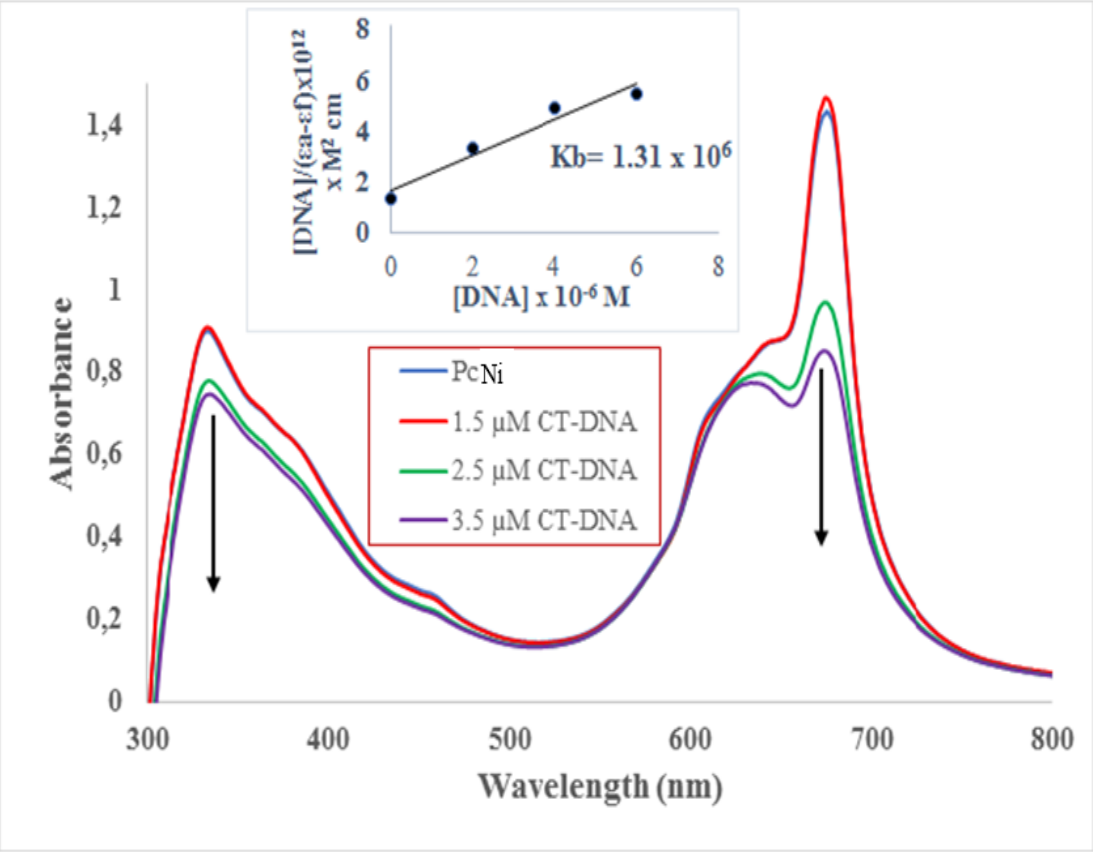


***Figure 4.*** *Absorption titrations of* ***PcNi*** *(20 µM) on increasing concentration of DNA (0 to 3.5 µM) in the buffer solution*

### 3.2. The Fluorescence Titration Studies

The fluorescence procedure is also conducted to explain DNA-medicine interaction activities by reason of fluorescence titration is susceptible technique in DNA binding probes. Besides, it can produce additional knowledge for the intercalation [26, 27]. Fluorescence titration was performed by adding CT-DNA for intercalative interaction to additional examine the binding activity of **PcNi** with DNA. When **PcNi** was connected to the DNA, the intensity of fluorescence spectrum were dropped slowly. The dropping of intensities of fluorescence spectra proved that **PcNi** interacts with CT-DNA using hypochromic mechanism. **PcNi** gives strong fluorescence spectra in the absence of the DNA around 500 nm as illustrated in Figure 5. The powerful fluorescence spectra may be originated from ligands [26,28, 29]. Upon increasing of DNA concentration, intensity of fluorescence spectrum drop for the compound **PcNi**. The finding demonstrated that **PcNi** connects to the DNA much probably via an intercalation, which was coincided with UV-Vis absorption titration results.

