ISTANBUL TECHNICAL UNIVERSITY ★INSTITUTE OF SCIENCE AND TECHNOLOGY

SYNTHESIS OF PYRIDINE-SUBSTITUTED PHTHALOCYANINES AND THEIR DNA BINDING PROPERTIES

M.Sc. THESIS

Maryam MOEINI ALISHAH

Department of Chemistry
Chemistry Programme

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Thesis Advisor: Prof. Dr. Zehra ALTUNTAŞ BAYIR

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ISTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

PİRİDİN SÜBSTİTÜE FTALOSİYANİNLERİN SENTEZİ VE DNA BAĞLANMA ÖZELLİKLERİNİN İNCELENMESİ

YÜKSEK LİSANS TEZİ

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ARALIK 2016

Maryam MOEINI ALISHAH, a M.Sc. student of İTU Graduate School of Science Engineering and Technology student ID 509131043, successfully defended the thesis entitled "SYNTHESIS OF PYRIDINE-SUBSTITUTED PHTHALOCYANINES AND THEIR DNA BINDING PROPERTIES", which she prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

Thesis Advisor : **Prof. Dr. Zehra ALTUNTAS BAYIR** ……...

ISTANBUL Technical University

Jury Members : **Prof. Dr. Ahmet GUL** ……

ISTANBUL Technical University

Prof. Dr. Ulvi AVCIATA ……

ISTANBUL Esenyurt University

Date of Submission : 25 November 2016

Date of Defense : 19 December 2016

To my brother,

FOREWORD

I would like to express my sincere gratitudes to my thesis advisor, Zehra ALTUNTAŞ BAYIR, for her interest and guidance.

Thanks are due to Ms. Yasemin YENİLMEZ, Mrs. B. Şebnem SESALAN, Mr. İbrahim ÖZÇEŞMECİ, and Mr. Barbaros AKKURT for their help in my thesis.My dear friends Merve PAMUKÇU POLAT and Nazlı FARAJZADEH helped a lot in my thesis studies, for that I am grateful.

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November 2016

Maryam MOEINI ALISHAH

(Chemist, MSc)

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SYNTHESIS OF PYRIDINE-SUBSTITUTED PHTHALOCYANINES AND THEIR DNA-BINDING PROPERTIES

SUMMARY

Phthalocyanines, being tetrapyrrole derivatives, constitute an important class of compounds both in basic researches and in applied sciences. Adding desired functional groups to the periphery of phthalocyanine rings changes its optical, electronic and catalytic properties and enables them to be used in different areas. Owing to these properties, phthalocyanines have been used in dyestuffs, photochromic and electrochromic materials, liquid crystals, catalysts, chemical sensors, gas sensors in the form of Langmuir-Blodgett films, and non-linear optical materials.

Adding water-soluble groups to phthalocyanines as functional groups has also enabled them to be researched in the therapy of cancer. Today, phthalocyanines are being used as second-generation photosensitizers. Compared with the first-generation photosensitizers such as hematoporphyrin derivative, metallophthalocyanines have a much higher extinction coefficient of the Q-band near 680 nm, which means that they are efficiently excited directly through tissue, whereas the introduction of hydrophilic groups into substituted phthalocyanine derivatives has been performed in order to achieve solubility in aqueous media. Phthalocyanines having water soluble groups have a strong influence on the bioavailability and *in vivo* distribution. While the ionic groups provide binding of phthalocyanines to DNA and proteins (*e.g.* BSA).

Within the scope of this thesis, suitable substituents were attached to the phthalocyanine rings and metallophthalocyanines were synthesized by incorporating different metal ions. For this purpose, iodophthalonitrile was synthesized as a first step and tetraiodozinc phthalocyanine was synthesized with zinc acetate as the metal ion in a suitable solvent. Under typical Sonogashira reaction conditions, the cross-coupling reaction between an excess of 2-ethynyl pyridine and tetraiodo-metallophthalocyanines in triethylamine with copper(I)iodide (CuI) and bis(triphenylphosphine)palladium(II)chloride [$Pd(PPh_3)_2Cl_2$] as catalysts at room temperature under nitrogen atmosphere produced 2,9(10),16(17),23(24)-tetra-(3-pyridylethynyl) phthalocyaninato zinc(II). For the synthesis of water-soluble phthalocyanine derivatives, pyridine groups were introduced into the reaction to synthesize quaternized derivatives. The phthalocyanines were reacted with excess amount of dimethyl sulfate. The same method was applied to obtain Co(II) phthalocyanine and its quaternary derivative. The new compounds were characterized ^{1}H-NMR, FTIR and UV-Vis techniques. The aggregation and binding to DNA and BSA properties were investigated.

PİRİDİN SÜBSTİTÜE FTALOSİYANİNLERİN SENTEZİ VE DNA BAĞLANMA ÖZELLİKLERİNİN İNCELENMESİ

ÖZET

Tetrapirol türevi olan ftalosiyaninler hem temel hem de uygulamalı çalışmalarda önemli bir bileşik sınıfını oluşturmaktadır. Ftalosiyanin halkasına istenilen özellikteki fonksiyonel grupların eklenebilmesi optik, elektronik ve katalitik özelliklerini değiştirmekte ve değişik uygulama alanlarında kullanılmasını sağlamaktadır. Bu özelliklerinden dolayı ftalosiyaninler boyar madde, fotokromik, elektrokromik malzemeler, sıvı kristaller, katalizör, kimyasal sensör, Langmuir-Blodgett filmlerinde gaz sensörü, fotokopi makinelerinde ve lazer yazıcılarda fotoaktif iletken, ve nonlineer optik maddeler gibi farklı alanlarda kullanılmaktadır.

Ftalosiyaninlere fonksiyonel grup olarak suda çözünebilen grupların eklenmesiyle kanser tedavisinde de kullanılabilmesinin önü açılmıştır. Günümüzde ftalosiyaninler ikinci nesil fotoalgılayıcılar olarak kullanılmaktadırlar. Hematoporfirin türevi gibi birinci nesil fotohassaslaştırıcılarla karşılaştırıldığı zaman, metalli ftalosiyaninlerin 680 nm civarındaki Q bandının molar soğurma katsayısı çok daha büyüktür, bunun anlamı doğrudan dokudan geçebilmeleridir, sübstitüe ftalosiyanin türevlerine hidrofil gruplar eklenmesi sulu ortamda çözünürlüğü sağlamak için üzerinde çalışılan bir konudur. Suda çözünen gruplar içeren ftalosiyaninler biyo-bulunurluk ve *in vivo* dağılım üzerine kuvvetli bir etkide bulunurlar. İyonik gruplar ise ftalosiyaninlerin DNA'ya ve proteinlere (örneğin BSA) bağlanma olanağı kazandırır.

Tez kapsamında ftalosiyanin halkasına çözünürlük sağlayacak uygun sübstitüe gruplar bağlanarak bunların çeşitli metal tuzlarıyla metaloftalosiyaninleri hazırlanmıştır. Bu amaçla ilk olarak iodophthalonitrile hazırlanarak $Zn(CH_3COO)_2$ la uygun çözücü ortamında tetraiyodoçinko ftalosiyanin sentezlenmiştir. Tipik Sonogashira tepkime koşulları altında, aşırı 2-etinilpiridin ve tetraiyodo ftalosiyanin arasındaki kenetlenme tepkimesi trietilamin içinde bakır(I) iyodür (CuI) ve bis(trifenilfosfin)paladyum(II) klorür ($[Pd(PPh_3)_2Cl_2]$) katalizörlüğünde oda sıcaklığında ve azot atmosferinde 2,9(10),16(17),23(24)-tetrakis(3-piridiletinil)ftalosiyaninatoçinko(II) elde edilmiştir.

Suda çözünen ftalosiyanin türevlerinin sentezi için, kuaterner türevlerin sentezlenebilmesi amacıyla reaksiyon ortamına piridin grupları ilave edilmiştir. Ftalosiyaninler aşırı miktarda dimetil sülfat ile reaksiyona sokulmuştur. Aynı yöntem uygulanarak Co(II) ftalosiyanin ve kuaterner türevi elde edeilmiştir. Sentezlenen yeni bileşiklerin yapıları ^{1}H-NMR, FTIR ve UV-Vis teknikleri kullanılarak aydınlatılmıştır. Ftalosiyaninlerin agregasyon ve DNA, BSA-bağlanma özellikleri incelenmiştir.

1. INTRODUCTION

First phthalocyanines (Pcs) were found accidentally in 1907 during the synthesis of *o*-cyanobenzamide as a side product. In Greek, the word phthalocyanine comes from naphtha (mineral oil) and cyanine (dark blue).

Phthalocyanines are the members of tetrapyrrolic compounds and in recent years, they are also used in both basic science and in practical works.

Phthalocyanines have 4 isoindole units having an 18-π electron aromatic system in the ring. Adding extra substituents to peripheral positions increases the distance between 18-π electron conjugation of phthalocyanines, thus providing easier solubility.

Delocalization of electrons in 18-π electron system of phthalocyanines is one of important reasons to use this compound in many areas from medicine to electronics. There are stable to heat, light, and non-oxidizing acids and bases and they are soluble in organic solvents if peripheral (or non-peripheral) substituents are added. Adding functional groups with different features to the Pc rings provide the desired changes in physical properties of phthalocyanines. When the solubility of the molecule and connected substituents start to rise, it is possible to synthesize the desired phthalocyanines through this method.

Phthalocyanines are able to accept more than 70 metallic and nonmetallic ions to ring vacancy. In producing metallophthalocyanines, the metal ion is connected to the ring by template effect and this is the reason of acceptable yields of reaction, So, metal-based phthalocyanines have been added with more yield than metal-free phthalocyanines. Some metal ions are used in MPcs because of their redox activity of the compound that has effects on the chemical, physical and thermal properties. The common metal ions used in the core are Co, Cu, Ni, and Zn because these complexes are able to be purified easily, give high yield in cyclotetramerizations, and ease of

synthesis. Redox richness makes phthalocyanines to have more potential in other practices.

In the chemistry of phthalocyanines, aggregation is a phenomenon that is seen commonly. However, some substituents on the peripheral positions of phthalocyanines, such as fluorinated substituents, are able to decrease the interaction between phthalocyanines, reduce the self-oxidation trends, and aggregation.

The electronic and optical properties for the phthalocyanines are the reasons to use these compounds in some technological applications. For example, solar cells, electrochromic display devices, molecular electronics, optical disks, photovoltaics, Langmuir –Blodgett films, liquid crystals, and gas sensors.

In recent years, phthalocyanines have been investigated in photodynamic cancer therapy (PDT) as photosensetizers. Solubility in water at different pH ranges is an essential factor for the tetrapyrrolic rings.

One of the obvious benefits of using cationic water soluble phthalocyanines in spite of porphyrins in PDT is their powerful absorption in the visible spectrum and long lifetimes. In materials, biology and catalysis practice, cationic phthalocyanines illustrate widespread category of compounds.

2. GENERAL INFORMATION

2.1 Tetrapyrrole Macrocycles

Macrocyclic tetrapyrrole derivatives include porphyrins, porphyrazines, tetrabenzoporphyrins, and phthalocyanines (Figure 2.1).

The complexes of natural compounds such as cytochromes and chlorophyll are attractive to the contents of macrocyclic complexes like porphyrins and corroles. As a result, there are several researches about derivatives of porphyrins and phthalocyanines.

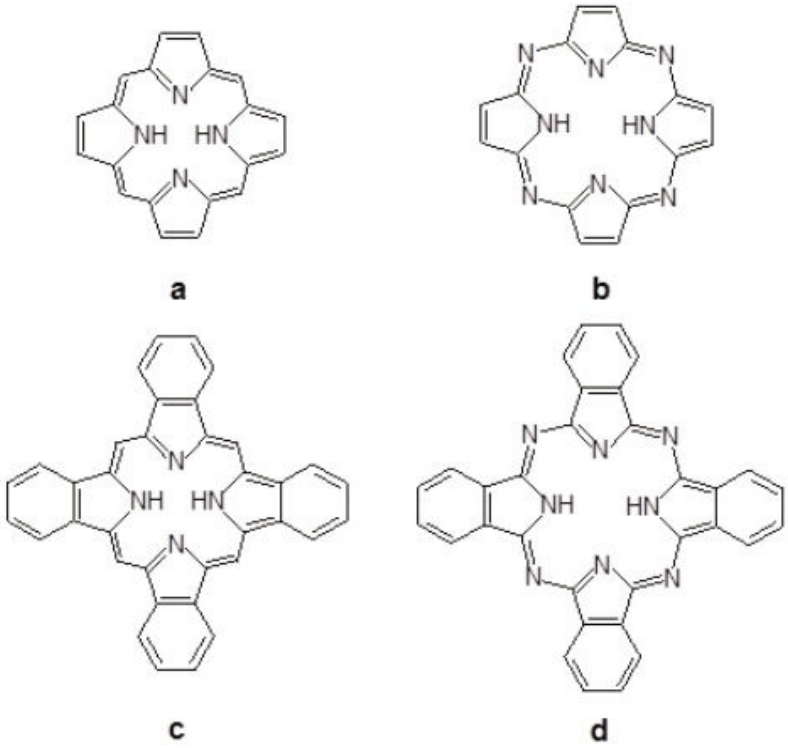


Figure 0.1 : a) Porphyrin, b) porphyrazine, c) tetrabenzoporphyrin, d) phthalocyanine.

2.2 Phthalocyanines

Phthalocyanines for the first time were found accidentally in 1907 by Braun and Tcherniac at South Metropolitian Gas Company (Great Britain) during the synthesis of o-cyanobenzamide from acetic acid and phthalimide. It was a side product which had a dark color (Figure 2.2) [1].

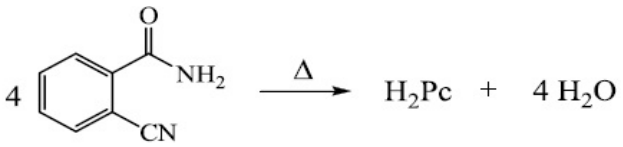


Figure 0.2 : Synthesis of Pc during the synthesis of o-cyanobenzamide.

Similarly in 1927, Diesbach and von der Weid at Fribourg University obtained a product with blue color when they heated dibromobenzene and copper(II) cyanide in pyridine to 200 °C, but they could not suggest any structure for the substance that formed. [2]

Then in Imperial College of London, Linstead and co-workers could present the structure of metal-based phthalocyanine and defined the molecular structure of phthalocyanine (Figure 2.2) [3-8].

The main compound is encircled by 16 hydrogens and another 2 hydrogens in the core center with the chemical formula of $C_{32}H_{18}N_8$ or $(C_8H_4N_2)_4H_2$. Linstead used the Greek name of phthalocyanine for this compound. The crystal structure for this bulky organic molecule unsubstituted then Robertson used X-ray diffraction on iron-phthalocyanine to explain that (Figure 2.3) [9-11].

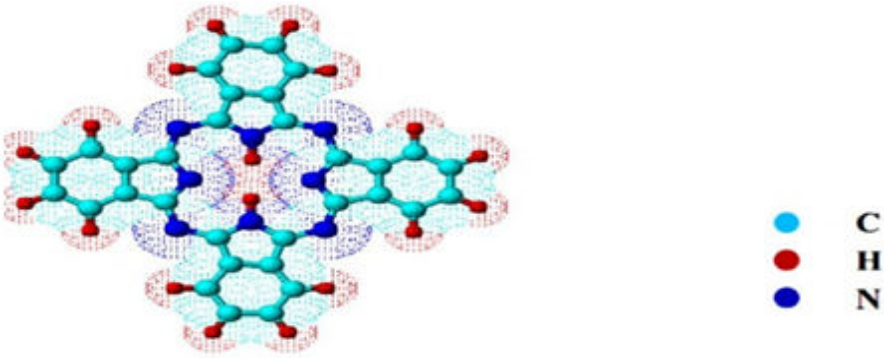


Figure 0.3 : Schematic representation of phthalocyanine [9].

The other features of phthalocyanines can be listed as follows:

- Pcs have chemical and thermal stability,

Pcs have sufficient stability against strong acids and bases, however, powerful oxidizers turn them to phthalic acid and phthalimide so the ring is broken down,

- Sublimation and crystallization of Pcs are simple so pure products are obtained,

- There is not significant breakdown in the air until 400-500 °C and the largest part of metal complexes are not broken down before 900 °C in the vacuum,

- Pcs are able to form complexes with most metals in the periodic table,

- The type of metal ions in Pcs has important effects in physicochemical features.

Adding various substituents to the peripheral positions have rendered Pcs have more properties. Between 1963 and 1975, many application area also were added and it will continue.

Nowadays, Pcs with blue and green color are used in several areas. For example, they are used in printing inks, aluminum, wall paintings, plastics, synthetic fibers and textiles, oxidation of hydrocarbons and sulfur-containing compounds, fuel tanks, catalysis of hydrogenation, medicine, liquid crystals, semiconductors, lasers, and lubricants. Having such specifications make Pcs more interesting.

2.3 Nomenclature of Phthalocyanines

Phthalocyanine ring system gets various names in different positions or locations. In (Figure 2.4), this system has been illustrated .

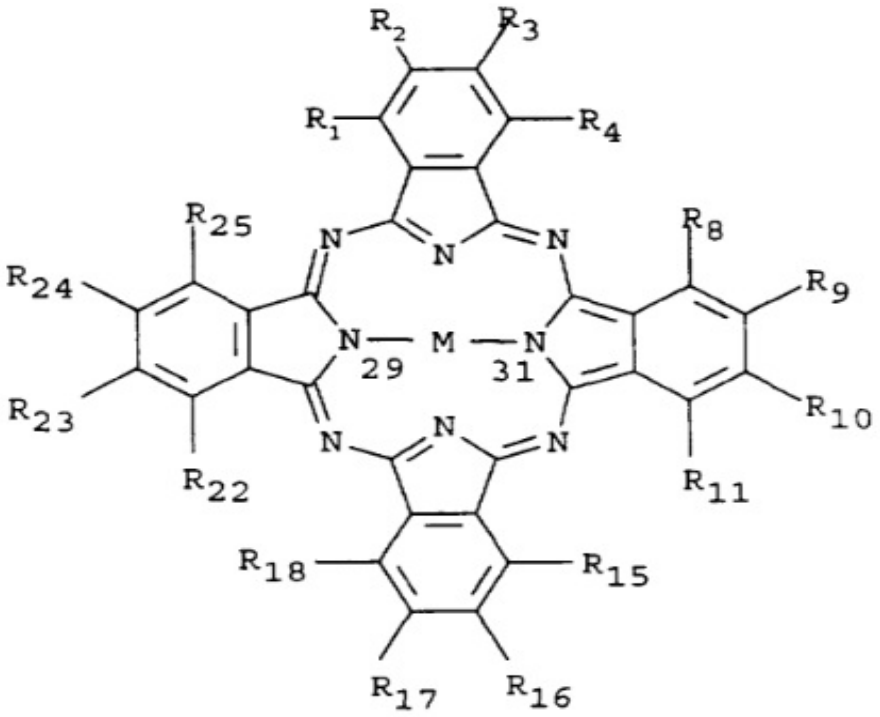


Figure 0.4 : Numbering system in PCs [12].

Phthalocyanines based on the benzene units have 16 different locations. (1-4, 8-9 ,15-18, 22-25). The location of benzo groups that are far from the rings are peripheral (p), close locations are non-peripheral (np), and also the bridging nitrogens are that connected to iso-imino-indoline units are named “meso”.

2.4 Structure of Phthalocyanines

“Tetrabenzotetraazaporphyrin” can be used as the other name for phthalocyanines. The concordance between the ligand and metal ion are obvious in dimensions of coordination.

Crystallography of X-ray in the evaluation of molecular geometry in solid state is the most reliable method. Robertson displayed non-metal phthalocyanine as a planar molecule with D_{2h} symmetry. There are differences between porphyrins and phthalocyanines due to the angles that are formed by nitrogen atoms in meso location. 16 membered inner macro rings are shorter than porphyrins, so the bridges that are made by meso nitrogen atoms are reduced. As a result, a decrease in bond angles and bond lengths cause the vacancy of coordination smaller than porphyrins. [13]

There are two hydrogen atoms at the center of metal-free phthalocyanines; they are able to be replaced with almost all the metal ions in the periodic table, and synthesis of new sorts of metal phthalocyanines is possible. Nowadays, more than 70 elements are utilized as the core metal ion (Figure 2.5) [14].

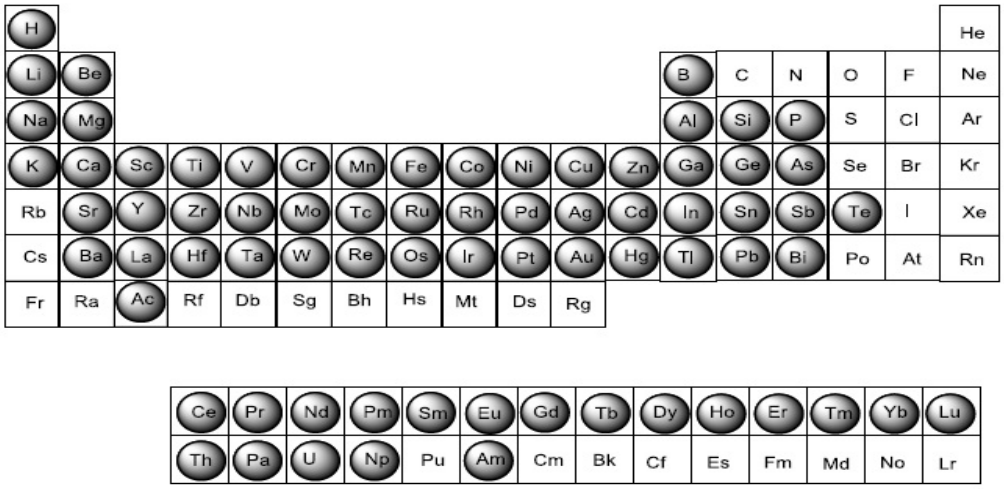


Figure 0.5 : The elements that can be used as the central element in Pcs.

Phthalocyanines normally form complexes with coordination number of four and square pyramidal structure. If some metals prefer high coordination number, they are also able to form square pyramid and octahedral structures. Phthalocyanines are able to form high-coordinated sandwich type (double decker, Pc_2M and triple decker, Pc_3M_2) complexes in the presence of alkaline earth metals (Figure 2.6) [15-17]. A small number of actinide phthalocyanines have been produced due to the difficulty in obtaining the metal ions and their radioactivity. These sandwich type of complexes are organic semi-conductors with attractive characteristics [18].

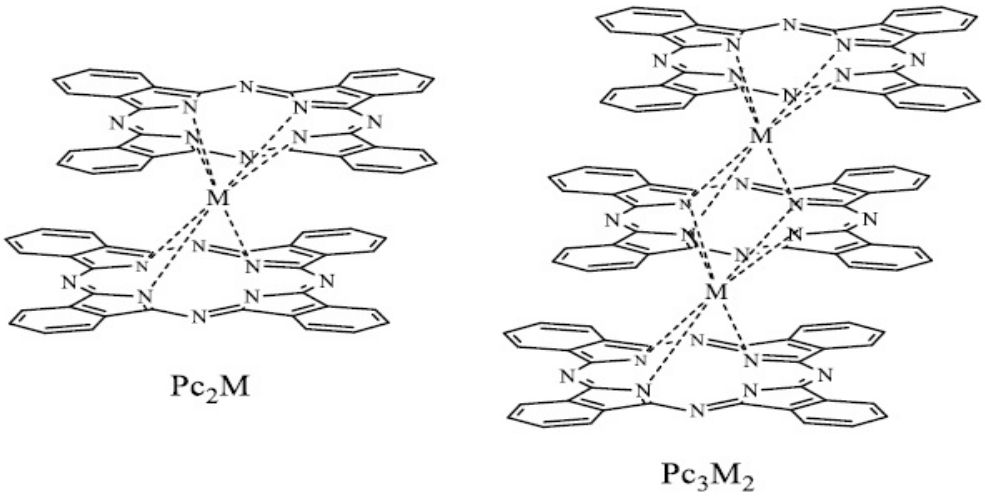


Figure 0.6 : Double decker and triple decker structures.

Additionally, when some axial ligands such as water, pyridine, and chlorine are used as central elements, the complexes with high coordination number can be formed (Figure 2.7) [19].

Among the derivatives of phthalocyanines, sub-phthalocyanines (sub-Pc) have a boron atom as central element and three isoindole units connected to this structure. The other one is super-phthalocyanines (super-Pcs) with uranyl cation at the center and five isoindole units connected to them (Figure 2.8).

Sub-phthalocyanines have the lowest similarity to phthalocyanines. Super phthalocyanines are conjugated macrocycles including 22-π electron structure, however, sub-phthalocyanines contain 14- π electronic system. Moreover, in super-phthalocyanines, the bond-length between uranyl and nitrogen is 2.5-2.6 Å but for phthalocyanines, this distance 1.85-2.05 Å. [18,20].

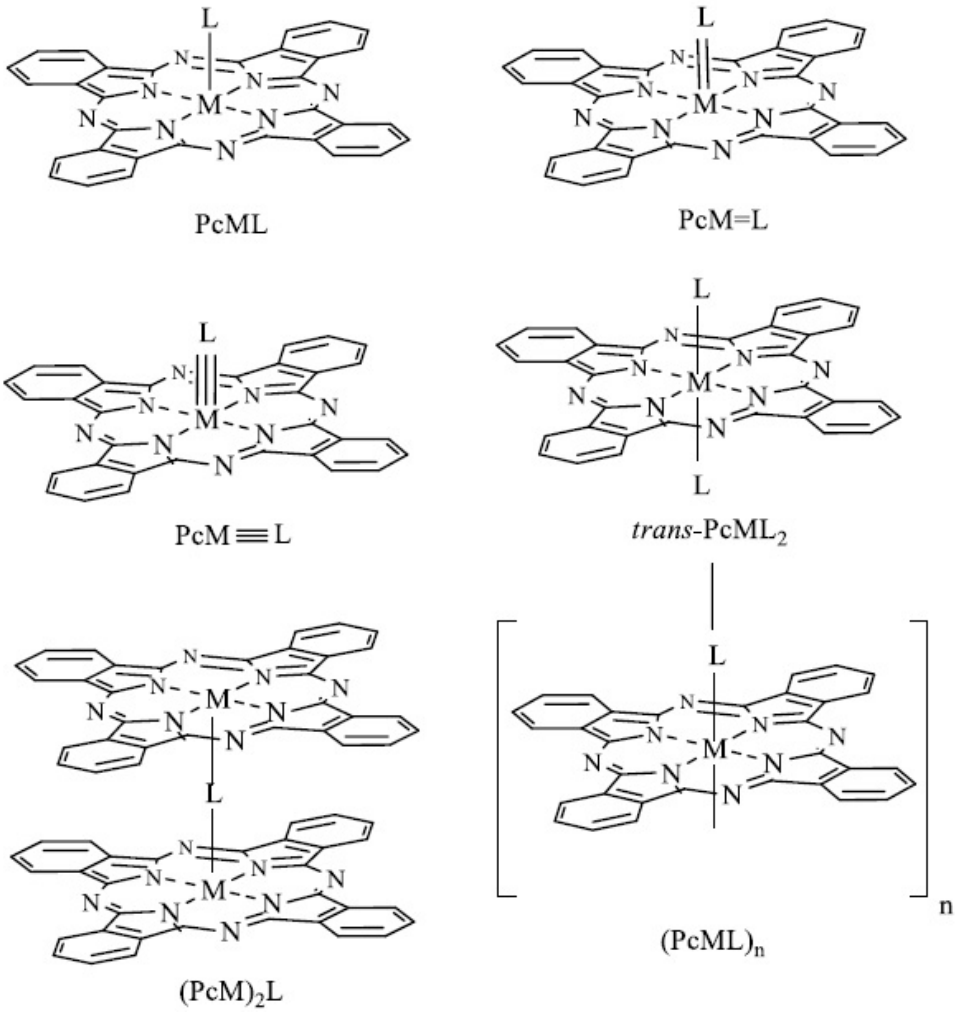


Figure 0.7 : The structure of axially substituted phthalocyanines.

