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Synthesis of Substituted N_4-macrocycle, Characterization and their Electrochemical Determination of Phenolic and Substituted Phenolic Compounds and Biological Applications

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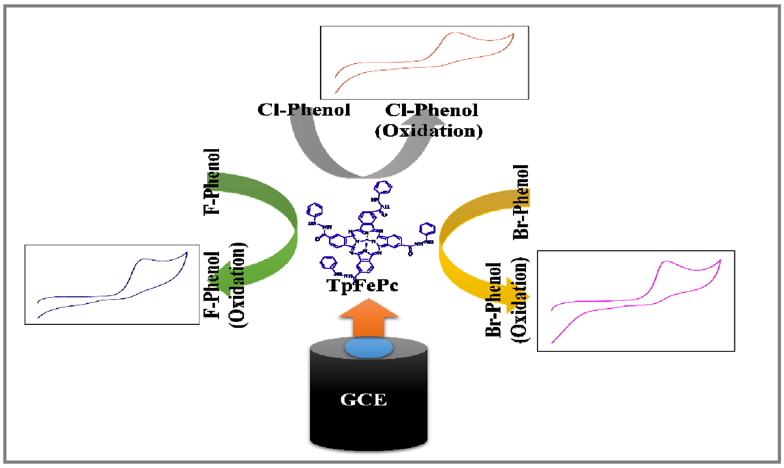
ABSTRACT

Novel polar, electroactive N_4 macrocycle with tetra phenyl hydrazine substituted metal phthalocyanine complexes (TpMPcs) have been reported for the first time and were characterized by elemental analysis, UV-Visible, FT-IR, and X-ray powder diffraction, thermo gravimetric (TGA) and differential scanning calorimetry (DSC) analysis. The synthesized N4- macrocycle compounds were screened for antimicrobial, antioxidant activity and electrochemical investigation using cyclic voltammetry. The TpCoPc modified glassy carbon electrode (GCE) was used for the detection of phenol and substituted phenols. Modified GCE increases the oxidation current of these species and increases the stability of the electrode and showed less fouling by the oxidation products than the unmodified GCE.

Graphical Abstract



Keywords: Metallophthalocyanines, Spectroscopy, Antimicrobial, Antioxidant, Phenol, Substituted phenols, Cyclic voltammetry.

INTRODUCTION

Phenolic compounds are typical hazardous and non-biodegradable effluents, which are commonly used as industrial chemicals and are by-products in many industrial productions [1]. Phenols and substituted phenolic compounds in natural waters have toxic effects on animals, plants and human beings cause undesirable taste and odor in drinking water even at low concentrations. Analysis of water for phenolic compounds is of vital important since many of these compounds have established a reputation for their toxicity and persistence in the environment. Phenols and substituted phenols are commonly employed in the manufacture and processing of plastics and plasticizers, resins, explosives, drugs, detergents, paper, herbicides, insecticides, algaecides, bactericides, molluscicides, fungicides, preservatives, dyes, paints, lubricants [2, 3]. Aromatic compounds are generally toxic and those having a quinine structure_exhibited very high toxicity. Removing phenol from aqueous effluents by liquid-liquid extraction is not economically viable. Furthermore, biological processes are slow, [4, 5] hence, an alternative and low cost techniques were needed to destroy these pollutant. Electrochemical methods have been successfully used in the detection of phenolic compounds because phenols are oxidized at readily accessible potentials. There have been several reports on the voltammetric behavior of phenol [6-8] and cresols. Mechanisms for the oxidation of cresols and phenols have been proposed [9, 10]. The free radical formed during the initial one-electron oxidation of phenol or cresol and the coupling of the free radicals followed by intermolecular rearrangements resulted in the formation of electroactive dimeric products and long-chain polymeric species [11]. This coupling is more favoured in lower concentration of acid media. The main disadvantage of the use of voltammetry for the detection of phenol compounds is the fouling of the electrode by the dimeric or polymeric oxidation products [12]. Thus the voltammetric response for the detection of the phenol compounds decreases on the second and subsequent scan, making the use of electrochemical methods problematic for accurate determination. Many investigations have been proposed for solving this problem. The best results were obtained by the modification of the electrode surface with polymeric substances. Substituted N_4-macrocyclic complexes were the best candidate for the detection of many substances including thiols, amino acids nitrates, and nitrites [13-14]. Electrochemical deposition is a good technique to prepare highly functionalized catalytic surfaces. GCEs modified using phthalocyanine compounds shows an increase in the electrocatalytic activity of the electrode and also the stability of the GCE [15, 16]. Phenyl hydrazine rings are the important class of high performance electroactive amide bridged complexes, which are of special interest for the production of advanced materials due to their high thermal oxidative stability, which is better known for the strong electron-withdrawing character. Many properties of Pcs change upon aggregation. In this consequence, a substantial number of Mpcs with sterically demanding substituent's have been reported. In the present work we report the synthesis of TpMPcs which shows enhanced solubility of Pcs in organic solvents and reduce their tendency to aggregate. Further, the title complexes were investigated for electrochemical detection of phenols and substituted phenols using modified GCE.

