Chapter-2: New water soluble metallophthalocyanine posture eight hydroxyphenyl moieties via 1,3,4-Oxadiazole bridge: Novel DNA Interactions, Biological Evolution, Electrochemical and Electrocatalytic activities.

Summary: The "bottom-up" strategy is an attractive and promising approach for the construction of novel water soluble phthalocyanine-architectures. These are mainly nickel, cobalt, copper, zinc and iron-phthalocyanine derivatives and synthesized metallophthalocyanines contains eight phenolic hydroxyl substituents via 1,3,4-oxadiazole unit at the periphery. Fourier transform infra-red spectroscopy (FTIR), Nuclear Magnetic Resonance (NMR), Electron spin ionization Mass spectra (ESI-MS) and elemental analysis confirmed the well-defined saddle like distorted structures. Supramolecular assemblies based on non-intercalation interactions have been explored in an attempt of effective DNA binding with calf thymus DNA (CTDNA) was monitored using UV-vis spectral titrations and cleavage pBR322 DNA conceded in the absence of reductant by agarose gel electrophoresis method. The results indicated that all these water soluble complexes significantly show excellent binding and modest cleavage sensitivities activity. It is noteworthy that 6, 7 and 8 exhibits potential antimicrobial and appreciable antioxidant activity. The measurement of electrochemical and non-noble metal
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Novel Water soluble Metallophthalocyanine

electrocatalytic behaviour of water soluble cobalt Pcs for O₂ reduction were evaluated by cyclic voltammetry (CV) and rotating disc electrode (RDE) techniques quantitatively obtained the oxygen reduction reaction (ORR) in acidic media. The results emphasized that new non-precious catalyst was leads to the outperformance and suggesting favorable conditions to work as cathode catalysts in fuel cells.

1. Introduction

In light of the considerable impact on the synthesis of supramolecules are expected to possess interesting physical and chemical properties. It is surprising that numerous advance researches has been carried out on these intriguing phthalocyanine (Pc) macromolecules due to their bright colors, conductivity, and chemical and thermal stability have made them very desirable for many applications. Phthalocyanines (Pcs) continually find their usefulness in contemporary and emerging technologies such as nonlinear optical (NLO) devices, liquid crystals, Langmuir–Blodgett films, electrochromic devices, photosensors and in the field of photodynamic therapy (PDT) of cancer. In recent years, the development of sensitive interaction and reaction of DNA with Pc complexes has become an active field of research because of their potential application in biotechnology, medicine and as antitumor agent due to cytotoxic effects of metals. Platinum (Pt)-based materials have long been regarded as the best cathodic oxygen reduction reaction (ORR) catalysts in fuel cells. However, the susceptibility of a Pt electrode to the crossover effect, together with its limited reserves and high cost, hinders it from large-scale commercialization. In this context, non-
precious catalysts for ORR, such as metal chalcogenides, modified carbon materials, and metal-N$_4$ compounds, have attracted great interest. Among them, metallophthalocyanine (MPc) is one of the most promising candidates for efficient ORR electrocatalysts due to its high catalytic activity. However, the stability of MPc has been a primary limiting factor to its practical application in fuel cells.

Oxadiazoles (OXDs) are five-membered cyclic compounds containing one oxygen and two nitrogen atoms in the ring. The oxadiazole (OXD) exists in different isomeric forms such as 1,2,4-, 1,2,5-, 1,3,4- and 1,2,3-oxadiazoles. Out of which thermally stable 1,3,4-oxadiazole is the only isomer not containing a nitrogen-oxygen bond.

The parent compound 1,3,4-oxadiazole is thermally stable neutral aromatic molecule and known since from eighty years and has been intensified due to the large number of uses and applications in most areas e.g. in dye synthesis, scintillation materials, and in drug synthesis. Moreover, ring cleavage reactions of 1,3,4-oxadiazoles have accelerated interest in various fields like medicinal chemistry, polymer chemistry etc., since they lead to new aliphatic nitrogen containing compounds and other ring systems.

2,5-disubstituted-1,3,4-oxadiazole compounds serves both as biomimetics and reactive pharmacophores with potential biological activities such as antibacterial, HIV-integrase inhibitor, a potent PDF inhibitor, anti hypertensive, pesticidal, anti peripheral vasomotility, CNS stimulant, hypoglycemic, analgesic, anticonvulsive,
antiemetic\textsuperscript{44}, diuretic\textsuperscript{45}, muscle relaxant\textsuperscript{46}. Some of them are strong enzyme inhibitors such as monomine oxidase cyclooxygenase\textsuperscript{47}, lipoxygenase\textsuperscript{48}, and succinate dehydrogenase\textsuperscript{49}.

It is appropriate, therefore, to review the important features of OXDs which have paved the way for the present study. Molecular and polymeric OXDs have been widely studied due to their high thermal and chemical stability, high photoluminescent quantum yields and the electron deficient nature of the 1,3,4-oxadiazone heterocycle\textsuperscript{50}. OXD derivatives are promising electron transport and hole blocking materials used for organic light-emitting devices\textsuperscript{51-55}. In recent studies of polymer light-emitting diodes, the OXD moieties have demonstrated to possess a high potentiality for electron transport\textsuperscript{56}. The charge drift mobility of OXD derivatives doped with polycarbonate were studied, with the time-of-flight technique\textsuperscript{57}, and discotic OXDs, with columnar mesophases, were proposed for applications in organic electronics\textsuperscript{58}. Electroluminescent polymers were prepared, in which thiophene and OXD moieties are connected alternately to form fully conjugated polymers\textsuperscript{59,60}. Likewise, liquid crystalline compounds containing OXD moieties were reported to exhibit a high electron transport capability and a blue electroluminescence emission\textsuperscript{61,62}. Polyoxadiazoles have been reported for proton conductive membranes\textsuperscript{63}. Tailored structures containing OXD groups and acid sites have been tested as fillers for dense fuel cell\textsuperscript{64}. This class of compounds can be synthesised efficiently by a variety of methods using readily available and cheap precursors. A variety of reaction conditions influence the cyclization reaction. Typically, the reaction is promoted by heat and anhydrous reagents including thionyl chloride\textsuperscript{65-67}, phosphorus oxychloride\textsuperscript{68}, phosphorus pentoxide\textsuperscript{69}, triphenylphosphine\textsuperscript{70}, and triflic anhydride\textsuperscript{71}. 
Alternative synthetic methods comprise reaction of carboxylic hydrazides with keteneylidene triphenylphosphorane\textsuperscript{72} or base-promoted cyclization reaction of trichloroacetic acid hydrazones\textsuperscript{73}. A wide range of functionality can also be attached at the peripheral aryl groups prior to, or after, the synthesis of the ring system to give novel small molecules or polymeric compounds.

OXDs were arose the special interest for the production of advanced materials due to their high thermal, chemical and specific properties which are determined by the electron withdrawing nature of 1,3,4-oxadiazole units. OXD derivatives restricts extensions of $\pi$-conjugation beyond the ring even if the molecule is co-planar\textsuperscript{74,75}. It has been shown that bipyridyl substituted OXD compounds have efficient electron transporting and hole-blocking properties with high thermal stability and practical operation durability\textsuperscript{76}. The 1,3,4-oxadiazole building block is an attractive unit, because it is a good electron-withdrawing group and is relatively very stable and is easy to build as part of a $\pi$-conjugated system.

Specificity in the applications of Pcs can be introduced by modification of the Pc ring or by changes in the central metal or substitution on the periphery. The literature survey reveals that very few reports on 1,3,4-oxadiazole substituted Pcs are available and the functionalization of Pc macrocycle with OXD and aryl system is highly desirable. There are several advantages to this type. The design allows for extensive electronic interaction of Pc macrocycle with OXD and aryl substituent which may lead to large bathochromic shifts in the ground-state electronic absorption spectra. Further, the synthetic scheme is very general and the possibilities are only highlighted by this preliminary study.
The scheme allows for the incorporation of OXD along with substituted phenyl rings, and could be extended to include heteroaromatic moieties. On the other hand, the investigation on these new type of Pc may be regarded as an important step in developing a viable sensor and semiconducting material with high chemical and thermal stability. OXD substituted Pcs showing excellent thermal resistance and good hydrolytic stability, they have rigid molecules due to the delocalization of π-electrons, which makes them insoluble in organic solvents and infusible, and therefore their processing and practical use is very limited. Improving the solubility of these Pcs is an imperative aspect, as the insolubility causes difficulties in their separation/identification and limits their potential applications in medicine. The most part of literature shows that to conquer the low solubility or insolubility of the unsubstituted MPcs in common organic solvents, substituents should be introduced at the periphery of Pc core. This can be achieved either by using appropriately substituted precursors or by postsynthetic modification of the Pc moiety. The former approach is preferred because it allows one to obtain well identified complexes. Postsynthetic modification of MPcs typically leads to a mixture of differently substituted complexes. Classical examples are long carboxylates, sulfonates, glucose, phosphonates, polyoxyethylene, amino, carboranyl, dimethylaminocinnamaldiminophenoxy, quaternized amino groups, are tailored at the peripheral positions and another kind of water-soluble Pcs contains hydrophilic groups as axial ligands coordinated to the central metal ion. In order to overcome the insolubility of 1,3,4-oxadiazole substituted MPcs, we made an attempt to substitute polar functional groups at the...
periphery of Pc for enhanced solubility. Recently, we have been engaged with the synthesis of MPcs with different functional moieties for diverse purposes.

2. The Present Work

Literature review reveals that a lot of work has been done on metal complexes of Pes. This is due to their wide range of applications in dyes, pharmaceutical, medical, polymer science, catalysis, and electronics; and also due to the binding ability of Pes to a wide range of transition metal ions.

In view of these applications, this chapter presents an investigation on the synthesis and characterization of substituted MPc with thermal and chemical stability, which is important since it presents an improvement in solubility of Pc. This substitution opens a route for further substitution by other ligands/groups. Encouraged by the above facts we designed a water soluble substituted MPc frame work in which highly polar groups compositions on a compact system of Pc core and we modified the Pc core with phenolphthalein unit via 1,3,4-oxadiazole moiety. The developed frame work of macrocycle is expected towards enhances photophysical and electrochemical properties. Generally, different MPcs were employed for DNA binding studies; the positively charged Pes are the most efficient ones in terms of binding and cleaving DNA as compared with either the neutral or negatively charged Pes\(^{95,104}\). In view of continuous interest to develop new photonucleus metal complex and believed that it is worth to investigate DNA binding and cleavage studies. In this perspective, we obtained the potential interaction with CT DNA and excellent cleavage studies and seems to be promising candidate in photodynamic therapy (PDT). The substitution on the periphery
of the planar structure of MPcs exhibited significantly a saddle like distortion structure with admirable yield. The characterization of the ultimate structure of MPcs was done by FTIR, $^1$H NMR, ESI-Mass spectrometry and elemental analysis. It also, addresses the efficiency in antioxidant activity as well as in biological evolution.