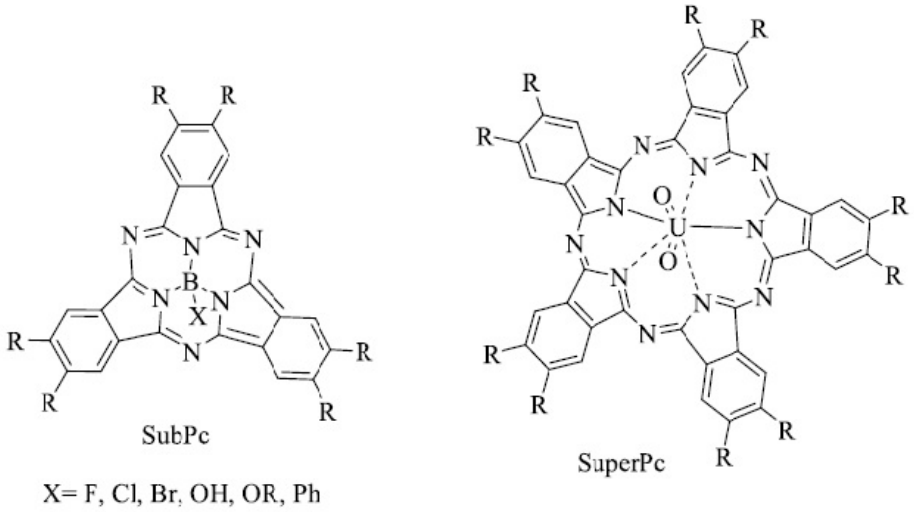


Figure 0.8 : The structure of sub-phthalocyanines and super phthalocyanines.

In addition, there are some other sorts of phthalocyanines which contain anthracene, naphthalene, and phenanthrene groups with extended π-system (Figure 2.9).

Due to additional π- electron system of naphthalic phthalocyanines (NPc) cause them to be one of the most attractive molecules with dark-green crystalline structures. The other reasons that are relevant to the additional π- electron system which make difference in the following [21, 22]:

- electrical conductivities,
- catalytic activities,
- photoconductivity,
- redox potentials.

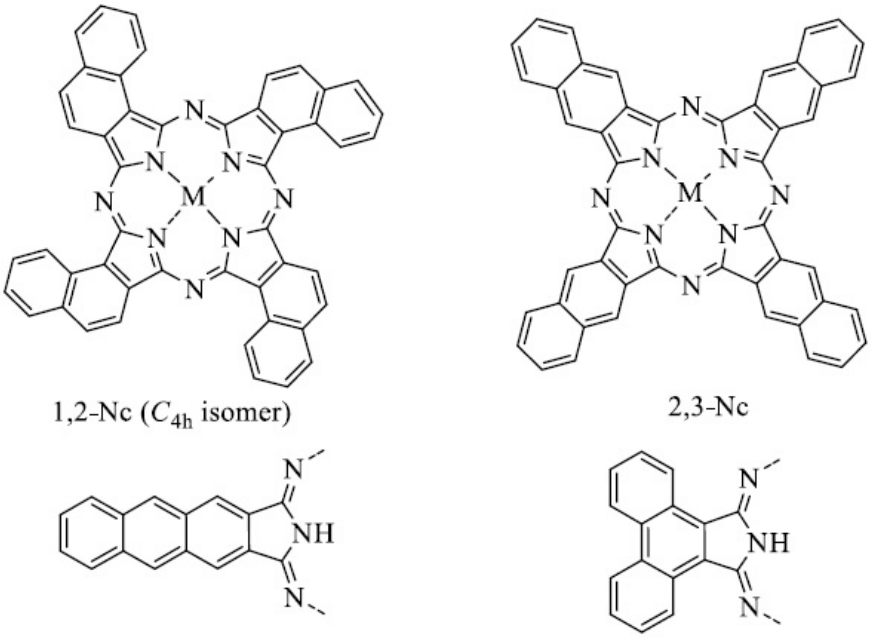


Figure 0.9 : Naphthalophthalocyanines, anthracophthalocyanines, and phenanthrophthalocyanines.

2.5 Physical and Chemical Properties of Phthalocyanines

The metal-based phthalocyanine compounds have D_{4h} symmetry and the structure is square plane (a).

When various molecules are axially connected to metal ions, the structure is a square pyramid with coordination number of five (b).

And if the coordination number is six ,the structure will be octahedral (c) (Figure 2.10) [23, 24]. Deflection from planarity is 0.3 Å.

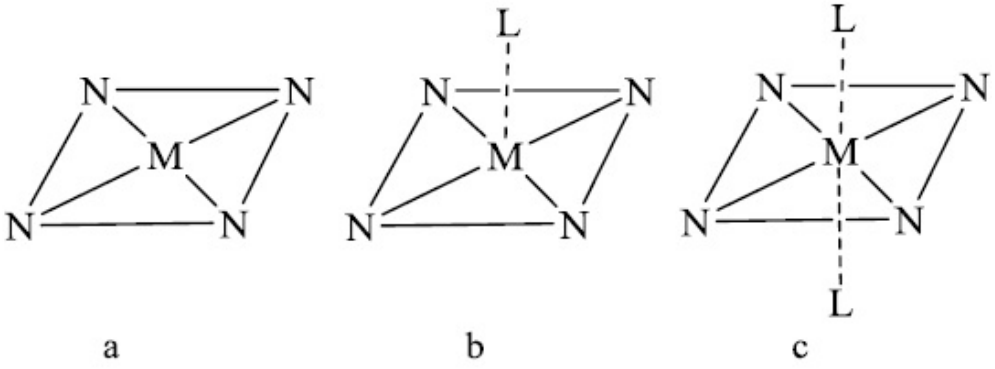


Figure 0.10 : Geometries of phthalocyanine compounds.

Several crystalline structures for phthalocyanines are available and it is completely relevant to the manufacturing form. [25]

One of the most important crystalline structure is the α-form and the other one is β-form. Thermodynamically, the latter is more stable. In β-form, metal atom with the two nitrogen atoms in neighbors prepare an octahedral structure. Also in the α-form, the phthalocyanines have stacked over and over (Figure 2.11) .

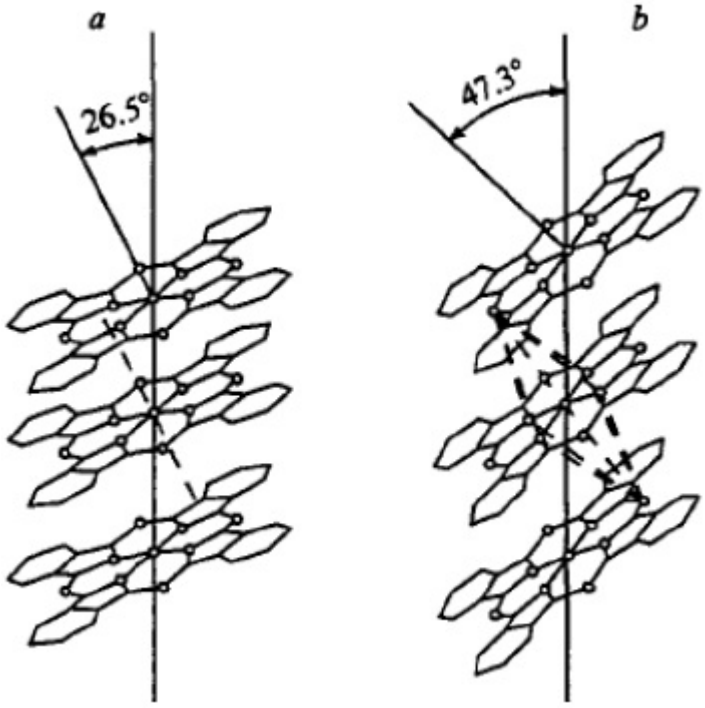
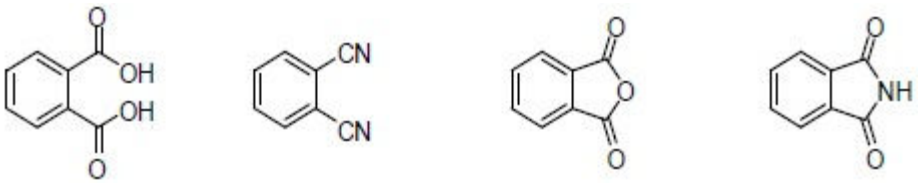


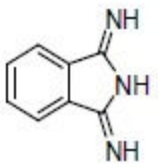
Figure 0.11 : Arrangement of α-MPc and β-MPc in crystalline form.

2.6 Starting Substances in Phthalocyanine Synthesis

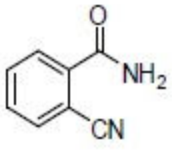
Finding many areas of use in several fields is a noticeable feature of phthalocyanines. In general, the starting substances are phthalic acid, phthalic anhydride, phthalimide, cyclo-1-en-dicarboxylic anhydride, phthalonitrile, o-cyanobenzamide, 2,3-naphthalene dicarbonitrile, iminothioamide, dithioamide, 1,3,3-trichloroisoindoline, and diiminoisoindoline (Figure 2.12). In the synthesis of phthalocyanines, starting substances must have ortho substitution and atoms with the functional groups must have double bond with each other or there must be an arrangment to provide double-bond during the condensation.



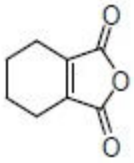
phthalic acid **phthalonitrile** **phthalic anhydride** **phthalimide**



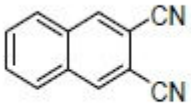
Diiminoisoindoline



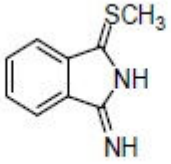
o-cyano benzamide



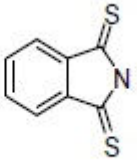
cyclo-1-en-dicarboxylic anhydride



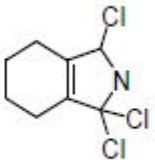
2,3- naphthalene dicarbonitrile



imino thioamide



dithioamide



1,3,3-trichloroisoindoline

Figure 0.12 : Starting compounds in phthalocyanine synthesis.

2.7 General Synthesis Method for Phthalocyanines

2.7.1 Synthesis of non- substituted phthalocyanines

2.7.1.1 Synthesis of metal-containing phthalocyanines

Several methods for synthesis of metal containing phthalocyanines have been shown in the literature (Figure 2.13). There is no difference in which one to use because they have common features:

- having multi-step reaction,
- taking place in high temperature,

Methods for using in synthesis are as follows:

i. heating the phthalonitrile and iminoisoindoline structures with DMF, pentanol and hexanol solvents, which have high boiling points.

ii. heating the phthalimide, phthalic anhydride, and urea in high boiling point solvents in the presence of molybdate catalyst.

iii. adding metal ions to the metal-free Pcs.

iv. Heating Li_2Pc in ethanol; here, it is possible to substitute Li with desired element.

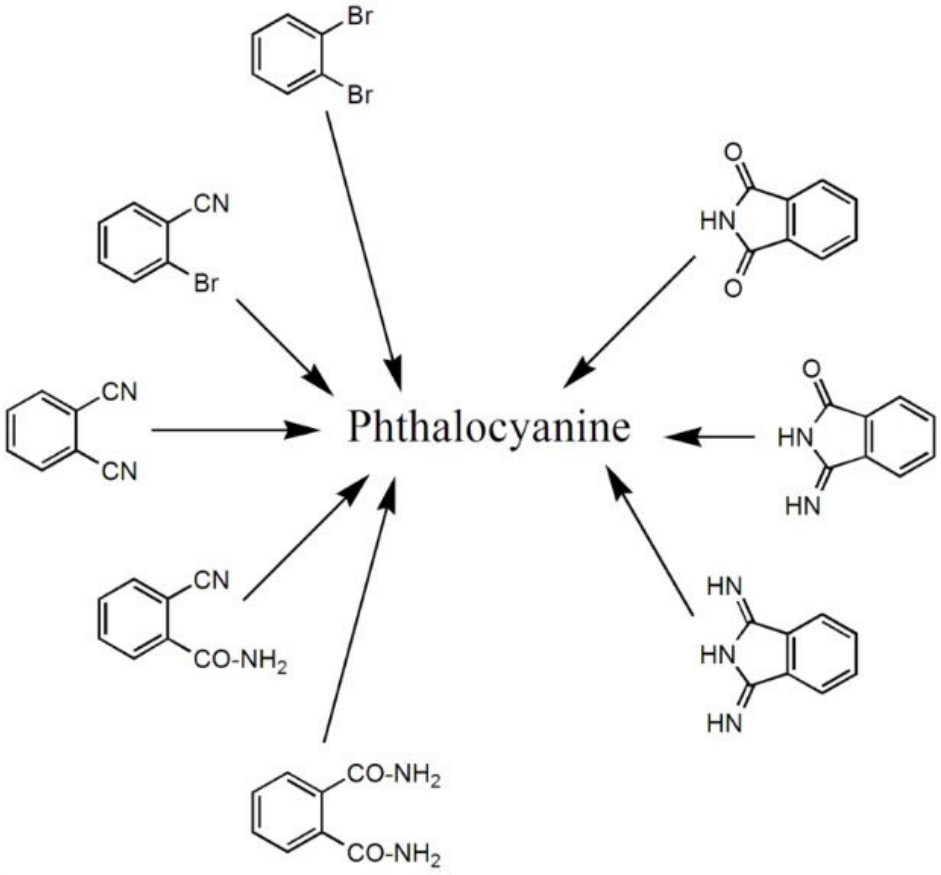


Figure 0.13 : Schematic form of MPc synthesis.

2.7.1.2 Synthesis of metal-free phthalocyanines

Synthesis of metal free phthalocyanines are similar to the synthesis of metal-containing Pcs, using same substances as starting subtance, but in this method of synthesis, it happens without using metal atom and through cyclotrimerization. [26]

In some metal-containing phthalocyanines such as Li, Pb, Na, Hg, and Bi that have large differences between diameter of phthalocyanine and diameter of metal ion in the cavity, it is possible to produce metal-free phthalocyanines (Figure 2.14) [26].

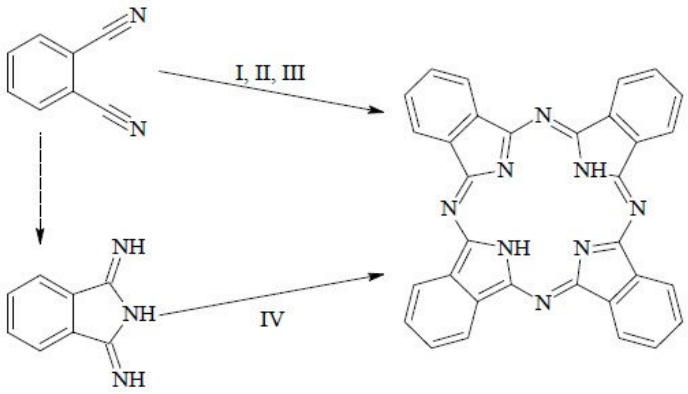


Figure 0.14 : Synthesis of metal-free Pcs.

i) Phthalonitrile and metal-free Pcs

In preparing metal-free Pcs through this method, adding bases such as NH_3, DBU, or DBN to the basic solvent of N-N-dimethylaminoethanol (DMAE) or n-pentanol, with heating of phthalonitrile through cyclotetramerization, metal-free Pcs are obtained .

ii) Extracting the metal ion in phthalocyanine cavity and then acidic treatment of phthalocyanines which have Li^+, Na^+, K^+, Mg^{2+}, Be^{2+}, Pb^{2+}, Hg^{2+} as the metal ion. Therefore, metal-free Pcs are produced.

Linstead method: Adding a primary alcohol (n-pentanol or pentan-1-ol) to the Li-, Mg-, and Na-alkoxide solvent and the phthalocyanine recursor is cyclotetramerized to have metal-containing Pcs. After treatment with acids , metal-free Pcs also are obtained.

iii) Using reducing agents to obtain meta-free phthalocyanines from phthalonitrile

To obtain metal-free phthalocyanines from phthalonitrile through cyclotetramerization, 2 electrons and 2 protons are needed.

With 1,2,3,6-tetrahydropyridine or hydroquinone which are appropriate organic reducing agents, metal –free Pcs are obtained in a melt. The melting point is higher than 180 C°.

iv) Obtaining metal-free Pcs from Diiminoisoindoline

- Adding NH_3 to the phthalonitrile causes production of cyclotetramerization of 1,3- diiminoisoindoline,

- Heating 1,3- diiminoisoindoline in dimethyloaminoethanol (DMAE) solvent

At the end, metal-free PCs are obtained.

2.7.2 Synthesis of substituted phthalocyanines

2.7.2.1 Axially substituted phthalocyanines

Ligands are able to connected to axial position of metal ions in metal-contained phthalocyanines. Axial substitution increases resolution and decrease the attraction between face to face molecules. So, these compounds are important due to optical and optoelectronic features.

Axial ligands with covalent bonds prefer metal ions with 3+ and 4+ oxidation states. Also in several cases suitable ligands creating coordination bonds with metal ions. The amount of these ligands increase solubility of pyridine and quinoline in MPcs. [27]

- **Axially substituted SiPc, GePc and SnPc :**

α-Cl_2SiPc can be obtained in the presence of silicon tetrachloride and diiminoisoindoline through cyclotetramerization. [28] Hydrolysis of α-Cl_2SiPc with aqueous sodium hydroxide yields **α-$(OH)_2$SiPc** .

If α-Cl_2SiPc reacts with alcohols, alkyl halides and chlorosilanes, the products will be interesting. They are normally soluble in several organic solvents (Figure 2.15) [29,30].

- The conditions for reaction are given in Figure 2.15.

i) Heating silicon tetrachloride in a high-boiling solvent,

ii) using a base as a catalyst then reacting with an alcohol and toluene in 80 °C,

iii) hydrolysis in acidic and basic conditions,

iv) heating solid pyridine with silyl chloride or alkyl halide.

The indicative techniques are applicable for naphthalo phthalocyanines. Synthesis of GePc and SnPc are also similiar to this method.

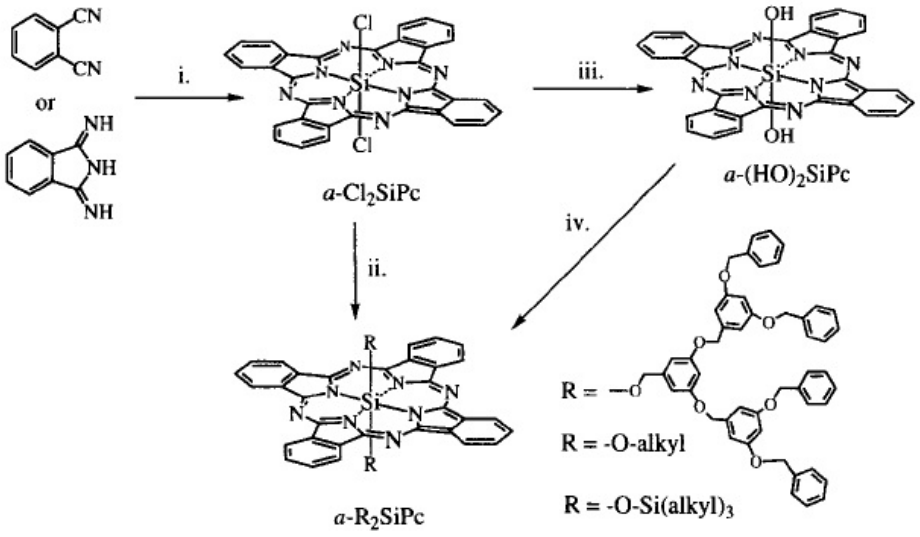


Figure 0.15 : Synthesis of axially substituted SiPc.

2.7.2.2 Synthesis of benzo-substituted phthalocyanines

Without having substituents on benzene groups, a large number of MPc and H_2Pc are insoluble in organic solvents, except for MgPc, Li_2Pc and axially substituted phthalocyanines.

If some substituents replace with the peripheral (p=2,3,9,10,16,17,23,24) and non-peripheral positions (np= 1, 4, 8, 11, 15, 18, 22, 25) on Pc ring, the solubility of phthalocyanine will increase significantly. (Figure 2.16).

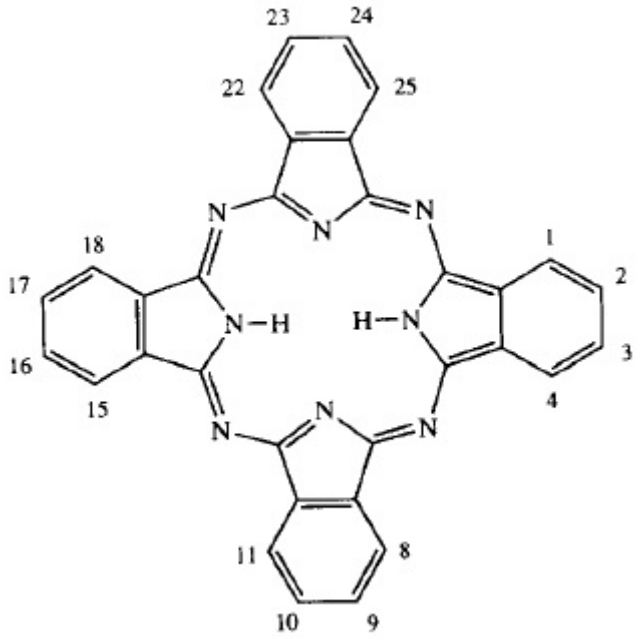


Figure 0.16 : Nomenclature of Pcs atoms for substitution.

2.7.2.3 Synthesis of tetra substituted phthalocyanines

Tetra substitution of phthalocyanines make them highly soluble in almost all organic solvents because of having four substitution in their structure. So they are used in biology, chemistry, physics, and electrochemistry. Moreover, tetra-substitution of Pcs are divided into peripheral and non-peripheral groups.

- For substitution on peripheral positions, the method mostly used is as follows:

Using phthalimide to synthesize 4-nitrophthalonitrile at the end of three steps then treating with basic catalyst of this compound with nucleophilic displacement reaction. (Figure 2.17). [31]

4-nitrophthalonitrile in the presence of polar solvents such as DMF and DMSO react with several nucleophiles. The acidic proton in nucleophiles are extracted by Na_2CO_3 or K_2CO_3. Nucleophile attact to the ring and remove nitro group in the form of sodium nitrite. [32, 33]

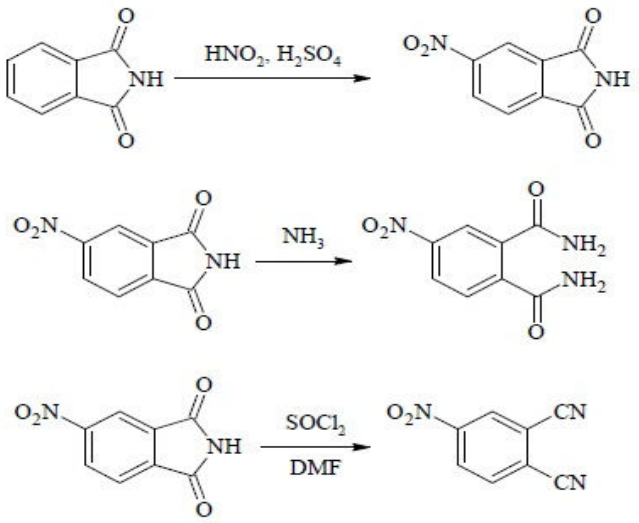


Figure 0.17 : Synthesis of 4-nitrophthalonitrile.

In the synthesis of tetrasubstituted phthalocyanines, monosubstituted phthalonitriles are used as the starting substances and at the end of cyclotetramerization, the symmetries are D_{2h}, C_{4h}, C_{2v}, C_s, and the statistical distribution of the isomers are 4:2:1:1 (Figure 2.18).

In the separation of these isomers chromatographic methods are used but the solubility and aggregation of isomers are similar to each other so it is difficult to separate them.

Non-peripheral tetrasubstituted phthalocyanines are newer than peripheral derivatives and 3-nitrophthalonitrile is the compound used mostly. (Figure 2.19) [34-36].

2.7.2.4 Synthesis of octasubstituted phthalocyanine

For obtaining phthalocyanines in peripheral position which contain 8 substituents, there are 2 different methods.

The method described here is more acceptable because the yield of reaction is higher. The starting substance is 4,5-dichlorophthalic acid. Obtaining 4,5-dichlorophthalonitrile is possible after 4 steps. (Figure 2.20). For the synthesis of derivatives of disubstituted phthalonitrile, 4,5-dichlorophthalonitrile must react with the nucleophiles like 4-nitrophthalonitrile in same conditions. [37]

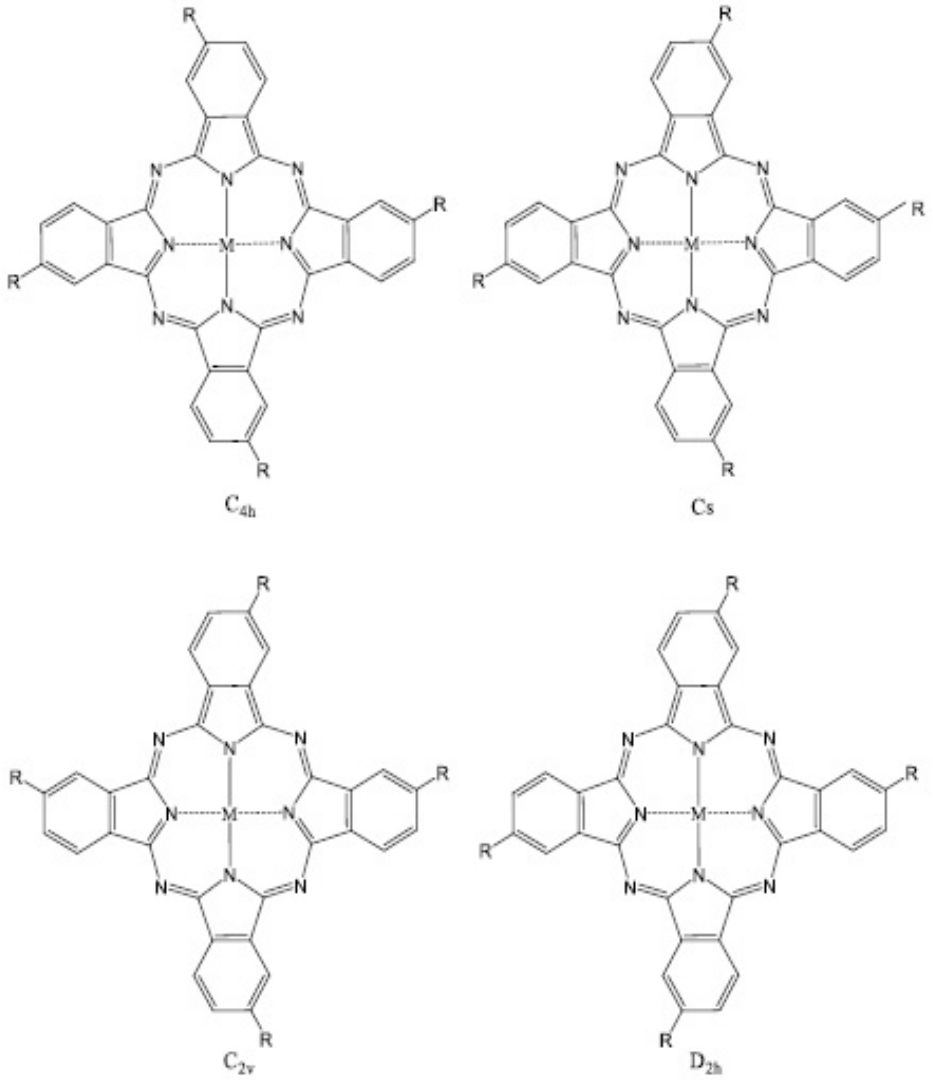


Figure 0.18 : Structural isomers of tetra substituted phthalocyanines.