MATERIALS AND METHODS

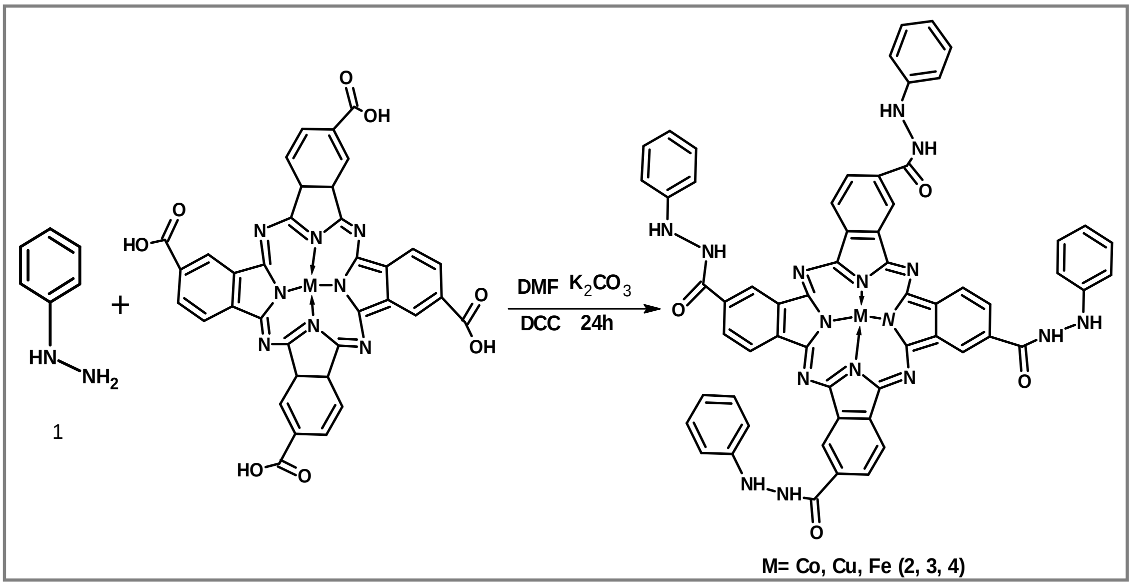
Materials: Metal salts, trimellatic anhydride (TMA:$C_9H_4O_5$), urea ($CO(NH_2)_2$), ammonium chloride (NH_4Cl), ammonium molybdate ($(NH_4)_6Mo_7O_{24}$. $4H_2O$), sodium hydroxide (NaOH), nitrobenzene ($C_6H_5NO_2$), methanol (CH_3OH), ethanol (C_2H_5OH), acetone (CH_3COCH_3) n-hexane (C_6H_{14}), dimethylformamide (DMF), dimethylsulphoxide (DMSO), potasiumcorbonate (K_2CO_3), DCC, phenylehydrazine, sulphuric acid (H_2SO_4) were purchased from sigma-Aldrich and used as received. 4-chlorophenol, 4-bromophenol, 4-flourophenol, 2,4-dichlorophenols where purchased from sigma-Aldrich and used without further purification.

The electrochemical analysis was carried out using electrochemical work station CH1620E with latest windows based acquisition s/w includes power supply 220V/50 Hz. The glassy carbon disks of surface area 0.02 cm^2 were used as working electrode in the modification of electrode and subsequent analyses. The GCE was polished using sequentially with 1.0 µm and 0.5 µm alumina, washed repeatedly in water and ethanol, sonicated for 5 min in an ultrasonic bath.

Synthesis of 2,9,16,23-tetra phenyl hydrazine substituted Co(II)phthalocyanine (2): 2, 9, 16, 23-tetraphenylhydrazine cobalt(II) phthalocyanines complex was synthesized using the reported procedure [17-19] by slight modifications. Phenylhydrazine (0.25 gm, 0.0023 mol), tetra carboxylic cobaltphthalocyanine (0.322 gm, 0.0004 mol), K_2CO_3 (1.19 gm, 0.0086 mol) and DCC as catalyst in DMF as solvent (20 mL) were charged into flask and stirred under nitrogen atmosphere for 24 h at room temperature. The progress of the reaction was observed by the colour change from blue to bluish green and the precipitate was removed by filtration. The crude product was washed thoroughly with hot water and repeatedly with hexane followed by acetone. The product was dried in the oven for 1 h at 65°C to yield bluish green solid. The copper and iron substituted metallophthalocyanines were prepared by taking respective metal salts (Scheme 1). Yield: (84 %) Found: anal. (%) calc. for [$C_{60}H_{40}O_4Co$]: C, 59.28; H, 3.16; N, 18.26; O, 6.12; Co, 4.79. Found: C, 58.18; H, 3.84; N, 18.13; O, 6.85; Co, 4.28. IR. (KBr, cm^{-1}): 3328 (-NH), 2926 - 2844 (Ar-CH), 1627 (C=O), 1619 (C=N), 1524 (C=C), 1521 (C-C), 1315, 1364, 1239 1256, 1148, 1000, 901, 737 and 606 were attributed to the various skeletal vibrations of Pc ring. UV-Visible (H_2SO_4, λ_{max} (nm)): 325, 685.

Synthesis of 2,9,16,23-tetra phenyl hydrazine Cu(II) metallophthalocyanines(3): Yield: (85 %) found: anal. (%) calc. for [$C_{60}H_{40}N_{16}O_4Cu$]: C, 58.49; H, 3.25; N, 18.20; O, 5.20; Cu, 4.77. Found: C, 58.26; H, 4.82; N, 18.16; O, 6.57; Ni, 18.16. IR (KBr, cm^{-1}) 3364-3373 (-NH), 2864- 2924 (Ar-CH), 1627 (C=O), 1626-1678 (C=N), 1545-1575 (C=C), 1436-1442 (C-C), 1364, 1321, 1248, 1128, 1029, 1050, 922, 876, 768, 745, 704 and 643 were attributed to the various skeletal vibration of Pc ring. UV-Visible (H_2SO_4, λ_{max} (nm)): 350, 714.

Synthesis of 2,9,16,23-tetra phenyl hydrazine Fe(II) metallophthalocyanines(4): Yield: (86 %) Found: anal (%) calc. for [$C_{60}H_{40}O_4Fe$]: C, 59.16; H, 3.14; N, 18.33; O, 5.87; Fe, 4.5365. Found: C, 58.76; H, 3.02; N, 18.14; O, 5.16; Fe, 4.26; IR, (KBr, cm^{-1}): 3454 (-NH), 3094, 2947 (Ar-CH), 1684 (C=N), 1592 (C=C), 1342 (C-C), 1246, 1128, 974, 856, 758 and 624.2 were attributed to the various skeletal vibration of substituted Pc ring. UV-Visible (H_2SO_4, λ_{max} (nm)): 348, 746,



Scheme 1. Synthesis of 2, 9, 16, 23, phenylhydrazine substituted phthalocyanine[Co,Cu, Fe].

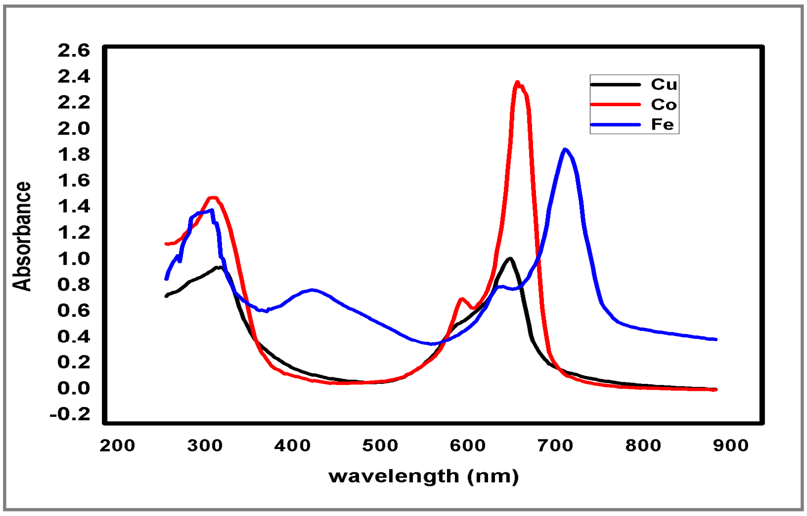


Figure 1. UV-Visible spectra of 2,9,16,23-tetraphenyl hydrazine substitute phthalocyanines(TpMPcs) using 0.1 mM in (28 N) H_2SO_4 (Cu, Co, Fe)