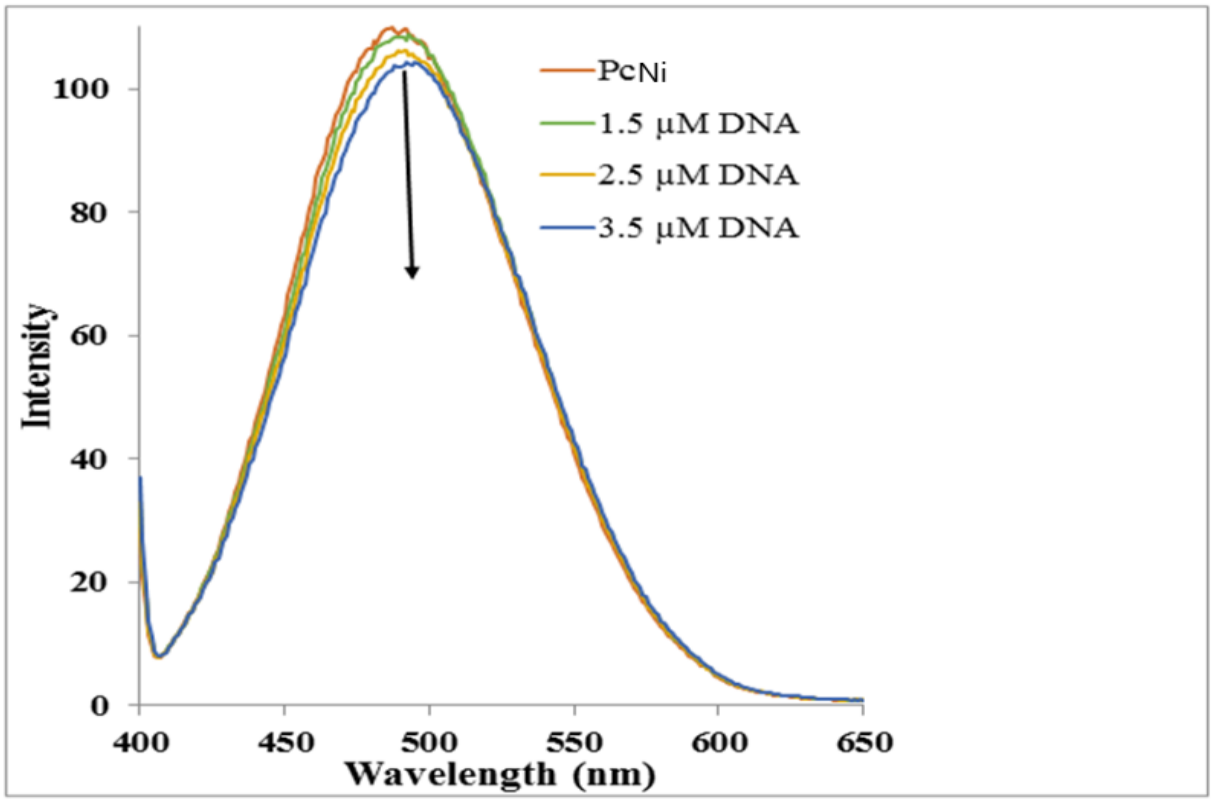


***Figure 5.*** Fluorescence *titration spectra of nickel (II) phthalocyanine complex (20 µM) in the buffer solution at 25°C in the absence and the presence of CT-DNA. Arrow represents changes in intensity on raising the concentration of CT-DNA*

### 3.3. Viscosity Studies for DNA Binding

In the current study for **PcNi** complex, viscosity experiments were conducted to explain binding property between **PcNi** and DNA. An intercalative binding mechanism of binding can yield in the elongation of the double helix of DNA molecule, which can incline rising in viscosity of DNA by the progressive adding of chemical compounds. Nevertheless, non-intercalation mechanism of binding activity can not yield almost rising in viscosity of DNA molecule [28-32]. The obtained results from the viscosity method for **PcNi** complex were plotted by the [Complex]/[CT-DNA] ratio. As illustrated in Figure 6, the relative viscosity of CT-DNA favorably with an intercalative interaction mode.

