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Chapter-3: New water soluble Core modified metal (II) phthalocyanines with 4,4'-[4-(1,3,4-oxadiazol-2-y1)butane-2,2-diyl]diphenol-substituents: Synthesis, characterization, interaction of DNA, biological Studies, electrochemical and electrocatalytic activities.

Summary: phthalocyanines (Pcs) are attractive class of functional dyes for the construction of highly polar groups for progress in the application of Pcs and that related both material and biological field. Herein we synthesized the 4,4'-[4-(1,3,4-oxadiazol-2-y1)butane-2,2-diyl]diphenol substituted metallophthalocyanines (MPcs) and the effect of the substituents on the phthalocyanine (Pc) periphery made its soluble in water. The supramolecular assembly of these components by innovative approach is of particular interest as this provides a facile route to build arrays with various architectures and tunable photophysical properties. We report herein a synthesis of new series, water soluble MPcs and the structure of the complexes had been proposed by elemental analysis, FTIR, $^1$HNMR, ESI-MS, UV-visible spectroscopy and thermal studies. The compounds manifested significantly the binding properties with calf thymus DNA through non-intercalative mode. The nuclease activity of the complexes with pBR322 plasmid deoxyribonucleic acid was evaluated by agarose gel electrophoresis. The result shows that all the complexes have completely cleaved the DNA. The in vitro
microbiological activity of complexes showed that compounds 6 and 7 exerted potent activity than 5 against microbial stain. In the study of free radical scavenging activity compound 6 and 7 possessed a broad spectrum of activity than the complexes 5 when compared with standard. Synthesized water soluble cobalt Pcs subjected to efficient non-precious catalyst in the oxygen reduction reaction (ORR). The resulting product exhibits superior ORR catalytic activity, which leads to the invention of a new non-precious catalyst for ORR in fuel cells.

1. Introduction

Phthalocyanines exhibit intriguing physico-chemical properties that render them important as a class of molecular functional materials. As manifested by the rapidly increasing number of related scientific publications in recent years, great progress has been made in the field of advanced Pc materials. Tremendous efforts have been paid toward the development of new Pc molecular materials as well as toward their applications. Recent emphasis in both academic researches and technical field has been put on the design and synthesis of novel Pc species, the structure property relationship, self assembly properties, molecular electronics and opto-electronics, and dye-sensitized solar cells. In this chapter, the fascinating development in the synthesis of novel Pc derivatives with near-infrared absorbing and emphasis is on the application relation-ship between the DNA binding and cleavage studies in biological application and electrochemical-electrocatalytic properties in fuel cell applications.
Pcs and MPcs complexes has been a strong interest in recent years in developing of new reactive groups that can efficiently cleave or chemically modify nucleic acids under physiological conditions is very important for the design of new therapeutic drugs. Although many synthetic compounds have been developed to explore a variety of DNA/RNA structures, few molecules can be used to image DNA\(^1\). DNA binding to MPcs plays a key role in biology and medicine as a steering mechanism in biological processes and leading to a large body of structural studies using both experimental and theoretical methods\(^2\). The DNA is highly negatively charged and it interacts strongly with oppositely charged species\(^3\)–\(^5\). Owing to the central role of DNA in replication and transcription, DNA has been a major target for antibiotic, anticancer, and antiviral drugs\(^6\). There has been a growing interest in the use of Pcs in a variety of new technological fields such as blue-green dyes\(^7\)–\(^8\), industrial\(^9\), technological\(^10\)–\(^11\), liquid crystals\(^12\), Langmuir–Blodgett films\(^13\), particularly attractive features of Pcs is the possibility of tuning its electrical, optical, catalytic and photochemical properties through slight changes on the nature of the peripheral substituents or using different central metal ions in the Pc core\(^14\)–\(^15\). Pcs and their analogs have long been of interest for their interactions with DNA because of their role in the human body, ability to accumulate in many kinds of cancer cells, as well as their magnetic and optical properties. Studies on the interaction of cationic Pcs and their analogs with DNA have received interest in recent years\(^16\)–\(^23\). Water soluble cationic Pcs and MPcs and their binding to DNA have become important subjects of interest in the search of new DNA-targeting drugs\(^24\)–\(^25\). It is time to provide a survey of a number of new important developments in this fascinating area of phthalocyanine chemistry.
In recent decades, most scientific activity in the search for outstanding catalysts has been driven primarily by the demands of fuel cell technology. The ORR at the cathode is of great importance for the overall performance of fuel cells. Platinum is currently the most effective electrocatalyst for the ORR, but its high price and extreme scarcity prevent the wide application of commercial fuel cells in our daily life. Its poor tolerance to crossover effect is another fatal factor for the whole performance of a fuel cell. Thus, it is necessary to find efficient, durable and cheap alternatives to Pt and Pt-based catalysts. Recent studies have implied that transition metal N4-macrocycles are promising catalysts for the ORR, and first row transition metal (notably Fe and Co) phthalocyanine complexes have proved excellent electrocatalysts. One of the means of tuning the redox properties of MPc complexes is by introducing substituents on the peripheral positions of the Pc rings.