X-ray diffraction studies: Powder X-ray diffraction patterns for the synthesized complexes were recorded with 2θ angle of 7 to 85°C. Highly noisy pattern and broader nature of the peaks indicates that the CoTcPc is amorphous in nature. Peaks with lesser intensities were observed at 2θ values 14.75^0, 27.22^0, 32.96^0, 40.17^0, and 45.02^0, for CoTcPc. d_{hkl} and 2θ values obtained were compared with the values reported in literature (ICDD-PDF Files) and found to be in good agreement for the carboxylic acid phthalocyanine complex. Powder X-ray diffraction patterns of 2,9,16,23-tetraphenyl hydrazine substituted phthalocyanine showed a peak at 7.33^0, 12.57^0, 19.07^0, 22.68^0, 28.906^0, 36.47^0, 48.23^0, 56.03^0, 81.42^0, with maximum intensities at lower 2θ values. TpFePc showed more intense, sharp, well-resolved peaks. In general, the observed patterns are very much similar to those of the unsubstituted phthalocyanines except for broadening of the peaks in case of TpFePc which may be due to the presence of substituent's which seems to play an important role in the stacking of the metal phthalocyanine derivatives (Figure 2).

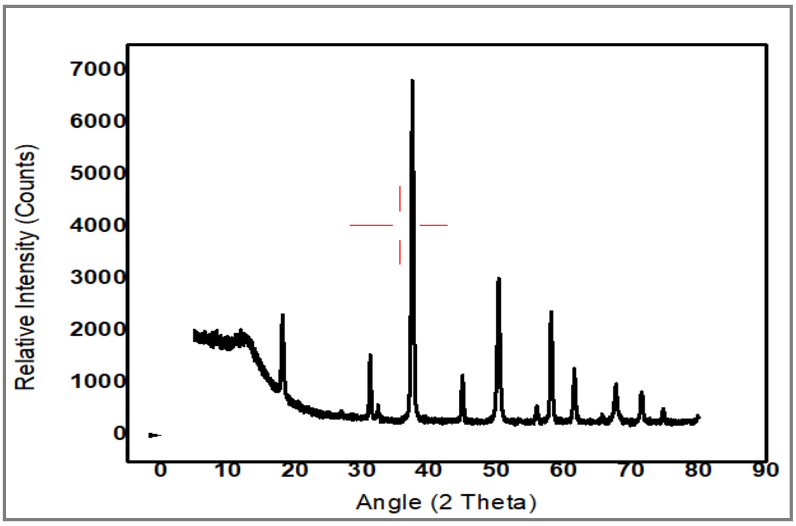


Figure 2. PXRD patterns of 2, 9, 16, 23-tetraphenyl hydrazine substituted iron phthalocyanine (Fe).

Thermogravimetric analysis (TGA)–differential scanning calorimetry (DSC) of the phenyl-hydrazine substituted phthalocyanine: The TGA-DSC technique was used to verify the thermal stability and the initial weight loss for FePc occurring between 25° and 100°C is caused by water evaporation and alcohol used during the preparation step. The second weight loss between 100° and 210°C corresponds to the elimination of residual alkoxide and hydroxyl groups. Above 200°C a gradual weight loss was observed up to 280°C, this corresponds to the complete dehydroxylation of the solids and the decomposition of organic matter, i.e., phthalocyanines and oxalates. The residual mass corresponds to iron oxide [20]. The DSC graphs of FePc showed two endothermic peaks at 134° and 240°C attributed to desorption of water and alkoxide and one exothermal process at 372°C attributed to oxidation of organic matter. As shown in figure 3.

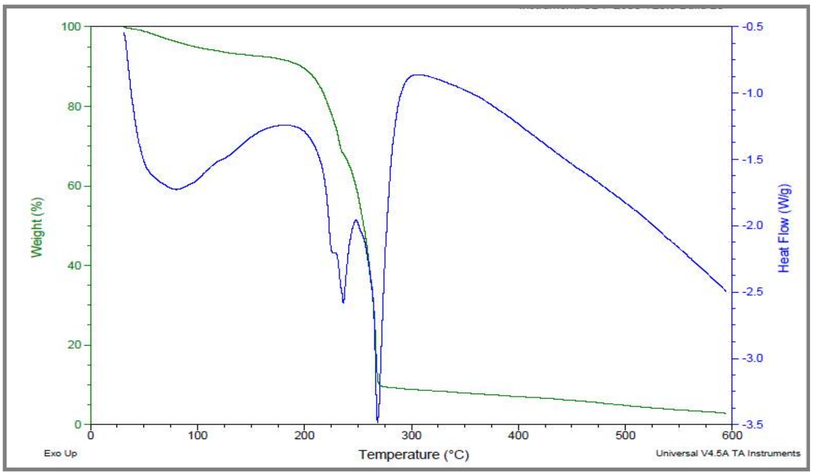


Figure 3. TGA and DSC thermo grams of phenylhydrazine substituted iron phthalocyanine (TpFePc) under nitrogen atmosphere.

RESULTS AND DISCUSSION

The FT-IR spectroscopy of TpMPcs is illustrated in figure 4. The spectrum showed a narrow and broadband at 3364-3373 cm^{-1} attributed to (Ar–NH) starching frequency of the primary amine, 2864-2924 cm^{-1} (Ar-CH) for starching, 1626-1678 cm^{-1} for (C=N), 1545-1557 cm^{-1}for (C=C), 1436-1442 cm^{-1} for (C-C). The peaks at 1328 cm^{-1}, 1248 cm^{-1}, 1074 cm^{-1}, 940 cm^{-1}, 855 cm^{-1}, 732 cm^{-1}, 656 cm^{-1} are attributed to the various skeletal vibrations of Pc ring and IR spectral data support the proposed target structure. The elemental analysis results are consistent with the expected one. Electronic absorption spectra of compound (2-4) are presented in the figure 1. The Q bands were observed at 674, 684, 714nm respectively which was attributed to π- π* transition. The B bands arising from deeper π levels to LUMO were observed at: 314 nm, 323nm, 364 nm respectively (Table 1).