Further the aim of this present work is to evaluate the possibility of improving ORR electrocatalytic activity by synthesizing new highly selective water soluble substituted cobalt Pc based non-precious oxygen reduction reaction (ORR) in fuel cell application. As a part of continuing efforts to developed a facile method for the synthesis of new water soluble substituted cobalt Pes, were evaluated as cyclic voltammetry and electrocatalytic behavior towards oxygen reduction were screened by linear sweep voltammetry (LSV) employing a rotating disk electrode techniques in acidic media. The resulting tafel and kouatecky-levich analyses revealed that water is the major product formed via four electron transfer and reaction showed outstanding switching the catalytic activity towards oxygen reduction reaction, which outstrips that of unsubstituted cobalt Pcs, formerly the most effective catalyst. This substituted cobalt Pc electrocatalytic behavior shows simple and economic method offers an alternative route to fabricate non-precious electrocatalysts with superior ORR performance compared to other unsubstituted and substituted cobalt Pcs and shows promising catalyst for the oxygen reduction application in acidic media.
3. Experimental Protocols

3.1. Chemicals

All chemicals used for the synthesis were of reagent grade and the intermediates were prepared as per the known literature procedure. Trimellitic anhydride or Benzene-1,2,4-tricarboxylic anhydride and ammonium molybdate from Sigma–Aldrich and used as received. Phenolphthalein, urea, ammonium chloride, metal salt: copper(II)chloride, nickel(II)chloride, cobaltous(II)chloride, zinc acetate, ferric chloride, hydrochloric acid, sodium hydroxide and nitrobenzene were purchased from HIMEDIA Chemicals, polyphosphoric acid was purchased from Spectrochem Pvt. Ltd., All the chemicals used for synthesis of the Pcs were of analytical grade. Ultrapure water (MilliQ, Millipore 18 MΩ cm) was used for electrochemical and electrocatalytic measurements, solutions were purged with ultra nitrogen or ultrapure O₂ for 30 min prior to each measurement, depending on the experiment. All the other chemicals used for the synthesis were of reagent grade and the intermediates were prepared as per the known literature procedures.

3.2. Phthalocyanines Characterization Techniques

The Pcs characterization methods described in this section covers only the general information on the procedures for carrying out the spectroscopic measurements. However detailed and specific protocols are given in the corresponding chapters.

3.3. Fourier Transform Infrared (FTIR) Spectroscopy

The vibrational states of a molecule can be probed in a variety of ways. The most direct way is infrared spectroscopy because vibrational transitions typically require an amount of energy that corresponds to the infrared region of the spectrum between 4000 and
400 cm$^{-1}$. Radiation in this region can be utilized in structure determination in coordination chemistry by making use of the fact that interatomic bonds in ligands absorb it. Infrared spectra of ligand and complexes were recorded in the region of 4000 cm$^{-1}$-400 cm$^{-1}$ on a Perkin Elmer Spectrum 100 spectrophotometer using ATR. Vibrational spectra contain vast amounts of molecular or microscopic structure information about Pc materials. Geometric and steric isomerism, molecular orientation, conformational regularity, crystallinity, and the local microscopic environment of specific functional groups of Pc materials can be elucidated with vibrational spectroscopic analysis. Particularly, Fourier transform infrared spectrometry is a convenient and powerful means of measuring vibrational spectra of Pc materials. The characteristic IR vibrations are influenced strongly by small changes in molecular structure, thus making it difficult to identify structural fragments from IR data alone. However, there are some groups of atoms that are readily recognized from IR spectra. IR chromophores (such as carbonyl, hydroxyl and other characteristic groups) are most useful for the determination of structure$^{105}$.

3.4. Nuclear Magnetic Resonance Spectroscopy (NMR)

The $^1$H and $^{13}$C-NMR spectra were recorded in DMSO-d$_6$ at 400 MHz Varian-AS NMR spectrometer with tetramethylsilane as internal standard at Sophisticated Instrumentation Facility, Indian Institute of Science, Bangalore and Karnataka. The extensive chemical applications of NMR lie in the elucidation of structure, identification of the compounds, study of molecular conformation, dynamic processes and the interaction between the molecules. In some cases, the NMR method alone may be sufficient for the obtaining the structure of an unknown compound, while in other cases the NMR
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spectrum may compliment the results obtained from the other methods. NMR spectroscopy is a powerful technique for studying the macromolecules at the atomic level.\textsuperscript{105}

3.5. Thermogravimetric Analysis (TGA)

Thermogravimetric analysis (TGA) is an analytical technique used to determine a material's thermal stability and its fraction of volatile components by monitoring the weight change that occurs as a specimen is heated.\textsuperscript{106,107} TGA and DTA were performed in Perkin-Elmer Thermal analyzer. The measurement is normally carried out in air or in an inert atmosphere, such as helium or argon, and the weight is recorded as a function of controlled temperature program. Because, mass is a fundamental attributing of any material, any change is more likely to be associated with a chemical change, which may, confine on the compositional change. Kinetic and thermodynamic parameters were calculated using Broido's method.\textsuperscript{108} Broido has developed a model and the activation energy associated with each stage of decomposition was also evaluated by this method. The Equation (1) used for the calculation of activation energy ($E_a$) is:

$$-\ln \left[ \ln \left( -\frac{1}{y} \right) \right] = \frac{E_a}{R} \frac{1}{T} + \text{Constant}$$

(1)

Where, \[ y = \frac{(w_t - w_\infty)}{(w_0 - w_t)} \]

'y' is the fraction of the number of initial molecules not yet decomposed; $W_t$ - the weight at time t; $W_\infty$ - the weight at infinite time (i.e., zero) and $W_0$ - the initial weight. A plot of $-\ln[\ln(-1/y)]$ v/s $1/T$ gives an excellent approximation to a straight line. The slope is related to the activation energy ($E_a$).
3.6. Electronic Absorption Spectroscopy

Electronic spectroscopy is the measurement of the wavelength and intensity of absorption of near ultraviolet and visible by a sample. UV-Vis spectra were measured on an ocean optics USB 4000.USA, using 1 cm path length cuvette at room temperature. Samples were prepared in dimethylsulphoxide at a concentration of $5.0 \times 10^{-5}$ mol dm$^{-3}$. UV-Visible spectroscopy is usually applied to organic ions or complexes. The absorption of UV or Visible radiation corresponds to the excitation of outer electrons.

There are three types of electronic transitions that can be considered for coordination compounds. These are transitions involving a) $\pi$, $\sigma$, and $n$ electrons of ligands b) charge-transfer electrons c) $d$ and $f$ electrons. Most absorption spectroscopy of ligands is based on $n$ to $\pi^*$ and $\pi$ to $\pi^*$ transitions. Many inorganic complexes show Ligand-to-Metal Charge Transfer (LMCT) transitions and Metal-to-Ligand Charge Transfer (MLCT) transitions (not as common as LMCT). Transition probability in ligand field transition ($d$-$d$ transition) is determined by the spin selection rule and the orbital (Loporte) selection rule.

To know the solvent effects of ligands and complex of synthesized compounds in various pH electronic spectra was recorded in various solvents like, DMSO, DMF, pyridine, MeOH, acetone, AcOH, acetonitrile, tertahydrofuran, and 1N NaOH with a SHIMADZU UV-Visible 1650 spectrometer in the wavelength range of 400-800 nm in Dept. of Chemistry, Kuvempu University, Shimoga, Karnataka.
3.7. Cyclic Voltammetry (CV)

Cyclic Voltammetry (CV) provides a sophisticated electroanalytical tool to study reaction systems which include electron transfers. The simultaneous investigation of the time dependence by means of sweep rate variation as well as the potential dependence allows recognizing the presence of different species. This recognition can be tedious based on current (i) vis time (t) gained from potential step experiments alone. A number of method have in the past been employed for the measurement of reaction kinetics but CV has stood out due to the ease of measurement and its effectiveness in observing redox behaviour over a wide potential window. The first step in characterization any electroactive material is to perform cyclic voltammetry over the chosen potential window.

Voltammetry is a potential sweep technique, which is widely used to study electrode processes, where in the electrochemical reactions occur on the working electrode (WE) and potential is measured relative to a reference electrode (RE) of known potential. The WE acts as a source or sink of electrons for exchange with the interfacial region. This region consists of electrolyte solution adjacent to the electrode surface where charge distribution differs from that in the bulk solution. The WE must be an electronic conductor and must be electrochemically inert, i.e. it does not generate current when potential is applied. There is a wide variety of materials used as WE, choice of material depends on the potential window required. The RE must be chemically and electrochemically reversible, i.e., its potential must be governed by Nernst equation and should not change with time. It must also be non-polarizable, i.e., its potential must remain constant when a small current passes through it; otherwise it should regain its original value after such current flow. Counter electrode (CE) should have a much larger...
surface area than the WE so that, it may be incapable of becoming polarized. In other words, its potential remains constant. In linear sweep voltammetry (LSV), potential is scanned from the initial potential \( E_i \) in one direction and stopping at the final potential \( E_f \), whereas cyclic voltammetry (CV) consists of scanning linearly the potential of a working electrode, using a triangular potential waveform (Figure 1).

![Figure 1. Potential-time excitation signals in a cyclic voltammetric experiment](image)

The scan direction can be either positive or negative depending on the reaction of interest. Application of potential results in the occurrence of oxidation or reduction reactions of electroactive species in solution. In addition, adsorption, deposition or polymerization may occur. A typical cyclic voltammogram (CV) of a general reduction-oxidation reaction \( O + ne^- \rightarrow R \) is shown in Figure 2.

![Figure 2. Typical cyclic voltammogram for a reversible redox process](image)

At the beginning of potential sweep at \( t=0 \), no reaction occurs and there is no concentration gradient, and the solution has the uniform bulk concentration. As the
potential is applied, the concentration of the oxidized species (O) is depleted at the surface. This lower concentration at the surface gives a higher concentration gradient so according to Fick’s law of diffusion, more flux to the surface exists and hence a higher cathodic current. As we continue to make the potential more negative, the concentration of the oxidized species at the surface will eventually go to zero. Simultaneously, the volume in the solution that is depleted of the oxidized species will increases and the concentration gradient will begin to decrease. As the concentration gradient decreases, we will have less flux to the surface and the will begin to decrease. As we reverse the voltage scan, we still have a layer depleted of the oxidized species, but the surface concentration begins to raise so current decreases further. Finally, when the potential approaches $E_{pa}$ the reduced species (R) begins to undergo re-oxidation to give O, subsequently moves into the bulk of the solution and generates concentration gradient at the electrode surface and hence the current profile.