- In the second method, Br connects to o-xylene and produces 4,5-dibromo-o-xylene as the product and connecting Br through N-bromosuccinimide (NBS), one obtains 1,2-bromomethyl-4,5-dibromobenzene at the end. For substitution, primer alkyl groups must react with nucleophiles, then Br groups in benzene ring are converted to nitrile groups by Rosenmund-von Braun method. [38]

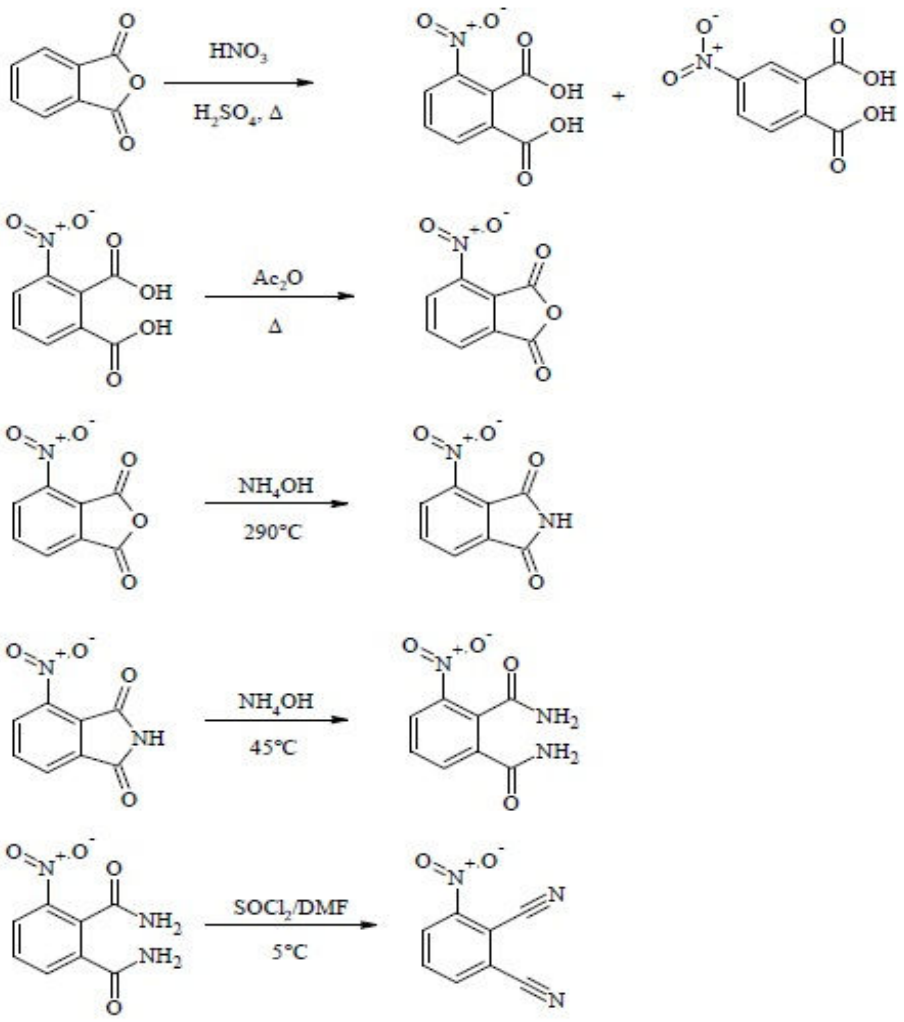


Figure 0.19 : Synthesis of 3-phthalonitrile.

Non-peripheral octasubstituted phthalocyanines (H_2Pc-*onp*-C_n) display liquid crystal features. Cook and co-workers presented 2 new methods .

- 3,6-dialkylphthalonitrile is needed in this method and it is possible to obtain this precursor from appropriate 2,5-dialkylfuran or thiophene. (Figure 2.21). Adding a Diels-Alder ring to phthalonitrile and five-membered hetero ring, is the reason of synthesis of the dinitrile derivatives. The thiophene process is more effective in producing simple MPc-*onp*-C_n .

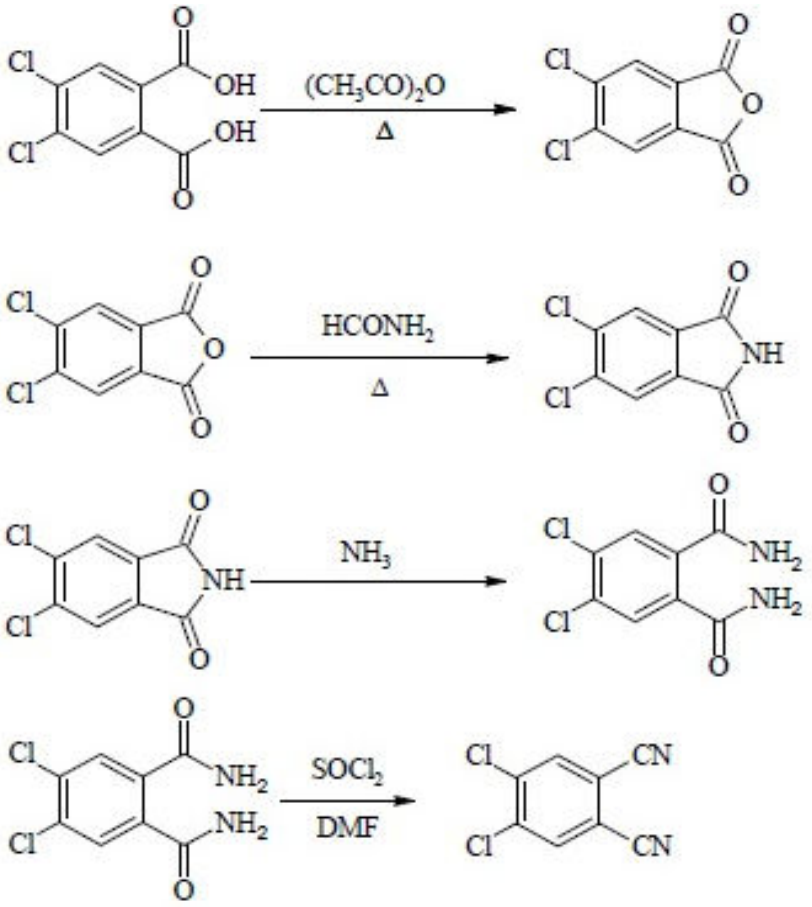


Figure 0.20 : Synthesis of 4,5-dichlorophthalonitrile.

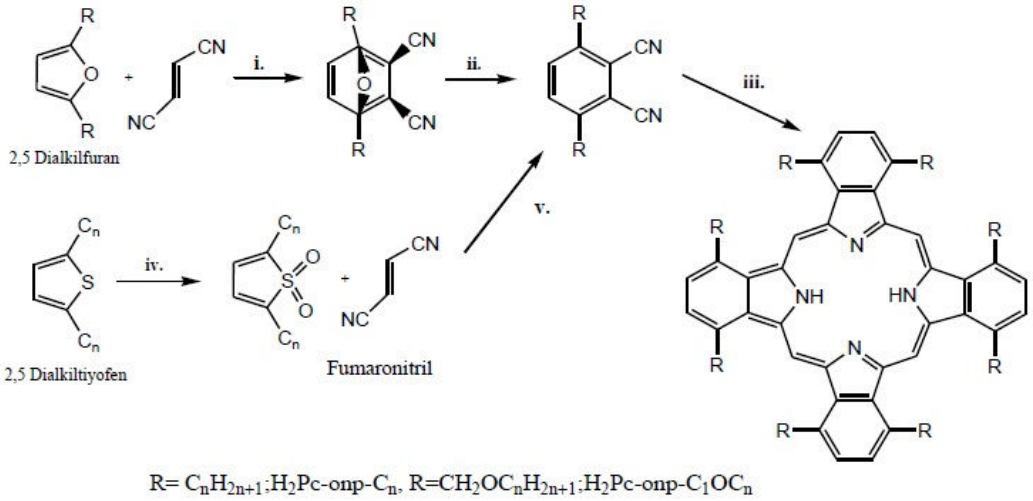


Figure 0.21 : Synthesis of non-peripheral octa substituted phthalocyanine(H_2Pc-onp-C_n).

However, furan process is flexible and functionally allows to obtain phthalonitriles which contain carboxylic acid and alcohols. This process is also used in the synthesis of unsymmetrical phthalocyanines. Furan method is used in the preparation of liquid crystals having MPc-*onp*-COC_n system.

2.8 Characterization of Phthalocyanine

Characterization of phthalocyanines resembles those of organic compounds; FT-IR and UV-Vis methods are used. In visible region, the position of Q-band is affected by substitution and the presence of metal atom, so this technique has an essential role in characterization of phthalocyanines.

For derivatives of soluble phthalocyanines, NMR is a convenient technique, but since phthalocyanine compounds exhibit powerful aggregation in solvents, expansion of peaks can be seen and obtaining a good spectrum is often difficult.

Recent developments show that mass spectrometry has an important role in characterization of phthalocyanines and with this technique, characterization is often easier.

In characterization of phthalocyanines with high molecular mass, fast atom bombardment (FAB) and matrix-assisted laser desorption/ionization (MALDI) are new methods that went widespread.

2.8.1 FT-IR spectrometry of phthalocyanines

A basic difference between metal-containing phthalocyanines and metal-free phthalocyanines can be seen in 3298 cm^{-1} which is N-H stretching band. For both metal-free and metal-containing Pcs, C-H stretching band appears between 3000-3050 cm^{-1}. C-C stretching bonds are observed between 1450-1600 cm^{-1} and C-H bending bonds are seen between 750-800 cm^{-1}.

2.8.2 H-NMR spectra of phthalocyanines

In non-substituted phthalocyanines, the protons in peripheral and non-peripheral positions display signals with same intensity.

Tetrasubstituted phthalocyanines show various isomers but octa-substituted phthalocyanines have only one isomer in their structure. Therefore, signals of tetrasubstituted Pcs are broad as compared to octa-substituted Pcs.

Ligands in axial positions and substitutions on the ring is the reason for complex ^{1}H-NMR spectra in metal containing phthalocyanines. According to the structure and position of substitutions, signals of magnetic field are able to shift downfield and topfield areas. In general, the signals of electron donor groups are shifted downfield but electron-withdrawing substitutients prefer to shift to upfield. In addition, electron donor non-peripheral substituted phthalocyanines prefer to slide to down areas if compare to derivatives of peripheral substituted Pcs with the same groups. [39] H-NMR spectra of metal-free phthalocyanines show N-H protons in negative field that is a reason diamagnetic anisotropy. [40]

2.8.3 UV-Vis spectra of phthalocyanines

The chemical and electrical properties of phthalocyanines come from 18- π electron system. In general, UV spectra of phthalocyanines have a sharp Q-band at 650-720 nm. Another characteristic band is distinguished at 300-400 nm as B (SORET) band. [41] The sharp Q-band is the result of π- π* transition from basic state (HOMO) to excited state (LUMO). B (SORET) band also is the reason of transition between a_{2u} or b_{2u} and e_g orbitals. (Figure 2.22)

The other bands in the spectrum are related to metal-ligand (MLCT) and ligand-metal (LCMT) charge transfer or interaction between π-system of dimeric complexes. [42].

The symmetry of molecule is effective in the form of Q-band. In metal-containing phthalocyanines with D_{4h} symmetry, only one band appears, while in metal-free phthalocyanines with D_{2h} symmetry, Q-band is split into two peaks. (Figure 2.23) [43].

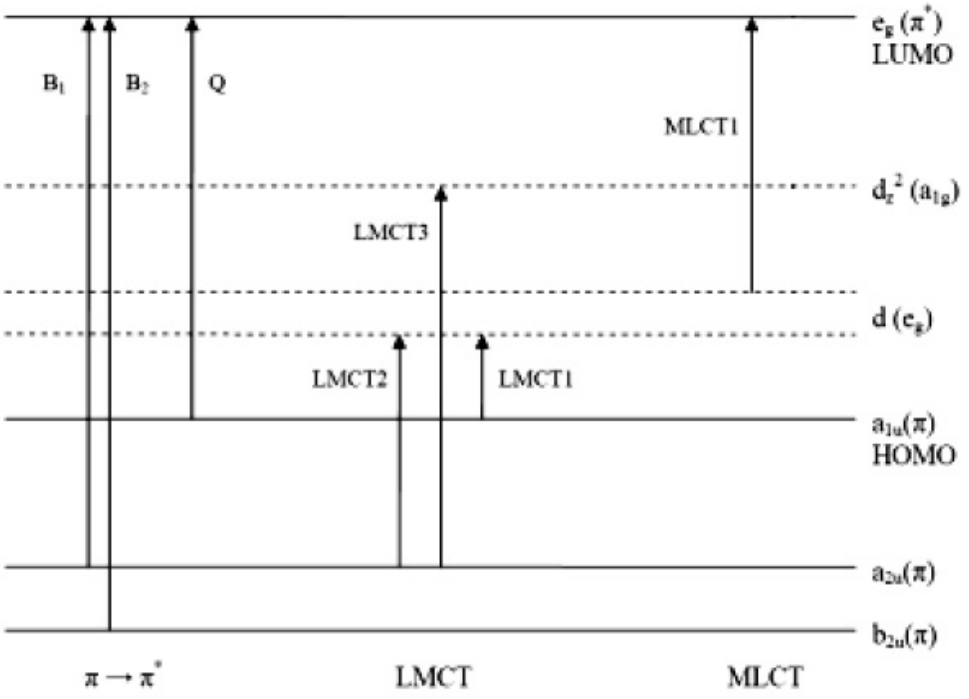


Figure 0.22 : Energy diagram of MPc.

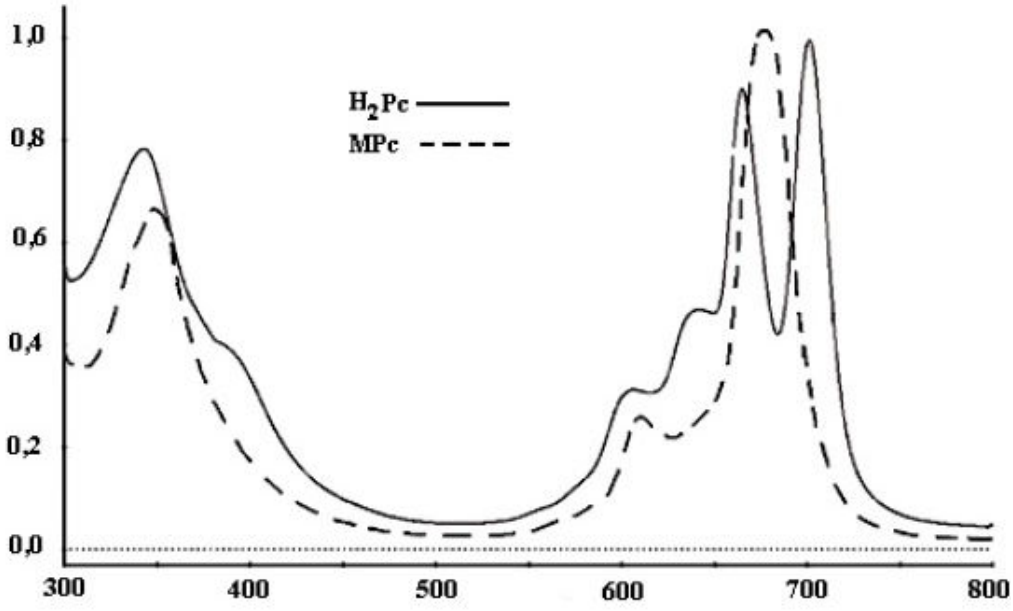


Figure 0.23 : UV-Vis spectrum of metal free and metal containing PCs. The x axis is wavelength in nanometers and the y axis is absorbance.

The location and intensity of Q-band in phthalocyanine compounds are important in terms of specific applications.

The factors that effect the spectral features of phthalocyanines:

- metal central atom,
- aggregation,
- π-conjugation,
- symmetry of molecule,
- solvent,
- groups that are connected to Pc ring (axial, peripheral, non-peripheral).

At high concentrations or situations that use polar solvents, a band appears on the left side of the Q-band because aggregation is increased, so a decrease of intensity of Q band is lowered. [44].

Moreover, geometry of ring is able to change the intensity of Q-band.

In 4-coordinated system, aggregation appears frequently, while in 6-coordinated systems, aggregation is not seen due to steric barrier.

Adding axial and peripheral groups to phthalocyanine compounds causes greater distance between molecules and facilitating solvation. As a result, aggregation will decrease.

Also attaching these groups to the ring will change the location of the Q-band. Electron donor groups in non-peripheral positions are the reason of bathochromic shift in Q-band. [45]. If the groups in peripheral position (naphthalo-phthalocyanines) do not join to π-conjugation, an intensity shift in Q-band can not be seen. Increasing π-conjugation in phthalocyanines cause to bathochromic shift in Q-band.

2.9 The Main Applications of Phthalocyanines

2.9.1 Dyes and pigments

In 1907, phthalocyanines were prepared by A. Braun and T.Cherniac with blue color, then in 1927, copper Pcs, naphthalophthalocyanine, and octamethyl copper phthalocyanine compound were synthesized by accident in Switzerland, but the real discovery of Pcs belongs to the investigations in Scottish Dyes company. In 1933, copper phthalocyanine was started to produce industrially for the first time with Monastral Blue name. They had produced α-type particles by precipitation from sulfuric acid, then brightness of phthalocyanines pigments had been increased. (Figure 2.24). The next step was utilization of halogenated phthalocyanines, because this sort of Pcs were stability supply, so it did not allow Pcs to convert to bigger and opaque particles (β-type). After a short time, several derivatives of copper phthalocyanines obtained with sulfo- groups which had high solubility [46]. For example, copper phthalocyanine with Direct Blue 86 sulfonic acid group is a sodium salt, while with Direct Blue 199 is an ammonium salt. Solvent paints are named for amine salts of copper phthalocyanines with sulfonic acid groups due to high solubility of these Pcs in several solvents. Examples include Solvent Blue 38 and Solvent Blue 48. Phthalogen Dye IBN is a derivative of cobalt phthalocyanine with amine groups.

Unique color of phthalocyanines allow them to be used in many other areas. For example, inkjets, pen inks, paper industry, and coloring of metals and plastic surfaces.

2.9.2 Sensors

Phthalocyanines are able to modify their optical, redox, and electrical properties with environmental conditions so used widely as sensors [47]. This alternation deserves investigation with other methods [48-52]. Phthalocyanines are resistant to chemicals and heat, compatible with microelectronic devices, and providing Langmuir-Blodgett films with thin films are the other properties of phthalocyanines that allow them to be used as sensors [53-55]. The metal atom and ligands in axial

position can affect on chemical properties of phthalocyanine. It is possible to synthesis various symmetrical and unsymmetrical phthalocyanines.

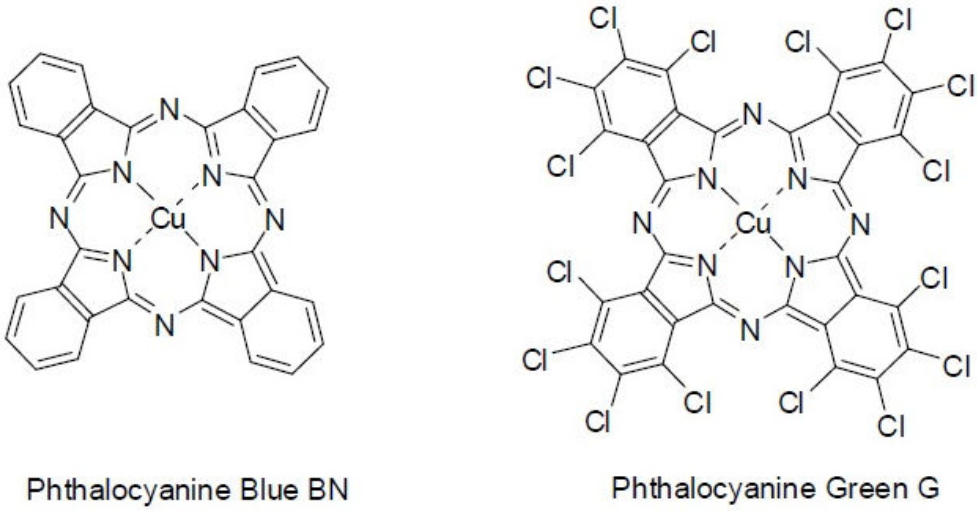


Figure 0.24 : Pigments of copper phthalocyanine.

Among several phthalocyanines, double-decker phthalocyanines have unique physical and chemical properties so they are the most appropriate compounds for sensors.

2.9.3 Electrochromic imaging

When an electrical field is applied, the color of material is changed. This procedure has double-way and known as electrochromic imaging.

Electrochromic materials are used in other areas.

- Controlling the amount of heat and light which pass through the windows,
- Changing the color of mirrors based on weather conditions in automobile industry.

Phthalocyanines exhibit electrochromic properties, so they are used in the structure of imaging panels and smart materials. The most used electrochromic Pcs are bis(phthalocyanine) compounds of alkaine earth metals.

$LnPc_2$ with green color are able to convert to $LnHPc_2$ with blue color.

[Pc_2-$LnPc^{1-}$] is a reducing agent of bis phthalocyanine with different spectral, electrochromic, electrochemical, and magnetic properties. The sandwich structure and attraction between π-electron system of both phthalocyanines are reason of those properties. Elecrochromic transformation in $LnPc_2$ are shown below [56]:

$$LuPc_2^- \rightleftharpoons LuPc_2 \rightleftharpoons LuPc_2^+$$

$$(Pc^{2-}Ln^{3+}Pc^{2-})^- \rightleftharpoons (Pc^{2-}Ln^{3+}Pc^-)^0 \rightleftharpoons (Pc^-Ln^{3+}Pc^-)^+ \quad \text{(Eq.1)}$$

Blue **Green** **Orange**

2.9.4 Photodynamic therapy (PDT)

For many years human has known about the effect of light in therapy with chemicals or alone. For example, solarium is a sample of native architecture of ancient Greek. In India, vtiligo (dermal cancer) is remediable with the compounds containing furocoumarin in sunlight [57]. Researches about phototherapy by Niels Ryberg Finsen show the efficiency of light in modern medicine. First, photodynamic effect was found by Raab in 1900. In 1913, Meyer Betz used an extravagant way to observe photodynamic therapy in human body. He injected 200 mg of hematoporphyrin to himself, then in sunlight he observed some damage on his hands and his face. In 1942 , Auler and Banzer investigated an animal by UV light. In the following years, Diamond and co-workers found a way to damage sensitive tumors by visible light so in 1976 , first clinical application was started. From 1993 on, in several countries such as, USA, Canada, Japan, and some European countries, photophyrin is used to treatment various cancers.

Photodynamic therapy (PDT), a treatment method without operation and minimum injury is an evolution in medicine. In pricipal, there is photosensitizer that is activated by a light with certain wavelength .

A photosensor must achieve the following:

- Synthesis must be convenient and toxic effects can not be seen in the presence of light .

- It must be selective to diseased cells and ability in cleaning immediately in comparison to normal cells.

- It must have high photodynamic effect and photostability.

In photodynamic therapy, the visible region of spectrum (Red) is used, because red light has the ability of penetrate into the tissue more than others. This light activates the drug to chemical, damage cancer cells with minimum harm to normal cells, and provide toxic form of oxygen.

PDT has more advantages than other treatment methods such as chemotherapy or operation, because this method does not have side effects. The only disadvantage of this method is that the photosensitizer is able to settle in normal cells, too, so the patient must stay in a dark place after treatment for a certain time.

There are 2 oxidative mechanisms for destruction of the cancer tissue , Type 1 and Type 2 [58].

Type 1: Photosensitizer attracts to biomolecule (or oxygen) and hydrogen atom (or electrons) are transferred for obtaining radicals.

Type 2: Transfering energy from excited triplet state to triplet state of dioxygen. So at the end, singlet state of oxygen are obtained (see Figure 2.25).

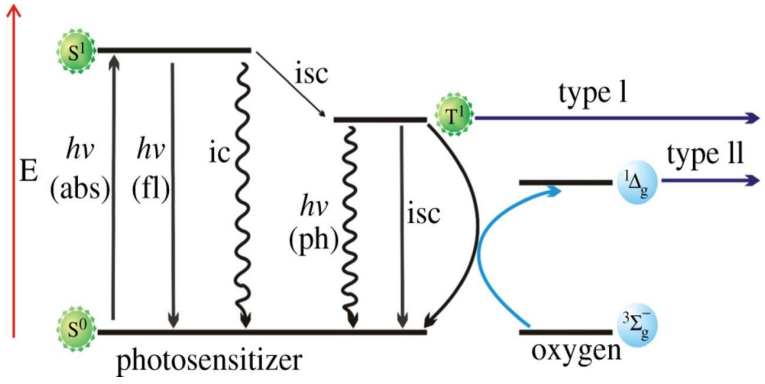


Figure 0.25 : Jablonski diagram for Type 1 and Type 2.

In general, porphyrin materials are used.

The advantages of these compounds are summarized as follows:

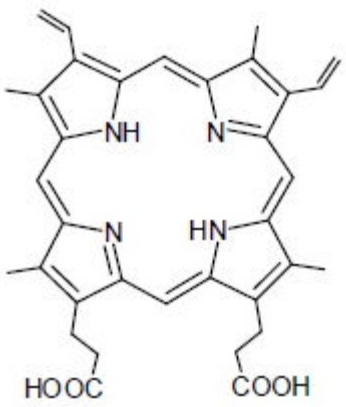
- Aromatic stability,
- high yield of quantum of singlet oxygen,
- having absorption in red region.

Hematoporphyrin (HpD), photophyrin (pure sort of HpD), photosan, and photohem are used in PDT in a widespread manner.

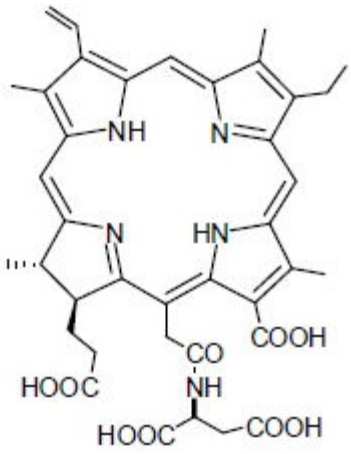
These sort of compounds have some disadvantages:

- Low selectivity,
- having low absorption in red region, so treating of cancer cells located in the deep is difficult.
- having complex state in photosensitizers.

Nowadays, many photosensitizers are obtained from phthalocyanines, derivatives of porphyrins, and texaphyrins (see Figure 2.26).