Table 1. The UV-Visible absorption data for synthesized compound

Compounds	Q-band λmax (nm)	B-band λmax (nm)
TpCuPc	674	314
TpCoPc	684	323
TpFePc	714	364

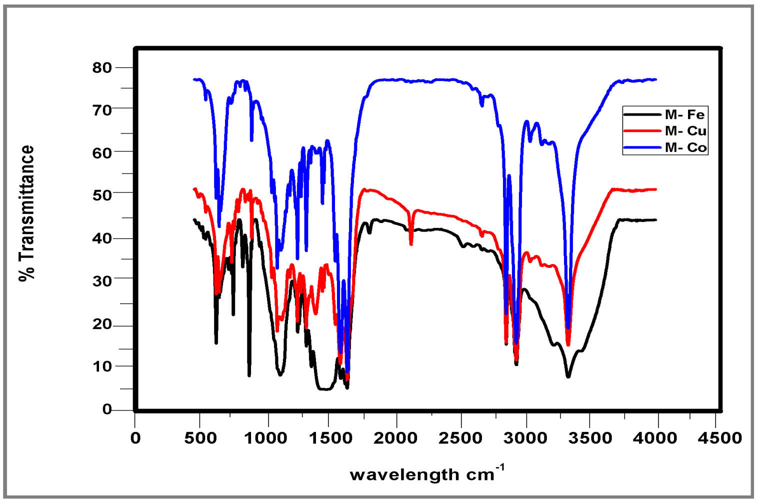


Figure 4. IR spectra of 2, 9, 16, 23-tetraphenyl hydrazine substituted phthalocyanine [Fe, Cu, Co].

Electrochemical behavior of phenol and substituted phenols using TpCoPc modified electrode: Cyclic voltammetry was carried out in a three electrode system consisting of silver /silver chloride and platinum electrodes as reference and counter electrodes, and modified GCE as working electrode or the bare GCE. The modified GCE was prepared by the drop dry cast method in which a drop of a saturated (CA. $1X10^{-3}$ mol^{-3}) solution of substituted phthalocyanine was placed on the glassy carbon electrode (area=0.06 cm^2) and dried in air. Prior to coating the GCE was polished with alumina on a buehler felt pad, followed by soaking in dilute nitric acid and rinsing in distilled water. All electrochemical experiments were performed in 0.05 mol dm^{-3} H_2SO_4 Oxidation potential of phenols and substituted phenols etc., the cyclic voltammograms for TpCoPc-GCE immersed in the blank 0.05 mol dm^{-3} H_2SO_4 solution was presented in figure 5. The TpCoPc-GCE exhibited a single anodic wave when scanned in the blank solution which is similar to CoPc carbon paste electrodes [21]. Small reductions were observed on the reverse scan. The anodic wave was observed at 1.056 V and its peak current is $1.231x10^{-6}$A, vs. Ag/AgCl and was associated with the oxidation of Co(II)Pc to Co(III)Pc species [22].The cyclic voltammogram of phenol in 0.05 mol dm^{-3} H_2SO_4 on bare GCE and on modified-GCE, respectively in figure 5(a) and (b). An irreversible oxidation crest was observed in both cases. Higher currents were observed for phenolic compounds on modified GCE than bare GCE. The bare GCE with phenol gives peak current and peak potential is 1.00 V, and $4.189x10^{-6}$A, but with modified GCE there was an enhanced crest current and crest

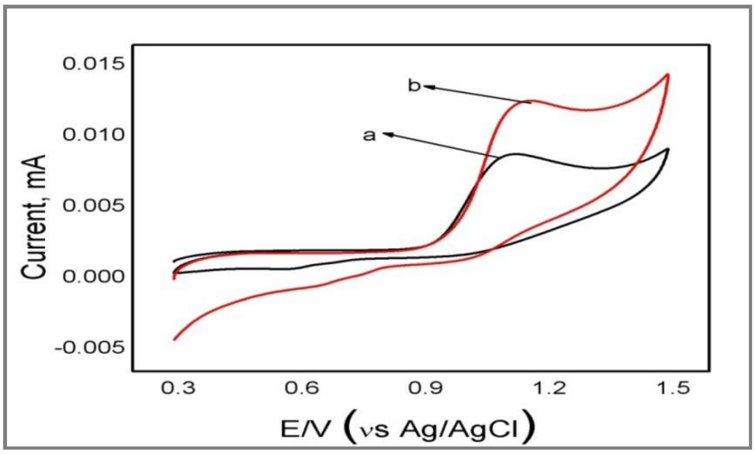


Figure 5. Cyclic voltammetry of (a) 0.00025 mol dm^{-3} phenol on an unmodified modified GCE and (b) 0.00025 mol dm^{-3} phenol on a modified GCE, scan rate 0.05 mV s^{-1}, 0.05 mol dm^{-3} H_2SO_4 electrolyte.

potential 1.005 V and 1.245x10^{-5}A. The small positive shift (0.02 V) was detected for all the phenol compounds under investigation. The cyclic voltammograms of phenol, 4-chlorophenol, 2.4-dichlorophenol, 4-flourophenol, 4-bromophenol, on a modified-GCE exhibits a irreversible oxidation peaks in all cases (Figure 8), but there was no difference in the shapes of the voltammograms of the phenols and its substituted phenols .The stability of the phenylhydrazine substituted modified-GCE (TpCoPc/GCE) towards the determination of phenols and its substituted phenols was illustrated by studying the variation of the peak current response with run number (Figure 6, 7 and 8). The stability of the TpCoPc modified GCE, towards the determination of phenolic and substituted phenolic compounds was carried out by studying the variation of the current response with the number of scans as shown in figure 6. For phenol, 4-bromophenol, and 4-chlorophenol a lessening in anodic peak current with scan number was observed on both the TpCoPc-GCE and the bare GCE (Table 2). However, higher currents were observed on the TpCoPc-GCE then on the bare GCE for similar concentrations of the phenolic compounds for all complexes, indicating a higher sensitivity and stability of TpCoPc-GCE, compared to bare GCE figure 5.

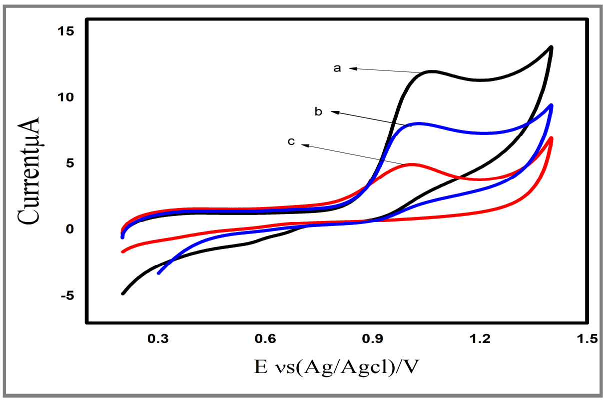


Figure 6. Cyclic voltammetry of (a) 0.00025 mol dm^{-3} phenol on a modified GCE (b) 0.00025 mol dm^{-3} of 2.4 dichloro phenol, and (c) 0.00025 mol dm^{-3} 4-clorophenol on a TpCoPc- GCE, scan rate 50 mV s^{-1}, 0.05 mol dm^{-3} H_2SO_4 electrolyte.