As the faradaic current depends mainly on the rate of electron transfer and the rate at which the redox species diffuses to the electrode surface. For any redox couples in which the kinetics of electron transfer are reasonably fast and the concentration of O and R can be described by the Nernst equation 2:

$$E = E^0 - \frac{RT}{nF} \ln \left( \frac{[R]}{[O]} \right)$$  \hspace{1cm} (2)

where $[R]$ and $[O]$ are activities of reduced and oxidized species respectively.
3.7.1. Data Interpretation

The cyclic voltammogram is characterized by several important parameters. Four of these observables, the two peak currents and two peak potentials provide the basis for analyzing the cyclic voltammetric response. Generally the following limiting cases of studied system do exist.

3.7.2. Reversible Systems

When surface concentrations of \( O \) and \( R \) are maintained at values required by the Nernst equation, the system is in equilibrium throughout the potential scan. Species formed in the forward reaction is regenerated in the reverse reaction, i.e., \( O + ne^- \leftrightarrow R \). The reaction is termed reversible and the peak current, \( I_p \) (A) is given by the Randles-Sevcik equation at 25°C:

\[
I_p = (2.69 \times 10^5)n^{3/2} AC D^{1/2} v^{1/2}
\]

(3)

Where \( n \) is number of moles of electrons transferred, \( A \) is electrode area (cm\(^2\)), \( C \) is molar concentration, \( D \) is diffusion coefficient (cm\(^2\)/s) and \( v \) is the scan rate (V/s). Thus we can see that the peak current density is proportional to the concentration of electroactive species and diffusion coefficient.

According to the equation 3, peak current is proportional to the square root of scan rate and the plot of \( I_p \) against \( v^{1/2} \) should be straight line for a reversible system. Although the peak current increases with scan rate, the potential at which the peak occurs is invariant and the difference in peak potential is equal to \( 59/n \) mV, and their peak current ratio, \( I_{pa}/I_{pc} = 1 \) at all scan rates.
3.7.3. Irreversible Systems

On the other hand, when the system is not in equilibrium and surface concentrations of O and R are not maintained at values required by the Nernst equation, it is said to be irreversible. The most common irreversible system has no return peak. The peak current of an irreversible processes is given by the Randles-Sevcik equation 4 at 25°C:

\[
I_p = \left(2.99 \times 10^5\right)n(\alpha n_a)^{1/2} AC D^{1/2} v^{1/2}
\]

(4)

Where the symbols have their usual meaning, \(\alpha\) is a electron transfer coefficient and \(n_a\) is number of electrons involved in a charge transfer step. As for the reversible case, the peak current density is proportional to the concentration and the square root of the sweep rate, but additionally to the square root of the transfer co-efficient. The most marked feature of a cyclic voltammogram of a totally irreversible system is the total absence of a reverse peak. The peaks are more spread and flatter, and depend on the scan rate.

Some diagnostic tests for totally irreversible systems are as follows:

1. \((an_a) = 48/(E_p-E_p/2)\)

2. \(E_p\) shifts -30/an_a mV for each decades increases in scan rate.

3.7.4. Quasi-reversible Systems

Some processes are intermediate between reversible and irreversible they are said to be quasi-reversible. In the quasi-reversible region both forward and back reactions make a contribution to the observed current. The peak current ratio, \(I_{pa}/I_{pc} = 1\) provided \(\alpha c = \alpha a = 0.5\) i.e., for one electron transfer reactions and the peak current increases with scan rate but not proportional to it. The difference in peak potential \(\Delta E_p\) increases with scan...
rate and is greater than $59/n$ mV. It can also be observed in quasi-reversible system that the $E_{pc}$ shifts in the negative direction with scan rate.

Electrochemical measurements were carried out using an electrochemical system (CH Instruments, 660C, USA). A three-electrode system was used, consisting of a glassy carbon working electrode, a platinum counter electrode, and a saturated calomel reference electrode (SCE). Ultrapure water (MilliQ, Millipore 18 MΩ cm) was used for electrochemical and electrocatalytic measurements, solutions were purged with ultra nitrogen or ultrapure $N_2$ and $O_2$ for 30 min prior to each measurement, depending on the experiment.

3.8. Electrocatalytic Oxygen Reduction Reaction

Oxygen ($O_2$) is the most abundant element in the Earth’s crust. The oxygen reduction reaction (ORR) is also the most important reaction in life processes such as biological respiration, and in energy converting systems such as fuel cells. ORR in aqueous solutions occurs mainly by two pathways: the direct 4-electron reduction pathway from $O_2$ to $H_2O$, and the 2-electron reduction pathway from $O_2$ to hydrogen peroxide ($H_2O_2$). In non-aqueous aprotic solvents and/or in alkaline solutions, the 1-electron reduction pathway from $O_2$ to superoxide ($O_2^-$) can also occur.

3.8.1. Electrochemical $O_2$ Reduction Reactions

Table 1 lists several typical ORR processes with their corresponding thermodynamic electrode potentials at standard conditions. The mechanism of the electrochemical $O_2$ reduction reaction is quite complicated and involves many intermediates, primarily depending on the natures of the electrode material, catalyst, and electrolyte.
Table 1. Thermodynamic electrode potentials of electrochemical O₂ reductions

<table>
<thead>
<tr>
<th>Electrolyte</th>
<th>ORR reactions</th>
<th>Thermodynamic electrode potential at standard conditions, V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidic aqueous solution</td>
<td>O₂ + 4H⁺ + 4e⁻ → H₂O</td>
<td>1.229</td>
</tr>
<tr>
<td></td>
<td>O₂ + 2H⁺ + 2e⁻ → H₂O₂</td>
<td>0.70</td>
</tr>
<tr>
<td></td>
<td>H₂O₂ + 2H⁺ + 2e⁻ → 2H₂O</td>
<td>1.76</td>
</tr>
</tbody>
</table>

In Table 1, the reduction pathways such as the 1-, 2-, and 4-electron reduction pathways have unique significance, depending on the applications. In fuel cell processes, the 4-electron direct pathway is highly preferred. The 2-electron reduction pathway is used in industry for H₂O₂ production. The 1-electron reduction pathway is of importance in the exploration of the ORR mechanism.

3.8.2. Kinetics Of The O₂ Reduction Reaction

It is desirable to have the O₂ reduction reaction occurring at potentials as close as possible to the reversible electrode potential (thermodynamic electrode potential) with a satisfactory reaction rate. The current-overpotential is given in Equation 5:

\[ I_c = I_{O_2}^0 \left( e^{\frac{n_a a_a F \eta_c}{RT}} - e^{\frac{n_a(1-a_a) F \eta_c}{RT}} \right) \]  

(5)

where \( I_c \) is the oxygen reduction reaction current density, \( I_{O_2}^0 \) is the exchange current density, \( n_a \) the number of electrons transferred in the rate determining step, \( a_a \) is the transfer coefficient, \( \eta_c \) is the overpotential of ORR, \( F \) is the Faraday constant, \( R \) is the gas constant, and \( T \) is the temperature in Kelvin. To obtain high current at low overpotential, the exchange current density \( I_{O_2}^0 \) should be large, and/or \( \frac{RT}{n_a a_a F \eta_c} \) should be small.
3.8.3. Tafel Slope

If the overpotential is large, the backward reaction is negligible and Equation 6 can be simplified as:

\[ \text{Tafel slope} = 2.303 \frac{RT}{n_a \alpha_a F} \]  

(6)

This slope is called the Tafel slope. Since all other parameters in the Tafel slope are known, the parameters determining the Tafel slope are actually \( n_a \) and \( \alpha_a \). The higher the Tafel slope, the faster the overpotential increases with the current density. Thus, for an electrochemical reaction to obtain a high current at low overpotential, the reaction should exhibit a low Tafel slope or a large \( n_a \alpha_a \). For ORR, usually two Tafel slopes are obtained, 60 mV/dec and 120 mV/dec, respectively, depending on the electrode materials used and on the potential range. Details for individual materials are given in later sections of this chapter. The electron transfer coefficient is a key factor determining the Tafel slope. For ORR, the transfer coefficient is dependent on temperature. On a Pt electrode, the transfer coefficient of ORR increases linearly with temperature in the range of 20–250°C, following:

\[ \alpha_a = \alpha_a^0 T \]  

(7)

Where \( \alpha_a \) is the electron transfer coefficient of ORR, \( \alpha_a^0 \) equals 0.001678, and \( T \) is temperature in Kelvin. Relative humidity (RH) has also been found to affect the transfer coefficient\(^{112} \). Our recent study showed that in PEMFCs, at 120°C the RH dependence of transfer coefficient change for ORR follows Equation 8:

\[ \alpha_a = (0.001552H_c + 0.000139)T \]  

(8)

Where \( RH_c \) is the relative humidity of the cathode compartment.
3.8.4. Techniques Used in Electrocatalytic O₂ Reduction Reactions

The most frequently used techniques for ORR catalysis studies are cyclic voltammetry and rotating disk electrode (RDE).

3.8.5. Cyclic Voltammetry

Cyclic voltammetry is the most useful technique in electrochemistry. It can quickly provide qualitative information about catalysts and electrochemical reactions, such as the electrochemical response of catalysts and the catalytic activity of the catalysts with respect to some electrochemical reactions.

3.8.6. Rotating Disk Electrode

Equation 9 used for RDEs are as follows\(^{109}\):
\[
\frac{1}{j} = \frac{1}{j_k} + \frac{1}{B \omega^2}
\]  

Where \(j\) represents the measured current density, \(j_k\) is the kinetic current density, and \(\omega\) is the rotation rate of the electrode. \(B\) could be calculated from the slope of K–L plots based on the levič equation 10 as follows:
\[
\frac{1}{j} = 0.62nF C_{O_2} D_{O_2}^2 \eta \frac{1}{\nu} \omega^\frac{1}{2} = B \omega^\frac{1}{2}
\]  

Where \(n\) is the overall electron transfer number, \(C_{O_2}\) is the concentration of dissolved O₂, \(D_{O_2}\) is the diffusion coefficient of O₂, \(\nu\) is the kinematic viscosity of the electrolyte solution, and \(\omega\) is the rotation rate represented by rpm.

For RDE data analysis, three non-electrochemical kinetic parameters, such as the diffusion coefficient of O₂, the kinematic viscosity of the electrolyte solution, and the
solubility of $O_2$ must be known accurately. These parameters are all temperature
dependent. Their values are also slightly dependent on the electrolyte used. Table 2.3 lists
these parameters at various conditions.

