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Efficacious Detection of Epinephrine in the Midst of an Interference (ASCORBIC ACID) Employing Designed Gold Electrode with Cobalt Octacarboxyphenoxy Phthalocyanines

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ABSTRACT

The modification of gold electrode with cobalt octacarboxyphenoxy phthalocyanines (CoOcPhOPc), for the pH selective detection of epinephrine was investigated. 1,4-phenylene diamine dihydrochloride (PA) was electrografted onto gold electrode and CoOcPhOPc was immobilized on the electrode via amide bond coupling. The modified electrode (Au-PA-CoOcPhOPc) was characterized with voltammetry and impedance studies. The modified electrode demonstrated high sensitivity and good selectivity with low limit of detection (LOD) of 0.27 nM at 3σ, limit of quantitation (LOQ) of 0.9 nM at 10σ and high sensitivity of 0.6 ± 0.01 nA nM^{-1} towards the detection of epinephrine (EP). The reusability, stability and reproducibility of the electrode were confirmed for the detection with cyclic voltammetry (CV). The calibration curves showed very good and strong positive linear relationship with correlation coeffient of +0.99. The calibration curve of the square root of the scan rate confirmed the detection and the process of the reaction to be diffusion controlled.

KEYWORDS: Amide bond, cobalt octacarboxyphenoxy phthalocyanines, neurotransmitters, Epinephrine, Voltammetry, Electrochemical impedance spectrometry.

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1.0: INTRODUCTION

Epinephrine (adrenaline) is a hormone and a neurotransmitter (NT), produced at a very small concentration by both the medulla of the adrenal glands and the neurons. Epinephrine is one of the major neurotransmitters in the neuron, plays important roles in fight-or-flight response of the brain and body. It maintains heart beat rate, sugar metabolism, regulate memory strength, heart and high blood pressure **[1]**. The abnormal level of epinephrine can lead to disagreeable body malfunctions and prompt high risk of disease and death. Early-stage detection could allow for effective treatment and control of the altered level of epinephrine in the body. The concentration of EP in the blood ranges from 0 to 140 pg. mL^{-1} (764.3 pmol.L^{-1}; 0.7643 µmol.L^{-1}) for healthy individual. The instability of the concentration results in anxiety, nervousness and affect memory **[2].**

Analytical methods for the detection and quantification of EP

Both in-vivo and in-vitro diagnostic methods had been developed for the determination of NTs. The in-vivo methods include the microdialysis-capillary, capillary electrophoresis-laser induced fluorescence (CE-LIFD) **[4]**, the fast scan voltammetry **[5]**, neurochips **[6]**, wearable wireless electrochemical instruments **[7]**, and carbon nanotube on carbon fibre microelectrodes (CFME) **[8].** The in-vivo methods involve the immersion of a microdialysis probe into the brain **[3]**. This is invasive, painful to patients and expensive. The only advantage is the in-vivo measurements are online determination of neurotransmitters in that they measure NTs as they are produced and in their natural environment. This is particularly important in monitoring the NTs is their natural environment. These techniques require expensive instrumentation and reagents, they are laborious, tedious and require expertise to carry out and analyse result.

In recent time, electrochemical devices have received research attention for the detection of NTs **[9]**. The electrochemical detection is characterised by its simplicity, fast analytical response and generation of reproducible qualitative and quantitative results. Moreover, the catecholamine NTs are electrocatalytically active making their electrochemical detection easy to pursue. The catecholamines can be oxidised electrochemically at a potential ranging from 200 mV to 700 mV (Ag|AgCl) at physiological conditions. The current intensity or density (i_p) is a measure of NT concentration and the potential where the oxidation can be used for identifying NT under investigation. Randle-Sevcik equation in **Equation 1.1** is useful in relating the current to the concentration of the NT.

$i_p = 2.69 \times 10^5 n^{3/2} ACD^{1/2} \upsilon^{1/2}$ ……………….**1.1**

In which n is the number of electrons transferred in the redox process, A is the electrode area in cm^2, C is the concentration of the NT in mol/cm^3, D is the diffusion coefficient in cm^2/s and υ is the scan rate in V/s.