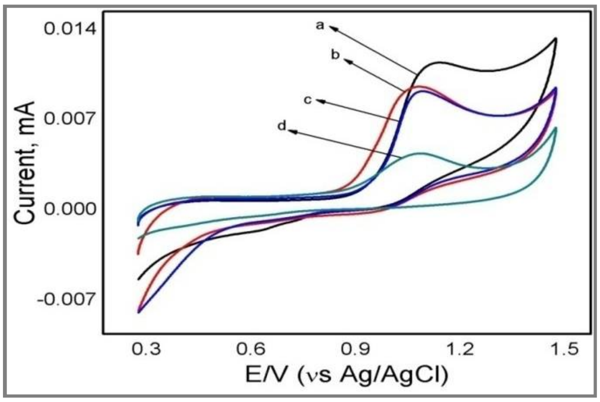


Figure 7. Cyclic voltammetry of (a) 0.00025 mol dm^{-3} phenol on a modified GCE (b) 0.00025 mol dm^{-3} of 4 flourophenol,(c) 0.00025 mol dm^{-3} 4-bromophenol (d) 4-chlorophenol on modified TpCoPc GCE, scan rate 50 mV s^{-1}, 0.05 mol dm^{-3} H_2SO_4 electrolyte.

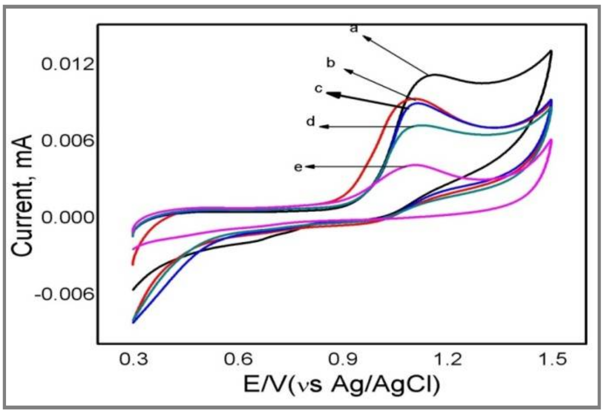


Figure 8. Cyclic voltammetry of (a) 0.00025 mol dm^{-3} phenol on an modified GCE (b) 0.00025 mol dm^{-3} of 4- flourophenol, and (c) 0.00025 mol dm^{-3} 4-Bromophenol (d) 0.00025 mol dm^{-3} 2.4-dichlorophenol (e) 4-chloro phenol on TpCoPc GCE. Scan rate = 50 mV s^{-1}, Electrolyte = 0.05 mol dm^{-3} H_2SO_4.

Generally, all the analysed phenolic compounds get oxidised at different potentials. The oxidation potential was arranged in the following order of Phenol > 4-Bromophenol > 4-chlorophenol>2.4-dichlorophenol > 4-Flourophenol.

Table 2. Peak potential for the oxidation of phenols and substituted phenols on TpCoPc

Compounds	**E_p (V vs. Ag/AgCl)**
Blank	0.84
Phenol	1.06
4-chlorophenol	0.1
2.4-dichlorophenol	0.99
4-Bromophenol	1.05
4-Flourophenol	0.98

Fouling of the electrode materials: All the phenolic compounds, the TpCoPc-GCE was found to be more stable compared to bare GCE for the oxidation of phenols and substituted phenols. The peak current became less than 0.25 µA after fewer runs on bare GCE than on modified GCE. In general there was a decrease in poisoning when the modified electrode was used. This was an important observation because the use of voltammetry for oxidative detection of phenolic compounds results in polymeric products which adhere to the electrode surface, thus causing a sudden decrease of current with increasing scan numbers on the conventional carbon electrodes. The modified GCE was found to be more stable towards the oxidation of phenol than the rest of the phenolic compounds. But there was a significant reduction in peak current after the first run on modified GCE for phenol, the rate of decline of the current slowed down greatly after about five runs (Figure 9, 10, 11). Anodic peak currents higher than 1.2 µA were obtained for phenol even after 180 scans. The oxidation peak for phenol on bare glassy carbon electrode became less than 0.2 µA after about 15 scans. The similar number of scans that could be observed for the oxidation of the phenolic compounds on modified GCE compared to the number of scans observed using bare GCE before the current became lower than 0.25 µA indicate that the stability of the former electrode relative to the later (Table 3). In general, it was apparent that modified GCE is steadier towards the oxidation of phenol since the difference between the number of scans that could be observed on modified GCE and bare GCE was much higher for phenol than for the rest of the compounds. The modified GCE showed the least stability for the oxidation of the 4-chloro phenol.

Table 3. Stability of modified against bare GCEs towards detection of different phenolic compounds (i.e. number of cyclic voltammetric runs before the current becomes less than 0.25 µA, vs Ag/AgCl at50mV S^{-1}, electrolyte = 0.05 mol dm^{-3} H_2S0_4.

Compound	**Modified GCE (No. of Scans)**	**Bare GCE**
Phenol	Over 192	25
4-bromophenol	155	68
4-flouro phenol	140	45
4-chloro phenol	126	14
2.4-dichloro phenol	114	18

The decreased fouling of the electrode when the phenolic and substituted phenolic compounds were determined on phenyl hydrazine substituted Cobalt phthalocyanine-GCE compared to the bare GCE, suggests that the substituted TpCoPcs on GCE inhibits the formation of the dimeric or polymeric oxidation products that are known to poison the electrode, rendering relatively more usable after many scans when compared to bare GCE. This decrease in electrode fouling could be due to some steric hindrance caused by the substituted cobalt phthalocyanine species, minimizing the adsorption of the oxidation products on to the substituted cobalt phthalocyanine modified electrode.