Table 2. Non-electrochemical kinetic parameter for RDE data analysis

<table>
<thead>
<tr>
<th>Experiment conditions (T, P electrolyte)</th>
<th>Diffusion coefficient of $O_2$, $cm^2.s^{-1}$</th>
<th>Kinematic viscosity of the Electrolyte solution, $cm^2.s^{-1}$</th>
<th>Solubility of $O_2$, mol.cm$^{-3}$</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 M $H_2SO_4$,</td>
<td>1.4X10$^{-5}$</td>
<td>0.010</td>
<td>1.1X10$^{-6}$</td>
<td>113</td>
</tr>
</tbody>
</table>

3.8.7. Synthesis of Catalysts

A glassy carbon (GC) disk electrode (3 mm diameter), Prior to every measurement, each
electrode was polished sufficiently with aqueous slurry of fine alumina powder (1 and
0.06 mm in grain size) on a polishing microcloth. Then they were ultrasonicated in Milli-
Q water for 10 min to remove remaining impurities and pre cleaned rotating disk
electrode (RDE) was coated with catalyst to form the catalyst layer. For catalyst
measuring in 0.5 M $H_2SO_4$, 1.0 mg of catalyst was added to 1.0 mL of tertahydrofuran /
Nafion solution (5%). This mixture was ultrasonicated for 10 min to form a of the
suspension were deposited onto a polished tip of a glassy carbon RDE of 3 mm diameter
and left dry at room temperature.

Prior to all measurements, the electrolyte was completely de-aerated using high-purity N2
or Ar gas to remove dissolved oxygen. ORR studies were performed in 0.5 M $H_2SO_4$ and
the solution was continuously purged with high-purity $O_2$ gas for 30 minutes to saturate
the electrolyte with $O_2$. The currents are normalized for the geometric area used. Rotating
disc electrode (RDE) measurements were performed using the RDE system obtained
from Pine instruments (Model AFMSRCE) by coupling with a galvanostat/potentiostat. All electrochemical experiments were carried out in a glass cell with provision for gas purging. Commercial GC electrodes (3 mm diameter) were used for RDE studies, the surface of which was coated with the catalyst material. All electrochemical measurements were performed at room temperature in the RDE electrochemical cell using a saturated calomel electrode reference electrode, a Pt wire counter electrode and 0.5M H$_2$SO$_4$ solution as electrolyte. The RDE voltammograms were recorded with a different scan rate.

3.9. DNA Binding And Cleavage Experiments

DNA binding experiments were carried out using CT DNA and stock solutions of nucleic acids was prepared by directly dissolving commercial CT DNA in double distilled water at 0-4°C. A minimum time of 24h was needed for the complete dissolution of DNA, although occasional shaking was done. CT DNA was made up to 5 mL in 10 mM Tris-HCl buffer solution (pH 7.5, 10 mM NaCl) and all experiments were performed at room temperature under ambient condition. The binding constant K$_a$ was determined by

\[
\frac{C}{\Delta \varepsilon_a} = \frac{C}{\Delta \varepsilon_a + 1/\Delta \varepsilon K_a}
\]

given in Equation 11:

\[
C/\Delta \varepsilon_a = C/\Delta \varepsilon_a + 1/\Delta \varepsilon K_a
\]  

(11)

C is the concentration of DNA, $\Delta \varepsilon_a = [\varepsilon_a - \varepsilon_f]$, $\Delta \varepsilon = [\varepsilon_b - \varepsilon_f]$, and $\varepsilon_a$, $\varepsilon_b$, and $\varepsilon_f$ corresponds to the apparent extinction coefficient, the extinction coefficient of the bound form and that of free of compound 5, 6, 7, 8 and 9 respectively. The electrophoresis experiments were performed on a horizontal gel electrophoresis system. Agarose gel electrophoresis experiments were carried out on pBR322 DNA. All samples in 5 mM Tris–HCl/50 mM
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NaCl buffer, at pH 7.4 in the absence of an external agent examined under physiological pH and temperature at 37°C for 2h.

3.10. Antimicrobial Activity

The infectious diseases caused by bacteria, fungi, viruses and parasites in an individual are due to a complex interaction between the host, the pathogen and the environment. The mortality and morbidity rates have been dramatically decreased after the discovery and use of antibiotics. However, indiscriminate use of antibiotics and the ability of microbes to transmit and acquire drug resistance genes led to the development of resistant microbes such as Streptomycin resistant e coli, Methicillin resistant *Staphylococcus aureus*, Vancomycin resistant *Staphylococcus aureus*, Vancomycin resistant *Enterococcus*, Multidrug resistant TB, Fluconazole resistant *Candida albicans* and others. There are considerable reports on the progress of drug resistance and is an alarming situation in developed as well as developing countries. The resistance development in microbes has even complicated the treatment of infectious diseases in immune compromised and cancer patients. In the scenario of emergence of multidrug resistant microbes, it has necessitated the search for new antimicrobials from other sources"115-118".

Antimicrobial evaluation involves testing the microbial susceptibility to chemotherapeutic agents. Determination of antimicrobial effectiveness against specific pathogens is essential to proper therapy. Testing can show which of the agents are more effective against a pathogen and give an estimate of proper therapeutic dose. The idea of the effectiveness of a chemotherapeutic agent against a pathogen can be obtained from the Minimum Inhibitory Concentration (MIC). The MIC is the lowest concentration of a drug, which prevents growth of particular pathogen. The synthesized compounds were
screened in vitro for their antimicrobial activity against *Gram positive Staphylococcus aureus*, *Gram negative, Klebsiella pneumonia, Pseudomonas aures* and *Escherichia coli* bacterial stain and *aspergillus niger* and *candida albicans* fungal strains using the agar well diffusion method\(^{119}\). The in vitro antibacterial activity measurements were carried out against 24h cultures of bacterial strains. The bacterial strains were collected from different infectious status of patients who had not administered any antibacterial drugs for at least two weeks with the suggestions of an authorized physician, in Kiran diagnostic health centre of Chitradurga, Karnataka state, India. Fungal strains were procured from the culture maintained at National College of Pharmacy, Shimoga. Sensitivity plates were inoculated with Gram negative bacteria and Gram positive bacteria and the well was loaded with 75 \(\mu\)l of test solution using a sterilized micropipette. The zone of inhibition was compared with standard drug after 24h of incubation at 37 ± 2°C for antibacterial activity and 72h at 25± 2°C for antifungal activity. Three replicas were made for each study. To evaluate the effect of concentration on antibacterial activity, three different concentrations (0-5000\(\mu\)g/\(\mu\)L) of the test compounds were used. DMSO, which exhibited no antibacterial activity, was used as a negative control.

### 3.11. Minimum Inhibitory Concentration

The MIC (minimum inhibitory concentration) value for the water soluble Pcs showed positive result towards antibacterial activity was further determined using the microdilution broth method. Sample solutions were added to the broth at different concentrations. Measured samples of each bacterial suspension were added to the serial dilution of the test substances\(^{120}\). The respective clinical strain was spread separately on the medium. The wells were created using a stainless steel sterilized cork borer under
aseptic conditions. The newly synthesized substituted MPcs (5-8) at different concentrations viz. (0-5000μg/μL) was dissolved respectively in 25, 50, 75, 100 and 125 μL of DMSO and later loaded into corresponding wells. The standard drug streptomycin (40 μg in100 μl) and fluconazole (40 μg in100μl) were used as standard drugs for comparison of antibacterial and antifungal activities respectively. The zone of inhibition was compared with standard drug after 24h of incubation at 37°C for antibacterial activity and 72h at 25°C for antifungal activity.

3.12. Antioxidant Activity

Antioxidants in food play an important role as a health protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. Primary sources of naturally occurring antioxidants are grains, fruits and vegetables. Plant sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids; phytate and phytoestrogens have been recognized as the potential antioxidants. Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties. Some compounds, such as gallates, have strong antioxidant activity, while others, such as the mono-phenols are weak antioxidants.

The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease. Antioxidants like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxy and thus inhibit the oxidative mechanisms that lead to degenerative diseases. The free radical
scavenging activity of antioxidants in foods has been substantially investigated and reported in the literature by Miller et al.,\textsuperscript{121,122} A rapid, simple and inexpensive method to measure antioxidant capacity of food involves the use of 2,2-Diphenyl-1-picrylhydrazyl (DPPH). DPPH is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors and to evaluate antioxidant activity of food. It has also been used to quantify antioxidants in complex biological systems in recent years. The DPPH method can be used for solid or liquid samples and is not specific to any particular antioxidant component, but applies to the overall antioxidant capacity of the sample. A measure of total antioxidant capacity helps to understand the functional properties of new drugs. Analytical methods measure the radical scavenging activity of antioxidants against free radicals like DPPH radical, the superoxide anion radical (O\textsubscript{2}'), the hydroxyl radical ('OH), or the peroxyl radical (ROO'). The various methods used to measure antioxidant activity of food products can give varying results depending on the specific free radical being used as a reactant.

The radical scavenging ability of synthesized and the ascorbic acid (standard) was tested on the basis of radical scavenging effect on DPPH free radical\textsuperscript{123}. Different concentrations (5, 10, 25, 50, 100 and 200 \(\mu\)g/ml) of the compounds and standard were prepared in methanol. In clean and labeled test tubes 2 ml of different concentration of compounds and standard separately. The tubes were incubated at room temperature in dark for 30 minutes and the optical density was measured at 517 nm using UV-vis spectrophotometer. The absorbance of the DPPH control was also noted. The suppression ratio for OH was calculated from the formula: Scavenge activity \(\% = (A - B)/ A \times 100\).
where A is absorbance of the DPPH and B is the absorbance of the DPPH in the standard combination.

3.13. Synthesis

3.13.1. Synthesis of tetracarboxy metallophthalocyanines as precursor

Scheme 1: (A) Urea, ammonium molybdate, ammonium chloride, nickel acetate, nitro benzene, 180-190°C 6h/microwave 30 min.

(B) 1 M KOH, 90°C 10-12 h/Hydrolysis.