In particular, very few reports on the array of 1,3,4-oxadiazole substituted Pcs are available with insolubility in most organic solvent, which encouraged us to investigate their structures in soluble form. However, it's very difficult for the Pc without solubility in finding their potential applications in medicine. Solubility is key characteristic specialist for Pcs and the majority of their behaviors are admirably explored in soluble form and it must be essentially designed in such a way so that the final Pc derivatives are adequately soluble to achieve the desired application. In order to overcome the insolubility of unsubstituted Pc parent molecule, peripheral groups have been extensively used to enhance solubility. In this sense, long alkyl, alkyloxy, alkylsulfanyl or bulky a polar groups lead to soluble products in common organic
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Solvents while anionic or cationic substituents (e.g., sulfo groups, carboxy groups, ammonium groups) result with products soluble in aqueous media.40,41

2. The Present Work

Tremendous efforts have been paid toward the development of new Pc molecular materials as well as toward their applications. Recent emphasis in both academic researches and technical field has been put on the design and synthesis of new water soluble Pc. Herein we embellished the synthesis of 2(3),9(10),16(17),23(24)-tetra-{bis(4-hydroxyphenyl)-butane-[1,3,4]-oxadiazole}-metallophthalocyanine and characterization by FTIR, 1H NMR, ESI-Mass spectrometry and elemental analysis. Substituted MPcs were bearing eight phenol groups which enable the molecules to dissolve in aqueous media. The water soluble MPcs applications have been especially investigated in water and in buffer solutions in order to follow the aggregation property of these compounds. The aim of our ongoing research is to synthesis water-soluble nickel(II), cobalt(II) and copper(II) Pc complexes were employed as an effective DNA binding with calf thymus DNA (CT DNA), since nickel(II), cobalt(II) and copper(II) Pc complexes show modest cleavage sensitivity activity. Our work suggests that these are highly water-soluble Pcs are promising sensitizers for the PDT of tumors studies. In vitro microbiological activity and free radical scavenging activity of MPcs were also performed.

Synthesized highly durable water soluble substituted cobalt Pc subjected for non precious ORR catalyst. The complete reduction of O₂ to water via four-electron transfer was confirmed by cyclic voltammetry and RDE voltammetry. This work demonstrates that
proper application of electrocatalyst can considerably improve the O₂ reduction efficiency.

3. Experimental Protocols

3.1. Chemicals

All reactions and purification processes were carried out according well known literature and all solvents were dried by molecular sieves or proper methods⁴². Valeric substituted MPcs was prepared according to the literature²⁶-²⁷. Precursors 4,4-bis(4-hydroxyphenyl)valeric acid (99%), hydrazine hydrate(99%), poly phosphoric acid, trimellitic anhydride (98%), dry methanol, sulphuric acid (98%) were purchased from Sigma-Aldrich and used without further purification. All chemicals used for the synthesis were of reagent grade and the intermediates were prepared as per the known literature procedure.

3.2. Phthalocyanines Characterization Techniques

We discussed in detailed already see chapter 2.

3.3. Synthesis

3.3.1. Synthesis of 4,4-bis(4-hydroxyphenyl)butane hydrazide

4,4-bis(4-hydroxyphenyl)pentanoic acid (1) (5 g, 0.018mmol) and moisture free ethanol (50ml) in H₂SO₄ (catalytic amount) were refluxed at 55°C for 10h. The reaction was cool to room temperature, poured to ice water, filtered off, to yield methyl 4,4-bis(4-hydroxyphenyl)pentanoate (2). Compound 2 (5 g, 0.018mmol), 10ml of dry methanol and hydrazine hydrate were refluxed for 10 h at 55°C. The reaction mixture allowed cool to
ambient temperature, washed with n-hexane and then filtered off. The crude product was evaporated and washed with ether and hexane to get 4.8 g of solid. This compound (3) is soluble in water, methanol, THF, DMF and DMSO. Anal. 4,4-bis(4-hydroxyphenyl)butane hydrazide: C_{17}H_{20}N_{2}O_{3}: Calc(%) C, 67.98; H, 6.71; N, 9.33; O, 15.98. Found: C, 67.89; H, 6.68; N, 9.30; O, 15.95. IR absorption bands (cm\(^{-1}\)): 3322 (ph-OH), 3318 (Aliphatic -NH), 2882 (Aliphatic CH), 2327 (Ar-CH), 1615(C=O), 1530 (C=C). \(^{1}\)H NMR (300 MHz; DMSO-d\(_{6}\)): \(\delta\)H, ppm 1.14 (3H, S, -CH\(_{3}\)), 1.758-2.186 (4H, T, -CH\(_{2}\)), 4.09 (2H, S, -NH\(_{2}\)), 6.6-6.9 (8H, m, Ar-H), 8.170 (1H, S, -NH), 8.880 (2H, S, -OH). LCMS: m/z Calc. 300.35; Found (M+H) 301.15.

3.3.2. General procedure for the synthesis of 2(3),9(10),16(17),23(24)-tetra-{bis(4-hydroxyphenyl)-butane-[1,3,4]-oxadiazole}-substituted MPcs

2(3),9(10),16(17),23(24)-tetra-{bis(4-hydroxyphenyl)-butane-[1,3,4]-oxadiazole}-substituted MPcs (4) was prepared by mixing a total of (100.0 mg 0.001 mmol) and 4,4-bis(4-hydroxyphenyl)pentanehydrazide (3) (12.0 mg, 0.006 mmol) in polyphosphoric acid (20 mL). The mixture was heated to 180°C for 24h. After completion the mixture was poured into ice cold water and precipitated by salt out method, filtered off and dried in a vacuum. Finally, the crude product was washed with methanol (3 x 5 mL) and acetone (3 x 5 mL), and vacuum dried finally.