Electrochemical detection of EP has been challenging for the following reasons:

- The redox characteristics of catecholamine (NTs) are similar at physiological pH; thus, makes it difficult to electrochemically distinguish between the different NTs. This is the major challenge especially working with unmodified or solid electrodes.
- NTs coexists with other electroactive interfering compounds such as ascorbic acid (AA) and uric acid (UA) with similar oxidation potential as NTs.
- The fouling and poisoning of electrode surfaces by the formation of insoluble polymers as the by-product of NTs electrochemical oxidation. The polymers inhibit reusability of the electrode surfaces during the electrochemical detection of NTs.

Therefore, the modification of the electrode surface with materials that could improve the selectivity, prevent electrode fouling, and screen-off interfering substance has been the leading research interest in recent times **[10]**. Various materials have been used for the fabrication of chemically modified electrodes. The materials that have been investigated for electrode modification include metallophthalocyanines (MPcs), metalloporphyrins (MPPs), electroactive polymers and nanomaterials. The electrocatalytic properties of electrode modifiers enhance the current and lowers the potential of the analytes during the detection process **[11]**.

The electrochemical sensor investigated in this study is a gold electrode modified with metallophthalocyanines complexes (MPc) film for the selective detection of Epinephrine in the midst of other Neurotransmitters and the other interference (ascorbic acid).

Ascorbic acid (AA) is an oxidizable metabolite like the neurotransmitter and have similar oxidation potential. The coexistence of AA and epinephrine in the neurofluids makes the accurate detection and quantification of epinephrine very difficult **[12]**. Several methods such as chemiluminescence, flow injection analysis, hromatographic methods and capillary electrophoresis have been reported for the detection of epinephrine. However, these techniques required bulky and expensive equipment, long analysis time and not suitable for point of care application. Electrochemical methods offer greater advantage for the detection of neurotransmitters due to the low cost, design simplicity and faster analysis time.

In this work, electrochemical methods were used, with electroactive cobalt octacarboxyphenoxy phthalocyanines as the electrocatalyst on the gold electrode surface and the electrode screens off the AA and detected epinephrine at ultra-low concentration. The electro-oxidation of epinephrine at the electrode surface was studied in detail with cyclic voltammetry potentiostatic (CV), differential pulse voltammetry (DPV), linear sweep voltammetry (LSV) and electrochemical impedance spectroscopy (EIS). The detection process was pH dependent and the experiments were carried out at the physiological pH of 7.4 **[3].**

Metallophthalocyanines (MPcs) Complexes

Metallophthalocyanines (MPc) are blue-green anisotropic complexes with planar structure.

MPc is aromatic compounds, highly conjugated with 18 π-electrons in the macrocyclic ring [**13]**. The metal ion centre can contain transition metals known for its excellent electroactive properties. The transition metals have varied oxidation states and therefore improve the electrocatalytic activity of MPc-based electrochemical sensors **[14]**.

The Electrochemical properties of MPc

The redox activity of metal-free phthalocyanines was due to the conjugated π-electrons ring system**.** The redox reaction at the ring involves the removal of π-electrons from the highest occupied molecular orbital (HOMO). It also involves the addition of electrons to the lowest unoccupied molecular orbital (LUMO). In the neutral state, phthalocyanines exist as dianion in the form of Pc^{-2}. Each could undergo a redox process due to its ability to gain and donate electrons. Phthalocyanines lose two electrons to be Pc^0 or one electron to Pc^{-1} cationic species.

In its reduced state, it becomes Pc^{-3}, Pc^{-4}, Pc^{-5} and Pc^{-6} due to the addition of electrons to the LUMO of the orbital **[15]**. The band energy between the first oxidation redox potential and the first reduction redox potential of the Pc ring is about 1.6 eV. The extended conjugation in the structure aids the electron transfers in the metallophthalocyanines ring. The bandgap can be tuned by the nature of the conjugation and the substituent attached to the Pc ring. As the conjugated Pi-system increases, the energy gap between the HOMO-LUMO decreases. The Q band also shifted to the longer wavelength **[16]**. The substituents affect the electrochemical properties of the MPc. The substituents that are electron-donating would thereby reduce the HOMO-LUMO gap. In addition, the electron-withdrawing substituents would increase the width of the HOMO-LUMO gap. The metallophthalocyanines modified electrode for detection of neurotransmitters

Modification of electrodes with MPcs protects the electrode surfaces from chemical fouling**,** provides enhanced catalytic properties**,** improves sensitivity and selectivity towards the analyte of interest [**17**].