Pure 2,9,16,23-tetracarboxylic acid nickel Pc has been synthesized by the documented procedure\textsuperscript{124} with some modification is as shown in scheme 1. The general procedure used is as follows: nickel sulfate hexahydrate (0.105 mol), trimellitic anhydride (0.40 mol), and excess urea were finely ground with a catalytic quantity of ammonium chloride and ammonium molybdate. The mixture was then placed in a three-necked flask containing nitrobenzene as solvent. The flask was provided with a thermometer, condenser, and mechanical stirrer. The temperature of the flask was slowly increased to 180°C and maintained at 180-185°C for 4h. The resulting deep colored solid cake was finely ground and purified with methanol until free from nitrobenzene. The cake was then repeatedly treated with boiling 2N hydrochloric acid, and followed by saturated sodium chloride solution. Finally the product obtained was washed with water until the washings
were free from chloride ions and dried at 100°C for 1h to get pure 2,9,16,23-tetra amido nickel (II) Pc. Then moved into 2.0 M sodium hydroxide solution saturated with sodium chloride in a 500-mL three-neck flask, and the hydrolysis reaction was performed at 90°C for 10h until no ammonia was evolved. The resulting mixture diluted with appropriate amount of deionized water was brought to pH < 3 with concentrated hydrochloric acid; then centrifugation and filtration were executed, followed with dissolving well with 0.5 M sodium hydroxide solution and filtering. The resulting solution was acidified and solid obtained was washed first with water and second with methanol until neutrality occurred. A dark blue solid with metallic luster was obtained via drying in a vacuum. Yield: conventional 56, Anal. Calc. for C₃₆H₁₆N₈NiO₈: C(57.86%); H(2.16%); N(15.00%), Found: C(56.93%); H(1.97%); N(14.01%); IR absorption bands (cm⁻¹): 737, 847, 943, 1088, 1148, 1530, 1614, 1697, 2854, 2935, 3371 cm⁻¹; ¹H NMR: (DMSO-d₆, δ ppm): 6.58-8.25 (m, ArH, 12H), 12.34 (s, COOH, 4H).

3.13.2. Synthesis of 2-[bis4-hydroxyphenyl)methyl]benzohydrazide as precursor 3

Firstly, compound 1 was synthesized according to the reported synthetic protocol¹², 2-[bis(4-hydroxyphenyl)methyl]benzoic acid 1 was used to synthesis compound 2 by heating(under reflux) a mixture of 1 (1g , 0.003 mmol), dried ethanol (10ml) in H₂SO₄(catalytic amount) at 55°C for 10h. The reaction was then allowed to cool to room temperature and poured to ice water and the product 2-[bis(4-hydroxyphenyl)methyl]benzoate obtained was then filtered and washed with copious amounts of water. Upon drying, the respective ester is taken for next step to synthesize 2-[bis(4-hydroxyphenyl)methyl]benzohydrazide. Compound 3 was prepared by refluxing mixture of compound 2, 10ml of dry ethanol and hydrazine hydrate for 10h at 55°C, the
solution was cooled to room temperature and the precipitate was filtered off, washed with water and methanol. The resulted final precursor 3 was dried at (60°C for 2h) and characterized by NMR (\(^1\)H & \(^{13}\)C), Mass and FT-IR spectroscopy. Yield (80%). Anal. Calcd. for C\(_{20}\)H\(_{18}\)N\(_2\)O\(_3\): C, 71.84; H, 5.43; N, 8.38; O, 14.35. Found: C, 71.81; H, 5.40; N, 8.35; O, 14.31. IR [(KBr) cm\(^{-1}\)]: 3326.2 (OH), 3228.3 (NH), 2850.7 (Ar-CH) 1626.5 (C=O), 1574.8 (C=C), 1449.1 (C-N), 1436.8 (C-H). \(^1\)H NMR (300 MHz; DMSO-\(d_6\)): \(\delta\)H, ppm 4.519 (1H, S, -CH), 6.225 (2H, S,-NH\(_2\)), 6.654 (5H, m, Ar-H), 6.877-6.904 (4H, m, Ar-H), 7.402-7.454 (2H, m, Ar-H), 7.835-7.852 (1H, m, Ar-H), 9.152 (1H, S, -NH), 9.38 (2H, S, -OH). \(^{13}\)C NMR (75 MHz; CDCl\(_3\); Me4Si): \(\delta\)C, ppm 67.47 (-CH), 120.94 to 133.67 (Ar-C), 147.10 (-C-N), 156.66 (-C-OH), 164.53 (C=O). MS (LCMS): m/z 334.654 (calcd. for [M + H]\(^{+}\)333.2).

3.13.3. General procedure for the Synthesis of 2(3),9(10),16(17),23(24)-tetra-2-[bis(4-hydroxyphenyl)methylphenyl-1,3,4-oxadiazole substituted metal phthalocyanine

Polyphosphoric (PPA) acid was added to the pulverized mixture of tetracarboxy MPc and 2-[bis (4-hydroxyphenyl) methyl]benzohydrazide, reaction mixture was maintained under moisture free nitrogen atmosphere stirred at 150°C for 24h, during the course of the reaction, the blue color suspension was changed into green color. Then the mixture was cooled to 80°C poured into 200 ml water (10°C). The green color product was soluble in water, due to the introduction of the phenolphthalein-1,3,4-oxadiazole bridge having hydrophilic character on the peripheral position of Pc ring. The green color product was precipitated by salt out method was collected and filtered off; the crude product was washed with cold ethanol and acetone. The same procedure was optimized by
maintaining the conditions in triplicate. Finally the pure green solid product was dried in vacuum.

3.13.4. Synthesis of 2(3),9(10),16(17),23(24)-tetra-[bis(4-hydroxyphenyl)methyl phenyl-1,3,4-oxadiazole substituted nickel(II)phthalocyanine (5)

Yield: 92%. Anal. calcd. for C_{116}H_{72}Ni_{16}O_{12}Ni: C, 71.79; H, 3.74; N, 11.55; O, 9.89; Ni, 3.02. Found: C, 71.64; H, 3.66; N, 11.48; O, 9.88; Ni, 3.00. IR (cm^{-1}, KBr): 3388 (ph-OH), 2903.8 (Aliphatic -CH), 2384.49 (Ar-CH), 1616 (C=N), 1513.7 (C=C), 1383.16, 1196.57, 1112.61, 1056.64, 972.67, 814.08 and 739. \textsuperscript{1}H NMR (D\textsubscript{2}O): \delta = 5.4 to 8.8 (m, 60H, Ar-H), \delta = 2.16 (s, 4H, CH). ESI-MS: m/z Calc. 1940.6; Found (M+H) 1941.7.

3.13.5. 2(3),9(10),16(17),23(24)-tetra-[bis(4-hydroxyphenyl)methyl phenyl-1,3,4-oxadiazole substituted Cobalt(II)phthalocyanine (6)

Yield: 91%. Anal. calcd. for C_{116}H_{72}Ni_{16}O_{12}Co: C, 71.79; H, 3.74; N, 11.55; O, 9.89; Co, 3.04. Found: C, 71.66; H, 3.64; N, 11.52; O, 9.87; Co, 2.92. IR (cm^{-1}, KBr): 3360.95 (ph-OH), 2931.80 (Aliphatic CH), 2437.36 (Ar-CH), 1607.06 (C=N), 1495.11 (C=C), 1411.25, 1233.89, 1168.59, 1121.94, 963.34, and 739.44. ESI-MS: m/z Calc. 1940.84; Found (M+H) 1942.3.

3.13.6. 2(3),9(10),16(17),23(24)-tetra-2-[bis (4-hydroxyphenyl) methyl phenyl-1,3,4-oxadiazole substituted copper(II)phthalocyanine (7)

Yield: 93%. Anal. calcd. for C_{116}H_{72}Ni_{16}O_{12}Cu: C, 71.62; H, 3.73; N, 11.52; O, 9.87; Cu, 3.27. Found: C, 71.53; H, 3.62; N, 11.49; O, 9.83; Cu, 3.18. IR (cm^{-1}, KBr): 3416.70 (ph-OH), 2888.27 (Aliphatic CH), 2813.63, 2384.49 (Ar-CH), 1619.50 (C=N), 1507.55 (C=C), 1376.94, 1339.62, 1209.01, 1097.06, 957.13, and 742.55. ESI-MS: m/z Calc. 1945.45; Found (M+H) 1946.5.
3.13.7. 2(3),9(10),16(17),23(24)-tetra-2-[bis (4-hydroxyphenyl) methyl phenyl-1,3,4-oxadiazole substituted zinc(II)phthalocyanine (8)

Yield: 89%. Anal.calcd. for C_{116}H_{72}N_{16}O_{12}Zn: C, 71.55; H, 3.73; N, 11.51, O, 9.86, Zn, 3.36. Found: C, 71.43; H, 3.62; N, 11.49, O, 9.81, Zn, 3.25. IR (cm\(^{-1}\), KBr): 3424 (ph-OH), 2892 (Aliphatic CH), 2379 (Ar-CH), 1624 (ON), 1512.2 (C=C), 1381.5, 1176.36, 1111.12, 1073.74, 971.12 and 803.19. ESI-MS: m/z Calc. 1947.32; Found (M+H) 1948.5.

3.13.8. 2(3),9(10),16(17),23(24)-tetra-2-[bis (4-hydroxyphenyl) methyl phenyl-[1,3,4]-oxadiazole substituted iron(III)phthalocyanine (9)

Yield: 87% Anal. calcd. for C_{116}H_{72}C_{16}N_{16}O_{12}Fe: C,70.61; H, 3.68; Cl, 1.80; N, 11.36; O, 9.73; Fe, 2.83. Found: C, 70.43; H, 3.58; Cl, 1.71; N, 11.32; O, 9.69; Fe, 2.72. IR (cm\(^{-1}\), KBr): 3382.71 (ph-OH), 2888.27 (Aliphatic CH), 2365.83 (Ar-CH), 1666.14 (C=N), 1507.55 (C=C), 1386.27, 1339.62, 1227.67, 1171.20, 1097.06 (Fe-Cl), 1050.42, 1013.42, 1013.10 and 798.53. ESI-MS: m/z Calc. 1973.12; Found (M+H) 1974.3.