3.3.3. Synthesis of 2(3),9(10),16(17),23(24)-tetra-{bis(4-hydroxyphenyl)-butane-[1,3,4]-oxadiazole}-nickelphthalocyanine (5)

Yield: 91%. Anal. for 2(3),9(10),16(17),23(24)-tetra-{bis(4-hydroxyphenyl)-butane-[1,3,4]-oxadiazole}-nickelphthalocyanine: C\(_{104}\)H\(_{86}\)N\(_{16}\)O\(_{12}\)Ni: Calc(%): C, 69.22; H, 4.47; N, 12.42; O, 10.64; Ni, 3.25. Found: C, 69.20; H, 4.44; N, 12.39; O, 10.60; Ni, 3.19. IR
absorption bands (cm\(^{-1}\)):
3200 (ph-OH), 2898 (Aliphatic -CH), 2044 (Ar-CH), 11602 (C=N), 1510 (C=C). \(^1\)H NMR (D\(_2\)O):
\(\delta\): 7.17 to 7.75 (44H, Ar-H), \(\delta\): 3.85-3.88 (8H, CH\(_2\)), \(\delta\): 3.56-3.60 (8H, CH\(_2\)), \(\delta\): 2.15 (3H, CH\(_3\)), \(\delta\): 2.07 (3H, CH\(_3\)), \(\delta\): 1.08-1.18 (6H, CH\(_3\)). ESI-MS: m/z Calc. 1804.5; Found (M+H) 1805.8.

3.34. Synthesis of 2(3),9(10),16(17),23(24)-teta-{bis(4-hydroxyphenyl)-butane-\[1,3,4\]-oxadiazole}-cobaltphthalocyanine

Yield: 90%. Anal. for 2(3),9(10),16(17),23(24)-teta-{bis(4-hydroxyphenyl)-butane-\[1,3,4\]-oxadiazole}-cobaltphthalocyanine: C\(_{104}H_{80}N_{16}O_{12}\)Co: Calc(%): C, 69.21; H, 4.47; N, 12.42; O, 10.64; Co, 3.27. Found: C, 69.19; H, 4.46; N, 12.39; O, 10.61; Co, 3.18. IR absorption bands (cm\(^{-1}\)): 3437 (ph-OH), 2896 (Aliphatic CH), 2327 (Ar-CH), 1618 (C=N), 1515 (C=C), 1105, ESI-MS: m/z Calc. 1804.8; Found (M+H) 1806.6.

3.3.5. Synthesis of 2(3),9(10),16(17),23(24)-teta-{bis(4-hydroxyphenyl)-butane-\[1,3,4\]-oxadiazole}-copperphthalocyanine

Yield: 91%. Anal for 2(3),9(10),16(17),23(24)-teta-{bis(4-hydroxyphenyl)-butane-\[1,3,4\]-oxadiazole}-copperphthalocyanine: C\(_{104}H_{80}N_{16}O_{12}\)Cu: Calc(%): C, 69.03; H, 4.46; N, 12.39; O, 10.61; Cu, 3.51. Found: C, 68.98; H, 3.43; N, 12.34; O, 10.58; Cu, 3.49. IR absorption bands (cm\(^{-1}\)): 3127 (ph-OH), 2882 (Aliphatic CH), 2341 (Ar-CH), 1603 (C=N), 1516 (C=C), 1221. ESI-MS: m/z Calc. 1809.4; Found (M+H) 1810.8.

3.4. Interaction with Calf Thymus DNA (CT DNA)

The interaction of metal complexes with CT DNA was studied by the absorption spectroscopic method. The absorption titrations were carried out in Tris–HCl buffer (5 mM Tris–HCl/50 mM NaCl, p\(H\) 7.5). Calf thymus DNA (CT DNA) was purified by centrifugal dialysis before use. CT DNA solution at p\(H\) = 7.5 gives a ratio of UV
absorbance at 260 and 280 nm of about >1.86, indicating that the DNA was sufficiently free from protein contamination\textsuperscript{43,44}. The concentration of CT DNA was determined by monitoring the UV absorbance at 260 nm using $\varepsilon_{260} = 6600 \text{ mol}^{-1}\text{cm}^2$. The stock solution was stored at 4°C and used within one day. The spectrophotometric titration was done by maintaining the concentration of the complex constant and varying the concentration of CT DNA in interaction medium. The binding constant $K_a$ was determined\textsuperscript{45} by given in Equation 1:

$$\frac{C}{\Delta\varepsilon_a} = \frac{C}{\Delta\varepsilon_a} + \frac{1}{\Delta\varepsilon K_a}$$

$C$ is the concentration of DNA, $\Delta\varepsilon_a = [\varepsilon_a - \varepsilon_d]$, $\Delta\varepsilon = [\varepsilon_b - \varepsilon_i]$, and $\varepsilon_a$, $\varepsilon_b$, and $\varepsilon_i$ correspond to the apparent extinction coefficient, the extinction coefficient of the bound form and that of free of compounds 5, 6 and 7 respectively.

3.5. Interaction with pBR322 plasmid DNA

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 NaCl buffer, at pH 7.4 in the absence of an external agent examined under physiological pH and temperature at 37°C for 2h.