2.1. MATERIALS AND METHODS

2.2. Materials

1,4-phenylenediamine dihydrochloride (PA), L – ascorbic acid, tetrabutylammonium tetrafluoroborate ($TBABF_4$), (±)- epinephrine hydrochloride, N-Ethyl-N-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), potassium ferrocyanide ($K_4[Fe(CN)_{6,}$ acetonitrile (ACN), and potassium chloride (KCl) were purchased from Sigma-Aldrich. Potassium dihydrogen orthophosphate and di-potassium hydrogen orthophosphate were purchased from Associated chemical enterprises (ACE). Potassium ferricyanide ($K_3[Fe(CN)_6]$) was purchased from United Laboratories Inc.(UNILAB). Absolute ethanol was purchased from SAARChem, hydrochloric acid (HCl) was purchased from Minema and alumina powder was purchased from Allied High Tech. Products. Ultra-pure Milli-Q Water (18.2MΩ) resistivity obtained from Milli – Q water system was used to prepare all chemical solutions.

Phosphate buffers saline were prepared with Milli-Q water, using appropriate amounts of KH_2PO_4, K_2HPO_4 and chloride salts depending on the pH and the molarity [24]. Potassium ferricyanide ($K_3[Fe(CN)_6]$), potassium ferrocyanide ($K_4[Fe(CN)_{6)}$, potassium chloride (KCl) and (±)-epinephrine hydrochloride was prepared with phosphate buffer saline (pH 7.4) solution. cobalt octacarboxyphenoxy phthalocyanines (Au-PA-CoOcPhOPc) was prepared and purified according to the reported procedure with slight modifications **[19].**

2.2. Electrochemical apparatus

All electrochemical measurements were carried out using Metrohm Autolab PGSTAT 302N, B.V. Modular electrochemical work-station interfaced to a proline desktop computer with a Nova 1.10 version software. Three-electrode electrochemical cell was used with gold electrode (1.6 mm diameter, 99.95% purity) MF 2014 purchased from Bio-analytical systems model (BASi) as the working electrode, Ag/AgCl sat'd KCl (3M) 6 mm diameter, 7.5cm long with coral por frit at the tip – (purchased from BASi) as the reference electrode and platinum wire as the counter electrode. pH measurements were performed using Metrohm 827 pH meter.

2.3. Electrode fabrication

The gold electrode was first polished with aqueous slurries of alumina powder (< 2 μm) on a SiC emery paper (type 2400 grit), mirror finished on a Buehler felt pad and then subjected to ultrasonic vibration in ethanol to remove residual alumina nanopowder at the electrode surface. The organic contaminant on the electrode surface was etched in a "Piranha" solution (1:3(v/v) 30% H_2O_2 and concentrated H_2SO_4) for about 2 minutes. It was then rinsed with copious amount of Milli-Q water and finally rinsed with absolute ethanol. The cleanliness was confirmed with cyclic voltammetry. The electrode was electrocleaned in 0.5 M H_2SO_4 and scanned at the potential between -0.5 and 1.0 V (versus Ag/AgCl) at a scan rate of 50 mV/s, until a reproducible scan was achieved. The electrode was then rinsed in absolute ethanol and immediately dipped into the deaerated solvent of ACN /1M HCl (96:4), containing 0.1 M $TBABF_{4,}$ and 1,4-phenylene diamine dihydrochloride (5 mM, 5.40 mg). The solvent was cooled in ice bath to 0^o C and stirred for 5 – 10 minutes. $NaNO_2$ (3 mM, 0.2096 g) was dissolved in milli-Q water (0.5 mL) and added drop wisely and stirred. After 15 minutes, 1,4-phenylenediamine dihydrochloride (PDD) was reductively electrodeposited on the electrode surface.**[18].**

Cyclic voltammetry of electrode modified with 1, 4-phenylene diamine dihydrochloride (PA)

Figure 1 shows the cyclic voltammograms for **(a)** electrochemical reduction of 4-nitrobenzene diazonium salt, and (**b**) electrochemical reduction of phenylnitro group to form phenylamino group. The first scan in the voltammograms in **Figure 1 (a)(i)** showed a cathodic peak at 0.31 V. This reduction peak corresponds to the electrochemical reduction of the diazonium and the formation of the phenylnitro radical with loss of N_2. The radical attached to the gold electrode and formed a thin film of phenylnitro (Au-PNO_2) on the electrode surface when the potential was scanned to the potential that is more negative. The cathodic peak disappeared during the subsequent scans due to the grafting of the radical onto the electrode surface. This confirmed the formation of phenylnitro layer on the Au electrode surface. The use of three CV scans was employed to achieve a thin of monolayer or few multilayers. The formation of a monolayer is idealistic but not achievable even though some reports have claimed the formation of a monolayer **[18]**. It is possible to form a thin multilayer film and a study of distinct number of layer deposition is ideal but not a subject of this work.