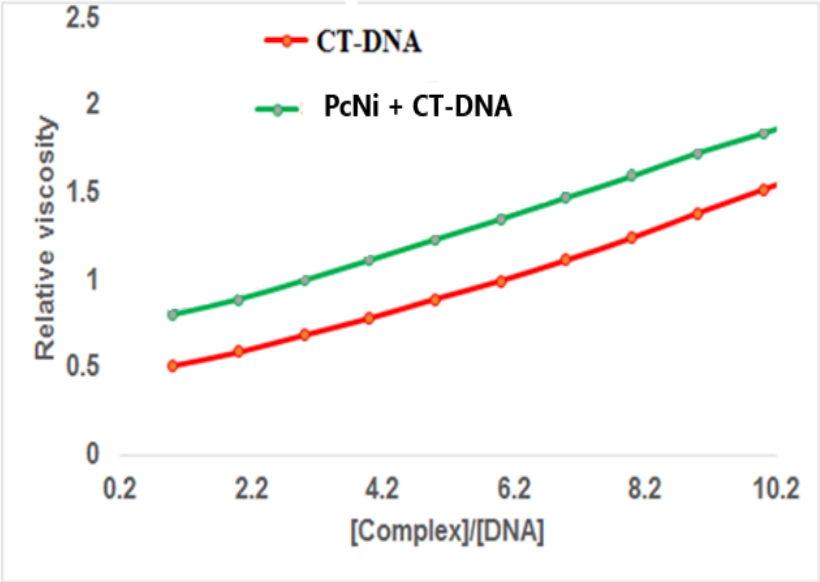


***Figure 6.*** *The impact of rising amount of* ***PcNi*** *complex over the viscosity of DNA (red) in the buffer solution at pH 7.02*

### 3.4. Thermal Denaturation Studies for DNA Binding

The melting temperature studies provide important information with regard to DNA binding activities of a tiny compounds. In this procedure, the solution of a particular amount of Calf Thymus-DNA and the complex was warmed up along 25 °C to 95 °C. The CT-DNA+ **PcNi** had been incubated at particular time each 5 ° C and the absorption titration spectra were registered. The absorbance versus temperature chart had been plotted as represented in Figure 7. Thermal melting points of the DNA was found as approximately 70.40 ºC as indicated in Figure 7. $T_m$ values of CT-DNA+complex had been determined as 77.28 ºC. Generally, if melting point deference of DNA and DNA+**PcNi** complex is big, interaction is considered to be an intercalative binding mode. If the value is not big, DNA interaction is considered as a non-intercalative [27]. For this study, the value of $T_m$ for CT-DNA and **PcNi** were observed as 70.40 ºC and 77.28 ºC, respectively. The findings proved that the **PcNi** connects to DNA using an intercalation.