3.6. Antimicrobial screening

The antimicrobial activities of the synthesized compounds were determined against Gram positive \textit{Staphylococcus aureus}, Gram negative, \textit{Klebsiella pneumonia}, \textit{Pseudomonas aures}, and \textit{Escherichia coli}. Against two fungal strains \textit{aspergillus niger} and \textit{candida albicans} by using agar well diffusion method\textsuperscript{46}. The in vitro antibacterial activity measurements were carried out against 24h cultures of bacterial strains. Sensitivity plates
were inoculated with microbes and the well was loaded with 75 µl of test compound solution using a sterilized micropipette. The zone of inhibition was compared with standard drug after 24 h of incubation at 37 ± 2°C for antibacterial activity and 72 h at 25± 2°C for antifungal activity. Three replicas were made for each treatment. To evaluate the effect of concentration on antibacterial activity, three different concentrations (100, 250 and 500 µg/ml) of the test compounds were used. DMSO, which exhibited no antibacterial activity, was used as a negative control.

3.7. Minimum Inhibitory Concentrations (MIC)

The MIC value for the water soluble Pcs which showed positive result towards antimicrobial 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. 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 (5-7) at different concentrations viz. (0-5000 µg/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 bacterial stain and 72h at 25°C for fungal stain.
3.8. Antioxidant activity

The radical scavenging ability of synthesized and the ascorbic acid (standard) was tested on the basis of radical scavenging effect on DPPH free radical. Different concentrations (5, 10, 25, 50, 100 and 200 µg/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 X 100 where A is absorbance of the DPPH and B is the absorbance of the DPPH in the standard combination.

4. Results and Discussion

4.1. Synthesis and Spectroscopic Characterization

The synthetic route for new water soluble compounds is depicted in scheme 1. The key compound 2(3),9(10),16(17),23(24)-tetra-[bis(4-hydroxyphenyl)-butane-[1,3,4]-oxadiazole]-substituted MPcs (5-7) were synthesized from 4,4-bis(4-hydroxyphenyl)pentanehydrazide (3) and tetracarboxy MPcs (4). Polyphosphoric (PPA) acid was added to the finely grounded mixtures of 4,4-bis(4-hydroxyphenyl)pentanehydrazide (3) and tetracarboxy MPcs (4). The reaction was stirred at 150°C for 24h. The blue color solution was changed into green color during complexation of substitution on the Pc core. The mixture was cooled to room temperature, the product was precipitated by salt out, the mixture was filtered and then
the green solid filtrate was washed three times successively with hot acetone, methanol, diethyl ether and n-hexane. The green product was soluble in water due to the introduction of eight phenol groups having hydrophilic character on the peripheral position of Pc ring. The resulting hygroscopic desired product was dried over phosphorous pentoxide. The characterizations of MPcs (5-7) were carried out by FTIR, $^1$H NMR, ESI MS, UV-Vis spectra and elemental analysis. All results of MPc complexes are consistent with the assigned formulations (Scheme 1). TGA was used to determine the thermal stability of these complexes.

Scheme 1 Synthesis of 2(3),9(10),16(17),23(24)-tetra-{bis(4-hydroxyphenyl)-butane-[1,3,4]-oxadiazole}-substituted metallophthalocyanine (5-7).

The IR spectra of (5-7) evidently connoted the presence of the OH groups in Pcs was confirmed by FT-IR is as shown in figure 1, which shows a broad peak at 3135.49-3444.92 cm$^{-1}$ (OH), 1608.61-1663.03 cm$^{-1}$ for C=N in plane skeletal vibrations of the Pc core. 1545-1505 cm$^{-1}$ for C=C stretching, 1418-1372.27 cm$^{-1}$ for C-C stretching in
isoindole, In the IR spectra of the MPcs, weak bands observed above 3000 cm\(^{-1}\) are due to aromatic C-H stretching. The IR absorption bands of synthesized complexes between 2892 and 2818 cm\(^{-1}\) are due to the aliphatic C-H stretching vibrations in macrocycles ring.

![Figure 1. IR spectra of compound (5-7).](image)

\(^1\)H NMR spectrum in the D\(_2\)O confirmed the proposed structure is depicted in the figure 2. The spectra for the compound (5) appeared broad signals at \(\delta\) 7.756-7.174 ppm, integrating for 44 protons for aromatic protons of Pc complex.

![Figure 2. \(^1\)H NMR spectrum of compound (5).](image)
In the $^1$H NMR spectrum -OH groups disappeared as expected, because spectral analysis
carried out in $\text{D}_2\text{O}$, signals at $\delta$ 3.886-3.851 and $\delta$ 3.600 to 3.565 ppm integrating for 16
$\text{CH}_2$ protons. The signals belong to methane (CH$_3$) total 12 protons were observed at $\delta$ =
2.15 ppm, $\delta$ = 2.07 ppm and $\delta$ = 1.08-118 ppm. The ESI mass spectral results of newly
synthesized Pes exhibited molecular ion species illustrated in the figure 3-5. Molecular
ion peaks for (5-7) showed good agreement with the calculated values; m/z = Calc.
1804.5; Found (M+H) 1805.8 for compound 5, m/z = Calc. 1804.8; Found (M+H)
1806.6, for compound 6, m/z = Calc. 1809.4; Found (M+H) 1810.8 for compound 7
respectively.

![Figure 3. ESI mass spectrum of compound (5).](image1)

![Figure 4. ESI mass spectrum of compound (6).](image2)
The elemental analysis of results were also consistent with the proposed structures of all synthesized 4,4-bis(4-hydroxyphenyl)pentanehydrazide and phthalocyanine compounds (5-7).

The starting precursor 4,4-bis(4-hydroxyphenyl)butane hydrazide is well characterized by $^1$H NMR spectroscopy and spectrum is illustrated in figure 6.