Figure 1 (b) shows the cyclic voltammograms for electrochemical reduction of phenylnitro to form phenylamino groups. The first scan in the voltammograms in **Figure 1 (b)(i)** showed intense cathodic peak at -0.92 V (labelled **II**). This cathodic peak corresponds to the reduction of the phenylnitro group (PNO_2) to phenylhydroxylamine group (PHA). The PHA oxidizes to phenylnitroso group and the CV in **Figure 1 (b)(ii)** showed an oxidation peak at -0.27 V (labelled **III**). The peak at -0.42 V in **Figure 1 (b) (iii)** was due to the reduction of PHA to phenylamino group (PA) thus the formation of Au-PA. On subsequent scans, the reduction peak at -0.92 V disappeared, confirming the conversion of the phenylnitro groups to phenylamino group. The peak at -0.92 V was preceded by a peak at -0.63 V (labelled **I**) attributed to the reduction of the physiosorbed phenylnitro on the phenylnitro modified surface.

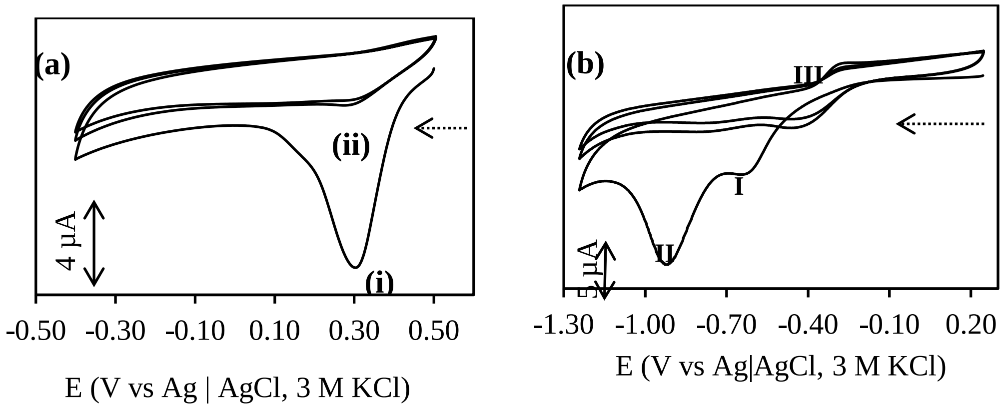
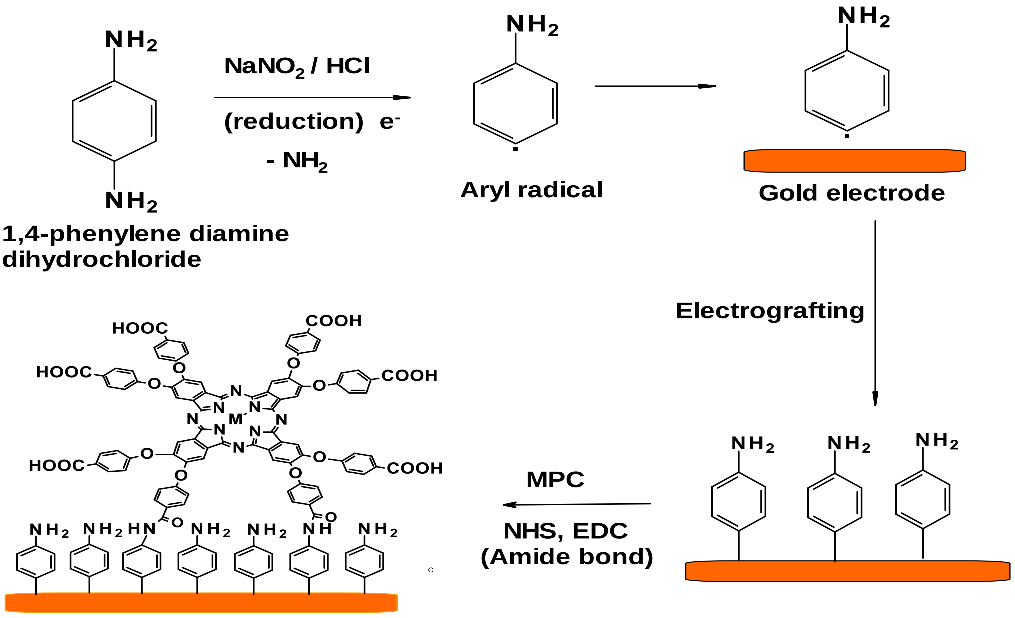