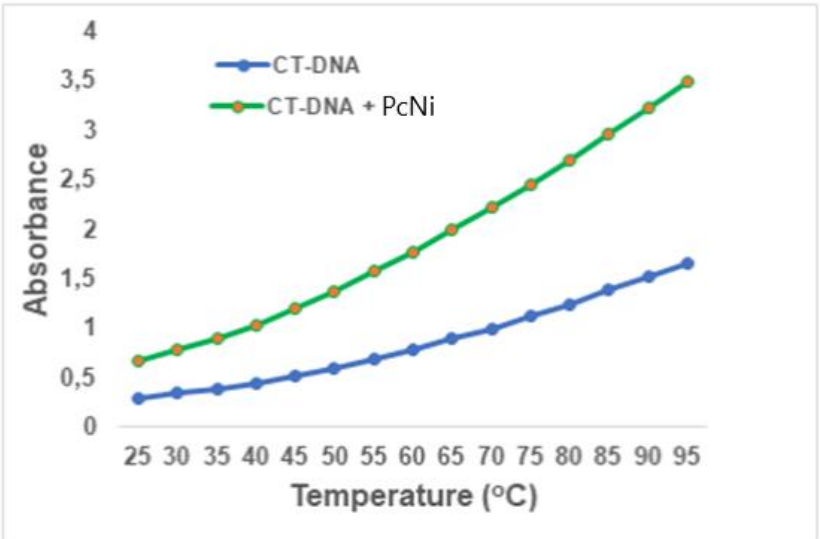


***Figure 7.*** *The $T_m$ of CT-DNA (blue line) and the $T_m$ of CT-DNA+***PcNi** *(green line)*

### 3.5. The DNA Binding Studies Using Agarose Gel Electrophoresis

As well above procedures, the DNA binding activities of **PcNi** was analyzed using the gel electrophoresis technique. The migration of CT-DNA+**PcNi** had been registered after the staining as illustrated in Figure 8. M lane represents DNA ladder. Bands 1, 2 and 3 belong to **PcNi** with different amount of the DNA. The concentration of CT-DNA enhances from the bands 1 to 3 and the amounts of the compound had been kept fixed at 25 μM whereas the concentration of CT-DNA was shifted between the range of 10 to 25 μM. Band intensities of DNA had been registered in the default of **PcNi** and then intensities of the DNA were studied in the presence of the compounds. As indicated in Figure 8, The band intensities of DNA were reduced and the migration of DNA bands had been slightly smeared due to the neutralization of DNA [30, 33]. These findings verified that **PcNi** link to DNA molecule.

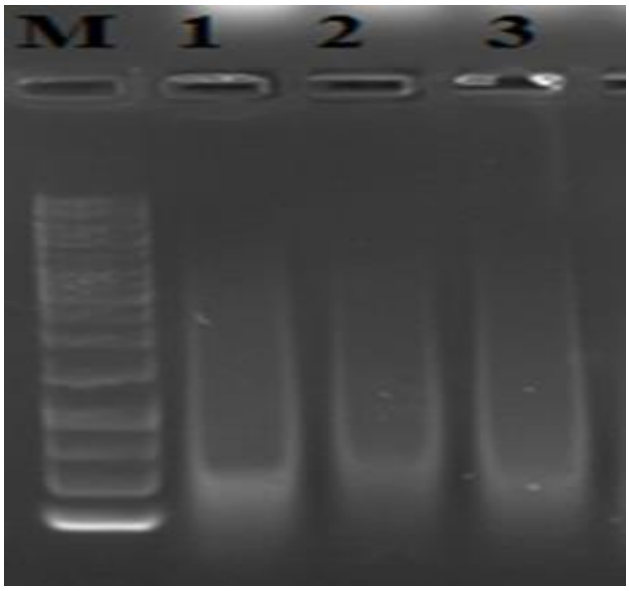


***Figure 8.*** *Agarose gel electrophoresis studies for* ***PcNi*** *(25 μM) in the buffer upon mounting concentration of the DNA (10-25 μM). Where M refers DNA ladder*

## 4. CONCLUSION

The synthesized tetra substituted the nickel (II) phthalocyanine was characterized with absorption titration, FT-IR and NMR. Interaction of the complex with CT-DNA were analyzed via absorption titrations, fluorescence titrations and viscosity measurement, melting temperature, and gel electrophoresis. The $K_b$ constant was attained using UV-Vis spectroscopy from CT-DNA titrations proposed an intercalation binding mechanism for **PcNi** complex. The values produced from fluorescence titrations suggested that **PcNi** binds to DNA via intercalative mode. The result from viscosity method indicated a rising inclination in viscosity of the DNA sample upon adding of **PcNi.** The result showed that the compound interacts with DNA. All of referred techniques proved that the binding mechanism was an intercalative interaction. DNA interaction activity of **PcNi** was also investigated via the agarose gel electrophoresis for DNA. The findings showed that **PcNi** interacts with DNA molecule.

## CONFLICTS OF INTEREST

No conflict of interest was declared by the authors

## ACKNOWLEDGMENTS

The research had been funded by Scientific Research Project Commission of Karabük University (Project No: KBÜBAP-18-DS-046).