4. Results and Discussion

4.1. Synthesis and Characterization

Synthetic route for water soluble 2(3),9(10),16(17),23(24)-tetra-2-[bis (4-hydroxyphenyl) methyl phenyl-1,3,4-oxadiazole substituted metal Pc is shown in scheme 1. The title complexes were prepared through the condensation reaction of 2-[bis (4-hydroxyphenyl) methyl] benzohydrazide and tetracarboxy MPcs with polyphosphoric acid. We conceded same reaction conditions to nickel, zinc, cobalt, copper and iron substituted Pcs with significant yields. The newly prepared symmetrically functionalized Pcs with eight
hydroxyphenyl peripheral units through the 1,3,4-oxadiazole bridges were extensively soluble in water and insoluble in all organic and inorganic solvents.

\[ \text{Scheme 1 Synthesis of 2(3),9(10),16(17),23(24)-tetra-[bis(4-hydroxyphenyl)methyl phenyl-[1,3,4]-oxadiazole substituted metallophthalocyanine (5-8) Conditions: (a) } H_2SO_4, \text{ dry ethanol, reflux at 55°C for 10h; (b) } N_2H_4, \text{ dry ethanol, reflux at 55°C for 10h; (c) Polyphosphoric acid, 150°C for 24h.} \]

The water soluble MPcs is well characterized by FT-IR spectroscopy. The FT-IR spectrum of water soluble MPcs is illustrated in Figure 3. The IR spectra reveal the substitution at peripheral of the Pcs by the appearance of -OH band at 3413 cm\(^{-1}\). The characteristic vibrations corresponding to OXD ring of the Pc C–O–C groups at 2234 cm\(^{-1}\) appears in all the metal complexes. The supramolecular vibrations observed around 2931 to 2880 cm\(^{-1}\) is assigned to (C-H) vibrations. The peak at 1016 cm\(^{-1}\) shifted towards the higher wave number assigned for Fe-Cl vibration in compound (9).
In $^1$H NMR the spectrum the signals corresponding to -OH groups disappeared as expected, because spectral analysis carried out in D$_2$O is presented in Figure 4. $^1$H NMR spectrum of 3 showed broad signals at $\delta$ 5.4 to 8.8 ppm for aromatic protons. The signals belong to methylene protons were observed at $\delta$ 2.16 ppm.

Figure 3. IR spectra of water soluble metallophthalocyanine (5-9).

Figure 4. $^1$H NMR spectrum of 2,9,16,23-tetra-{[bis(4-hydroxyphenyl)methyl]-phenyl-[1,3,4]-oxadiazole}-substituted nickel(ii)phthalocyanine (5).
The ESI-MS mass spectrum of compounds (5-9) is depicted in Figure 5-9 and confirmed the proposed structure. The molecular ion peaks at m/z = Calc. 1940.6; Found (M+H) 1941.7 confirmed the proposed structure for complex 5, m/z = Calc. 1940.8; Found (M+H) 1942.3 for complex 6, m/z = Calc. 1945.4; Found (M+H) 1946.5 for complex 7, m/z = Calc. 1947.32; Found (M+H) 1948.2 for complex 8 and m/z = Calc. 1973.1; Found (M+H) 1974.3 for complex 9 respectively.

**Figure 5.** ESI mass spectrum of 2,9,16,23-tetra-{2-[bis(4-hydroxyphenyl)methyl]-phenyl-[1,3,4]-oxadiazole}-substituted nickel(ii)phthalocyanine (5).

**Figure 6.** ESI mass spectrum of 2,9,16,23-tetra-{2-[bis(4-hydroxyphenyl)methyl]-phenyl-[1,3,4]-oxadiazole}-substituted cobalt(ii)phthalocyanine (6)
Figure 7. ESI mass spectrum of 2,9,16,23-tetra-{2-[bis(4-hydroxyphenyl)methyl]-phenyl-[1,3,4]-oxadiazole}-substituted copper(ii)phthalocyanine (7)

Figure 8. ESI mass spectrum of 2,9,16,23-tetra-{2-[bis(4-hydroxyphenyl)methyl]-phenyl-[1,3,4]-oxadiazole}-substituted Zinc(ii)phthalocyanine (8)

Figure 9. ESI mass spectrum of 2,9,16,23-tetra-{2-[bis(4-hydroxyphenyl)methyl]-phenyl-[1,3,4]-oxadiazole}-substituted iron(iii)phthalocyanine (9)
The starting precursor tetra carboxymetllophthalocyanines were characterized by $^1$H NMR and FT-IR spectroscopic. The tetra carboxy nickel Pcs structure was confirmed by both $^1$HNMR spectrum presented in Figure 10. In the spectrum, the chemical shift peaks at 8.25 to 6.58 ppm were assigned to the 12 aromatic protons of the Pc core and the COOH was observed at 12.34 ppm for 4 protons.

![Figure 10. $^1$H-NMR spectrum of tetracarboxy nickel phthalocyanine.](image)

FT-IR spectra of tetra carboxymetllophthalocyanines was shown in Figure 11. The broad spectrum band at 3371 cm$^{-1}$ was observed due to the –COOH. The peak at 2854 and 2935 cm$^{-1}$ was observed due to aromatic -CH stretching vibrations. Carbonyl stretching of group observed at around 1697 cm$^{-1}$ and exhibited a series of absorptions at 737, 847, 943, 1088, 1148, 1530 and 1614 cm$^{-1}$ respectively.
Figure 11. FT-IR spectra of tetracarboxy metallophthalocyanine (4).

The starting precursor 2-[bis (4-hydroxyphenyl) methyl] benzohydrazide was well characterized by NMR (\(^1\)H & \(^{13}\)C), Mass and FT-IR spectroscopic techniques. The FT-IR spectrum of 2-[bis (4-hydroxyphenyl) methyl] benzohydrazide was depicted in the Figure 12. The spectrum showed a two broad band at 3326.2 cm\(^{-1}\) due to the -OH and 3228.3 cm\(^{-1}\) due to -NH stretching vibrations, the respectively. Carbonyl stretching of group observed at around 1626.5 cm\(^{-1}\).

Figure 12. FT-IR spectrum of 2-[bis4-hydroxyphenyl)methyl]benzohydrazide.
The $^1$H NMR spectrum of 2-[bis (4-hydroxyphenyl) methyl] benzohydrazide was illustrated in Figure 13. Chemical shifts of aromatic protons appeared around at 7.852 to 6.654 ppm for 12 protons and the -NH & -NH$_2$ peak observed at 9.152 and 6.225 ppm respectively. The chemical shifts of -CH peak observed at 4.519 ppm and for -OH group observed at 9.38 ppm.

Figure 13. $^1$H-NMR spectra of 2-[bis (4-hydroxyphenyl) methyl] benzohydrazide.
The $^{13}$C NMR spectrum of 2-[bis (4-hydroxyphenyl) methyl] benzohydrazide was presented in Figure 14. -OH attached to aromatic carbon appears at 156.66 ppm and aromatic carbons observed at 114 to 133 pm. The peak at 147.10 and 164.53 ppm corresponds to the -C-N and C=O respectively.

Figure 14. $^{13}$C-NMR spectrum of 2-[bis (4-hydroxyphenyl) methyl] benzohydrazide.
Mass spectrometry (LCMS) analysis of the compound 2-[bis (4-hydroxyphenyl) methyl] benzohydrazide spectrum is depicted in Figure 15. The molecular ion peak of 2-[bis (4-hydroxyphenyl) methyl] benzohydrazide was good agreement with calculated 333.2 and found 334.654.

**Figure 15.** Mass spectrum of 2-[bis (4-hydroxyphenyl) methyl] benzohydrazide.
4.2. Electron Absorption Spectroscopy

The best indications for the Pc system are given by their UV-Vis spectra in aqueous media. The synthesized water soluble MPcs (5, 6, 7, 8 and 9) dominated by two intense bands, one at B band around 370 nm and a distinct absorptions value for the extinction coefficient ($\varepsilon$) of the Q band around 700 nm at the visible region were presented in Figure 16. The arrangement of the planar molecules in close facial proximity with sufficient intermolecular interaction would occur as extended $n$-conjugate system along with the presence of eight phenol groups through the OXD bridge in peripheral position tuning the wavelength of Q band absorption, Herein all the metal Pcs have explicate nearly equivalent value around 600 to 720 nm. The elevated number of polar and massive phenolphthalein OXD substituent has to be responsible for this remarkable feature.

![Figure 16. Electronic absorption spectra of compound (5-9).](image-url)
4.3. Thermal Analysis (TGA)

Thermogravimetric analysis provides information about the thermal stability and the kinetic decomposition behavior of the complexes at different temperatures. The TGA curves were evaluated by using Broido’s method and kinetic and thermodynamic parameters are evaluated. The thermogravimetric curve for compounds 5, 6, 7, 8, and 9 shows a mass loss near 100°C due to moisture from the sample with an exothermic decomposition were presented in Figure 17.

![TGA graph of compound (5-9).](image)

These complexes were highly stable in air even at high temperatures. The derivative of the mass signal clearly shows that there was only one step decomposition process foremost decomposition start between 340 to 520°C for compound 5, 335 to 500°C for compound 6, 340 to 550°C for compound 7, 350 to 520°C for compound 8, and 340 to 530°C for compound 9 respectively. From the plot (Figure 18) the activation energy ($E_a$) and frequency factor (ln A) were evaluated. The enthalpy ($\Delta H$), entropy ($\Delta S$) and free energy ($\Delta G$) have been calculated. This clearly indicates that the substitution of bulky
group at the periphery of the Pc ring leads to the higher thermal stability of the macrocycle. The variation in thermal stability was also observed for Pc complexes with different central metal ions. The changes in entropy of activation are negative for all the compounds varied from $-110$ to $113$ (kJmol$^{-1}$) and kinetic decomposition temperatures obtained from the derivative of the mass-signal is summarized in Table 3. The decomposition temperature for the different complexes increases in the following $7>8>9>5>6$ order.

**Figure 18.** Plots of $-\ln (\ln (-1/y))$ versus $1/T$ for thermal degradation of compound (5-9).
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Table 3 The loss of weight at the major decomposition temperature was 99% for Pc

Thermodynamic parameters for 1,3,4-oxadiazole substituted MPcs compound (5-9).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Temperature of decomposition (°C)</th>
<th>DTA_{max} (°C)</th>
<th>E_u (kJmol^{-1})</th>
<th>ΔH (kJmol^{-1})</th>
<th>ΔS (kJK^{-1})</th>
<th>ΔG (kJmol^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-Ni (5)</td>
<td>330-530</td>
<td>430</td>
<td>0.017341</td>
<td>-5.8274004</td>
<td>-160.98</td>
<td>112.12</td>
</tr>
<tr>
<td>P-Co (6)</td>
<td>340-550</td>
<td>445</td>
<td>0.017845</td>
<td>-5.9516068</td>
<td>-160.41</td>
<td>113.35</td>
</tr>
<tr>
<td>P-Cu (7)</td>
<td>335-495</td>
<td>415</td>
<td>0.017054</td>
<td>-5.7029776</td>
<td>-161.04</td>
<td>110.79</td>
</tr>
<tr>
<td>P-Zn (8)</td>
<td>335-520</td>
<td>420</td>
<td>0.017845</td>
<td>-5.7926123</td>
<td>-160.55</td>
<td>111.15</td>
</tr>
<tr>
<td>P-Fe (9)</td>
<td>340-530</td>
<td>430</td>
<td>0.017522</td>
<td>-5.8269012</td>
<td>-160.88</td>
<td>112.23</td>
</tr>
</tbody>
</table>