(**1**)



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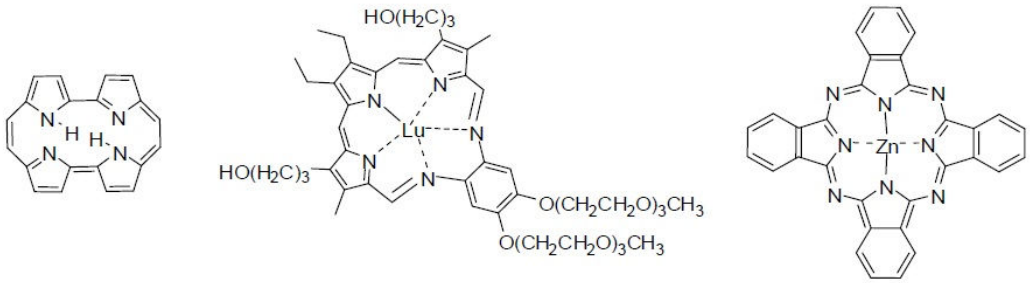


(**3**) (**4**) (**5**)

Figure 0.26 : Commonly used photosensitizers, protoporphyrin (left) (**1**) and monoaspartylchlorin (right) (**2**). Porphyrin (left) (**3**), lutetium texaphyrin (lu-tex; middle) (**4**), and zinc phthalocyanine (right) (**5**).

The compounds of this group belongs to second generation of photosensitizers. Aggregation tendency of phthalocyanines in solvent decrease their photosensitizing ability. To solve this problem, connecting certain ligands to the axial position of silicon, germanium and tin phthalocyanines or adding a bulky substituent to the peripheral positions (see Figure 2.27).

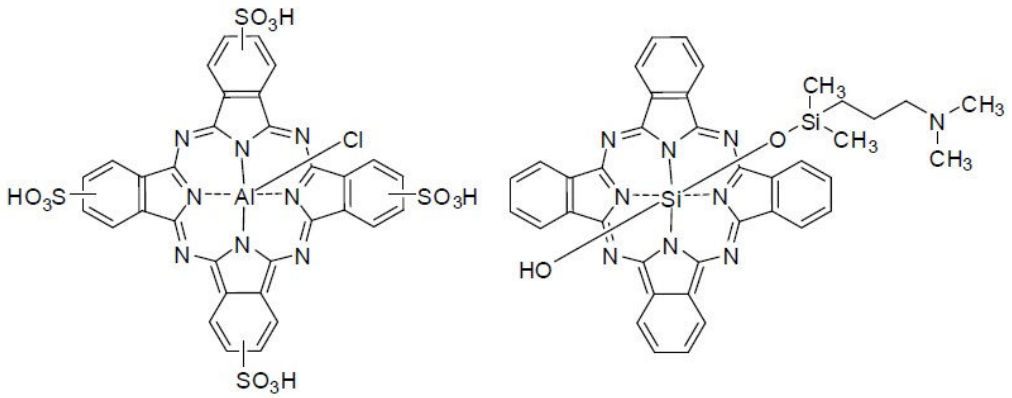


Figure 0.27 : Photosensitizing phthalocyanines with substitution on peripheral or axial positions.

In addition, diamagnetic elements such as zinc and aluminum are highly preferred to use in PDT (see Figure 2.28).

All compounds that are used as photosensitizer must be soluble in water and oil because of this factor, it helps compounds flow conveniently in patient's body. Because of this goal, in recent years, many unsymmetrical phthalocyanines have been synthesized.

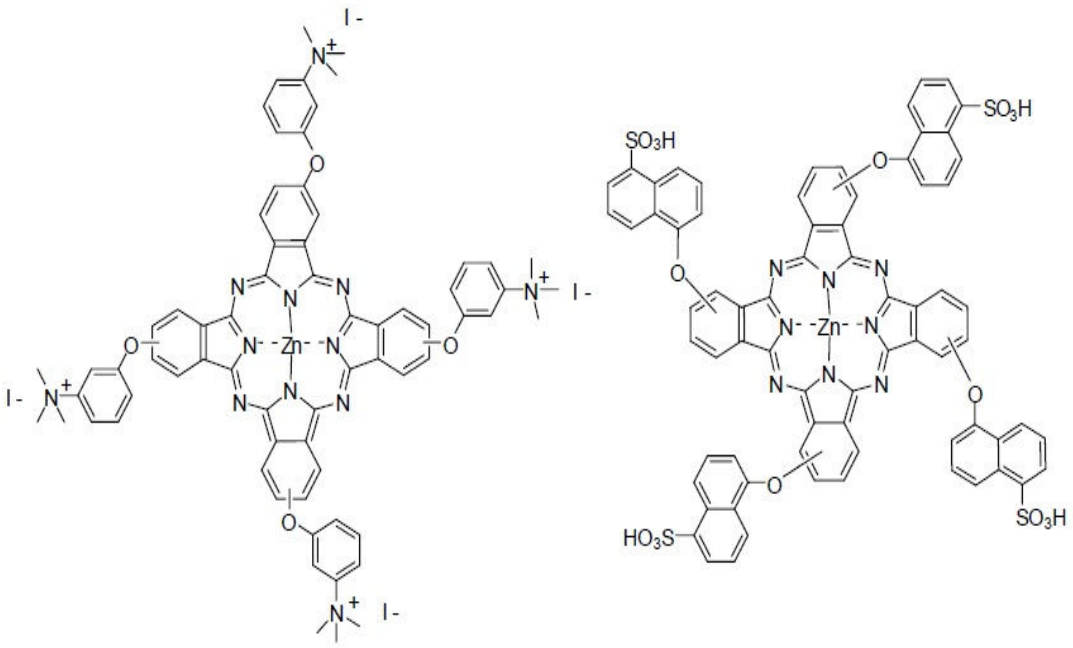


Figure 0.28 : Photosensitizing zinc phthalocyanines.

2.10 Water-soluble Phthalocyanines

Phthalocyanines can be rendered water-soluble by attaching anionic, cationic, zwitter-ionic or non-ionic side groups to the benzene rings.

2.10.1 Cationic phthalocyanines

They usually have quaternary pyridinium or ammonium groups on the side chains. Cationic side groups like quaternary ammonium, pyridinium, and quinolinium usually provide high solubility in water [59].

12.10.2 Anionic phthalocyanines

Carboxylate and sulfonate are used as anionic side groups and they especially provide high solubility in high pH values by incorporating alkaline metal cations. Anionic phthalocyanines containing phosphorus and sulfonate are known in the literature [61].

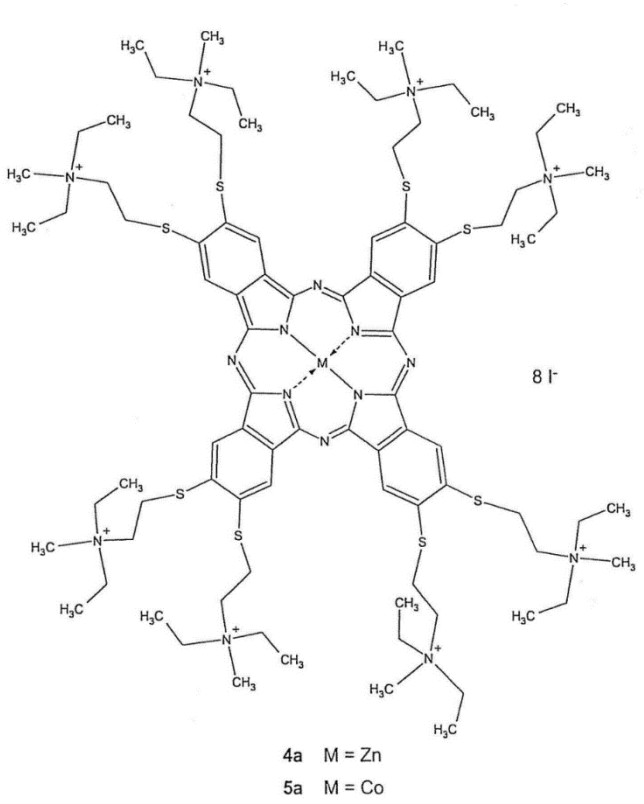


Figure 0.29 : Quaternarized metallophthalocyanines [60].

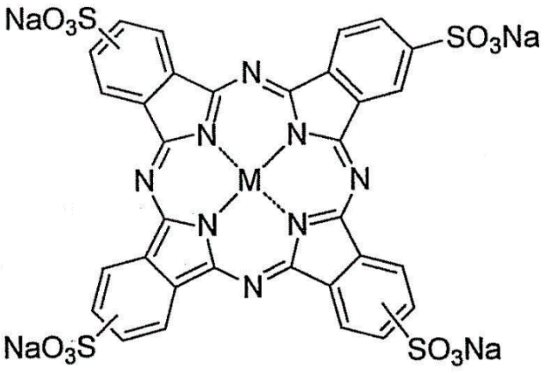


Figure 0.30 : Tetrasulfonated phthalocyanine [61].

2.10.3 Zwitterionic Phthalocyanines

Zwitterionic side groups contain acidic and basic side groups and form inner salts. Phthalocyanines with these side groups behave as anionic dyestuffs in neutral and basic media [59]. In many studies, the pyridine nitrogens in the phthalocyanine side groups were quaternized with propanesultone. Zwitterionic zinc, germanium, and silicon phthalocyanines were synthesized [62,63].

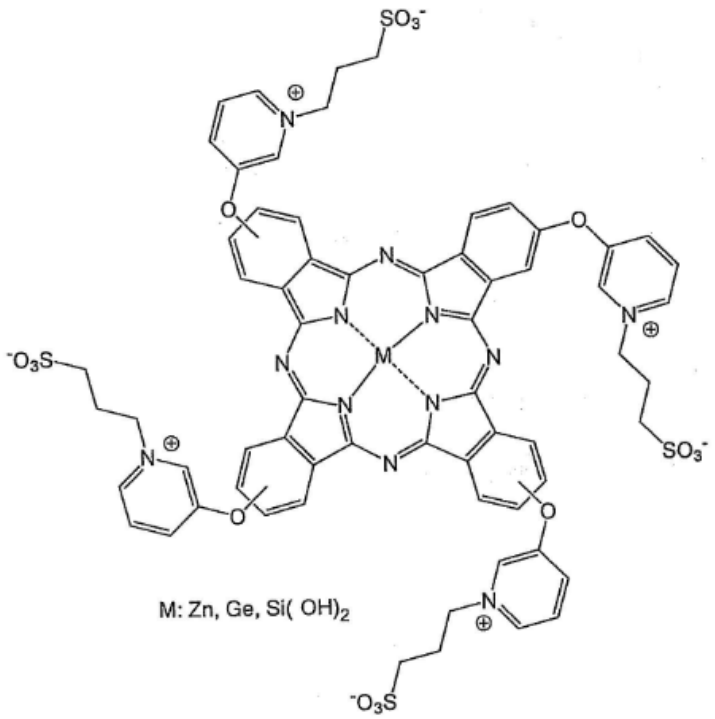


Figure 0.31 : Zwitterionic phthalocyanines [62].

2.10.4 Non-ionic phthalocyanines

Long polymeric chains (such as PEG 600) or sugar derivatives (glucose, galactose, solketal, etc.) can render the phthalocyanine compound soluble in water.

2.10.5 Conjucated phthalocyanines

Adding saccharides to non-natural organic compounds has been regarded as an important research area and especially, their conjugation to porphyrins has been widely studied for PDT [65, 66]. Glucose is the oldest example added to a phthalocyanine [67]. Maillard *et al*. linked four glucose molecules onto a zinc(II) phthalocyanine core (ΦΔ ZnPc = 0.55 in DMF [68]) to prevent aggregation and to increase the solubility in water. Only the synthesis and characterization of **1** (Fig 2.34) were reported [67].

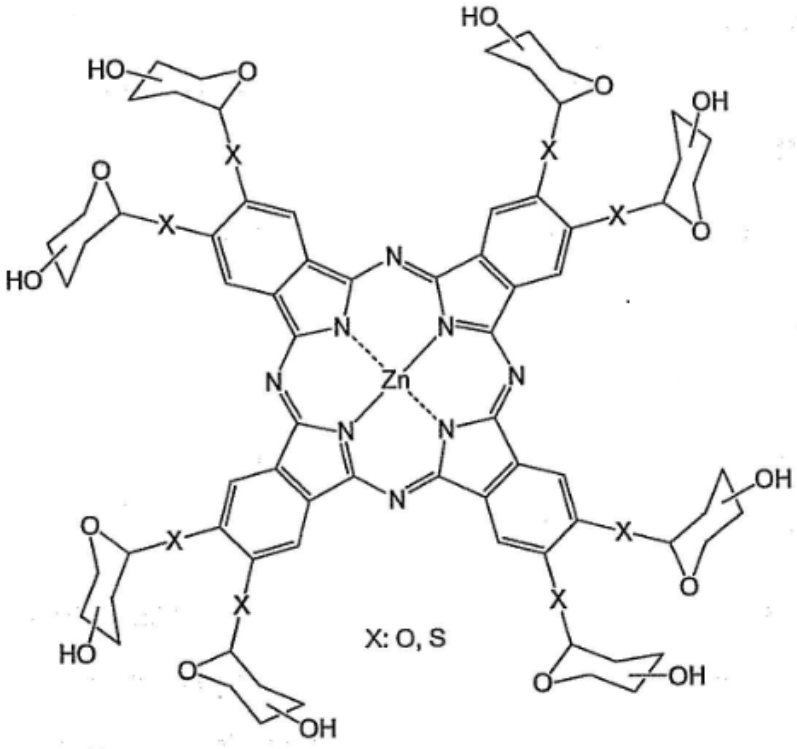


Figure 0.32 : Octaglycosilated zinc phthalocyanines [64].

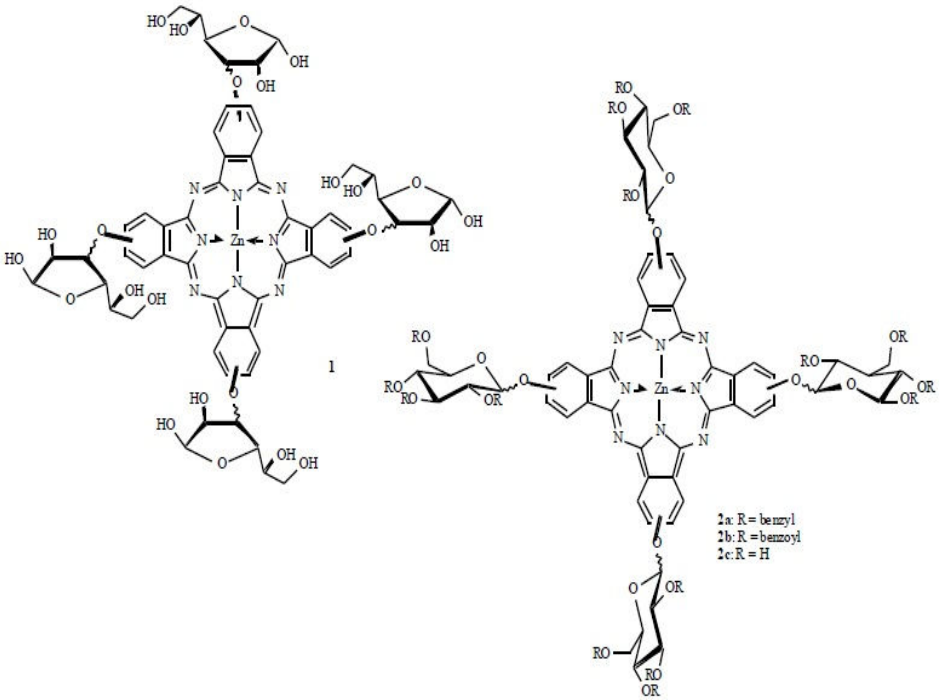


Figure 0.33 : Functionalized zinc phthalocyanines coupled with four glucose moieties.

2.10.6 Oligodeoxyribonucleotide and Nucleobases

There are large numbers of nucleobase porphyrins (they are of interest for antiviral and anticancer activities) [69,70], yet phthalocyanine nucleobase dyads reported in the literature are rare. Koval *et al*., in 2001, have reported for the first time about the synthesis of oligonucleotide-phthalocyanines for specific DNA modifications *in vitro* and *in vivo*. This is interesting for the development of sequence specific gene targeting reagents [71]. Later, Hammer *et al*. prepared asymmetrical water-soluble Pcs **19**-**20** conjugated with an oligonucleotide carrying an isothiocyanate function (Fig. 2.35). The dyes synthesized show favorable photophysical properties and excellent water solubility and these make them excellent fluorescent tags for genetic assays [72].

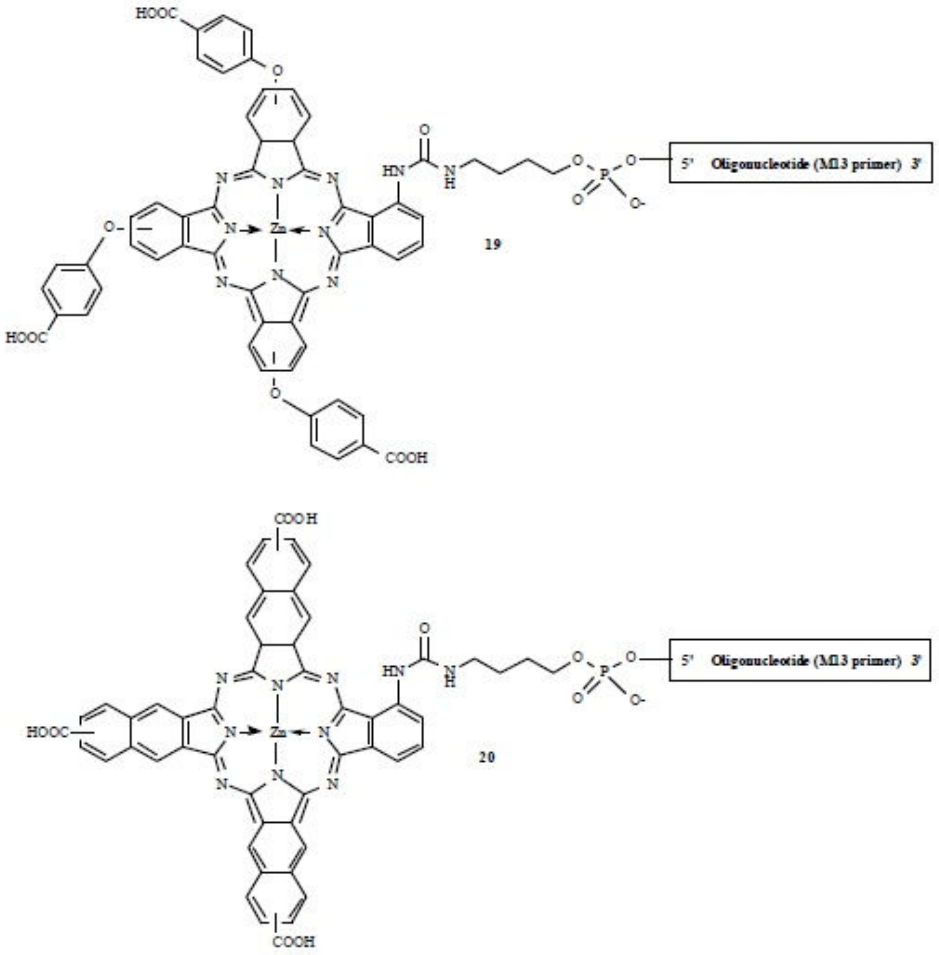


Figure 0.34 : Conjugated zinc phthalocyanines with an oligonucleotide.

2.10.7 Octacationic phthalocyanines

One important issue related to water-soluble phthalocyanine is that they show aggregation, which may have a strong influence on the bioavailability, *in vivo* distribution, and the singlet oxygen production efficiency. Lipophilic phthalocyanine derivatives have reportedly a higher tumor affinity, but associated with cutaneous phototoxicity, [73] probably due to overall lower excretion, and water-soluble phthalocyanines are considered the best targets for a new generation of photosensitizers [74]. Among water-soluble compounds, the initial focus was paid on the synthesis of anionic derivatives, such as sulfo-, carboxy- *etc*. and phosphono-derivatives, [75] mainly evaluated to treat tumors and cationic phthalocyanine derivatives for the treatment of both tumors and infectious diseases [76]. Only tetracationic constitutional isomers or poorly defined dicationic phthalocyanines [76, 77] were obtained and described so far. Since it is known that cationic compounds may specifically target mitochondria, [78] there is evidence that cationic photosensitizers are effective to gram-negative bacteria [79] and photoinactivation of yeast, [80] the authors prepared an octacationic phthalocyanine derivative for evaluation as a photosensitizer aimed at the inactivation of antibiotic-resistant microorganisms.

The synthesis of compound **3** is reported (see Figure 2.36).

The 4-[1,3-bis-(dimethylamino)2-propoxy]1,2-dicyanobenzene **1** can be easily prepared from 4-nitrophthalonitrile and 1,3-bis-(dimethylamino)-2-propanol.

Cyclization of compound **1** was best performed by using neat 1,8 diazabicyclo-[5.4.0]-undec-7-ene (DBU), in the presence of anhydrous zinc acetate at 150–180 °C to produce 18% yield of compound **2** by following a procedure already described. Compound **3** was obtained by reaction of **2** with an excess of methyl iodide in N-methyl-2-pyrrolidinone as solvent at rt (yield=82%). A shorter reaction time was achieved by using this solvent compared to other solvents such as N,N-dimethylformammide, methanol, chloroform, or by using neat methyl iodide at different temperatures.

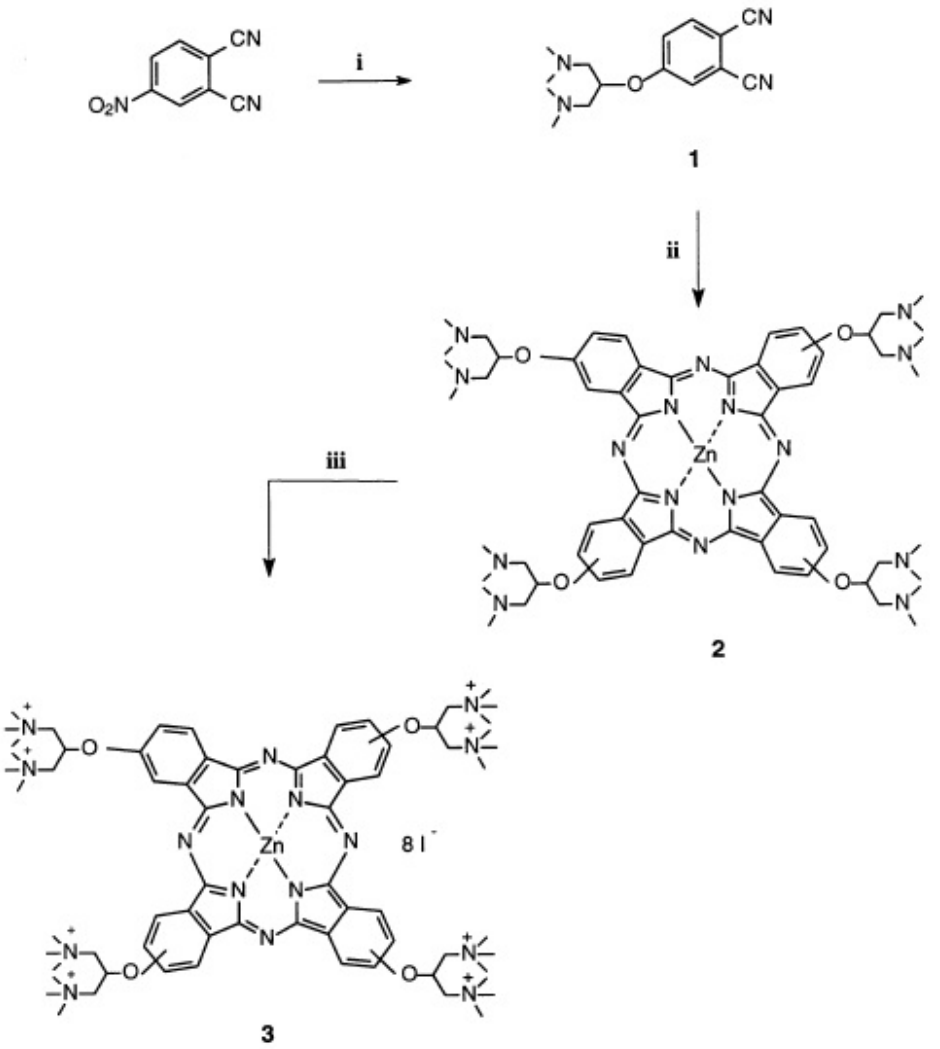


Figure 0.35 : i. 1,3-Bis-(dimethylamino)2-propanol, K_2CO_3, DMSO, rt (74%); ii. DBU, $Zn(OAc)_2$, N_2, 150–180 °C (18%); iii. MeI, N-methyl-2-pyrrolidinone, rt (82%).

3. PURPOSE OF THE STUDY

Phthalocyanines, having a wide use as dyestuffs, are also used as electrochromic materials, liquid crystals, optical recording media, photosensitizers in photodynamic therapy, gas sensors in the form of Langmuir-Blodgett films, in photocopiers, and photoactive conductors in laser printers, and non-linear optical materials.

Attaching several functional materials to phthalocyanines and using different metal salts in the synthesis allows one to prepare a large number of compounds for application purposes. Water-soluble groups are added as functional groups to phthalocyanines to allow us to use them in PDT. Today, phthalocyanines are used as second-generation photosensitizers. In order for phthalocyanines to be used as photosensitizers, substitutients that would allow them to do so are added into peripheral or non-peripheral positions of the macrocycle. Sulfonic acid, carboxylic acid, phosphonic acid, and quaternized ammonium groups could be substituted to obtain water solubility.

In the thesis, ethynylpyridine-substituted Zn(II) and Co(II) phthalocyanines were synthesized and quaternized water-soluble derivatives were prepared. Firstly, by using the method described in the literature, 4-aminophthalonitrile was synthesized and it was converted to 4-iodophthalonitrile. By using zinc acetate, reacting with 4-iodophthalonitrile in 2-dimethylaminoethanol yielded 2,9(10),16(17),23(24)-tetrakis(3-pyridylethynyl)phthalocyaninatozinc(II) was obtained. Under typical Sonogashira reaction conditions, the cross-coupling reaction between 2-ethynylpyridine and tetraiodo-metallophthalocyanines in triethylamine with copper(I) iodide and bis(triphenylphosphine) palladium(II) chloride as catalysts at room temperature under nitrogen atmosphere produced 2,9(10),16(17),23(24)-tetra-(3-pyridylethynyl) phthalocyaninato zinc (II). For the synthesis of water-soluble phthalocyanine derivatives, pyridine groups were introduced into the reaction to synthesize quaternized derivatives. The pthalocyanines were reacted with excess amount of dimethyl sulfate. The same method was also applied for the preparation of

Co(II) phthalocyanine and its quaternary derivative. The structures of new compounds were elucidated with ^{1}H-NMR, FTIR and UV-Vis techniques. The aggregative behavior of the complexes was examined at different concentrations in DMF. Water-soluble Zn(II) and Co(II) phthalocyanines were investigated with respect to their DNA and BSA binding properties.