## REFERENCES

[1] Yılmaz, F., Ozer, M., Kani, I., Bekaroglu, O., "Catalytic activity of a thermoregulated, phase-separable Pd(II)-perfluoroalkyl phthalocyanine complex in an organic/fluorous biphasic system: hydrogenation of olefins", Catalysis Letters, 130: 642-647, (2009).

[2] Leznoff, C.C., Lever, A.B.P., "Phthalocyanines properties and applications", VCH Publisher, New York, Vol. 2, (1993).

[3] Leznoff, C.C., Lever, A.B.P., "Phthalocyanines Properties and Applications", VCH Publisher, New York, 1, (1989).

[4] Parra, V., Bouvet, M., Brunet, J., Rodríguez-Mendez, M.L., Saja, J.A., "On the effect of ammonia and wet atmospheres on the conducting properties of different lutetium bis-phthalocyanine thin films", Thin Solid Films, 516: 9012-9019, (2008).

[5] Al-Raqa, S.Y., Khezami, K., Kaya, E.N., Durmus, M., "A novel water soluble axially substituted silicon(IV) phthalocyanine bearing quaternized 4-(4-pyridinyl)phenol groups: Synthesis, characterization, photophysicochemical properties and BSA/DNA binding behavior", Polyhedron, 194: 114937, (2021).

[6] Rosenthal, I., "Phthalocyanines as photodynamic sensitizer", Photochemistry and Photobiology, 53: 859-870, (1991).

[7] Leznoff, C.C., Lever A.B.P., "Phthalocyanines, properties and applications", VCH Publisher, New York, (1996).

[8] Hadjiliadis, N.D., Sletten, E., "Metal complex–DNA interactions", Wiley-Blackwell, New York, (2009).

[9] Van Holst, M., Grant, M.P., Aldrich-Wright, J., "Metallointercalators-synthesis and techniques to probe their interactions with biomolecules", Springer, Wien, New York, (2011).

[10] Lukyanets, E.A., "Phthalocyanines as photosensitizers in the photodynamic therapy of cancer", Journal of Porphyrins and Phthalocyanines, 3: 424–432, (1999).

[11] Vummidi, B.R., Noreen. F., Alzeer J., Moelling, K., Luedtke, N.W., "Photodynamic agents with anti-metastatic activities", ACS Chemical Biology, 8: 1737–1746, (2013).

[12] Yildiz, B.T., Sezgin, T., Cakar, Z.P., Uslan, C., Sesalan, B.S., "The use of novel photo bleachable phthalocyanines to image DNA", Synthetic Metals, 161: 1720–1724, (2011).

[13] Amitha, G.S., Vasudevan, S., "DNA/BSA binding studies of peripherally tetra substituted neutral azophenoxy zinc phthalocyanine", Polyhedron, 175: 114208, (2020).

[14] Ali, A., Bhattacharya, S., "DNA binders in clinical trials and chemotherapy", Bioorganic Medicinal Chemistry, 22: 4506–4521, (2014).

[15] Alam, M.D.F., Varshney, S., Khan, M.A., Laskar, A.A., Younus, H., "In vitro DNA binding studies of therapeutic and prophylactic drug citral", International Journal of Biological Macromolecules, 113: 300–308, (2018).

[16] Palchaudhuri, R., Hergenrother, P.J., "DNA as a target for anticancer compounds: methods to determine the mode of binding and the mechanism of action", Current Opinion in Biotechnology, 18: 497–503, (2007).

[17] Bağda, E., Yabaş, E., Bağda, E., "Analytical approaches for clarification of DNA-double decker phthalocyanine binding mechanism: As an alternative anticancer chemotherapeutic", Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 172: 199–204, (2017).

[18] Rescifina, A., Zagni, C., Varrica, M.G., Pistarà, V., Corsaro, A., "Recent advances in small organic molecules as DNA intercalating agents: synthesis, activity, and modeling", European Journal of Medicinal Chemistry, 74: 95–115, (2014).

[19] Ozluer, C., Satana Kara, H.E., "In vitro DNA binding studies of anticancer drug idarubicin using spectroscopic techniques", Journal of Photochemistry and Photobiology B: Biology, 138: 36–42, (2014).