Figure 1: Cyclic voltammograms of (a) the electroreduction and grafting of phenylnitro from 1.0 mM 1, 4-phenylene diamine dihydrochloride (diazonium salt) tetrafluoroborate in ACN containing 0.10 M $TBABF_4$ in 3 cycles and (b) reduction of NO_2 to NH_2 in ethanol/water (1:9) solution containing 0.10 M KCl. Scan rate 100 $mV.s^{-1}$.

Immobilization process of CoOcPhOPc

A mixture of CoOcPhOPc (6.0 µmol, 10 mg), NHS (1.0 µmol, 115 mg) and EDC (1 µmol, 192 mg) was dissolved in PBS pH 7.4 (10 mL) and left for one hour (to convert the -COOH group at the terminal end of the metallophthalocyanines to active carbodiimide esters. The solution pH was then adjusted to pH 8.0 with 0.1 M NaOH to deprotonate the NH_2 group (active carbodiimide esters react faster with deprotonated amino groups than the protonated form) on the electrografted electrode which was immersed in the solution and kept in the dark for 24 hours. The active carbodiimide esters undergo condensation reaction with the deprotonated amino group, resulting in the formation of amide bon**ds [21].** The modified electrode was stored in a nitrogen saturated phosphate buffer pH 4.0 at room temperature.

The schematic representation of the electrografting of 1,4-phelenylene diamine dihydrochloride (PA) compound and its amide bond coupling formation with the prepared and characterized cobalt octacarboxyphenoxy phthalocyanines (CoOcPhOPc) on gold electrode surface, forming Au-PA-CoOcPhOPc is shown in **scheme 1.**



Scheme 1: Schematic representation of the electrografting and amide bond coupling formation of 1, 4-phenylene diamine dihydrochloride (PA) and CoOcPhOPc on gold electrode.

Amide coupling technique used in modifying the gold electrode is of two-step in which the electrode was designed with electrografting of 1,4-phenylene diamine dihydrochloride coupled with cobalt octacarboxyphenoxy phthalocyanines via condensation reaction known as amide bond coupling. The CoOcPhOPc was covalently immobilized on the electrografted gold electrode following the method previously reported **[17].** At pH 7.4, -COOH group at the terminal end of the CoOcPhOPc was converted to active carbodiimide esters. The pH was adjusted to 8.0 at which the active carbodiimide esters undergo condensation reaction with the deprotonated amino group, resulting in the formation of amide bonds CoOcPhOPc was prepared and purified according to the reported procedure with slight modifications **[18].**

2.3. Electrochemical measurements

Cyclic voltammograms (CV) and Electrochemical impedance spectroscopy (EIS) were carried out in $K_3[Fe(CN)_6$ /$K_4[Fe(CN)_6]$ – 0.1 M KCl, 0.5 M H_2SO_4 and PBS pH 7.4 for the electrode surface before and after the modification to confirm the blocking capacity of the {Au-PA-CoOcPhOPc}**.** Other analyses were carried out with cyclic voltammetry at the potential window between the range of -0.2 to 0.6 V. The electrochemical impedance spectroscopy (EIS) measurements were performed with Autolab FRA software at the frequency range of 10 kHz to 100 mHz between the frequency scan of 1 – 50 and the data were analyzed with Nova software. The DPV analysis were carried out at differential pulse potential between -0.60 to 0.00 V and at -0.20 to 0.60 V at the interval time of 0.5 s with the estimated number of points of 260 and modulation time of 0.05 s.