4. DEVICES AND MATERIALS USED IN THIS STUDY

4.1 Devices

- Ultraviolet–visible (UV-Vis) spectrophotometer: Scinco LabProPlus UV/Vis spectrophotometer.
- Infrared spectrometry : Perkin-Elmer spectrum One FT-IR with UATR
- ^{1}H-NMR spectroscopy : Varian Unity Inova 500 MHz

4.2 Chemicals

Dimethyl formamide (DMF), dimethyl sulfoxide (DMSO), ethanol, methanol, acetone, hexane, chloroform ($CHCl_3$), zinc acetate, cobalt(II) chloride, sulfuric acid (H_2SO_4), hydrochloric acid (HCl), 4-nitrophthalonitrile, Fe powder, 4-aminophthalonitrile, sodium nitrite ($NaNO_2$), potassium iodide (KI), sodium thiosulfate ($Na_2S_2O_3$), sodium sulfate (Na_2SO_4), dichloromethane (CH_2Cl_2), dimethylaminoethanol, 2-ethynylpyridine (%98), bis(triphenylphosphine)palladium(II) chloride (15.2% Pd), copper(I) iodide, triethylamine, dimethylsulfate.

5. EXPERIMENTAL PART

5.1 Synthesis of 4-nitrophthalimide [81]

200 mL of sulfuric acid and 50 mL of fuming nitric acid were cooled on an ice bath and 40 g (0.272 mol) phthalimide was added in portions within 1-1.5 hours so as not to exceed the internal temperature beyond 10-15 °C. The mixture was stirred over ice bath for half an hour and the internal temperature was raised to 35 °C, and yellow particles were observed to dissolve. The mixture was stirred for an additional 1 hour, then cooled to 0 °C again and poured onto 1 kg of ice water mixture. Yellow 4-nitrophthalonitrile precipitated, it was filtered and washed with water until the filtrate was neutral to blue litmus paper. It was crystallized from 850-900 mL of ethyl alcohol. Bright yellow crystals were filtered, washed with cold ethyl alcohol, and dried in a vacuum oven at 80-90 °C. Molecular formula: $C_8H_4N_2O_4$, yield: 36.5 g (70%). Mp: 195 °C.

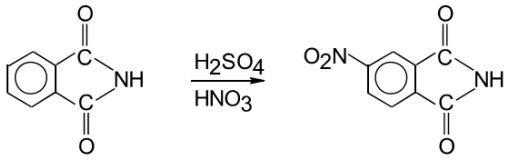


Figure 0.1 : Synthesis of 4-nitrophthalimide.

5.2 Synthesis of 4-nitrophthalamide [82]

30 g (0.156 mol) 4-nitrophthalimide was stirred in 168 mL 32% ammonia solution for 24 hours. Then it was filtered, washed with cold water, and tetrahydrofuran (THF). The color of phthalimide was yellow and it turned to white as reaction proceeds. Molecular formula: $C_8H_7N_3O_4$. Yield: 24 g (73%). Mp: 197 °C.

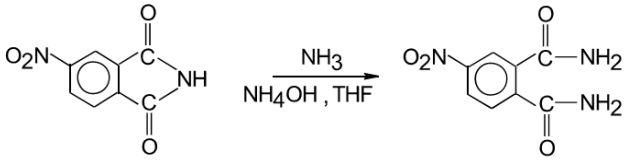


Figure 0.2 : Synthesis of 4-nitrophthalamide.

5.3 Synthesis of 4-Nitrophthalonitrile [83]

In a three necked flask, 70 mL of dry dimethylformamide (DMF) was cooled to 0 °C under a stream of nitrogen and 7.3 mL of thionyl chloride was added so that the internal temperature did not go beyond 5 °C. After addition, nitrogen flow was ceased and a calcium chloride tube was added to the top of flask. Meanwhile, the color of the medium was observed to be yellow. Then, 10 g (0.048 mol) of 4-nitrophthalamide was slowly added so that the internal temperature did not go beyond 5 °C. The mixture was stirred over ice bath for 1 hour. The mixture was stirred at room temperature for 2 hours and then poured over 500 g of ice-water. The precipitate was filtered and washed successively with water, 250 mL 5% sodium hydrogencarbonate solution, and water again and dried in a vacuum oven at 110-120 °C. Molecular formula: $C_8H_3N_3O_2$. Yield: 7.4 g (90%). Mp: 141 °C.

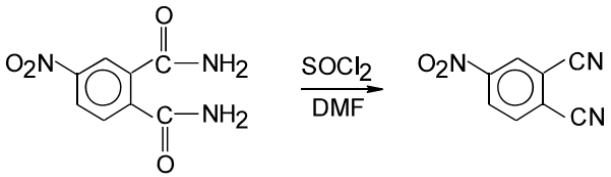
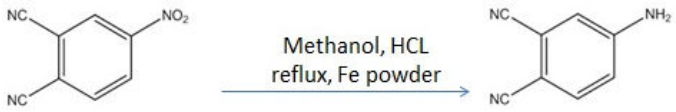


Figure 0.3 : Synthesis of 4-nitrophthalonitrile.

5.4 Synthesis of Aminophthalonitrile [84]

45 mL of methanol and 9.6 mL of concentrated HCl were stirred in a round-bottomed flask. 2.02 g (145 mmol) of 4-nitrophthalonitrile was added into this medium. The mixture was heated to reflux. 4-Nitrophthalonitrile dissolved in this step. Iron powder (2.21 g, 39.2 mmol) was added in portions within an hour. The color of solution changed to yellow-brown. After the addition of iron has been completed, the reaction medium was continued to stir at reflux temperature for another hour. The reaction medium was cooled to room temperature. It was

precipitated with 60 mL of water. The precipitate was filtered and washed with copious amount of water. Molecular formula: H2NC6H3-1,2-(CN)2. Yield: 1.4 g (81.59%).



(1)

Figure 0.4 : Synthesis of aminophthalonitrile **(1).**

5.5 Synthesis of iodophthalonitrile [85]

1.6 g (0.011 mol) 4-aminophthalonitrile was dissolved in 22.4 mL of 2.5 M H_2SO_4. The mixture was cooled to 0 °C. To the solution was slowly added 0.9 g (0.013 mol) of $NaNO_2$ dissolved in 3.5 mL of water. After the mixture was stirred for 30 minutes, 2.08 g (0.013 mol) of KI dissolved in 12.8 mL of water was added. The mixture was further stirred for 45 minutes at room temperature. After this period, the brown solid was filtered and washed with water. It was dissolved in a minimal amount of chloroform. The solution was washed first with saturated $Na_2S_2O_3$ and then with water. It was dried over Na_2SO_4. After the solution was filtered, it was dried in vacuum and purified with column chromatography over silica gel by eluting with dichloromethane. Molecular formula: IC6H3-1,2-(CN)2 (254.03 g/mol). Yield: 2.37 g (85%).

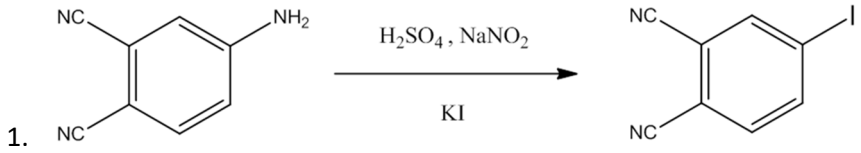


Figure 0.5 : Synthesis of iodophthalonitrile (left: **1**, right: **2**).

5.6 2,9(10),16(17),23(24)-tetraiodophthalocyaninatozinc(II) (3) [86]

4-Iodophthalonitrile (**3**) (100 mg, 0.394 mmol) was heated at reflux in 2-dimethylaminoethanol (1.5 mL) under nitrogen for 24 h in the presence of 30 mg (0.16 mmol) of $Zn(CH_3COO)_2$. After cooling to room temperature, the reaction mixture was treated with 1:1 (v/v) water/methanol mixture to precipitate the product which was filtered off. The resulting dark green solid was washed several times with

methanol and acetone. The desired compound was obtained after drying *in vacuo* at 75 C. Molecular formula: $C_{32}H_{12}I_4N_8Zn$ (1081,4892 g/mol). Yield: 0.0345 g (32.5%).

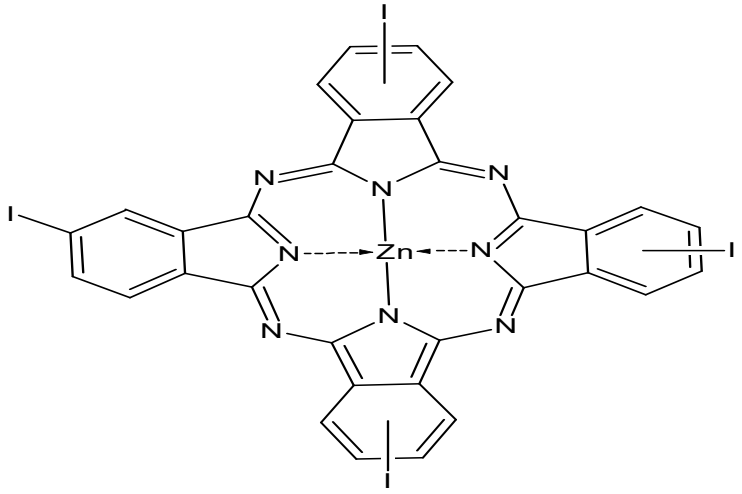


Figure 0.6 : Tetraiodo zinc phthalocyanine **(3).**

5.7 2,9(10),16(17),23(24)-tetra-(2-pyridylethynyl) phthalocyaninato zinc (II) (4)

Tetraiodozinc phthalocyanine (100 mg, 0.093 mmol) was reacted with 2-ethynylpyridine (0.038 g, 0.372 mmol) in the presence of bis(triphenylphosphine)palladium(II) chloride [$Pd(PPh_3)_2Cl_2$] (5 mg, 0.007 mmol) and copper(I) iodide (5 mg, 0.026 mmol) in 2 mL of triethylamine. The reaction mixture was stirred at room temperature for 12 h under nitrogen atmosphere. The reaction mixture was treated with 1:1 (v/v) water/methanol mixture to precipitate the product which was filtered off. The resulting dark green solid was washed several times with methanol and acetone. The desired compound was obtained after drying *in vacuo* at 75 C. Molecular formula: $C_{60}H_{28}N_{12}Zn$ (981.38 g/mol). Yield: 0.053 g (58.35%).

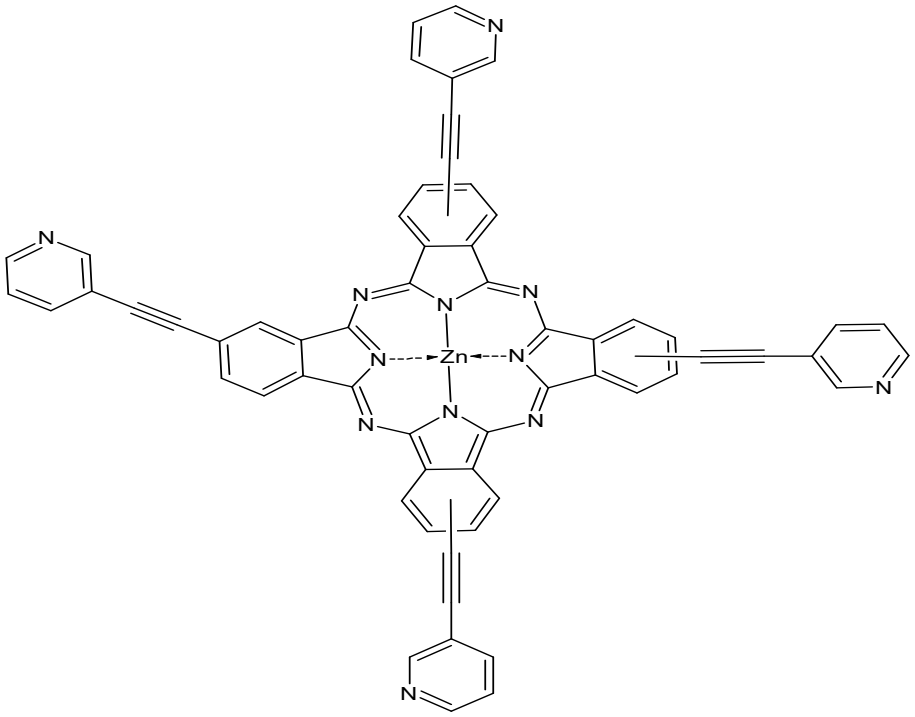
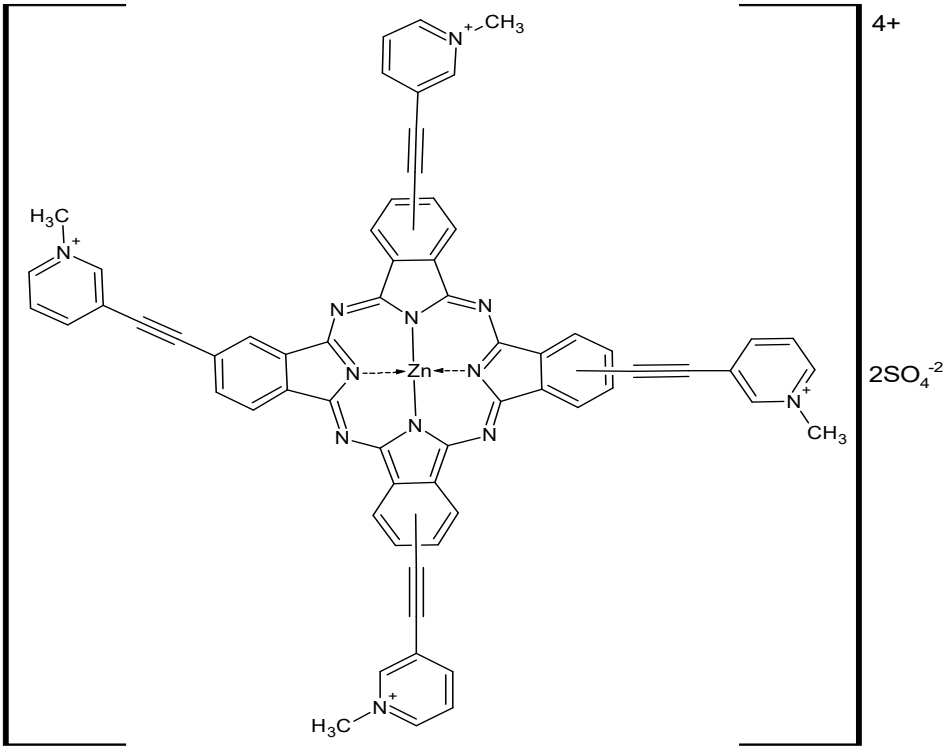


Figure 0.7 : Phthalocyanine with a N-donor base (pyridine) (**4**).

5.8 Quaternized Zinc phthalocyanine (5)

Compound **4** (100 mg, 0.14 mmol) was heated to 120 °C in DMF (5 mL) and excess dimethyl sulfate (0.1 mL) was added drop wise. The reaction mixture was stirred at 120 °C for 12 h. At the end of this period, the mixture was cooled to room temperature and the product was precipitated with acetone and collected by filtration. The green solid product was washed successively with hot ethanol, ethyl acetate, THF, chloroform, hexane, and diethyl ether. Molecular formula: $C_{64}H_{40}N_{12}O_8S_2Zn$ (1233.42 g/mol). Yield: 0.020 g (15.89%).



5

Figure 0.8 : Quaternized Zn-phthalocyanine (**5**).

5.9 2,9(10),16(17),23(24)-tetraiodophthalocyaninatocobalt (II) (6)

4-Iodophthalonitrile **(3)** (100 mg, 1.6 mmol) was heated at reflux in 2-dimethylaminoethanol (1.5 mL) under nitrogen for 24 h in the presence of 30 mg of $CoCl_2$ (0.23 mmol). After cooling to room temperature, the reaction mixture was treated with 1:1 (v/v) water/methanol mixture to precipitate the product which was filtered off. The resulting dark blue solid was washed several times with methanol and acetone. The desired compound was obtained after drying *in vacuo* at 75 °C. Molecular formula: $C_{32}H_{12}CoI_4N_8$ (1074.53 g/mol). Yield: 0.059 g (55.78%).

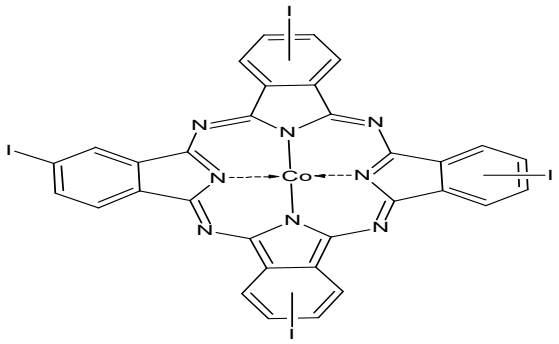


Figure 0.9 : Cobalt phthalocyanine compound **(6).**

5.10 2,9(10),16(17),23(24)-tetrakis(2-pyridylethynyl)phthalocyaninatocobalt (II) (7)

Tetraiodocobalt(II) phthalocyanine (100 mg, 0.093 mmol) was reacted with 2-ethynylpyridine (0.038 g, 0.372 mmol) in the presence of bis(triphenylphosphine)palladium(II) chloride [$Pd(PPh_3)_2Cl_2$] (5 mg, 3.5 mmol) and copper(I) iodide (5 mg, 0.026 mmol) in 2 mL of triethylamine. The reaction mixture was stirred at room temperature for 12 h under nitrogen atmosphere, then it was treated with 1:1 (v/v) water/methanol mixture to precipitate the product which was filtered off. The resulting dark green solid was washed several times with methanol and acetone. The desired compound was obtained after drying *in vacuo* at 75 C. Molecular formula: $C_{60}H_{28}CoN_{12}$ (974.93 g/mol). Yield: 0.022 g (24.26%).

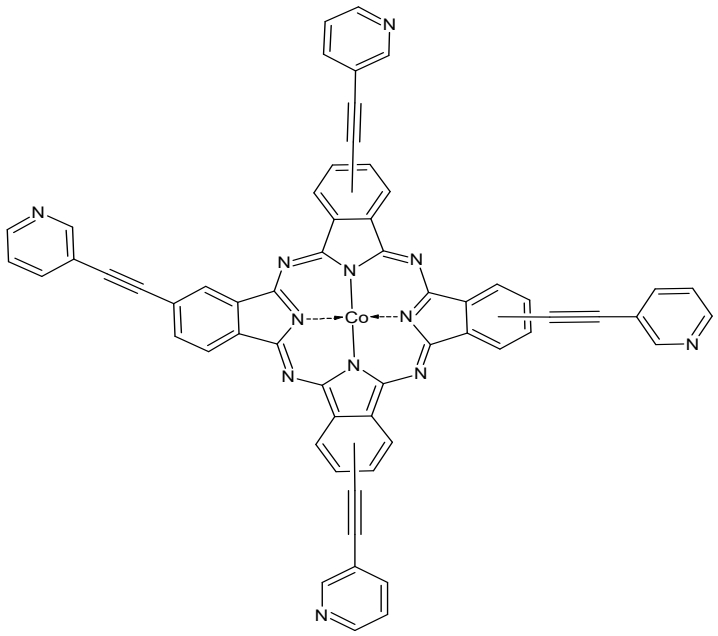


Figure 0.10 : Pyridine-based phthalocyanine with cobalt as metal salt **(7).**

5.11 Quaternized cobalt phthalocyanine (8)

Compound **7** (100 mg, 0.14 mmol) was heated to 120 °C in DMF (5 mL) and excess dimethyl sulfate (0.1 mL) was added drop wise. The reaction mixture was stirred at 120 C for 12 h. At the end of 12 h, the mixture was cooled to room temperature and the product was precipitated with acetone and collected by filtration. The green solid product was washed successively with hot ethanol, ethyl acetate, THF, chloroform, hexane, and diethyl ether. Molecular formula: $C_{64}H_{40}CoN_{12}O_8S_2$ (1226.97 g/mol). Yield: 0.026 g (20.56%).

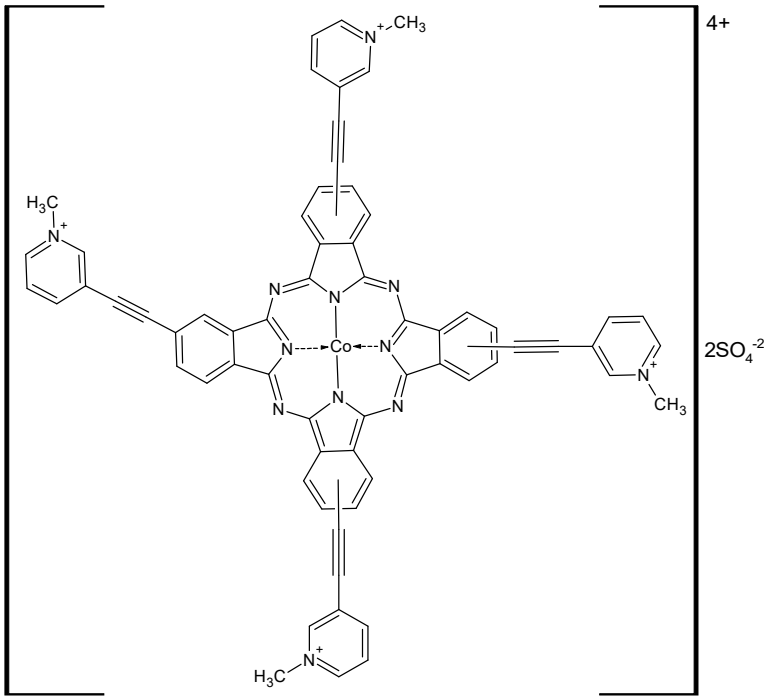


Figure 0.11 : Quaternized cobalt phthalocyanine **(8).**

6. RESULTS AND DISCUSSION

6.1 Synthesis and Structural Characterization

The starting material of phthalocyanine, namely 4-nitrophthalonitrile, was synthesized as detailed in the literature. In the first phase of our work, we started to study the synthesis of the iodophthalonitrile required for the phthalocyanines. This compound was synthesized in two steps from 4-nitrophthalonitrile. In the first step, the nitro moiety is reduced to amine. In the second step, the amine was converted into the water-soluble diazonium salt, and reacted with KI to synthesize 4-iodophthalonitrile (**2**).

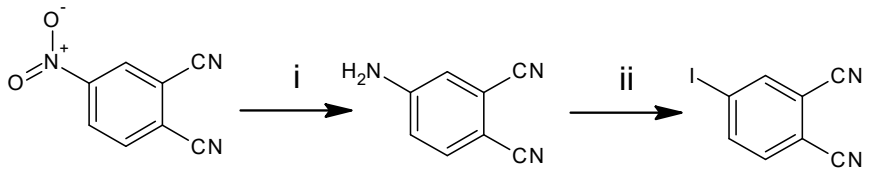


Figure 0.1 : Synthesis of 4-iodophthalonitrile (i: MeOH/HCl, Fe powder; ii: H_2SO_4, $NaNO_2$, KI)

Substituted-phthalocyanines could be synthesized by two different methods. First, the cyclotetramerization is carried out after the dinitrile derivative of the desired substituent is synthesized. The second method is cyclotetramerization followed by the reaction of the desired groups with the ring. In our previous study, we know that phthalocyanines bound by ethynyl bridge have problems regarding purification. Therefore, we aimed to synthesize the desired phthalocyanines by the second method. Firstly, tetraiodocobalt- and zinc-phthalocyanines were synthesized according to the literature [87]. The synthesized phthalocyanine derivatives were purified by washing and by precipitation.

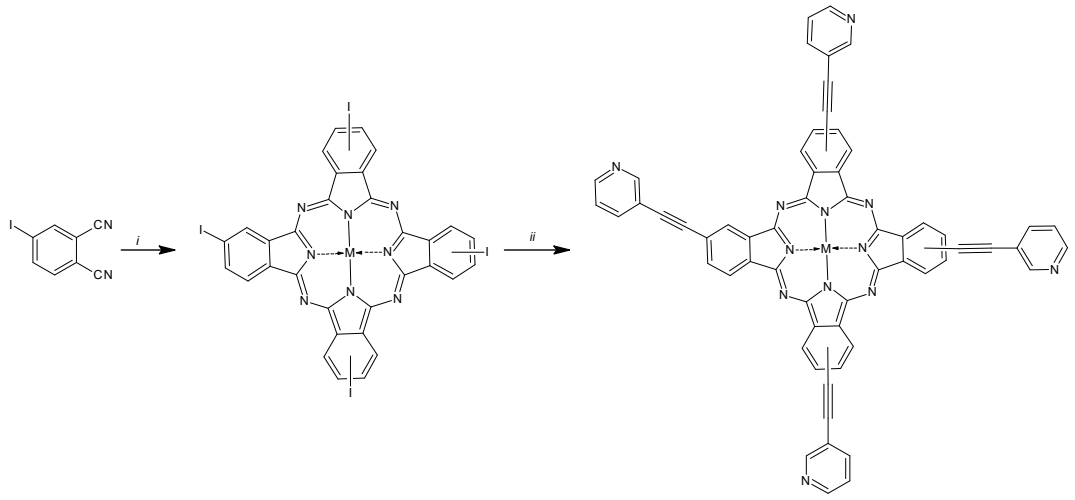


Figure 0.2 : Synthesis of pyridinethynyl substituted metallo-phthalocyanines.

In this part of the work, the bonding of the 2-ethynyl pyridine group to the pthalocyanine ring was performed. Under typical Sonogashira reaction conditions, the cross-coupling reaction between an excess of 2-ethynylpyridine and tetraiodometallophthalocyanines in triethylamine with copper(I) iodide (CuI) and bis(triphenylphosphine) palladium(II) chloride [$Pd(PPh_3)_2Cl_2$] as catalysts at room temperature under nitrogen atmosphere produced **4** and **7**. Then the compounds **4** and **7** were separated from the starting materials by washing and precipitation methods, pyridylethynyl- substituted phthalocyanine derivative was synthesized.

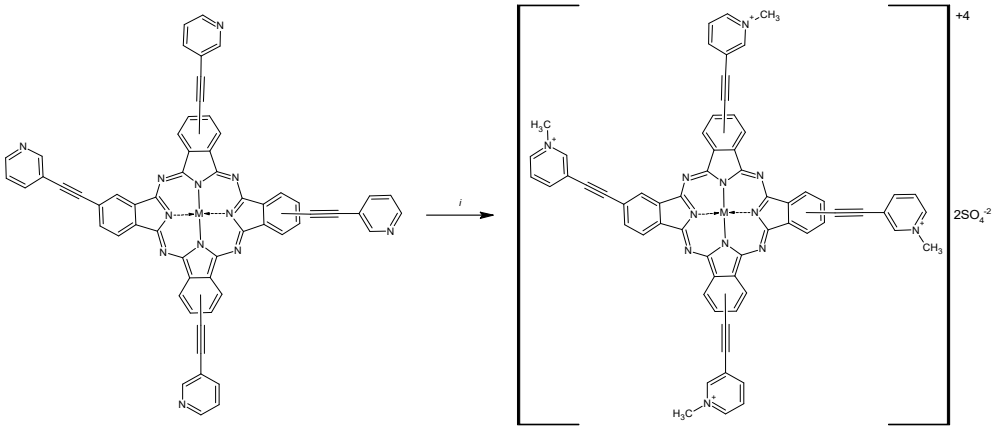


Figure 0.3 : Synthesis of the quaternized metallo-phthalocyanine.

For the synthesis of water-soluble phthalocyanine derivatives, pyridine groups were introduced into the reaction to synthesize quaternized derivatives. The pthalocyanines were reacted with excess amount of dimethyl sulfate in DMF at 120 °C. The reaction product was purified by washing with ethanol, ethyl acetate, THF,

chloroform, hexane and diethyl ether, respectively. Thus, water soluble pyridine substituted phthalocyanine derivatives were synthesized.