**Figure 5.** ESI mass spectrum of compound (7).

**Figure 6.** $^1$H NMR spectrum of 4,4-bis(4-hydroxyphenyl)butane hydrazide.
Chemical shifts of aromatic protons appear at around 6.60-6.97 ppm for 8 protons, for CH$_2$ protons were assigned appear at around 1.758-2.186 ppm for 4 protons and for CH$_2$ proton observed at 1.14 ppm at 3 protons. The proton of the -NH$_2$ & -NH were observed at 4.09 & 8.170 ppm respectively. For -OH slightly broad humped peak observed at 8.880 ppm for 2 protons.

The 4,4-bis(4-hydroxyphenyl)butane hydrazide structure was also confirmed by mass spectrum presented in figure 7. The molecular ion peak of 4,4-bis(4-hydroxyphenyl)butane hydrazide good agreement with calculated 300.35 and Found (M+H) 301.15.

![Mass spectrum of 4,4-bis(4-hydroxyphenyl)butane hydrazide.](image)

The IR spectra of 4,4-bis(4-hydroxyphenyl)butane hydrazide was depicted in the figure 8. The spectrum showed a two broad band at 3422 cm$^{-1}$ due to the -OH and 3318 cm$^{-1}$ due to –NH stretching vibrations, the respectively. Carbonyl stretching of group
observed at around 1615 cm\(^{-1}\) and the bands appeared at 2882 \& 2327 cm\(^{-1}\) for Aliphatic and aromatic -CH stretching.

**Figure 8.** Mass spectrum of 4,4-bis(4-hydroxyphenyl)butane hydrazide.

### 4.2. Electronic Absorption Spectra

UV-vis experiments were performed in water and its preeminent indications for Pc system by their UV-visible absorption spectra of nickel (II), cobalt (II) and copper (II) Pc complexes were recorded at room temperature in solution is demonstrated in the figure 9. The absorption spectrum of MPc complexes (5-7) showed characteristic peaks observed in the Q-band region in the range 638 and 696 nm for compound 5, 634 and 689 nm for compound 6, 643 and 703 nm for compound 7 were responsible for the observed green colour of the complexes and provide information about the complete substitutions on the periphery of the Pc core. B-bands at around 298-337 nm were observed due to the transitions from the deeper levels to the LUMO of all the complexes. The UV-vis spectra of MPcs in solution display a strong absorption Q-band, but the Q band absorption is shifted to the longer wavelength as a result of the electron-donating phenolic hydroxyl
substituents and the effect of peripheral substitution on the MPc complexes (5-7). The splitting of the peak observed in the Q-band region may due to the vibronic fine structure in addition to dimeric species in solution.\(^9\)

![Figure 9. Electronic absorption spectra of compound (5-7).](image)

4.3. Thermal stability of Metallophthalocyanines (TGA)

To evaluate the thermal studies of these MPcs (5-7) provided the information about the thermal stability and the decomposition behaviour of the complexes at different temperatures summarized in the figure 10. This analysis was carried out under the nitrogen atmosphere used for compounds (5-7). The thermogravimetric curve for (5-7) shows a mass loss below 100°C due to the loss of water and moisture from the sample with an exothermic decomposition. Thermal stabilities of these compounds were monitored and maximum reductions in mass were at 320-540°C for (5-7) and the TGA curves of -Ln (Ln (-1/\(y\))) versus 1/T obtained are exposed in Figure 11. It was observed that the above said compounds were quite stable up to these temperatures. The changes in
entropy of activation are negative for all the compounds varied from -1 to 1 (kJmol\(^{-1}\)) and kinetic decomposition temperatures obtained from the derivative of the mass-signal is given in Table 1. The observed thermal stabilities of these compounds indicated that they were stable significantly at high temperatures. The thermal stability of these Pcs is an important requirement for many catalytic applications.

![TGA graph of compound (5-7)](image)

Figure 10. TGA graph of compound (5-7)
Figure 11. Plots of -Ln (Ln (-1/y)) versus 1/T for thermal degradation of compound (5-7).

Table 1 The loss of weight at the major decomposition temperature was 99% for Pc
Thermodynamic parameters for 2(3),9(10),16(17),23(24)-tetra-{bis(4-hydroxyphenyl)-butane-[1,3,4]-oxadiazole}-substituted MPcs (5-7).

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<th>Pc</th>
<th>Temperature of decomposition(°C)</th>
<th>DTA&lt;sub&gt;max&lt;/sub&gt; (°C)</th>
<th>E&lt;sub&gt;a&lt;/sub&gt; (kJmol&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>ΔH (kJmol&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>ΔS (kJK&lt;sup&gt;-1&lt;/sup&gt;)</th>
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4.4. DNA-Binding Studies