For each of the phenolic compounds under investigation, values of $i_p/v^{1/2}$ (where i_p is the peak current and ν is the scan rate) were constant for scan rates ranging from 10 to 250 mV s^{-1}, showing that the electrode process involved was diffusion of the phenol. The Cvs of all the phenols and substituted phenols showed a characteristic shift of the peak potential with increasing scan rate was typical of totally irreversible systems [23]. Phenol was oxidized at higher potentials (1.06 V) than the 2.4-dichlorophenol, flouro phenol and bromo phenol. The difference in the potentials for the oxidation of these species was a result of the electron-donating and withdrawing abilities of the substituents. Earlier studies [11, 24] on the cyclic voltammetry of the cresols on carbon electrodes have shown that these species exhibit one oxidation peak during the first scan. For O-cresol, m-cresol and phenol, a new oxidation couples on second and subsequent scans was observed by Sharma *et al* [25]. On bare GCE, T. Mafatle et al, observed two new oxidation couples for phenol, m-cresol and o-cresol on the second scan [22]. But in our case we observed one oxidation peak during the first scan for phenols with modified TpCoPC-GCE. In acidic media, the initial electrode process for the oxidation of cresols and phenols is expected [26] be a one-electron oxidation forming free radicals. Free radical pairing is favored in lower concentrated acidic media. Merging of the free radicals of the phenolic compounds are expected to give new electroactive products, following intermolecular rearrangements into dimeric and polymeric products. The new redox couples observed after the first scan for o-cresol, m-cresol and phenol by Sharma *et al*., [25, 27] were assigned to such couplings. Since no new oxidation couples were observed on returns scans when a substituted CoPc-GCE was employed for the

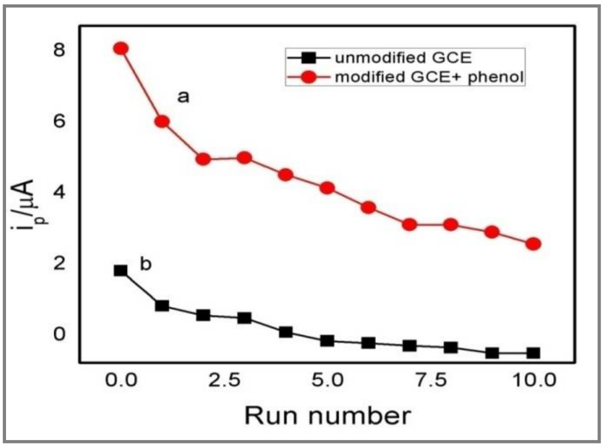


Figure:9. The variation of peak currents with run number for the voltammetric response of 0.00025 mol dm^{-3} of phenol using unmodified GCE and modified GCE with TpCoPc. Scan rate = 50mV s^{-1}, electrolyte = 0.05 mol dm^{-3} H_2SO_4.

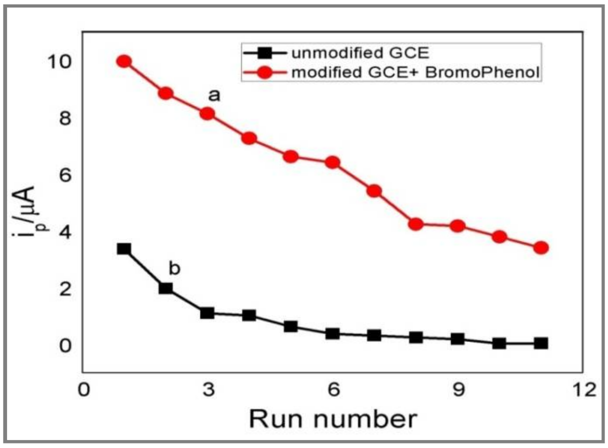


Figure 10. The variation of peak current with run number for the voltammetric response of 0.00025 mol dm^{-3} of 4-Bromo phenol with unmodified GCE and modified GCE Scan rate = 50mV s^{-1}, electrolyte = 0.05 mol dm^{-3} H_2SO_4.

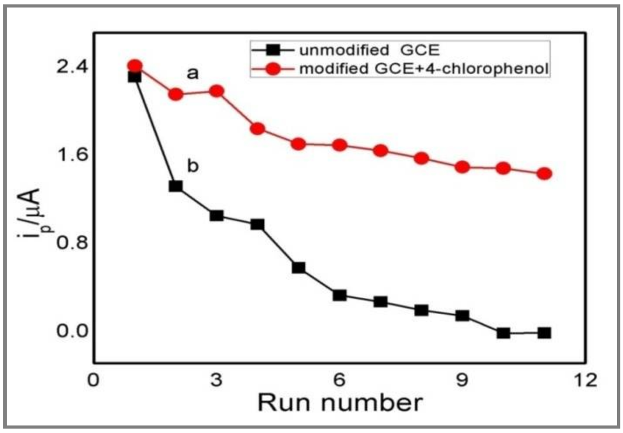


Figure 11. The variation of peak current with run number for the voltammetric response of 0.00025 mol dm^{-3} of (a) 4-chlorophenol with unmodified GCE and (b) modified GCE. Scan rate = 50 mV s^{-1}, electrolyte = 0.05 mol dm^{-3} H_2SO_4.

oxidation of flourophenol, bromophenol, it is likely that the TpCoPc complex on the electrode inhibits coupling of the flour phenol, bromophenol radicals.

The phenol showed higher current ($8.161x10^{-5}$A) on the first scan than flourophenol (5.552 $x10^{-5}$A), bromophenol ($5.463x10^{-5}$A) and 2.4-dichlorophenol ($3.87x10^{-5}$A). Figure 8 for the same concentration. In general, the phenol exhibited higher current responses then 4-florophenol, 4-bromophenol, 2.4-dichlorophenols.

We compared the catalytic activity of TpCoPc towards the oxidation of the phenols and substituted phenols in acidic media to the catalytic activity of other first row transition metal phthalocyanines complexes and of metal-free phthalocyanine. For the same concentrations, the currents detection for the oxidation of the phenols and substituted phenols when the electrode was modified with substituted phthalocyanine much greater currents observed then the unmodified GCE. Also, all the MPc complexes studied show considerable current responses than the unmodified GCE. Table 2 showed that the oxidation peak potentials of the phenolic compounds which detect at more positive potentials than observed for the $CO^{(II)}PC \rightarrow CO^{(III)}$ Pc peak. But based on the fact that enrichment of the oxidation currents of the phenolic compounds on TpCoPc modified GCE, it was suggested that the electron transfer reaction takes place between the phenolic compounds and MPc complexes

$$M^{(II)}\,Pcs \rightarrow (\,[M^{(III)}pcs]^{+} + e^{-} \qquad ..\,(1)$$

$$[M^{(III)}pcs]+ + Ph \rightarrow M^{(II)}pcs + Ph\ \text{oxidation products} \qquad ..(2)$$

Where Ph = 4-bromophenol, 4-Flurophenol, 4-chlorophenol, phenol, and 2.4-di chlorophenol.