Spectral data (MALDI-TOF, ^{1}H NMR, UV-Vis, and FT-IR) for all new products are consistent with the proposed structures. The peak of 12 protons in the NMR spectrum of compound **3** was observed at 7.57-7.77 ppm. In the NMR spectrum of compound **4**, peaks belonging to pyridine groups were observed in the range of 7.46-7.61 ppm, protons on the ring were observed in the range of 8.34-9.11 ppm. The NMR spectrum of both compounds supports the formation of the compound.

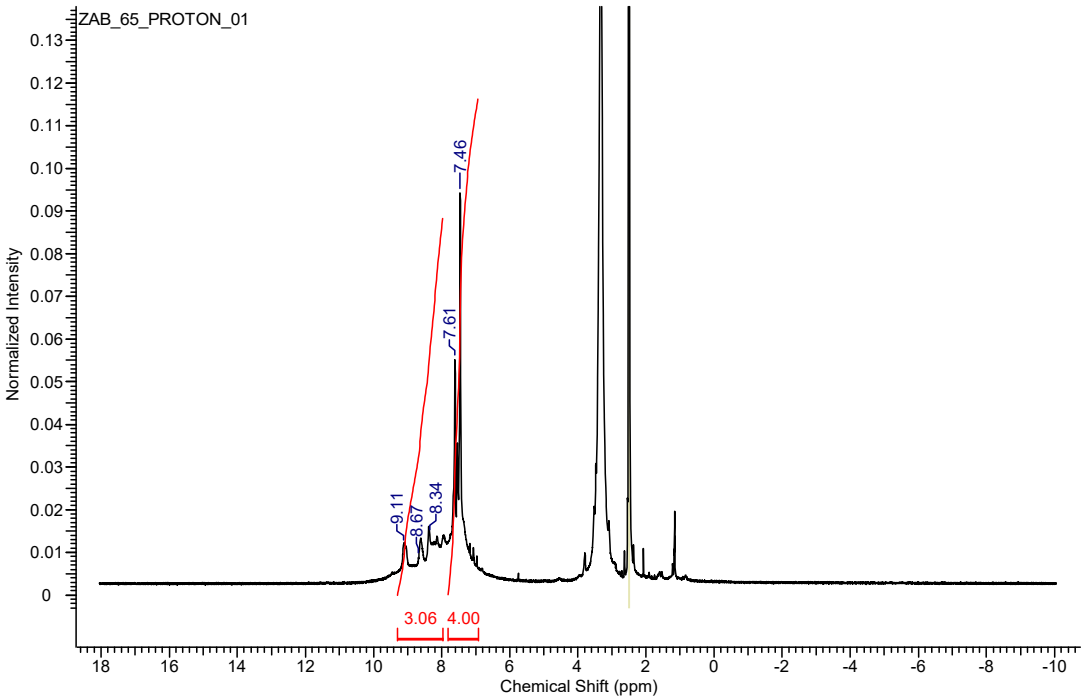


Figure 0.4 : ^{1}H NMR spectrum of the quaternized zinc phthalocyanine.

In the definition of phthalocyanines, UV-Vis spectra have a distinguishing property. Transition between π- π* display 2 separate bands , a Q-band in the region of 600-700 nm and a B-band in the 300-350 nm region. The zinc phthalocyanines (**3-5**) were observed around Q band 677-681 and B band 334-359 nm. In the case of the cobalt phthalocyanine derivatives (**6-8**), Q bands were observed around 664 while B bands were observed around 333 nm. These results show that the Pc ring has been formed.

Table 0.1 : Q and B bands of the metallophthalocyanine in DMF.

	B Band	**Q Band**
I_ZnPc (3)	351	677
Py_ZnPc (4)	359	681
Q_ZnPc (5)	334	679
I_CoPc (6)	333	664
Py_CoPc (7)	333	665
Q_CoPc (8)	333	664

Aggregation is relevant to concentration, substituent, solvent, temperature, and the metal ion. The aggregative behavior of the complexes was examined at different concentrations in DMF. The intensity of absorption of the Q band also increased with an increase in concentration. It was observed that a new band did not occur due to the aggregated species. The Beer–Lambert law was obeyed for all these compounds for the concentrations ranging from 2.00×10^{-6} to 14.00×10^{-6} M. The phthalocyanine derivatives **3**–**8** did not show a detectable aggregation in DMF.

The FT-IR spectra of the phthalocyanines **4**, **7** are very similar. In the FT-IR spectra of compounds **4** and **7,** stretching vibrations of C≡C groups observed at 2209 cm^{-1} and 2207 cm^{-1}, respectively. The aromatic groups appeared at 3057–3060 cm^{-1} and the aromatic C–H bending vibrations observed 1597–1468 cm^{-1} at expected frequencies. The FT-IR spectra of the quaternizated phthalocyanines are very similar to their Pcs. No major change was found in the FT-IR spectrum of **5** and **8** after quaternization. However, stretching vibrations were observed around 1385-1395 and 738-743 cm^{-1} for S=O and S-O bonds, respectively.

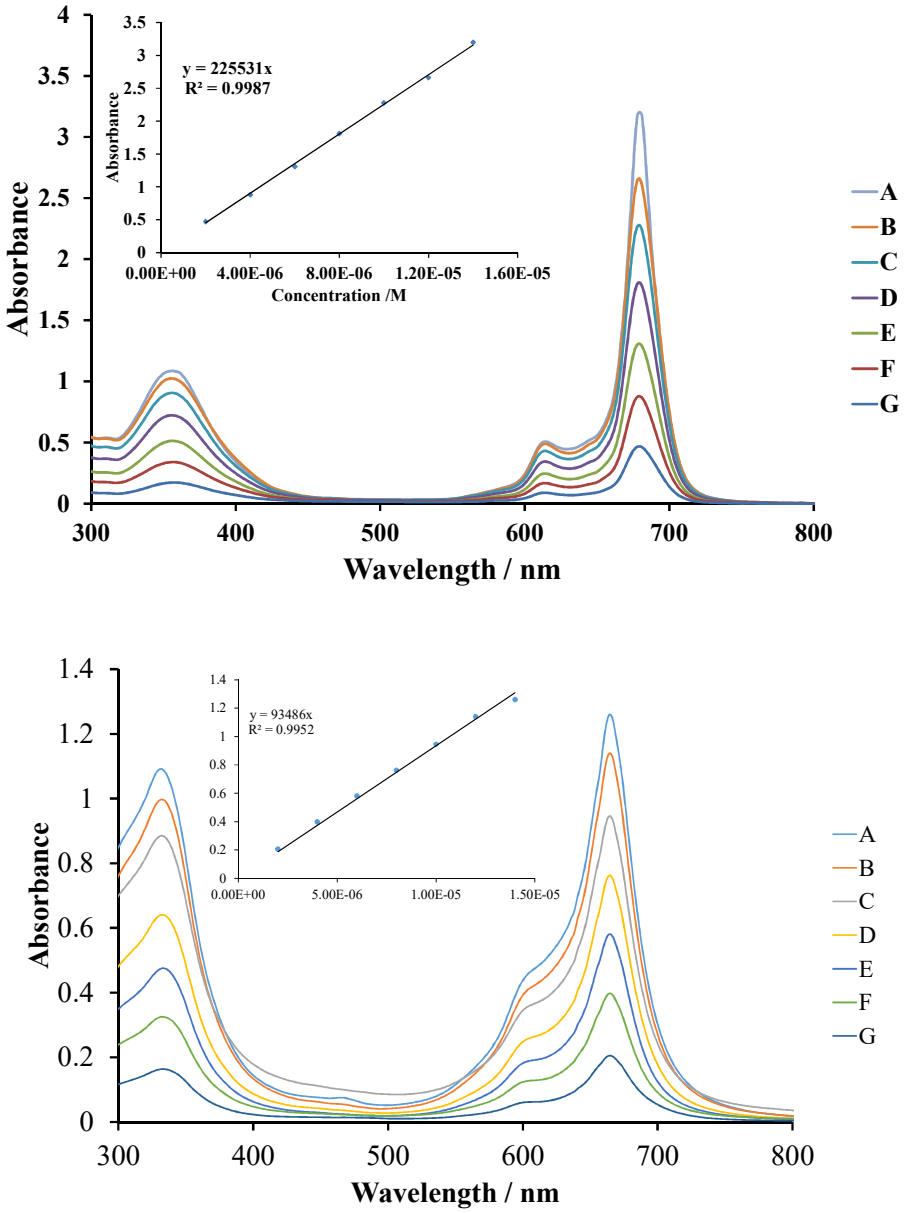


Figure 0.5 : Aggregation behavior of the MPc (**4**, **7**) in DMF at different concentrations. (14 $\times 10^{-6}$ (A), 12 $\times 10^{-6}$ (B), 10 $\times 10^{-6}$ (C), 8$\times 10^{-6}$ (D), 6 $\times 10^{-6}$ (E) and 4 $\times 10^{-6}$ (F) M).

6.2 Determination of Binding of ZnPc and CoPc to DNA using UV/Vis Titrations

All titrations of Pcs with CT-DNA were performed at room temperature in buffer solution. The concentrations of CT-DNA per nucleotide phosphate ([DNA]) was calculated from the absorbance at 260 nm using ε_{DNA} = 13200 M^{-1} cm^{-1} [88]. DNA was stored at 4 ºC overnight and used within 2 days. The stock solutions of 20 µM quaternized Pcs (**Q_ZnPc** and **Q_CoPc**) and 50 µM DNA were prepared in buffer solution. First the absorption spectrum of a 3 mL buffer solution of Q_**ZnPc (5)** and

Q_CoPc (8) was recorded and then 6 x 30 µL for **Q_ZnPc** (4 x 30 µL for **Q_CoPc**) injections of DNA were added manually. Absorption spectra were collected from 500 nm to 800 nm. The titrations were carried out until Pcs' Q bands remain at a fixed wavelength upon the successive additions of CT-DNA. To determinate the binding constants K_{bZnPc} and K_{bCoPc}, Eq. 2 [89] (Table 6.2);

$$[DNA]/(\varepsilon_a-\varepsilon_f) =[DNA]/]/(\varepsilon_b-\varepsilon_f) + 1/[K_b((\varepsilon_b-\varepsilon_f)] \quad \text{(Eq. 2)}$$

was employed where the apparent absorption coefficient ε_a, ε_f and ε_b correspond to A_{obsd}/[Pc], the extinction coefficient of the free Pc and the extinction coefficient of the Pc when fully bound to DNA, respectively. In plots of $[DNA]/(\varepsilon_a-\varepsilon_f)$ versus [DNA], K_b is given by the ratio of slope to intercept [90]. The experiments were repeated at three times (see Figures 6.6-6.8).

6.3 Determination of binding of ZnPc and CoPc to BSA using UV/Vis titrations

All titrations of Pcs with BSA were performed at room temperature in distilled water. 25 µM BSA, 20 µM quaternized Pcs (**Q_ZnPc** and **Q_CoPc**) stock solutions were prepared in distilled water. First the absorption spectrum of a 3 mL aqueous solution of **Q_ZnPc** and **Q_CoPc** was recorded. Then 5 x 30 µL injections of BSA were added manually and successively. Absorption spectra were collected from 500 nm to 800 nm. The titrations were carried out until the Q bands of Pcs remain at a fixed wavelength upon the successive additions of BSA [91] (see Figures 6.9 and 6.10).

6.4 Determination of the change in thermal denaturation profile of DNA

Melting temperatures were determined for CT-DNA (50 µM, 2.5 mL) and quaternized Pcs (**Q_ZnPc** and **Q_CoPc**) (25 µM, 0.3 mL) in buffer by heating from 20 to 90 °C at a rate of 0.6 °C/min, and recording the UV absorbance at 260 nm every 10 s (Figure 6.11). The absorbance measurements were repeated five times, and standard deviations were calculated.

6.5 Determination of thermodynamic parameters

The equilibrium constants of DNA:Pc complexes were determined by analyzing the absorbance of Pc-DNA solutions at varying temperatures (293.15 K, 303.15 K, 313.15 K, 323.15 K, 333.15 K). For the reversible binding reaction of a ligand that is binding to a DNA molecule with a single site to form a ligand-DNA complex, we can write the binding reaction as shown in Eq. 3 [92].

$$L + DNA \leftrightarrow LDNA \qquad K= [LDNA]_{eq} / ([L]_{eq}X[DNA]_{eq}) \qquad (Eq.\ 3)$$

where, [L] is the concentration of the ligand or DNA-binding domain (in present work, L represents **Q_ZnPc** and **Q_CoPc**), $[DNA]_{eq}$ and $[LDNA]_{eq}$ are the concentrations of DNA and bound complex at equilibrium, respectively. The stability of the bound complex is determined by the differences in the noncovalent interactions between the Pc and the DNA as temperatures varied using nonlinear least-squares algorithm [93]. At these temperatures, DNA does not undergo any structural degradation. The absorption spectra were analyzed by assuming phthalocyanine:DNA molar ratios as 1:1 and 2:1. The results show that the best fitting corresponds to the 1:1 model complex at studied temperatures.

The energetics of DNA–phthalocyanine equilibrium can be conveniently characterized by three thermodynamic parameters, standard Gibbs free energy, ΔG^o, the standard molar enthalpy, ΔH^o and the standard molar entropy, ΔS^o. ΔG^o can be calculated from the equilibrium constant, K, using the familiar relationship, $\Delta G^o = -RT \ln K$, in which R and T refer to the gas constant and the absolute temperature, respectively [93] (Table 6.3).

The van't Hoff equation gives a linear plot of lnK versus 1/T (see Figure 6.12), if the heat capacity change for the reaction is essentially zero:

$$d \ln K/d(1/T) = -\Delta H^o / R \qquad (Eq.\ 4)$$

The ΔH^o can be calculated from the slope of the straight line, $-\Delta H^o/R$ and the standard entropy by the following Eq. (4):

$$\Delta S^o = (\Delta H^o - \Delta G^o) / T \qquad \text{(Eq. 5)}$$

6.6 Aggregation Properties of Phthalocyanines

Aggregation behavior of phthalocyanines is described as the coplanar association of rings progressing from monomers to dimers and to higher order complexes. It depends on the concentration, nature of solvent, substituents, metal ions, and temperature [94].

In UV-Vis spectra of compounds **Q_ZnPc (5)** and **Q_CoPc (8)** in DMF, while the absorptions at around 636 and 600 nm corresponded to h-aggregates of **Q_ZnPc** and **Q_CoPc**, the more strong absorptions at 679 and 664 nm were assigned to Q-bands of monomeric **Q_ZnPc** and **Q_CoPc** respectively. These results indicated that strong interactions between π-clouds in Pcs gave rise to aggregates of quaternized **Q_ZnPc** and **Q_CoPc**. Especially, heterocyclic cationic structures similar to imidazolium or pyridinium rings, delocalization of positive charge ease the aggregation [95]. In buffer solutions, absorptions were enhanced quite a lot at shorter wavelengths and disappeared completely around Q band region which were observed in DMF (see Figures 6.6 and 6.7). In this work, non-ionic surfactant Triton-X was used to eliminate aggregation but the aggregates were prevailing over monomers. This attitude could be explained in such a way that Pc planes resemble to the "pages of a book" in which all the pages stick to each other with a strong π-π interaction working as "glue". In other words, in case of freshly synthesized **Q_CoPc** and **Q_ZnPc** compounds, the enhanced planarity of pc molecules provided with addition of rigid C≡C bonds in peripheral positions brought them into close proximity to aggregate on top of another and 18 π electrons stick them together as a glue.

6.7 The Evaluation of Binding of ZnPc and CoPc with CT-DNA by UV-Vis Titrations

Metal ion coordination to nucleic acids is not only required for charge neutralization, it is also essential for the biological function of nucleic acids. The structural impact of different metal ion coordinations on DNA helices is questionary [96a]. The interactions may be either through direct metal ion coordination or mediated through water molecules of the metal ion's hydration shell. It is known that

the interaction of purines and/or pyrimidines with chelating compounds destabilizes the nature of DNA [96b].

The more increase in interaction, the more decrease in maximum absorbances of **Q_ZnPc (5)** and **Q_CoPc (8)**, the more stable the Pc-DNA complex. Last lines in (Figure 6.6 and 6.7) recorded on top of each other, after the last addition of DNA to the solutions of **Q_ZnPc** and **Q_CoPc**, the Q band absorbances of **Q_ZnPc** and **Q_CoPc** remained constant which meant that stable pcs-DNA complexes were formed.

In **Q_ZnPc**, zinc could coordinate with both oxygens and nitrogens in DNA bases and behaves a metal-finger structure [97,98]. According to (Figure 6.6) , the absence of absorption close to 679 nm proved the superiority of h- aggregates over monomers.

In addition to hydrophobic interactions due to π-stacking of aggregates screened as absorption at shorter wavelength in (Figure 6.6) . Zinc(II) might be bound especially with N7 in guanidines and oxygens in phosphate skeleton to stabilize monomeric forms of **Q_ZnPc**. K_b value supported that **Q_ZnPc** could bind to DNA strongly and be as a possible minor groove binder (Figure 6.8).

Cobalt is an interesting bioelement which dominates and interferes with many biological reactions. It was reported that in crystals, Co(II) ion binds exclusively at the N7 position of guanine bases by direct coordination. The coordination geometry around Co(II) is octahedral and could induce significant conformational changes on A-DNA and B-DNA [96a]. Our K_b value is bigger than the one of **Q_ZnPc** which is consistent with a stronger interaction than **Q_ZnPc** (Figure 6.7). Since the bases in DNA axially coordinated with cobalt and zinc in **Q_CoPc** and **Q_ZnPc** respectively, an octahedral complex could be formed and this would be superior over the **Q_ZnPc** which had a possible square planar or planar geometry as a result of interaction with nitrogen or oxygen bases in DNA (Figure 6.8).

6.8 The Evaluation of binding of ZnPc and CoPc with BSA by UV-vis titrations

The more the interaction increases, the more maximum absorbances of Pcs decreases and the more stable is the BSA-Pc complex [97]. Last lines in (Figures 6.9,6.10) mean that after addition of 150 μL of BSA to **Q_ZnPc (5)** and **Q_CoPc (8)**, the Q band absorbance remained constant. They showed the end of titration which means maximum interaction between Pcs and BSA occurred. The large spectral perturbation might indicate that pc planes in freshly synthesized Pc compounds could bind to BSA strongly. According to results, water-soluble quaternized **Q_ZnPc** and **Q_CoPc** strongly bind to blood plasma proteins such as BSA and hence they can easily be transported in the blood [98].

6.9 The Evaluation of Thermodynamic Parameters

The data presented in (Table 6.3) deduced that the favorable free energy changes of the binding process for **Q_ZnPc (5)** and **Q_CoPc (8)** arose from the large positive entropy changes. Entropy-driven binding process took place according to thermodynamic parameters given in (Table 6.3). When $\Delta H° > 0$ and $\Delta S° > 0$, the effective force is hydrophobic [99]. In water, the hydrophobic effect is the driving force for the formation of aggregates. A large positive entropic value due to the removal of water from hydrophobic parts, resulting from aggregation [100].

Incorporation of zinc had a great effect on energetics of binding process. In **Q_ZnPc**, axial coordination of zinc with oxygens or nitrogens in DNA heterogeneous bases, influenced the thermodynamic driving forces; enthalpy and entropy. As the temperature increased, more water molecules were released around **Q_ZnPc**-DNA complex resulting an increase in entropy. Hydrophobic interactions were mainly responsible for positive entropy [101]. Together with UV-Vis spectra and K_b value, **Q_ZnPc** could be regarded as a minor groove binder.

Zinc finger domains are structures that mediate sequence recognition for a large number of DNA-binding proteins These domains consist of sequences of amino acids containing cysteine and histidine residues (which have sulfurs and nitrogen donors respectively) tetrahedrally coordinated to a zinc ion. However, it was reported that cobalt(III) Schiff-base complexes could selectively inhibit a zinc finger transcription

factor [97]. ^{1}H NMR spectroscopy was used to confirmed the structure of a zinc finger peptide which is disrupted by axial ligation of the cobalt(III) complex to the nitrogen of the imidazole ring of a histidine residue. [102]

According to thermodynamic parameters given in (Table 6.3), **Q_CoPc** was also entropy-driven binding process. When the formation of octahedral complex-DNA fragments was considered, more ordered structure required increase in enthalpy but also the exposion of water molecules around DNA increased entropy more. Thus, a more negative Gibbs free energy was released during the interaction. The binding mode of **Q_CoPc** could be attributed to minor groove.

Table 0.2 : K_b and K_{sv} values of Q_ZnPc (**5**) and Q_CoPc (**8**) with standard deviations (± STD).

	K_b ($x10^5$)(L x mol^{-1})	K_{sv} ($x10^4$)(L x mol^{-1})
ZnPc	2.6± 0.1	-
CoPc	11.7± 0.1	-

Table 0.3 : Calculated thermodynamic parameters for binding of **Q_ZnPc (5)** and **Q_CoPc (8)** to ct-DNA (± STD).

T (K)	(LnK ± ΔK)	ΔG^o (kJ·mol^{-1})	ΔH^o (kJ·mol^{-1})	$T\Delta S^o$ (kJ·$mol^{-1}K^{-1}$)
		ZnPc		
293.15	06.21 ± 0.05	-15.48 ± 0.1	26.01 ± 0.1	41.49 ± 0.30
303.15	06.65 ± 0.05	-16.89 ± 0.1	26.01 ± 0.1	42.90 ± 0.30
313.15	07.14 ± 0.05	-18.31 ± 0.1	26.01 ± 0.1	44.32 ± 0.30
323.15	07.48 ± 0.05	-19.72 ± 0.1	26.01 ± 0.1	45.73 ± 0.30
333.15	07.73 ± 0.05	-21.14 ± 0.1	26.01 ± 0.1	47.15 ± 0.30
		CoPc		
293.15	12.01 ± 0.05	-28.25 ± 0.1	34.99 ± 0.1	63.22 ± 0.30
303.15	12.10 ± 0.05	-30.41 ± 0.1	34.99 ± 0.1	65.37 ± 0.30
313.15	12.36 ± 0.05	-32.56 ± 0.1	34.99 ± 0.1	67.53 ± 0.30
323.15	12.65 ± 0.05	-34.72 ± 0.1	34.99 ± 0.1	69.68 ± 0.30
333.15	12.99 ± 0.05	-36.88 ± 0.1	34.99 ± 0.1	71.84 ± 0.30

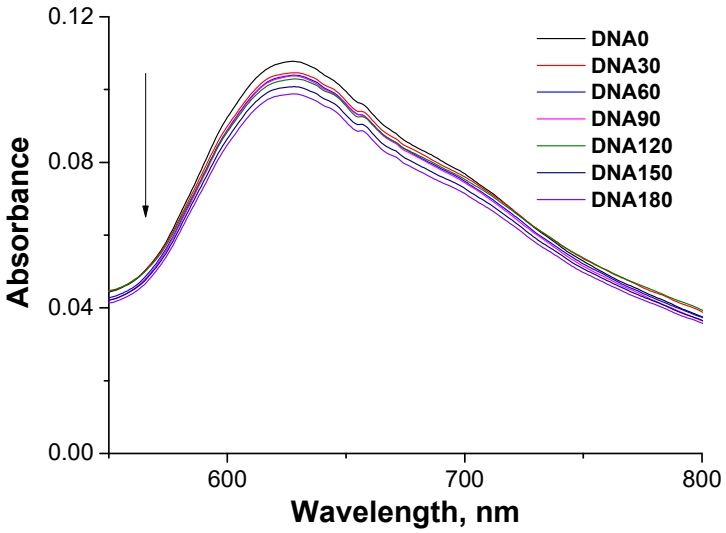


Figure 0.6 : The spectral changes in UV-Vis absorption spectrum of **Q_ZnPc (5)** in buffer solution upon addition of DNA.

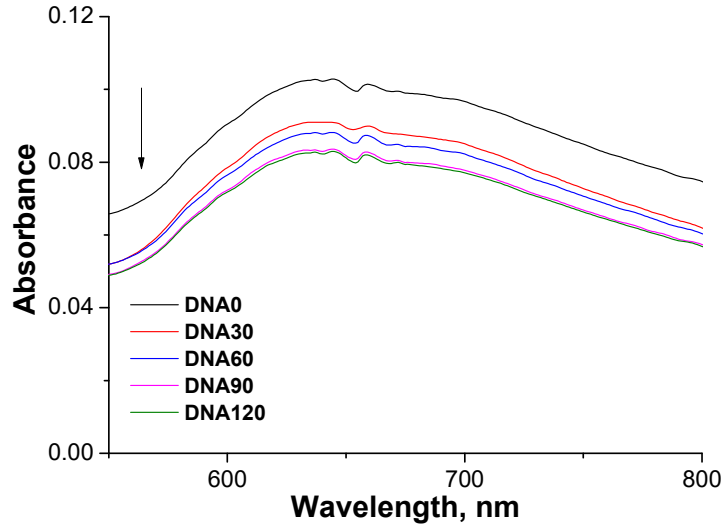


Figure 0.7 : The spectral changes in UV-Vis absorption spectrum of **Q_CoPc (8)** in buffer solution upon addition of DNA.

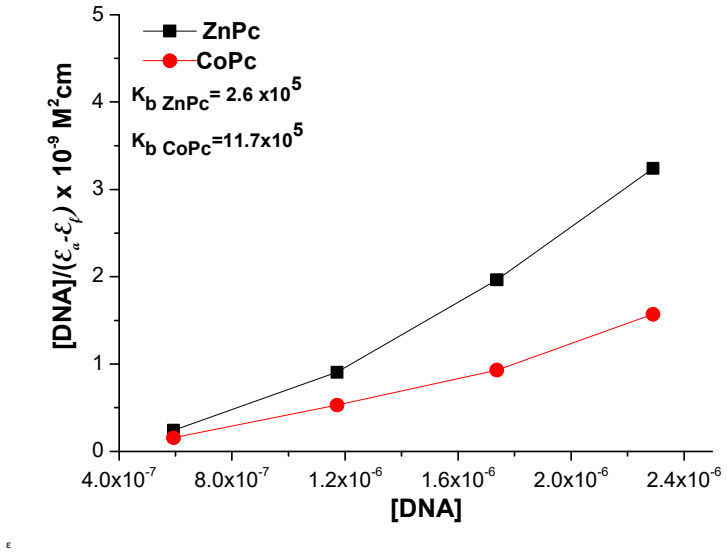


Figure 0.8 : Wolfe–Shimer equation plot of CT-DNA binding constant (K_b) of **Q_ZnPc (5)** and **Q_CoPc (8).**

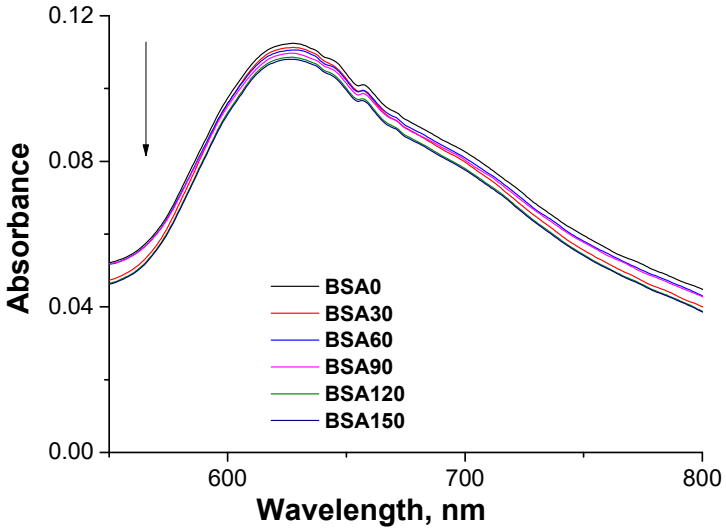


Figure 0.9 : The spectral changes in UV-Vis absorption spectrum of **Q_ZnPc (5)** in buffer solution upon addition of BSA.