Titration with electronic absorption spectroscopy is universally employed and an effective method to investigate the binding mode of DNA with a metal complex<sup>50</sup>. The spectra were recorded as a function of the addition of the buffer solutions of pre-treated...
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CT DNA to the buffer solutions of the complexes. The electronic absorption spectra of water soluble MPcs in the absence and presence of different concentration of buffered CT DNA. By adding of DNA, the absorption intensities of MLCT band gradually increased. Moreover, addition of increasing amounts of CT DNA resulted in a decrease of absorbance for each investigated water soluble MPcs representative spectra illustrating this hypochromicity effect with petite red shift and decreases in the absorption maxima of water soluble MPcs with CT-DNA. Compound 5 shows red shift from 635 to 640 nm (5 nm), compound 6 red shift from 630 to 632 nm (2 nm) and compound 7 red shift from 640 to 644 nm (4 nm) respectively. (Figure 12) Metal intercalators bound to DNA usually result in hypochromism and a red shift, due to the non intercalation mode involving a strong π-π stacking interaction between aromatic chromophores and the base pairs of DNA. The extent of the hypochromism in the visible 1 MLCT band is usually consistent with the intercalative binding strength. In order to compare quantitatively, the apparent binding constants (K_a) of the complexes were determined by monitoring the decrease in absorbance with increasing concentration of CT DNA. The K_a values of the complexes 2.29 x 10^5 M^-1, 2.26 x 10^5 M^-1 and 2.2 x 10^5 M^-1 were respectively. These values suggest that all water soluble MPc compounds 5, 6 and 7 has stronger binding affinity for CT-DNA. These values are lower than the apparent binding constant normally associated with intercalation (K_a<10^6), It can be realized from the high percent of hypochromicity that the high strength binding of the prepared complexes with DNA. The investigated complexes may bind to DNA via non-intercalative mode.
Figure 12. Absorption spectral traces of compound (5-7) at room temperature in Tris HCl buffer (pH 7.4) upon increasing amounts of CT DNA (0.179 mM) 13 successive injections. The decrease in absorbance continued (Line 2 (5 mL) to line 7 (35 mL)) until a stable Pc complex formed. Line 9 (35 mL) Line 13 (65 mL) (bold black lines): A stable Pc complex formed when 35 mL(5 x 25) 0.174 mM CT DNA was added to compound 5; Q band absorbance remained constant after 8th addition.
4.5. Interaction With pBR322 Plasmid DNA

It is known that DNA cleavage is controlled by relaxation of supercoiled circular conformation of pBR322 DNA into nicked circular and linear conformations. When electrophoresis is applied to circular plasmid DNA, fastest migration will be observed for DNA of closed circular conformation (Form I). If one strand is cleaved, the supercoil will relax to produce a slower moving nicked conformation (Form II). If both strands are cleaved, a linear form (Form III) that migrates between Forms I and II will be generated. The cleavage abilities of pBR322 plasmid DNA were monitored by agarose gel electrophoresis in a medium of 5 mM Tris-HCl/50 mM NaCl buffer, at 37°C under physiological pH for 2 h. The new series of water soluble MPc complexes (5-7) showed considerable DNA cleavage ability at concentrations (80-100 μM) in the absence of reductant examined. Firstly, the concentration dependent DNA cleavage by the complexes (5-7) was performed. Incubating plasmid pBR322 DNA while an increase in intensities of positive bands which indicate strong conformational changes. Figure 13 show the no cleavage of pBR 322 DNA occurred after incubation at concentrations (80 μM). Figure 14 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. These results
indicate that the process of DNA without the addition of external agents and the cleavage may be closely related to strong dependence on the concentration of the complex.

**Figure 13.** Agarose gel electrophoresis pattern for the showing Cleavage of pBR322 DNA (100µg) incubated with phthalocyanine compound, C, DNA alone; 5, 6 and 7 (DNA + complex).

**Figure 14.** Agarose gel electrophoresis pattern for the showing Cleavage of pBR322 DNA (120µg) incubated with phthalocyanine compound, C, DNA alone; 5, 6 and 7 (DNA + complex).
4.6. Antimicrobial Activity

Currently much attention has been focused to the synthesis of new metal complexes and the evaluation of these agents for antimicrobial activity. In the present work, we wished to test the activity of water soluble MPc complexes against bacterial and fungal stain. Streptomycin (antibacterial) and Flucanazole (antifungal) was used as a standard drug for comparison. The in vitro antibacterial activity of the water soluble MPcs, solvent (buffer) and their all complexes were evaluated against Gram-positive *staphylococcus aureus* and Gram-negative *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Escherichia coli* and in vitro antifungal activity against *A.niger*, and *Candida albicans*. Table 2 illustrate the antimicrobial activity of the synthesized compounds, buffer (blank) and streptomycin, which was used as a control. The results revealed that complex (5–7) was found to be more active as antibacterial than as antifungal as it shows a good zone of inhibition against all tested bacterial isolates. Among the intact synthesized MPc complexes 6 and 7 exhibited pronounced activity against bacterial stain and fungal stain as compared with standard, but the complex 5 least activity observed when compared with standard. The biocidal activity data of the investigated compounds are summarized in table 2. The increase in biological activity of complexes may be due to the enhanced penetration of complexes into the lipid membranes. A possible mode of toxicity increase may be considered in the light of chelation theory which considerably reduces the polarity of the metal ion because of partial sharing of its positive charge with the donor groups and possible p-electron delocalization over whole chelate ring. Such chelation could enhance the lipophilic character of central metal ion and its subsequent permeation through
semipermeable lipid layers the cell which blocks the metal binding sites in the enzymes of microorganisms\textsuperscript{57}.

\textbf{Table 2:} Anti-microbial activity of compound (5-7).