4.4. DNA-Binding Studies Using UV/vis Titration.

New water soluble Pcs were titrated with CT DNA to determine the binding parameters at room temperature under ambient conditions in phosphate buffer (pH 7.5). The concentration of CT DNA was measured from its absorption intensity at 260 nm using molar absorption coefficient $\varepsilon_{DNA} = 6600 M^{-1}$. CT DNA was stored at 4°C overnight and used within 2 days. Absorption spectra were recorded from 300 to 800 nm. The titrations were carried out until MPcs Q-bands remained at a fixed wavelength upon successive additions of CT DNA. To setup the stage for our investigation binding studies of novel water soluble with CT DNA as per the literature. The wavelengths and intensities of the three most prominent Pcs vibronic absorption maxima were appreciably altered; the results suggested that water soluble Pc was modestly engaged in intercalation and hence the binding study of individual MPcs. On absorption, in all cases a decrease in the intensity of the Q-band of Pcs was detected upon increasing the concentration of CT DNA.
DNA and the significant absorbance decreases, i.e., the hypochromism effect suggests an intercalation binding mode of the Pc with CT DNA, displayed appreciable red shift with increasing CT DNA around Q band region. Compound 5 shows red shift from 634 to 640 nm (6 nm), compound 6 red shift from 629 to 631 nm (2 nm), compound 7 red shift from 623 to 627 nm (4 nm), compound 8 red shift from 614 to 618 nm (4 nm) and compound 9 red shift from 596 to 598 nm (2 nm) respectively as shown in figure 19. The spectra reveals that substantial hypochromic and red shifts in the absorption spectrum of the chromospheres are produced by electronic effects involving π-π stacking and dipole-dipole interactions with DNA base pairs. The strength of these electronic interactions is inversely proportional to the cube of the distance separating the chromospheres and the bases. The apparent binding constants ($K_a$) were obtained for the association of water soluble MPc compounds 5, 6, 7, 8 and 9 with CT DNA was found to be $2.4 \times 10^5$ M$^{-1}$, $2.5 \times 10^5$ M$^{-1}$, $2.5 \times 10^5$ M$^{-1}$, $2.9 \times 10^5$ M$^{-1}$, and $3.1 \times 10^5$ M$^{-1}$ respectively. These values are lower than the apparent binding constant normally associated with intercalation ($K_a < 10^6$), the results may suggest that decreasing absorption of water soluble MPcs shows partial non-intercalate mode of binding that involves a stacking interaction between the complex and the base pairs of DNA through electrostatic mode.
Figure 19. Electronic spectra of compound (5-9) (0.001 mM) upon increasing amounts of CT DNA (0.174 mM). 12 successive injections at pH 7.45 mL in 10 mM Tris-HCl buffer solution (pH 7.5, 10 mM NaCl): The decrease in absorbance continued (Line 2 (5 mL) to line 6 (30 mL)) until a stable Pc complex formed. Line 8 (30 mL) Line 12 (60 mL) (bold black lines): A stable Pc complex formed when 30 mL (5 x 25) 0.174 mM CT-DNA was added to compound 5; Q band absorbance remained constant after 7th addition.
4.5. pBR322 DNA Cleavage

The DNA cleavage ability of water soluble Pc was studied by agarose gel electrophoresis using pBR322 plasmid DNA as a substrate in a medium of 5 mM Tris-HCl/50 mM NaCl buffer, at pH 7.4 in the absence of an external agent examined under physiological pH and temperature at 37°C. When plasmid pBR322 DNA was incubated with water soluble Pc in aqueous buffer solution for 2h, no DNA cleavage was observed for controls in which the complex was absent (lane C). The result indicated the importance of the metal complexes for observing the DNA cleavage activity. All the complexes cleaved the pBR322 DNA from its supercoil form SC (form I). The supercoil will relax to generate a slower-moving open circular form (Form II). If both strands are cleaved, a linear form (Form III) that migrates between Form I and Form II will be generated, even in the absence. An effective chemical cleavage activity is diminished significantly by the complex without the addition of external agents and the result reveals that cleavage of DNA by the complex has strong dependence on the concentration of the complex (60-100 µM). The DNA cleavage activities of the complexes are obviously concentration-dependent. With the increase of complex concentration, the supercoiled DNA decreases and nicked circular DNA gradually increases as shown in figures 20-22. The results show that the complex can effectively cleave the double-stranded DNA without the addition of external agents and the cleavage of DNA by the complex has strong dependence on the concentration of complex.
Figure 20. Agarose gel electrophoresis pattern for the Cleavage of pBR322 DNA incubated with Pc compound (60 μM), C, DNA alone; 5, 6, 7, 8 and 9 (DNA + complex).

Figure 21. Agarose gel electrophoresis pattern for the Cleavage of pBR322 DNA incubated with Pc compound (80 μM), C, DNA alone; 5, 6, 7, 8 and 9 (DNA + complex).

Figure 22. Agarose gel electrophoresis pattern for the Cleavage of pBR322 DNA incubated with Pc compound (100 μM), C, DNA alone; 5, 6, 7, 8 and 9 (DNA + complex).
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4.6. Antimicrobial Activity

Water soluble MPcs were screened against bacterial and fungal stain. The results revealed that complex (5-9) was found to be more active as antibacterial than as antifungal. The biocidal activity data of the investigated compounds are summarized in table 2. Results shown in table 2 clearly indicate that inhibition is much larger. In which 6, 7 and 8 complexes show higher activity which is probably due to effective chelation. This activity is quite comparable to the reference drugs streptomycin/flucanazole for bacterial/fungal test, respectively. The results in table 4 show that 6, 7 and 8 had the stronger effect against bacteria and fungi than 5 and 9 complexes used.

Table 4: Anti-microbial activity of compound (5-9).

<table>
<thead>
<tr>
<th>bacterial strains</th>
<th>S.aureus</th>
<th>P.aeruginosa</th>
<th>K.pneumoniae</th>
<th>E.coli</th>
<th>A.niger</th>
<th>C.albicans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Zone of inhibition test (in cm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>3.8</td>
<td>2.9</td>
<td>2.8</td>
<td>2.8</td>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td>6</td>
<td>3.8</td>
<td>2.8</td>
<td>2.9</td>
<td>2.9</td>
<td>1.8</td>
<td>1.9</td>
</tr>
<tr>
<td>7</td>
<td>3.8</td>
<td>2.8</td>
<td>2.8</td>
<td>2.9</td>
<td>1.9</td>
<td>1.8</td>
</tr>
<tr>
<td>8</td>
<td>3.8</td>
<td>2.8</td>
<td>2.9</td>
<td>2.8</td>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td>9</td>
<td>3.8</td>
<td>2.8</td>
<td>2.9</td>
<td>2.8</td>
<td>1.8</td>
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<tr>
<td>DMSO</td>
<td>0.0</td>
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<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
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<tr>
<td>Standard</td>
<td>4.1</td>
<td>3.4</td>
<td>3.7</td>
<td>3.6</td>
<td>2.2</td>
<td>2.3</td>
</tr>
</tbody>
</table>

Standard – Streptomycin (antibacterial)
Standard – Flucanazole (antifungal)

4.7. Minimum Inhibitory Concentrations (MIC)

The MIC of 2(3),9(10),16(17),23(24)-tetra-2-[bis(4-hydroxyphenyl)methylphenyl-[1,3,4]-oxadiazole substituted MPc (5-9) was defined as the lowest antibacterial concentration of the test samples that inhibit more than 99% of the bacterial population.
The experiments were repeated three times and the results were expressed as average values. The solvent showed no antibacterial action. The MIC results consistently show that 2 (MIC = 125 µg/ml) is the most active complex against E. aerogenes compared to 1 (MIC = 250 µg/ml) and 3 (MIC = 250 µg/ml). The MIC results of (5-9) summarized in Table 5.

**Table 5.** (MIC) of compound (5-9).

<table>
<thead>
<tr>
<th>Compound</th>
<th>S.aureus</th>
<th>P.aeruginosa</th>
<th>K.pneumoniae</th>
<th>E.coli</th>
<th>A.niger</th>
<th>C.albicans</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>40</td>
<td>40</td>
<td>30</td>
<td>40</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>6</td>
<td>40</td>
<td>30</td>
<td>30</td>
<td>40</td>
<td>30</td>
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</tr>
<tr>
<td>7</td>
<td>40</td>
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<td>30</td>
<td>40</td>
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<tr>
<td>8</td>
<td>30</td>
<td>40</td>
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</tr>
<tr>
<td>9</td>
<td>40</td>
<td>30</td>
<td>40</td>
<td>40</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Control</td>
<td>DMSO</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Control: DMSO (Dimethyl sulphoxide)

**4.8. Antioxidant Activity**

The radical scavenging activity of the Pcs was measured using the ascorbic acid as standard according to the established method. The DPPH radical is a stable free radical having $\lambda_{max}$ at 517 nm. Different concentration (5, 10, 25, 50, 100 and 200 µg/ml) of compounds and standard were prepared in methanol. In clean and labeled test tubes 2ml of DPPH solution (0.002% in methanol) was measured at 517 nm using UV-visible spectrophotometer. The absorbance of the DPPH control was also noted. The complexes exhibit good DNA binding affinity and DNA cleavage activity and its worthwhile to study the free radical scavenging activity of these compounds. The antioxidant properties of water soluble MPcs (5-9) results indicate that the sensible suppression ratio increased with increasing concentration of the complex. The compounds exhibited marked
antioxidant activity by scavenging DPPH\(^*\) (free radical) and converting into DPPH and the activity was found to be dose dependent. Figure 23 reveal that newly synthesized MPcs 6, 7 and 8 exhibited fruitful DPPH radical scavenging activity than the complexes 5 and 9 when compared with standard. So, the antioxidant activity assay results clearly indicate that the newly synthesized MPc is solely responsible for the scavenging activity and results were tabulated in table 6.