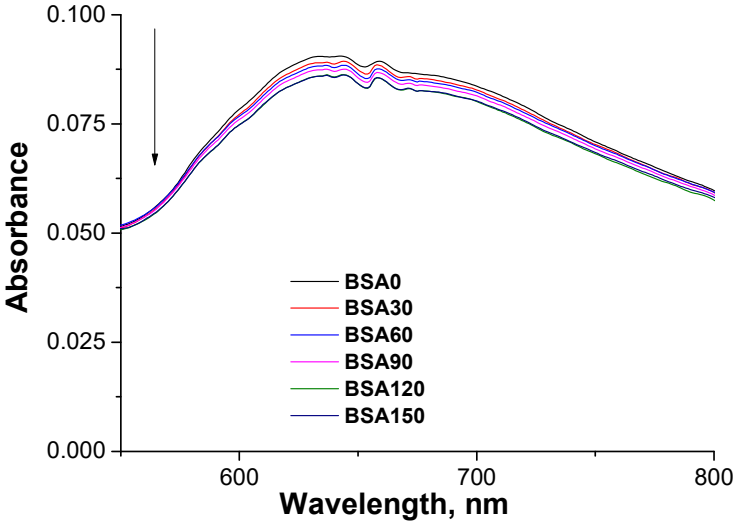


Figure 0.10 : The spectral changes in UV-Vis absorption spectrum of **Q_CoPc (8)** in buffer solution upon addition of BSA.

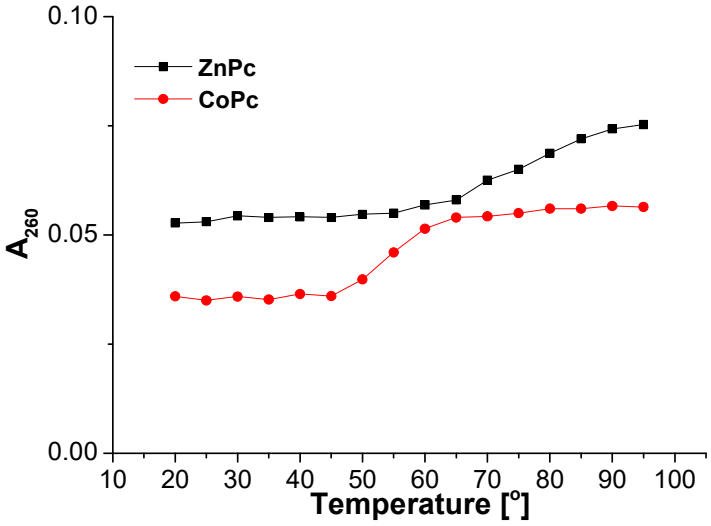


Figure 0.11 : The thermal denaturation profiles of CT-DNA in the presence of **Q_ZnPc (5)** and **Q_CoPc (8).**

[T_m values for **ZnPc** and **CoPc** are 77°C and 58°C, respectively]

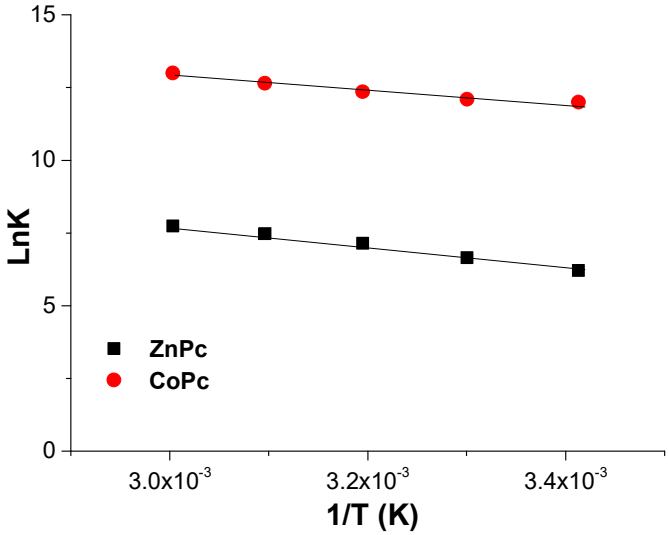


Figure 0.12 : Van't Hoff plots: Temperature dependence of equilibrium constant for **Q_ZnPc (5)** and **Q_CoPc (8)** -DNA interactions**.**

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APPENDICES

APPENDIX A. Spectral analyses of compounds synthesized

Appendix A.1 : IR spectrum of Tetraiodophthalocyaninatozinc(II).

Appendix A.2 : IR spectrum of Tetrakis(pyridine)phthalocyaninatozinc(II).

Appendix A.3 : IR spectrum of Tetrakis(methylpyridinium)phthalocyaninatozinc(II).

Appendix A.4 : IR spectrum of Tetraiodophthalocyaninatocobalt(II).

Appendix A.5 : IR spectrum of Tetrakis(pyridine)phthalocyaninatocobalt(II).

Appendix A.6 : IR spectrum of Tetrakis(methylpyridinium)phthalocyaninatocobalt(II).

Appendix A.7 : UV-Vis spectrum of tetraiodophthalocyaninatozinc(II) in DMF (14 x 10^{-6} M (A), 12x10^{-6} M (B), 10x10^{-6} M (C), 8x10^{-6} M (D), 6x10^{-6} M (E), and 4x10^{-6} M (F)

Appendix A.8 : UV-Vis spectrum of tetrakis(pyridine)phthalocyaninatozinc(II) in DMF (14 x 10^{-6} M (A), 12x10^{-6} M (B), 10x10^{-6} M (C), 8x10^{-6} M (D), 6x10^{-6} M (E), and 4x10^{-6} M (F).

Appendix A.9 : UV-Vis spectrum of tetrakis(methylpyridinium) phthalocyaninatozinc(II) in DMF (14 x 10^{-6} M (A), 12x10^{-6} M (B), 10x10^{-6} M (C), 8x10^{-6} M (D), 6x10^{-6} M (E), and 4x10^{-6} M (F).

Appendix A.10 : UV-Vis spectrum of tetraiodophthalocyaninatocobalt(II) in DMF (14 x 10^{-6} M (A), 12x10^{-6} M (B), 10x10^{-6} M (C), 8x10^{-6} M (D), 6x10^{-6} M (E), and 4x10^{-6} M (F)

Appendix A.11: UV-Vis spectrum of tetrakis(pyridine)phthalocyaninatocobalt(II) in DMF (14 x 10^{-6} M (A), 12x10^{-6} M (B), 10x10^{-6} M (C), 8x10^{-6} M (D), 6x10^{-6} M (E), and 4x10^{-6} M (F).

Appendix A.12 : UV-Vis spectrum of tetrakis(methylpyridinium) phthalocyaninatocobalt(II) in DMF (14 x 10^{-6} M (A), 12x10^{-6} M (B), 10x10^{-6} M (C), 8x10^{-6} M (D), 6x10^{-6} M (E), and 4x10^{-6} M (F).

Appendix A.13 : ^{1}H-NMR spectrum of tetraiodophthalocyainanozinc(II).

Appendix A.14 : ^{1}H-NMR spectrum of tetrakis(pyridine)phthalocyaninatozinc(II).

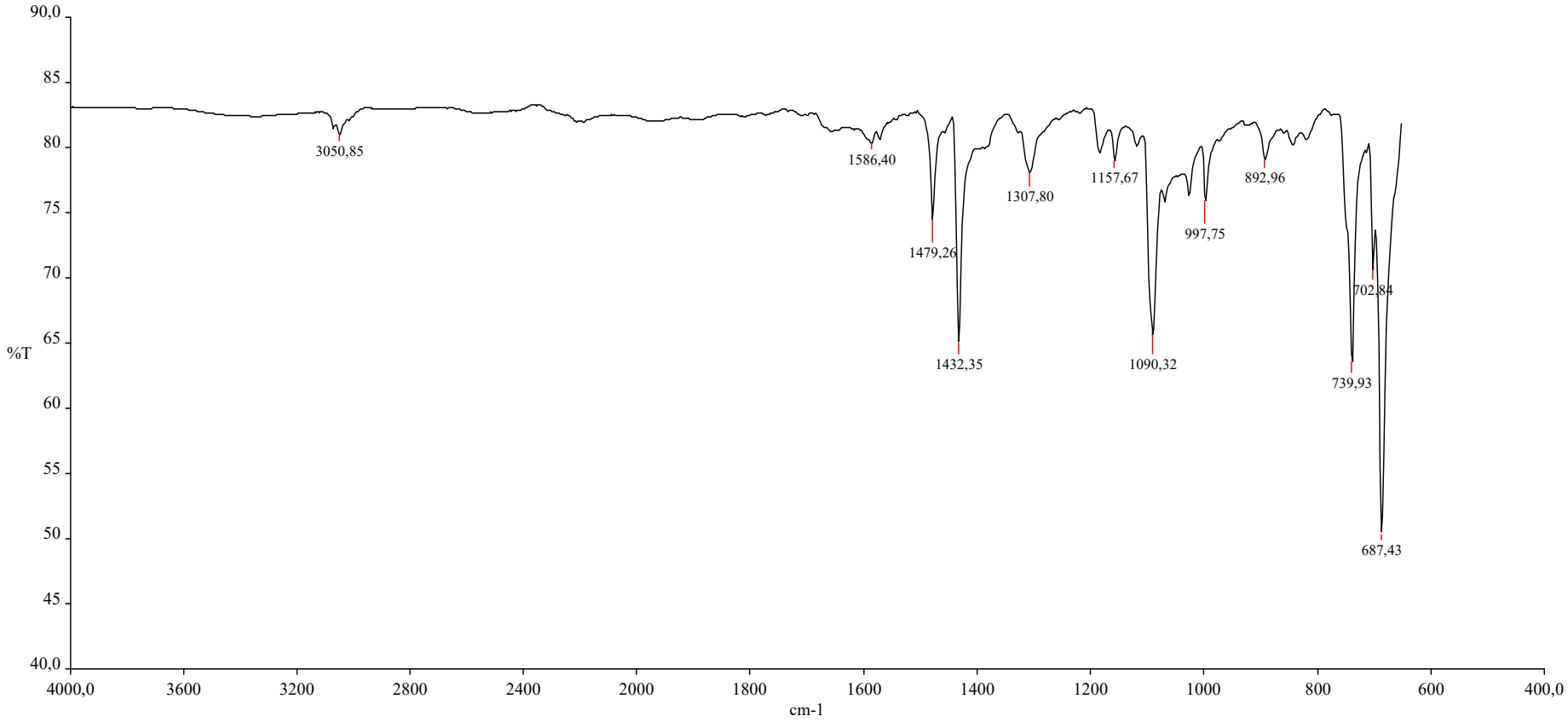

Figure A. 1 : IR spectrum of Tetraiodophthalocyaninatozinc(II).

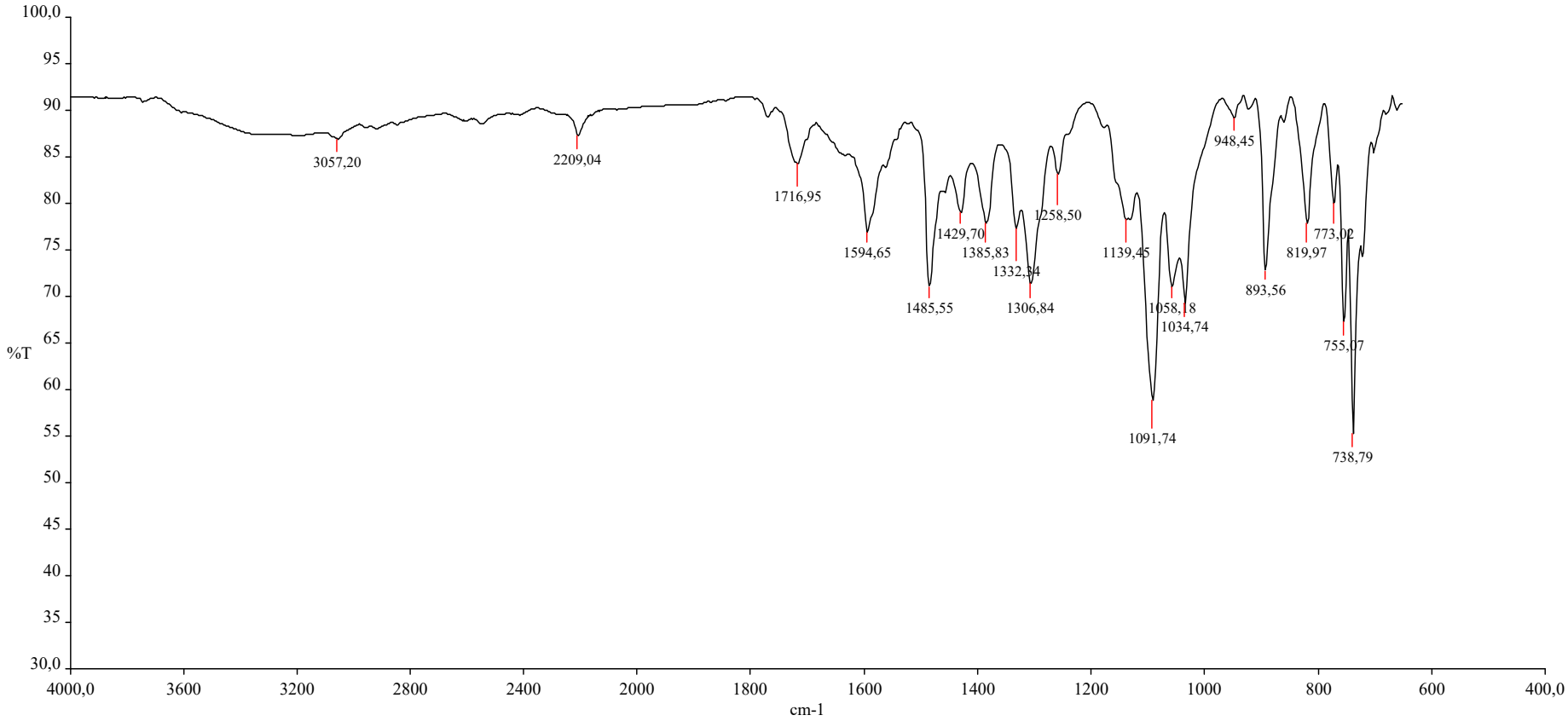

Figure A. 2 : IR spectrum of Tetrakis(pyridine)phthalocyaninatozinc(II).

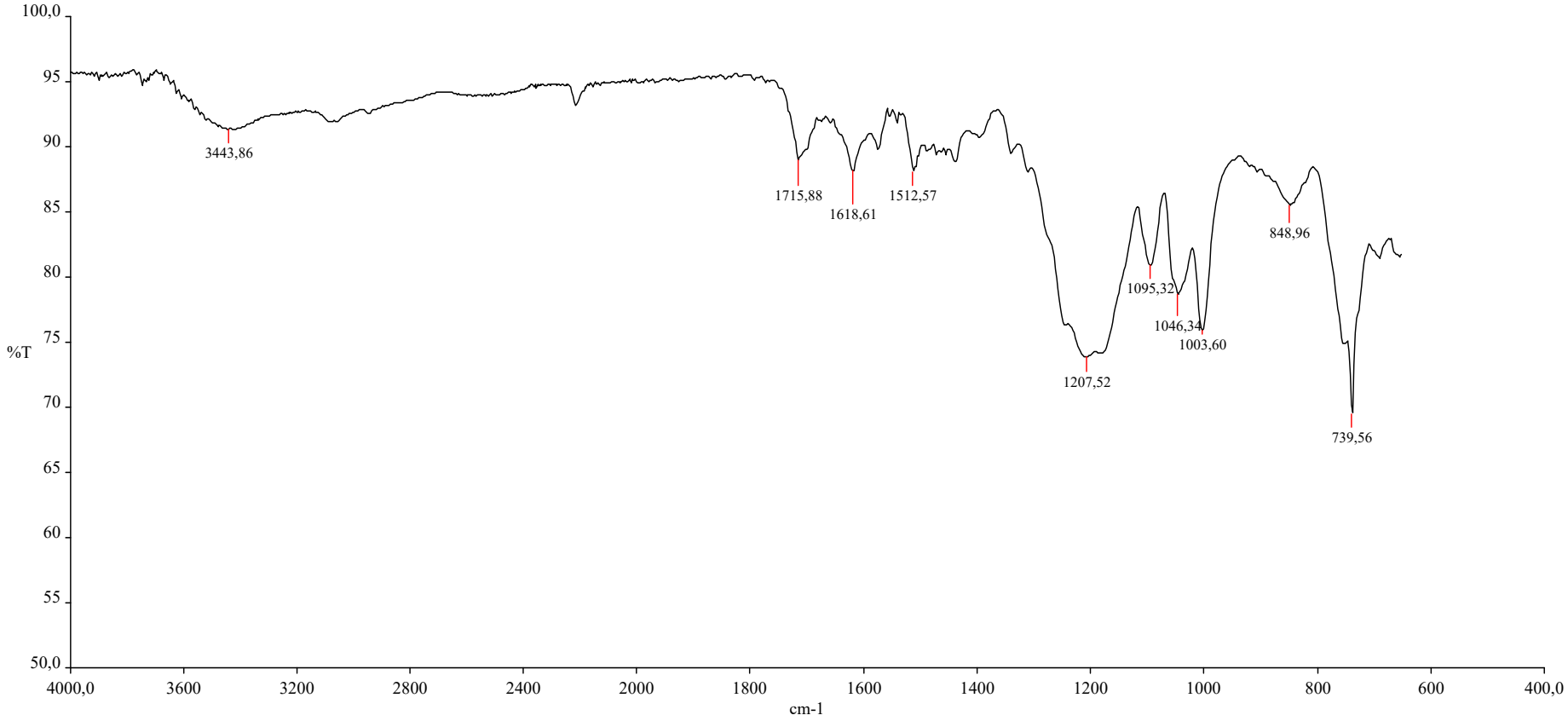

Figure A. 3 : IR spectrum of Tetrakis(methylpyridinium)phthalocyaninatozinc(II).

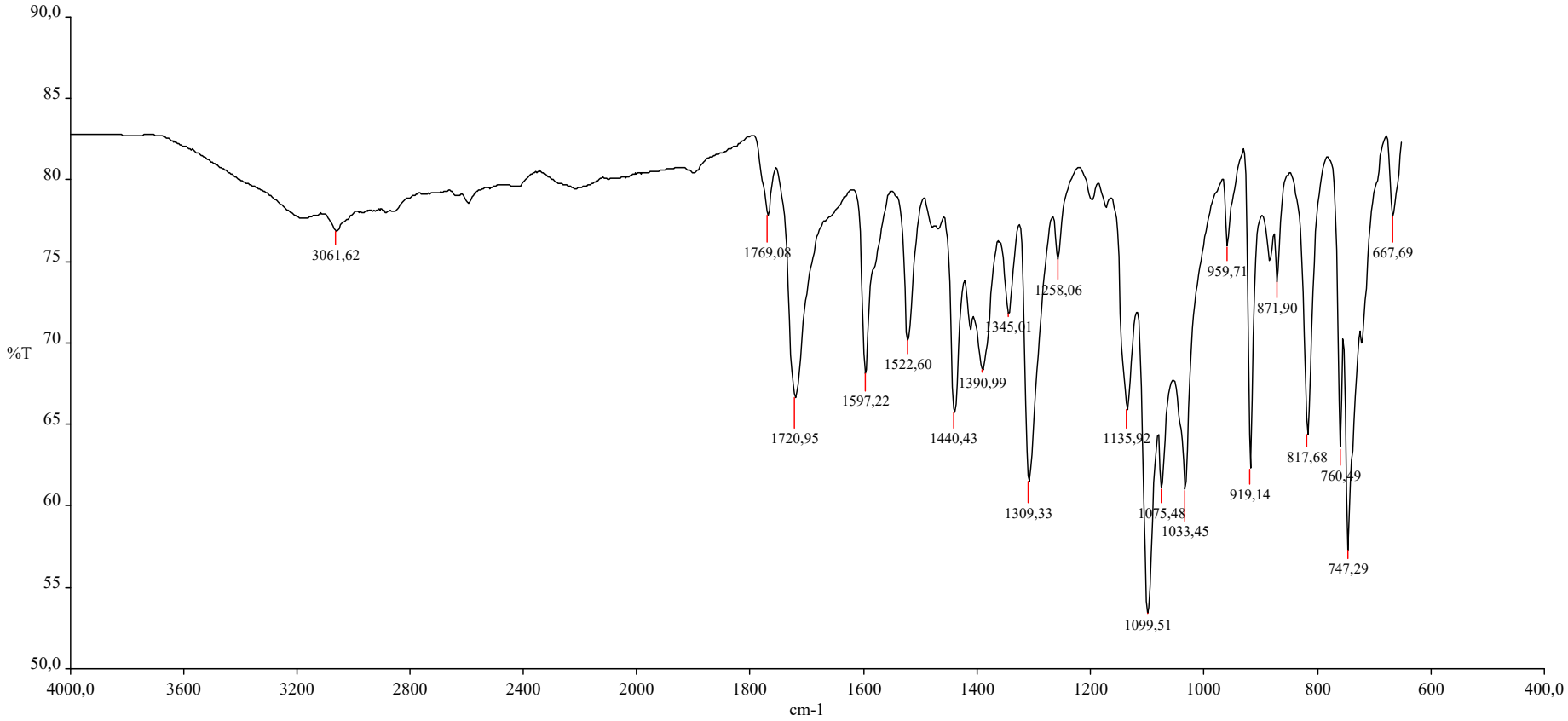

Figure A. 4 : IR spectrum of Tetraiodophthalocyaninatocobalt(II).

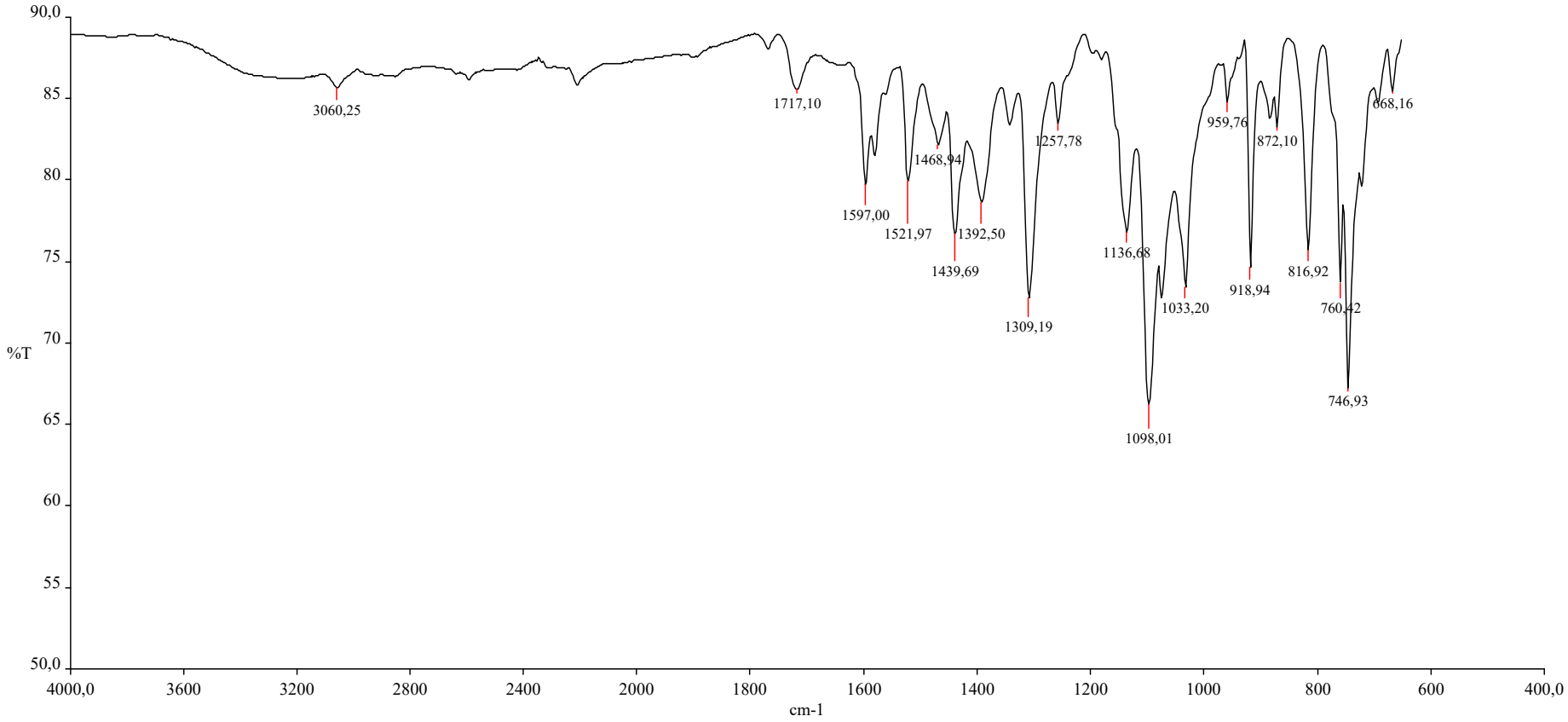

Figure A. 5 : IR spectrum of Tetrakis(pyridine)phthalocyaninatocobalt(II).

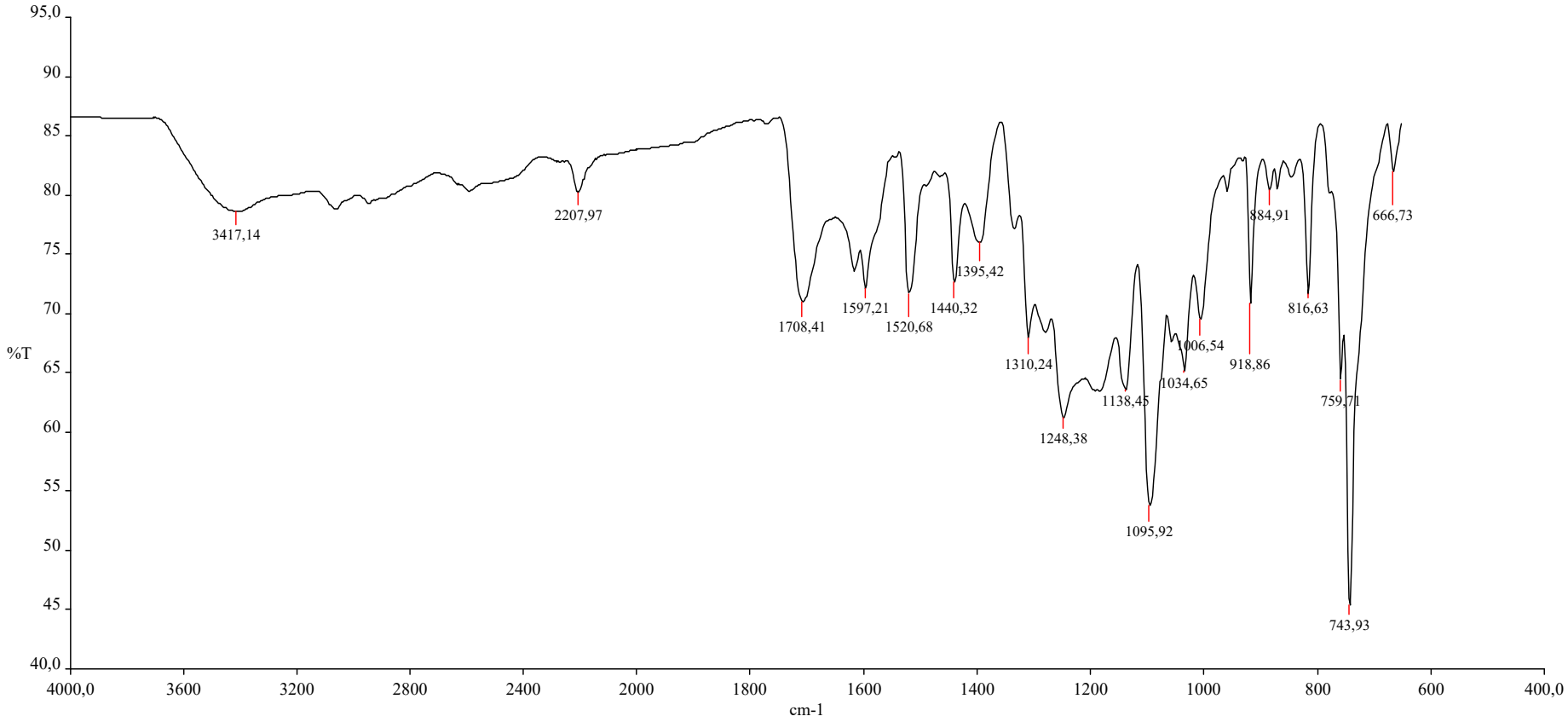

Figure A. 6 : IR spectrum of Tetrakis(methylpyridinium)phthalocyaninatocobalt(II).