<table>
<thead>
<tr>
<th>Compound</th>
<th>\textit{S. aureus}</th>
<th>\textit{P. aeruginosa}</th>
<th>\textit{K. pneumoniae}</th>
<th>\textit{E. coli}</th>
<th>\textit{A. niger}</th>
<th>\textit{C. albicans}</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>3.7</td>
<td>2.8</td>
<td>2.7</td>
<td>2.8</td>
<td>1.8</td>
<td>1.7</td>
</tr>
<tr>
<td>6</td>
<td>3.8</td>
<td>2.7</td>
<td>2.8</td>
<td>2.8</td>
<td>1.9</td>
<td>1.8</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>DMSO</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Standard</td>
<td>4.1</td>
<td>3.4</td>
<td>3.7</td>
<td>3.6</td>
<td>3.2</td>
<td>3.3</td>
</tr>
</tbody>
</table>

Standard – Streptomycin (antibacterial)

Standard – Fluconazole (antifungal)

4.7. Minimum Inhibitory Concentrations (MIC)

The MIC was defined as the lowest antimicrobial 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 antimicrobial action. The MIC results consistently show that 250 \( \mu \text{g/ml} \) to 7500 \( \mu \text{g/ml} \) caused high inhibitions at low concentration and The MIC values are summarized in table 3.

\textbf{Table 3.} (MIC) of compound (5-7).

<table>
<thead>
<tr>
<th>Compound (5.0 ( \mu \text{g/L} ))</th>
<th>\textit{S. aureus}</th>
<th>\textit{P. aeruginosa}</th>
<th>\textit{K. pneumoniae}</th>
<th>\textit{E. coli}</th>
<th>\textit{A. niger}</th>
<th>\textit{C. albicans}</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>50</td>
<td>200</td>
<td>200</td>
<td>150</td>
<td>600</td>
<td>600</td>
</tr>
<tr>
<td>6</td>
<td>50</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>500</td>
<td>600</td>
</tr>
<tr>
<td>7</td>
<td>75</td>
<td>200</td>
<td>250</td>
<td>200</td>
<td>500</td>
<td>550</td>
</tr>
<tr>
<td>Control</td>
<td>0</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

Owing to the complexes exhibit good DNA binding affinity and DNA cleavage activity, it is considered worthwhile to study the free radical scavenging activity of these compounds. The antioxidant properties of substituted MPc (5-7) derivatives have attracted a lot of interests and have been extensively investigated, mainly in the in vitro systems. The radical scavenging activity of the complex in cell free system is examined with reference to diphenyl-1-picrylhydrazyl (DPPH) radicals, for all the assays, ascorbic acid served as positive control. The plots of concentration of the complex versus suppression ratio (%) A DPPH radical was depicted in the figure 15. The 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 in to DPPH and the activity was found to be dose dependent. Results reveal that newly synthesized MPc complexes 6 and 7 exhibited fruitful DPPH radical scavenging activity than the complexes 5 when compared with standard. So, the antioxidant activity assay results clearly indicate that the newly synthesized MPc is only responsible for the scavenging activity and results were tabulated in table 4.

Table 4. DPPH radical scavenging activity of compound (5-7).

<table>
<thead>
<tr>
<th>Compounds (μg/ml)</th>
<th>Radical scavenging activity (%) of different concentrations (μg/ml) of compound (4-7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>64.96</td>
</tr>
<tr>
<td>6</td>
<td>58.44</td>
</tr>
<tr>
<td>7</td>
<td>58.49</td>
</tr>
<tr>
<td>5</td>
<td>51.36</td>
</tr>
<tr>
<td>5</td>
<td>58.48</td>
</tr>
<tr>
<td>5</td>
<td>58.49</td>
</tr>
<tr>
<td>5</td>
<td>64.96</td>
</tr>
</tbody>
</table>
4.9. Voltammetry Studies

The redox properties of compound 6 were examined by cyclic voltammetry (CV), connected to a standard three electrode electrochemical systems equipped with gas flow system and technique measured in phosphate buffer (pH-7). Figure 16 (a) shows the CV of complex 6 at 0.050 to 0.250 Vs$^{-1}$ scan rate, 16 (b) comparing with buffer and 16 (c) changing the potential range at 0.050 to 0.250 Vs$^{-1}$ scan rates. Complex 6 displayed two reductions process and three oxidation process in the phosphate buffer 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$^{58,59}$. This is because electron-donating group should increase the average electron density of the conjugated 18π-electron system of the Pc ring. From figure 16 (a) shows the CV of new water soluble cobalt Pc complex undergoes two reduction process at -0.73 V and -0.33 V with anodic scan and two oxidation process at -0.54 V and -0.23 V. The reduction and oxidation behavior of compound 6 is due to the interaction between the Pc ring and the central metal ions$^{60,61}$, in this V-Co display oxidation at 0.79 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$^{62}$ and these studies clearly...
point out that all of these processes could be ascribed to the Pc ring electron transfer reactions. According to diffusion controlled studies as demonstrated 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. the redox processes of P-Co recorded in this study are indicated as 2\(^+\) Co-V (2\(^-\))/ (+) Co-V (2\(^-\)) and (+) Co-V (2\(^-\))/ (+) Co-V (3\(^-\)) couples, respectively.

**Figure 16 (a), (b) & (c).** Cyclic voltammograms of water soluble P-Co with anodic scan in Phosphate buffer solution containing (pH=7), Scan rates = 0.050-0.250 V s\(^{-1}\).
4.10. Electrochemical Properties of Catalyst

4.10.1. Oxygen Reducing Reaction

The reduction region of the cyclic voltammograms (CVs) of the investigated V-Co (6) catalysts in O₂-saturated 0.5 M H₂SO₄ solution is shown in Figure 17 and 18. The CV for oxygen reduction on MPc modified electrodes shows two reduction peaks due to oxygen reduction to hydrogen peroxide and subsequent hydrogen peroxide reduction to water.⁵⁴ In highly alkaline solutions, the reaction goes to completion with only one peak observed due to four electron reduction of oxygen to water.⁵⁵ In this study, a similar trend in activity was observed. From figure 17 ORR run under the saturated N₂ and saturated O₂ atmosphere. The results 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 -0.004 V for P-Co electrode versus saturated calomel electrode with a scan rate 5 mVS⁻¹ in the saturated O₂ acidic electrolyte.