[20] Williams, A.K., Dasilva, S.C., Bhatta, A., Rawal, B., Liu, M., Korobkova, E.A., "Determination of the drug-DNA binding modes using fluorescence-based assays", Analytical Biochemistry, 411: 66–73, (2012).

[21] Özkay, Y., Işıkdağ, İ., İncesu, Z., Akalın, G., "Synthesis of 2-substituted-N-[4-(1-methyl-4,5-diphenyl-1H-imidazole-2-yl)phenyl]acetamide derivatives and evaluation of their anticancer activity", European Journal of Medicinal Chemistry, 45: 3320–3328, (2010).

[22] Uslan, C., Sesalan, B.Ş., "The synthesis, photochemical and biological properties of new silicon phthalocyanines", Inorganica Chimica Acta, 394: 353–362, (2013).

[23] Ballı, Z., Arslantaş, A., Solğun Güngördü, D., Ağırtaş, M.S., "DNA binding studies of the 2,10,16,24–tetrakis (phenoxy-3-methoxybenzoic acid) phthalocyaninato) Co(II) and Cu(II) compounds", Springer Nature Applied Sciences, 2: 844-853, (2020).

[24] Barone, G., Terenzi, A., Lauria, A., Almerico, A.M., Leal, J.M., Busto, N., García, B., "DNA-binding of nickel(II), copper(II) and zinc(II) complexes: Structure–affinity relationships", Coordination Chemistry Reviews, 257(19–20): 2848-2862, (2013).

[25] Wolfe, A., Shimer, G.H., Meehan, T., "Polycyclic aromatic hydrocarbons physically intercalate into regions of denatured DNA", Biochemistry, 26: 6392–6396, (1987).

[26] Liu, X.W., Shen Y.M., Li, Z.X., Zhong, X., Chen, Y.D., Zhang, S.B., "Study on DNA binding behavior and light switch effect of new coumarin-derived Ru (II) complexes", Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 149: 150–156, (2015).

[27] Barton, J.K., Goldberg, J.M., Kumar, C.V., Turro, N.J., "Binding modes and base specificity of tris(phenanthroline)ruthenium (II) enantiomers with nucleic acids: tuning the stereoselectivity", Journal of American Chemical Society, 108: 2081–2088, (1986).

[28] Amitha, G.S., Vasudevan, S., "DNA binding and cleavage studies of novel Betti base substituted quaternary Cu (II) and Zn (II) phthalocyanines", Polyhedron, 190: 114773, (2020).

[29] López Zeballos, N.C., Gauna, G.A., García Vior, M.C., Awruch, J., Dicelio, L.E., "Interaction of cationic phthalocyanines with DNA. Importance of the structure of the substituents", Journal of Photochemistry and Photobiology B: Biology, 136: 29-33, (2014).

[30] Barut, B., Yalçın, C.Ö., Sari, S., Çoban, Ö., Keleş¸ T., Biyiklioglu, Z., Abudayyak, M., Demirbaş, Ü., Özel, A., "Novel water soluble BODIPY compounds: Synthesis, photochemical, DNA interaction, topoisomerases inhibition and photodynamic activity properties", European Journal of Medicinal Chemistry, 183: 111685, (2019).

[31] Çoban, Ö., Barut, B., Yalçın, C.Ö., Özel, A., Biyiklioglu, Z., "Development and in vitro evaluation

of BSA-coated liposomes containing Zn (II) phthalocyanine-containing ferrocene groups for photodynamic therapy of lung cancer", Journal of Organometallic Chemistry, 925: 121469, (2020).

[32] Satyanarayana, S., Dabrowiak, J.C., Chaires, J.B., "Neither.DELTA.- nor.LAMBDA.-tris(phenanthroline)ruthenium(II) binds to DNA by classical intercalation", Biochemistry, 31: 9319–9324, (1992).

[33] Ji, L.N., Zou, X.H., Liu, J.G., "Shape- and enantioselective interaction of Ru (II)/Co (III) polypyridyl complexes with DNA", Coordination Chemistry Reviews, 216–217: 513– 536, (2001).