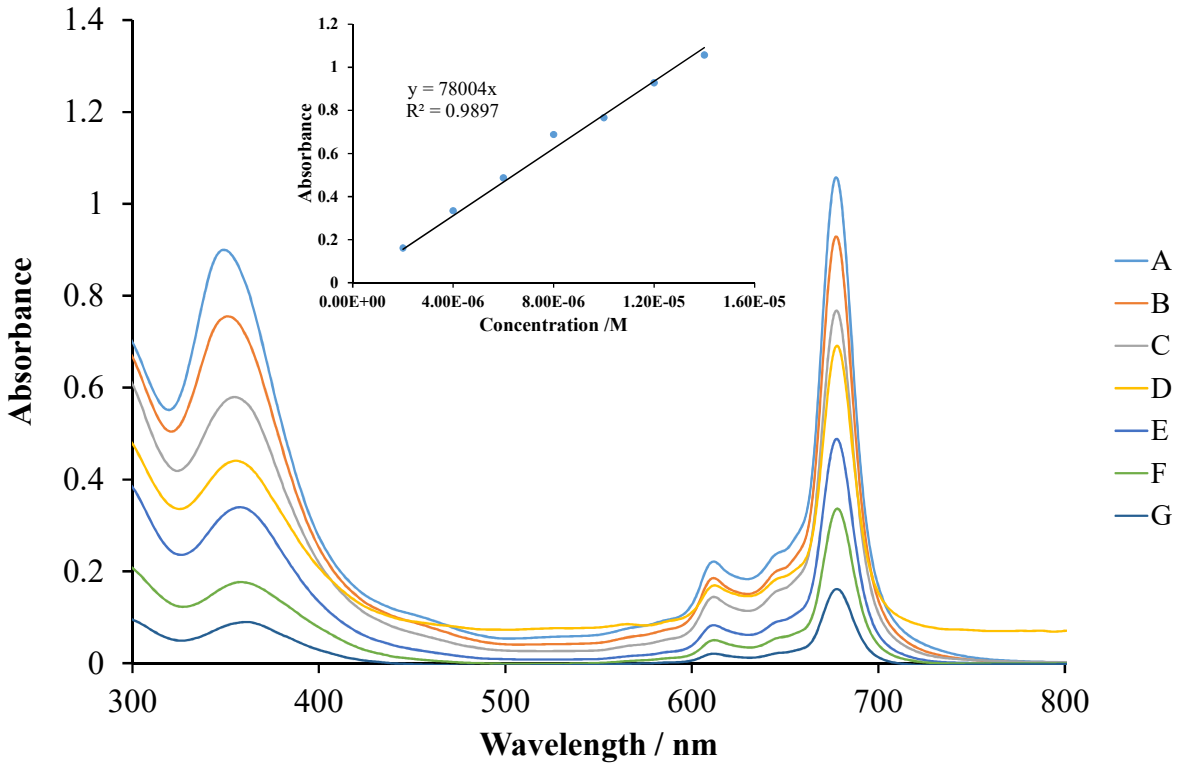

Figure A. 7 : UV-Vis spectrum of tetraiodophthalocyaninatozinc(II) in DMF (14 x 10^{-6} M (A), 12x10^{-6} M (B), 10x10^{-6} M (C), 8x10^{-6} M (D), 6x10^{-6} M (E), and 4x10^{-6} M (F)

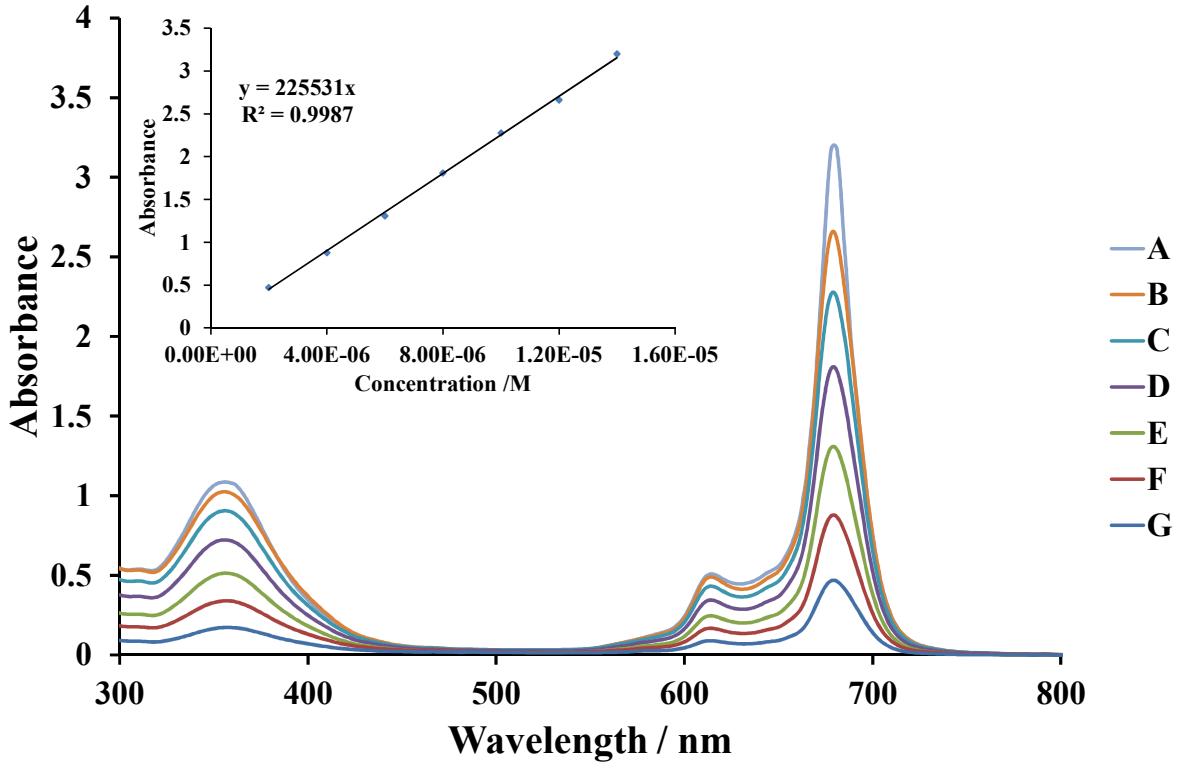

Figure A. 8 : UV-Vis spectrum of tetrakis(pyridine)phthalocyaninatozinc(II) in DMF (14 x 10^{-6} M (A), 12x10^{-6} M (B), 10x10^{-6} M (C), 8x10^{-6} M (D), 6x10^{-6} M (E), and 4x10^{-6} M (F).

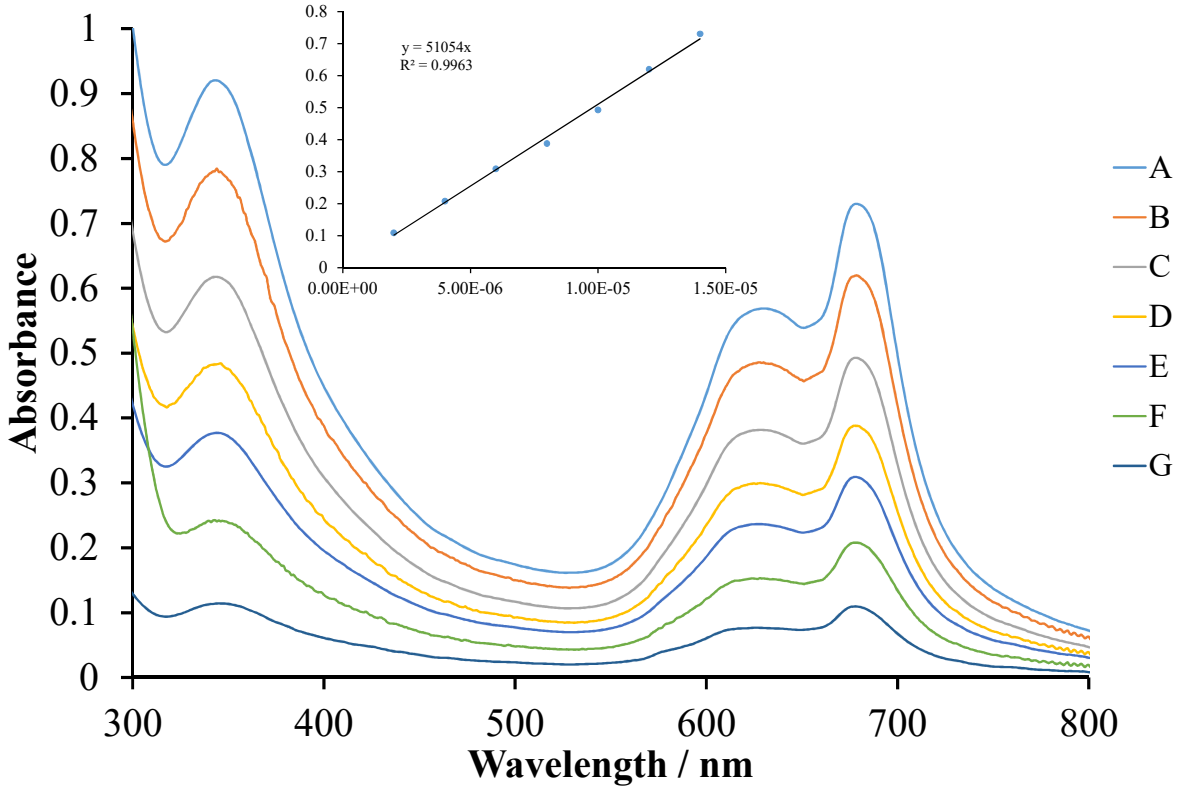

Figure A. 9 : UV-Vis spectrum of tetrakis(methylpyridinium) phthalocyaninatozinc(II) in DMF (14 x 10^{-6} M (A), 12x10^{-6} M (B), 10x10^{-6} M (C), 8x10^{-6} M (D), 6x10^{-6} M (E), and 4x10^{-6} M (F).

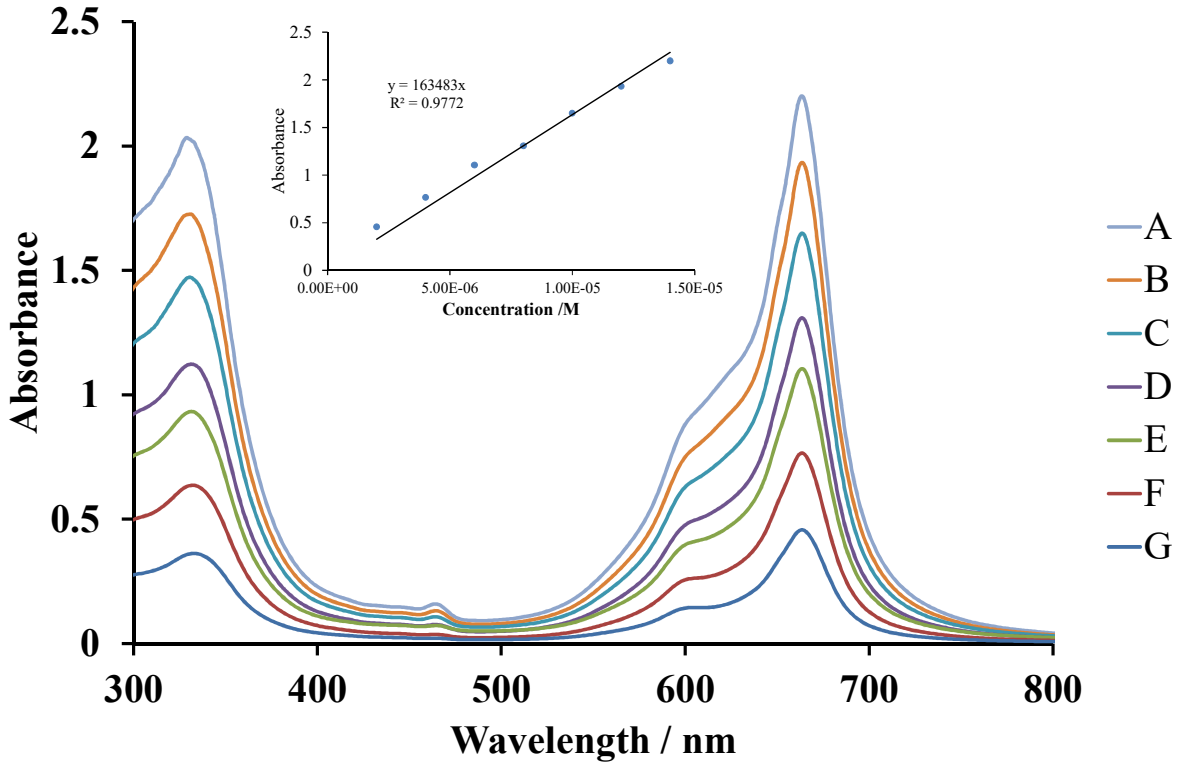

Figure A. 10 : UV-Vis spectrum of tetraiodophthalocyaninatocobalt(II) in DMF (14 x 10^{-6} M (A), 12x10^{-6} M (B), 10x10^{-6} M (C), 8x10^{-6} M (D), 6x10^{-6} M (E), and 4x10^{-6} M (F)

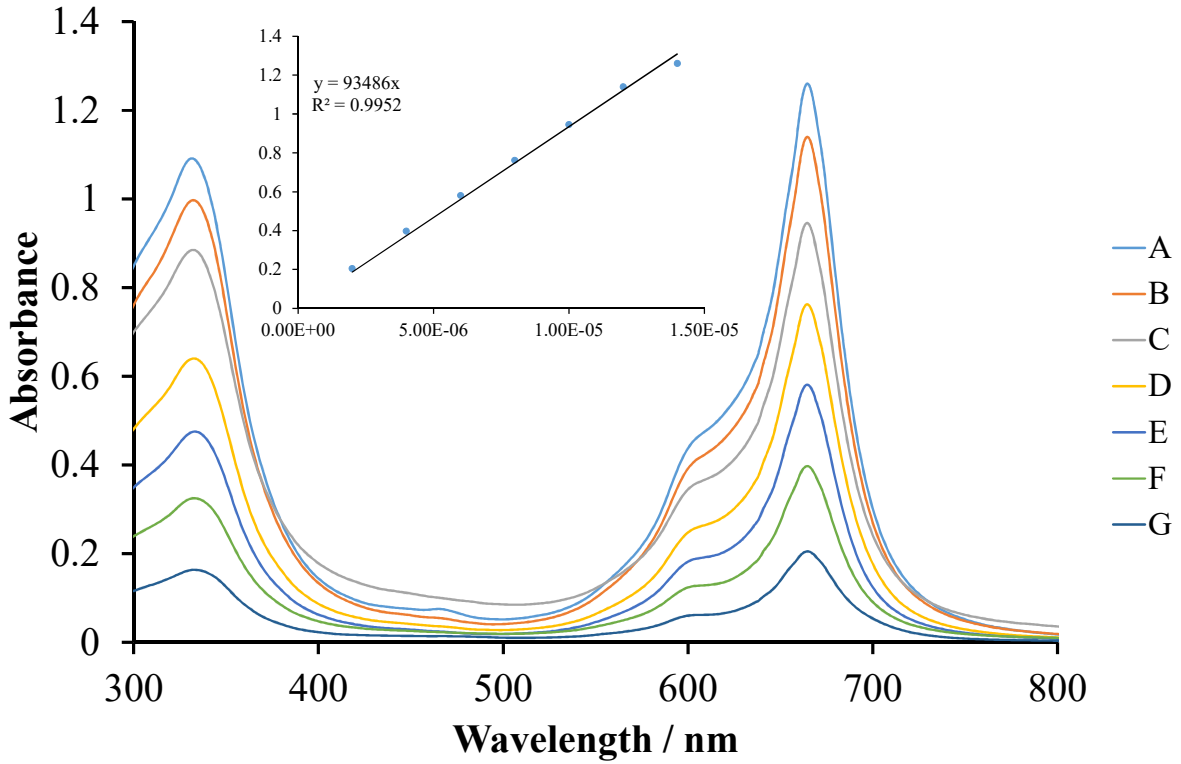

Figure A. 11 : UV-Vis spectrum of tetrakis(pyridine)phthalocyaninatocobalt(II) in DMF (14 x 10^{-6} M (A), 12x10^{-6} M (B), 10x10^{-6} M (C), 8x10^{-6} M (D), 6x10^{-6} M (E), and 4x10^{-6} M (F).

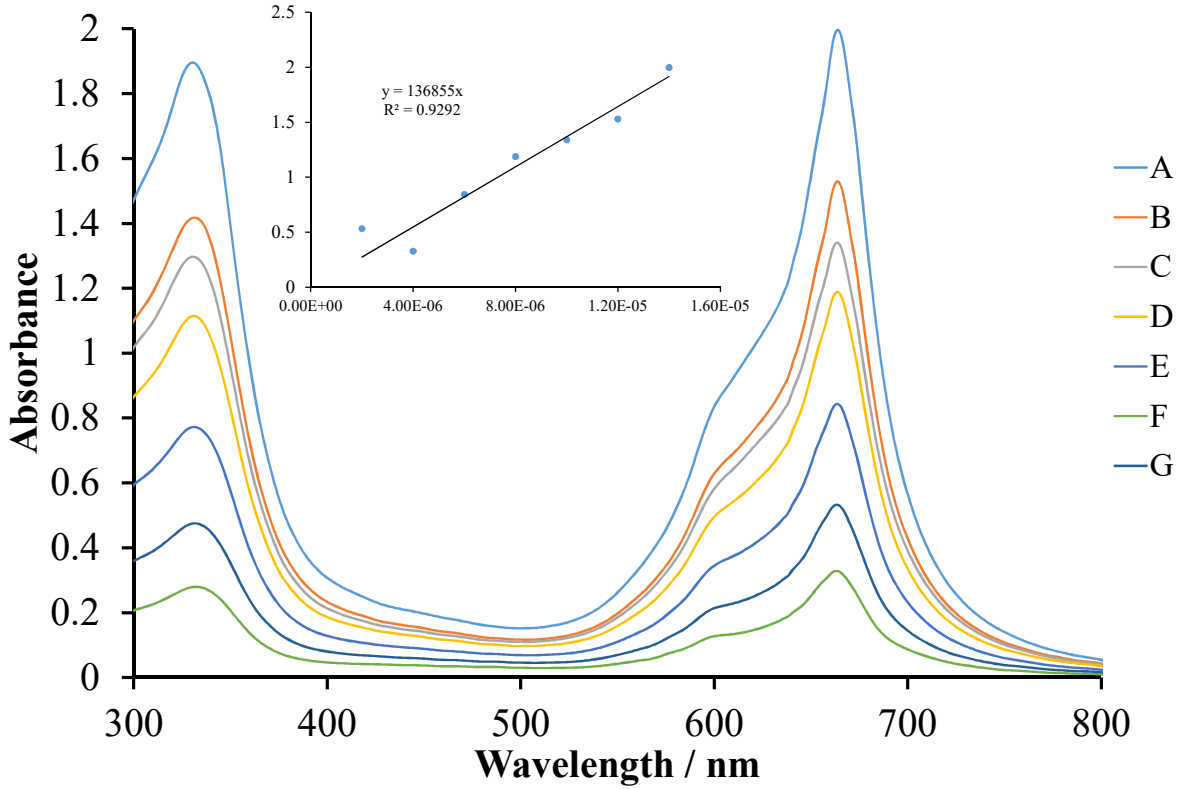

Figure A. 12 : UV-Vis spectrum of tetrakis(methylpyridinium) phthalocyaninatocobalt(II) in DMF (14 x 10^{-6} M (A), 12x10^{-6} M (B), 10x10^{-6} M (C), 8x10^{-6} M (D), 6x10^{-6} M (E), and 4x10^{-6} M (F).

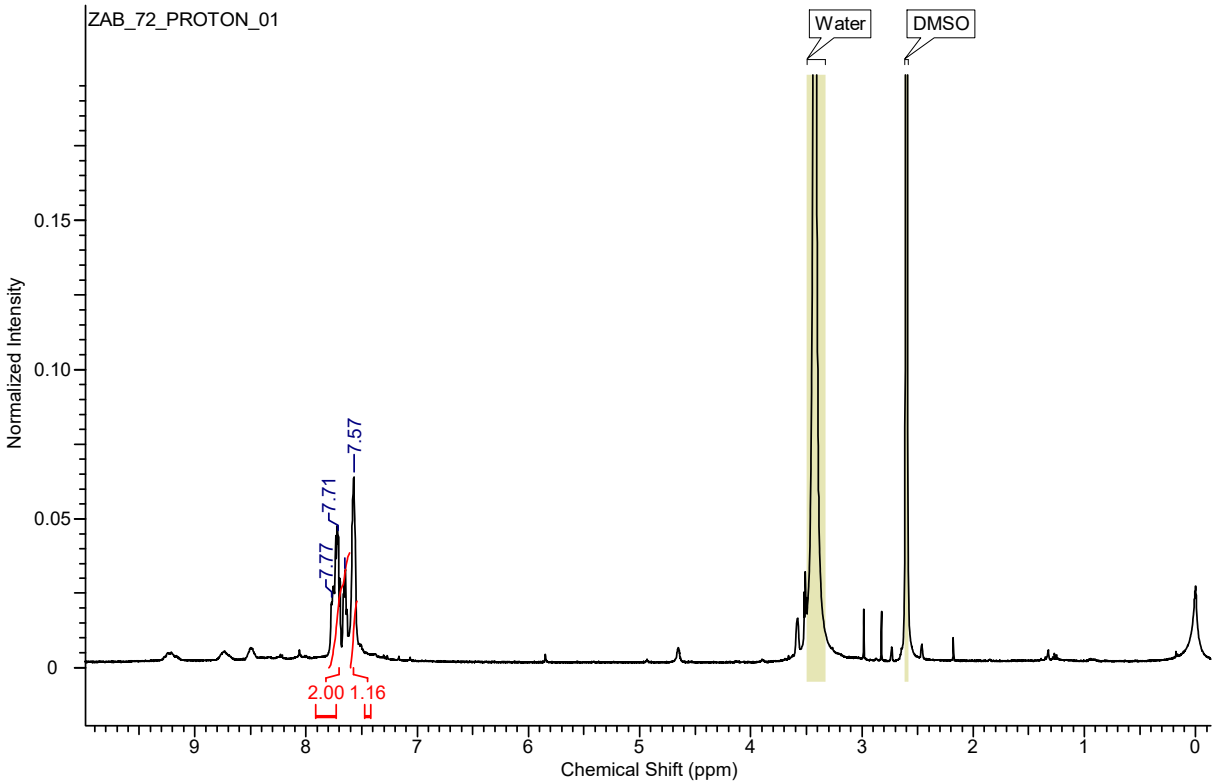

Figure A. 13 : ^{1}H-NMR spectrum of tetraiodophthalocyainanozinc(II).

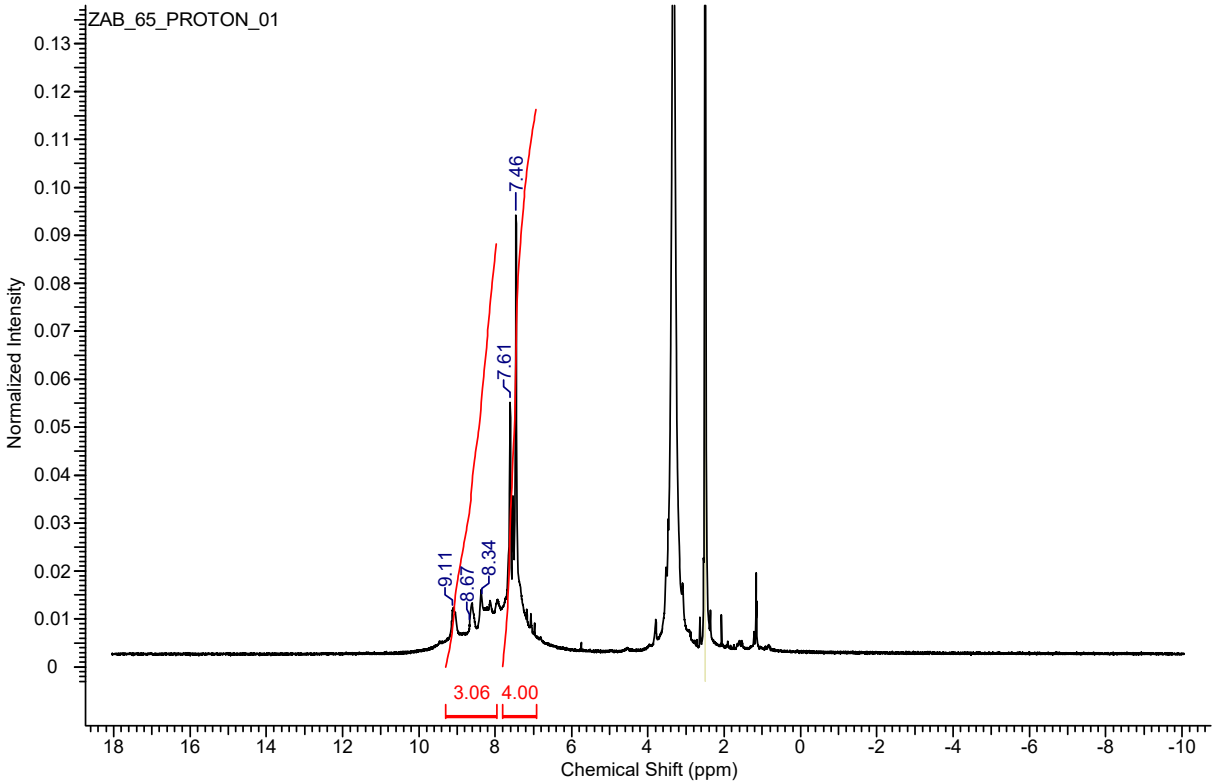

Figure A. 14 : ^{1}H-NMR spectrum of tetrakis(pyridine)phthalocyaninatozinc(II).

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