### Table 6. DPPH radical scavenging activity of compound (5-9)

<table>
<thead>
<tr>
<th>Compounds (µg/ml)</th>
<th>Radical scavenging activity (%) of different concentrations (µg/ml) of compound (5-8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>55.54</td>
</tr>
<tr>
<td>6</td>
<td>59.98</td>
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<tr>
<td>7</td>
<td>59.75</td>
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<tr>
<td>8</td>
<td>59.88</td>
</tr>
<tr>
<td>9</td>
<td>58.25</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>64.96</td>
</tr>
</tbody>
</table>

**Figure 23.** Plots of the radical scavenging effects (%) of compound (5-9) at various concentrations.
4.9. Cyclic Voltammetric Studies

Electrochemical measurement of compound 6 (P-Co) was investigated using electrochemical workstation, connected to a standard three electrode electrochemical systems equipped with gas flow system and technique measure in phosphate buffer (pH-7). Figure 24 (a) comparing with buffer, (b) shows the CV of complex 6 at 0.050 to 0.250 Vs\(^{-1}\) scan rate, and (c) changing the potential range at 0.050 to 0.250 Vs\(^{-1}\) scan rates. In this work our P-Co exhibited two reductions and three oxidations with the corresponding anodic waves. Introduction of electron-donating groups to MPc ring is expected to lead to a thermodynamically easier oxidation and a more difficult reduction of the MPc complex\(^{130,131}\). This is because electron-donating group should increase the average electron density of the conjugated 18\(\pi\)-electron system of the Pc ring. From Figure 24 shows the CV of new water soluble cobalt Pc\(_5\) complex undergoes two reduction process at -0.78 V and -0.32 V with anodic scan and two oxidation process at -0.6 V and -0.26 V, The reduction and oxidation behavior of P-Co is due to the interaction between the Pc ring and the central metal ions\(^{132,133}\), in this P-Co display oxidation at 0.78 V for phenol with quasi-reversible corresponding anodic wave displaced in saturated calomel electrode system at 0.250 Vs\(^{-1}\) scan rates versus SCE with glassy carbon electrode\(^{134,135}\) and these studies clearly point out that all of these processes could be ascribed to the Pc ring electron transfer reactions. The electrode processes were found to be diffusion controlled as demonstrated\(^{136}\) by the linearity of a plot of peak current versus square root of scan rate for scan rate ranging from 0.050 to 0.250Vs\(^{-1}\). The controlled potential coulometric (CPC) study indicated that the number of electrons transferred for electrochemical reactions of the complex was one for each oxidation and reduction processes. These
reduction processes were assigned to \((2^+)\) Co-P \((2^-)/(-)\) Co-P \((2^-)\) and \((+)^{\text{Co-P}_2}/(-)^{\text{Co-P}_3}\) couples, respectively.

Figure 24. (a), (b) & (c) Cyclic voltammograms of water soluble P-Co with anodic in Phosphate buffer solution containing (pH=7), Scan rates = 0.050-0.250 V s\(^{-1}\).
4.10. Electrochemical Properties of Catalyst

4.11. Oxygen Reducing Reaction

Figure 25 (a) and (b) illustrates the typical cyclic voltammograms (CVs) obtained at P-Co modified GC electrodes in 0.5 M H₂SO₄ under saturated N₂, dissolved O₂ and saturated O₂ atmosphere with scan rate 20-120 mVs⁻¹. From Figure 25 showed a substantial reduction process in the presence of oxygen, whereas no obvious response was observed under nitrogen. The CVs were carried out by scanning the disk onset potential displaced at 0.03V and the current densities at -2.50 V of ORR for P-Co electrode versus saturated calomel electrode with a scan rate 5 mVs⁻¹ in the saturated O₂ acidic electrolyte.

![Cyclic voltammograms of P-Co catalysts in 0.5 M PbSC₄ with Saturated N₂, Dissolved O₂ & Saturated O₂ at scan rate: 5 mVs⁻¹. (b) Saturated O₂ at scan rate: 20-120 mVs⁻¹.](image)

*Figure 25. (a) Cyclic voltammograms of P-Co catalysts in 0.5 M H₂SO₄ with Saturated N₂, Dissolved O₂ & Saturated O₂ at scan rate: 5 mVs⁻¹. (b) Saturated O₂ at scan rate: 20-120 mVs⁻¹.*
4.12. RDE Voltammetry and Electron Number For $O_2$ Reduction

Figure 26 shows the linear sweep voltammetry (LSV) at different rotation rates from 200 to 1000 rpm for P-Co coated Electrode, whereas the onset potential of the P-Co catalyst was significantly positively shifted to 0.03 V and kept almost constant on the same catalyst with the rotation rate. From this result we were obtained quantitative reaction parameters such as Tafel slopes ($b_a$), cathodic transfer coefficients for the rate limiting step ($n_aq_a$), the observed overall number of electrons involved in the reaction occurring on the disk surface ($n$), and the kinetic rate constant for the ORR was evaluated using the below Koutecky–Levich (K–L) equation 9.

\[
\frac{1}{j} = \frac{1}{j_k} + \frac{1}{B\omega^2}
\]  

(9)

Where $j$ represents the measured current density, $j_k$ is the kinetic current density, and $\omega$ is the rotation rate of the electrode. $B$ could be calculated from the slope of K–L plots based on the levich equation 10 as follows:

\[
B = 0.62nFD^2\nu^{-\frac{1}{6}}c_0\omega^{-\frac{1}{2}}
\]  

(10)

Where $n$ is the overall electron transfer number, $F$ is the Faraday constant ($F = 96 485$ C mol$^{-1}$), $C_0$ is the Concentration of dissolved $O_2$ (1.1X10$^{-6}$ mol.cm$^{-3}$), $D$ is the diffusion coefficient of $O_2$ (1.4X10$^{-5}$ cm$^2$.s$^{-1}$), $\nu$ is the kinematic viscosity of the electrolyte solution (0.010 cm$^2$.s$^{-1}$) and $\omega$ is the rotation rate represented by rpm. From figure 27 the slopes of the K-L plots depicts at potentials in the 0.3 to 0.4 V vs. SCE range, we could estimate the number of electron ($n$) involved in the pathway of $O_2$ reduction. This result indicates presence of the anchoring P-Co catalyst increases the amount of electrons per oxygen molecule transferred, and particularly for the process seems to proceed almost entirely via 4-electrons. In general earlier studies on the ORR catalytic behavior of
CoN₄ compounds suggest that the ORR catalyzed on CoN₄ based-electrodes is a 2e⁻ reduction process leading to H₂O₂.

![Graph showing RDE Curves of P-Co in catalysts in 0.5 M H₂SO₄ with Saturated O₂: With different speed at a Scan rate: 5 mVs⁻¹.](image)

**Figure 26.** RDE Curves of P-Co in catalysts in 0.5 M H₂SO₄ with Saturated O₂: With different speed at a Scan rate: 5 mVs⁻¹.

![Graph showing K–L plots of the P-Co electrode derived from RDE measurements.](image)

**Figure 27.** The K–L plots of the P-Co electrode derived from RDE measurements.
The present results suggest a possible two-step catalytic activity with first the $2e^-$ reduction of $O_2$ to $HO_2$, then the disproportionation of $HO_2$ into $OH^-$, which allows an apparent $4e^-$ reduction of $O_2$ in acidic media. And depicted the highest activity for $O_2$ reduction as compared to unsubstituted Pc; in terms of number of electrons per $O_2$ molecule transferred, because there are two possible effects that influence this observed change in ORR. First is the substituent effect that is substituted macromolecule ligand and central metal ion, substituent can be attributed to a larger delocalized $\pi$-system that probably decreases the reorganizational energy of the whole system, decreasing the activation energy and second is the substituents containing highly affinity and electron donating phenolic OH groups should show improved ORR catalytic activity because the substituents can increase the binding energy between $O_2$ and the metal center. This is obviously enhanced more favorable headed for ORR comparatively than unsubstituted.

4.13. Tafel Analysis

Figure 28 shows Tafel plots obtained in the linear region of log (i) vs. E plots were used to calculate approximate values for $b_a$ and $n_a\alpha_a$ and are summarized in Table I. These tafel slopes at the lower overpotential region (where $E > 0$ V vs SCE) for P-Co catalysts are 72 mV dec$^{-1}$ and indicates that the first electron transfer is the rate-determining step at the low overpotentials. At the higher overpotential region (where $E < 100$ mV vs SCE), the Tafel slope for P-Co was 116.9 mV dec$^{-1}$ which is little higher than that of Pt/C and imply that the rate-determining step of the ORR results are very close commercial Pt/C catalysts. Tafel slopes ($b_a$), cathodic transfer coefficients for the rate limiting step ($n_a\alpha_a$), number of electrons ($n$) and the kinetic rate constant were summarized in the Table 7.
Figure 28. Tafel plots of log \( i_k \) vs. \( E \) (V) for the ORR on P-Co catalysts

Table 7. Tafel slopes \( (b_a) \), cathodic transfer coefficients for the rate limiting step \( (n_a\alpha_a) \), number of electrons \( (n) \) and the kinetic rate constant were summarized.

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>No of electron</th>
<th>rate constant ( (cm^3/mol*s) )</th>
<th>( V_{onset} ) (V vs.SCE)</th>
<th>Tafel slope ( b_a ) (mV/dec)</th>
<th>( n_a\alpha_a )</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-Co</td>
<td>4.0</td>
<td>( 7.7 \times 10^{-4} )</td>
<td>0.03</td>
<td>77</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>142</td>
<td>0.38</td>
</tr>
</tbody>
</table>

Temperature- Room Temperature
Weight % (1 mM) - 5\( \mu l \)

5. Conclusion

We have successfully demonstrated the synthesis of new water soluble substituted MPcs appended eight hydroxyphenyl via 1,3,4-oxadiazole units and well characterized by elemental analysis, FT-IR, \(^1\)H NMR, ESI mass spectrometry and UV-vis spectroscopic techniques. The complexes display efficient binding to CT DNA and UV-vis titrations
have been carried out to find the binding with CT DNA and it decreasing in absorbance and red shift in the wavelength maxima at Q band, percentage of hypochromicity and the calculated binding constant values reveals that the non-intercalate mode of binding behavior of this novel water soluble substituted MPcs with CT DNA. The cleavage reaction on pBR322 DNA has been investigated by agarose gel electrophoresis. Remarkably, the results indicate that the complexes exhibit an efficient DNA-cleavage function. In addition, the complex 6, 7 and 8 also were showed considerable antimicrobial activity and exhibited varying degree of inhibitory effects on the growth of bacterial and fungal strains and exhibited good radical scavenging activity against DPPH radicals.

Anchored P-Co electrocatalyst increasing the amount of electrons per oxygen molecule transferred and particularly for the process seems to proceed almost entirely via 4-electrons. In this present studies depicted that uppermost activity for O₂ reduction as compared to unsubstituted cobalt Pc, both in terms of number of electrons per O₂ molecule transferred and by the decrease in the overpotential of the reaction by more than 0.2V as compared to unsubstituted cobalt Pc. P-Co electrocatalyst depicted results were very close to commercial Pt/C and has higher electrocatalytic activity for ORR in acidic medium than its unsubstituted cobalt Pcs.

So, that synthesized water soluble Pcs compounds were found to be biologically potent-fused heterocycles and selectively cobalt Pc shows highly effective and non-precious catalyst for ORR.
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