**Figure 17.** Cyclic voltammograms of V-Co catalysts in 0.5 M H₂SO₄ with Saturated N₂ & Saturated O₂. Scan rate: 5 mVS⁻¹.
4.11. RDE Voltammetry And Electron Number For O₂ Reduction

RDE current-voltage curves recorded in an O₂ saturated acidic solution using the V-Co catalyst were recorded in 0.5 M H₂SO₄ aqueous electrolyte solution with oxygen purging for 30 min. The potential scanning range was from 0.70 to -0.50 V v/s SCE with a scan rate of 0.005 Vs⁻¹. Figure 19 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 V-Co catalyst was significantly positively shifted to -0.004 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 (bₐ), cathodic transfer coefficients for the rate limiting step (n₁αₐ), 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 2.
\[
\frac{1}{j} = \frac{1}{j_k} + \frac{1}{B \omega^2}
\]  

(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 levich equation 3 as follows:

\[
B = 0.62nFD^2v^{-\frac{1}{6}}C_0\omega^2
\]  

(3)

Where \( n \) is the overall electron transfer number, \( F \) is the Faraday constant \((F = 96 485 \text{ C mol}^{-1})\), \( C_0 \) is the Concentration of dissolved \( O_2 \) \((1.1 \times 10^{-6} \text{ mol.cm}^{-3})\), \( D \) is the diffusion coefficient of \( O_2 \) \((1.4 \times 10^{-5} \text{ cm}^2.s^{-1})\), \( v \) is the kinematic viscosity of the electrolyte solution \((0.010 \text{ cm}^2.s^{-1})\) and \( \omega \) is the rotation rate represented by rpm. From figure 20 the slopes of the K–L plots depicts at potentials in the 0.3 to 0.4 V v/s SCE range, we could estimate the number of electron \((n)\) involved in the pathway of \( O_2 \) reduction. This result indicates ORR in acidic medium showing high catalytic activity of the anchoring V-Co catalyst increases the amount of electrons per oxygen molecule transferred. It is desirable that the reaction goes to completion and forms water via a four-electron transfer mechanism\(^{66-69} \).
Figure 19. RDE Curves of V-Co in catalysts in 0.5 M H₂SO₄ with Saturated O₂: With different speed at a Scan rate: 5 mVs⁻¹.

Figure 20. The K–L plots of the V-Co electrode derived from RDE measurements.

ORR is effectively improved catalytic performance is ascribed to the electron accepting ability of substituted Pc²⁰,²¹ and favoring the major production of water in direct four-
electrons pathway product of the ORR. V-Co showed that more potent than unsubstituted and substituted cobalt Pc.

4.12. Tafel Analysis

Tafel slopes of ORR catalyzed by V-Co complexes using plots obtained in the linear region of log (i) vs E plots were used to calculate approximate values for $b_a$ and $n_a\alpha_a$ was depicted in figure 21. These tafel slopes at the lower overpotential region (where $E > 0 \text{ V vs SCE}$) for V-Co catalysts are 77 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 $E < 100 \text{ mV vs SCE}$), the Tafel slope for V-Co was 140 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 5.

**Table 5.** 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>Catalysts</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></td>
<td></td>
<td></td>
<td></td>
<td>Low Potential</td>
<td>Higher Potential</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Low Potential</td>
<td>Higher Potential</td>
</tr>
<tr>
<td>V-Co</td>
<td>4.0</td>
<td>$7.75 \times 10^{-4}$</td>
<td>-0.004</td>
<td>70</td>
<td>142</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.69</td>
<td>0.39</td>
</tr>
</tbody>
</table>

Temperature- Room Temperature, Weight % (1 mM) - 5μl
5. Conclusion

In the presented work, the syntheses of new peripherally phenol substituted Ni(II), Co(II) and Cu(II) Pc complexes were described and these new complexes were characterized by FT-IR, $^1$H-NMR spectroscopy, electronic spectroscopy and mass spectra as well as elemental analysis. All studied Pc complexes (5-7) have good solubility in water. The in vitro DNA binding profile of water soluble MPcs were carried out by UV-visible absorption techniques to examine its effect on DNA binding propensity. These studies reveal that non intercalative mode of binding. The complex has been found to promote cleavage ability of pBR322 plasmid DNA. In addition, the complex also exhibited good radical scavenging activity against DPPH radicals. Further, the complexes were showed considerable in vitro antimicrobial activity and exhibited varying degree of inhibitory effects on the growth of bacterial and fungal strains.
Compound 6 was known to be the most active for the oxygen reduction reaction in acidic medium. RDE experiments and Tafel slope analysis suggested that both are four-electron reaction pathways for O₂ reduction. Signifying a good alternative catalyst for Pt-based, unsubstituted and cobalt Pcs for the oxygen reduction reaction.

Reference

42. Armarego WLF, Chai CLL. Purification of Laboratory Chemicals, third ed, Butterworth/Heinemann, Tokyo, 2003.