Biological Activity of the Substituted Phthalocyanine:

Antibacterial screening: The antibacterial activity of the compounds was tested against gram-positive bacteria *staphylococcus aurous, staphylococcus epidemidis* and *bacillus cereus* and gram-negative bacteria p*seudomonas aeruginosa, Vibrio cholerae and Escherichia coli* by agar well diffusion method. 24 h old Muller-Hinton broth cultures of test bacteria were swabbed on sterile Muller-Hinton agar plates using sterile cotton swab followed by punching wells of 6 mm with the help of sterile cork borer. The standard drug (chloramphenicol, 100 µg mL^{-1} of sterile distilled water), three different concentrations (100, 50 and 25 µg mL^{-1} in 10% DMSO) and control (10% DMSO) were added to appropriate labelled wells. The plates are allowed to stand for 30 min. and were incubated at 37°C for 24 h in upright position and the zone of inhibition was recorded [28]. During this period, the test solution diffused and zone of inhibition were recorded using vernier callipers.

Antimicrobial activity in various concentrations of substituted phthalocyanine complexes were acknowledged in table 4. The inhibitory activity of substituted phthalocyanine complexes was related to the cell wall structure of the microbes which is crucial to the survival of bacteria and some antibiotics are able to kill the bacteria by inhibiting the synthesis of peptidoglycan. Moreover, the low activity of these complexes may be due to their low lipophilicity, because of which penetration of the substituted phthalocyanine through the lipid membrane may decrease and hence, they could neither block nor inhibit the growth of the microorganism. The size of the inhibition zone depends upon the culture of the medium, incubation condition.

Antifungal screening: Antifungal activity of the compounds was evaluated against *Aspergillus aureus* and *Aspergillus fumigates fungus*, using the sabouraud dextrose agar diffusion method [29, 30]. Wells were made (6 mm diameter) with a sterile cork borer. The standard drug (Fluconazole, 100 µg mL^{-1} of sterile distilled water) and control (10 % DMSO) were added to respective labelled wells. To these wells, 140 µL each (100, 50 and 25 µg mL^{-1} in 10 % DMSO) of the test stock solution

Table 4. Antimicrobial activity of the isolated ligand and its metal complexes

Zone of inhibition against bacteria measured in mm																		
Synthesized Compound	***P. aeruginosa*** **(µg mL^{-1})**			***S.aureus*** **(µg mL^{-1})**			***V. cholera*** **(µg mL^{-1})**			***S. epidermidis*** **(µg mL^{1})**			***B. subtilis*** **(µg mL^{-1})**			***E.coli*** **(µg mL^{-1})**		
	25	**50**	**100**	**25**	**50**	**100**	**25**	**50**	**100**	**25**	**50**	**100**	**25**	**50**	**100**	**25**	**50**	**100**
Fe	13	15	17	15	17	18	12	14	16	10	13	16	12	13	14	09	10	12
Co	12	15	17	10	13	15	12	14	16	11	13	15	09	11	13	08	10	13
Cu	10	13	15	12	15	16	10	13	16	09	10	12	13	15	17	11	13	16
Std. (100 µg mL^{-1})	28.00			17.15			16.05			26.03			27.05			22.10		

Std. =Ciprofloxacin

were added and the plates were allowed to cool for an hour to facilitate the diffusion (Table 5). The plates were then incubated at 37°C for 48 h at the end of the incubation period the diameter of zone inhibition around the wells was measured using vernier calipers.

Table 5. Antifungal activity of the substituted phthalocyanines complexes

Synthesized Compound	***A. aureus*** **(µg mL^{-1})**			***A. fumigates*** **(µg mL^{-1})**		
	25	**50**	**100**	**25**	**50**	**100**
Fe	07	09	11	06	07	08
Co	08	10	11	07	09	11
Cu	13	16	18	11	13	18
Std. (100 µg mL^{-1})	29.005			26.50		

Std.- Fluconazole

An interesting observation made during the investigation was the change in the color of fungus sporulation. *A. niger* was known for its black color, however, in the presence of substituted matallophthalocyanine complexes, the fungus displayed a pale brown sporulation. It was confirmed by a parallel experiment both with and without addition of 2 mL DMSO in the medium that the change in color of the fungus was not due to the presence of DMSO in the medium. The color change of the fungus may be due to the effect of substituted phthalocyanine complexes on spore pigmentation [31]. The data showed that the substituted phthalocyanine complexes have the capacity of inhibiting the metabolic growth of the bacterial and fungal strains.

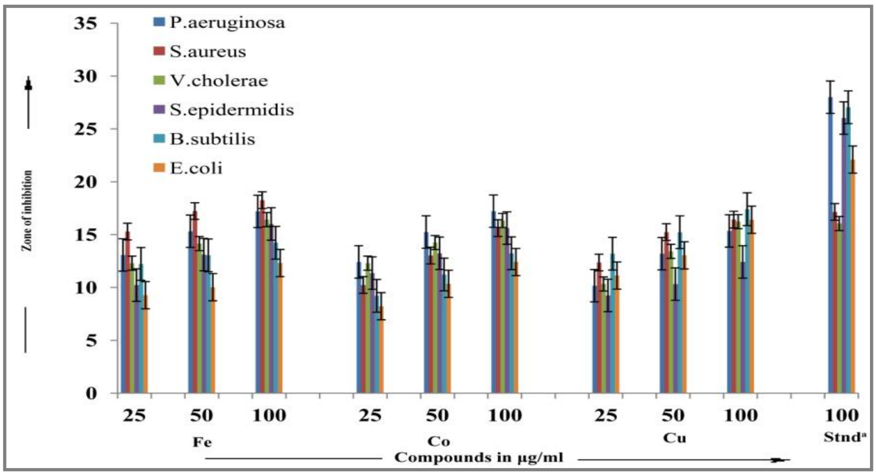


Figure 12. Antibacterial activity of the ligand and its substituted Phthalocyanines complexes.

In the present study, the substituted phthalocyanine complexes are active against the bacteria and fungi. As a result of this, the primary screening against the bacterial strains in various concentrations showed good zone of inhibition in figure 12. The substituted phthalocyanine [Fe, Cu, Co] complexes showed excellent antibacterial activity towards *B. cereus*, *S aureus* and *E. coli* respectively [32], the substituted copper phthalocyanine complexes performs highest antifungal activity against *A. aureus* and *A. fumigates*, the primary screening against the fungal strains in different concentrations showed good zone of inhibition as shown in figure 13. Substituted cobalt phthalocyanine complex showed the least activity against *B. subtilis* bacteria and both fungal strains.

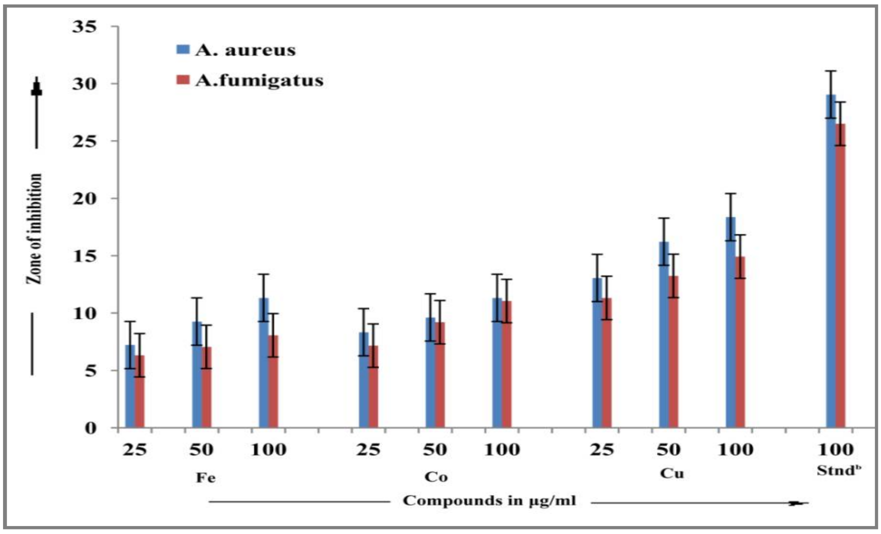


Figure 13. Antifungal activity of the substituted phthalocyanines complexes

Antioxidant activity: This activity for the synthesized compounds was performed using dipenyl-1-picrylhydrazyl (DPPH) method as per literature [33]. The compounds of different concentrations are dissolved in methanol and were introduced to each vial of 5 mL. To this test vials 3 mL of 0.004 % diphenyl-1-picrylhydrazyl (DPPH) in methanol was added and the mixtures was incubated in dark condition at ambient temperature for 30 min. Ascorbic acid is used as the standard. The absorbance reduced while the DPPH is scavenged by way of an antioxidant, through the contribution of hydrogen to shape a strong DPPH molecule. DPPH scavenging activity calculated by the use of the following equation and absorbance measured at 517 nm (Figure 14).

Antioxidant calculation formula:

$$\text{Antioxidant} = \frac{\text{Absorbance of sample} \ \mathbf{-} \ \text{Absorbance of control}}{\text{Absorbance of control}} \times \mathbf{100}$$

Standard DPPH (Control) absorbance will be **0.56**

$$\text{Scavenging ratio (\%)} = [(Ai–Ao)/(Ac–Ao)] \times 100\ \%$$

Where, **Ai** is the absorbance within the presence of the check compound. **Ao** is absorbance of the clean inside the absence of the check compound. **Ac** is the absorbance within the absence of the test compound.

The DPPH radical scavenging activity data represented in table 6, gives strong absorbance at 517 nm. If DPPH abstracts a hydrogen radical from an external source, the absorption decreases stoichiometrically depending on the number of electrons or hydrogen atoms [34, 35] the substituted phthalocyanine complex exhibited highly persuade scavenging activity almost close to the standard ascorbic acid (AA) (Vitamin-C) and Fe^{+2} and Cu^{+2}substituted phthalocyanine complex showed better inhibitions activity and Co^{+2} substituted phthalocyanine showed moderate activity.

Table 6. Scavenging activity

Concentration in mL	**Fe**	**Co**	**Cu**	**Ascorbic acid**
0	-	-	-	-
5	52.33±0.20	25.63±0..5	40.24±0.25	49.10±0.25
10	57.08±0.14	28.40±0.09	44.68±0.26	55.23±0.02
15	60.20±0.12	31.20±0.15	46.63±0.35	62.53±0.15
20	62.30±0.23	38.35±0.12	50.00±0.03	70.31±0.04
25	64.13±0.15	41.84±0.08	53.36±0.10	78.10±0.08

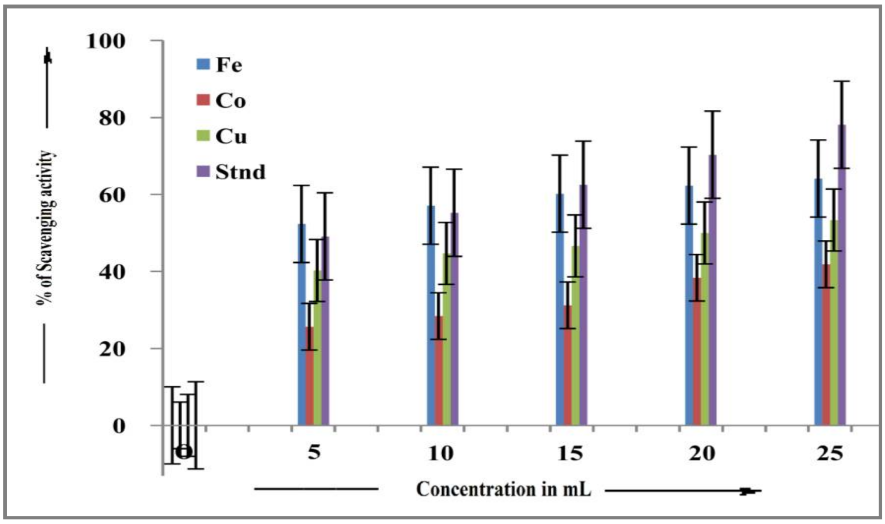


Figure 14. Plots of the radical scavenging effects (%) of substituted phthalocyanines complexes at various Concentrations.

APPLICATION

Control of puling rate after modification of phthalocyanine electrodes phthalocyanine molecules used in bimosensing applications. Phthalocyanine Derivatives are used in Antimicrobial Photodynamic Activity. Phthalocyanine was a versatile complex finds many applications like solar cells, solid-state batteries, chemical sensors, liquid crystals, photovoltaic cells, PDT for cancer therapy.

CONCLUSION

In conclusion, a new series of 2, 9, 16, 23 tetra phenyl hydrazine substituted phthalocyanine of Co, Cu, Fe, Cu, Fe, was synthesized. The FT-IR spectrum confirms the formation of substituted complexes. XRD pattern confirms the amorphous nature. TGA and DSC confirm improved thermal stability. All synthesized complexes shown good activity against the tested bacteria and the catalytic activity of metallophthalocyanines using modified GCE towards phenol and substituted phenols shows excellent results.

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