3.2. Characterization of modified electrode

3.2.1. Cyclic voltammetry analysis

The modification was confirmed in Figure 1 showing the cyclic voltammogram of (a) bare gold electrode (b) electrografted gold electrode (c) modified surface in **(A)** $K_3[Fe(CN)_6$ /$K_4[Fe(CN)_6]$ – KCl (a fast electron transfers redox couple) **(B**) electrocleaning and studied in 0.5 M H_2SO_4 - at the potential range of -0.2 to 1.5 V at the scan rate of 50mV/s. In Figure 1 (A), the half-wave potentials ($E½ = Ep_a + Ep_c /2$) for the clean gold electrode was measured and calculated to be 1.1330 V with the potential difference ($\Delta E = E_a - E_c$) of 0.449 V.

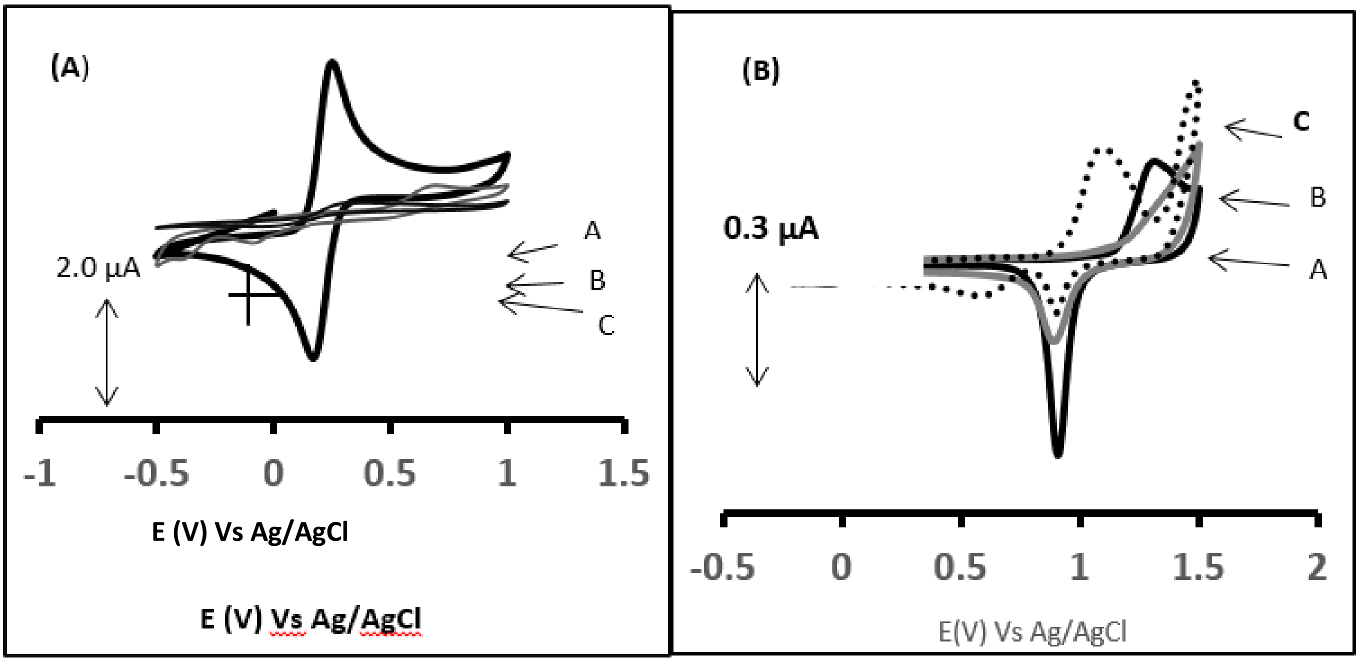


Figure 2: Cyclic voltammogram of (a) bare gold electrode (b) electrografted gold electrode (c) modified surface in (A) $K_3[Fe(CN)_6$ /$K_4[Fe(CN)_6]$ – KCl (B) in 0.5 M H_2SO_4 - using the potential range of -0.5 to 1.0 V and -0.5 to 1.5 V respectively at the scan rate of 50mV/s.

The modification process was confirmed with reduction of ΔE from 0.449 V (bare electrode) to 0.3142 V (electrografted) and further reduced to 0.1009 V (after modification) confirming the blocking capacity of the metallophthalocyanines, on the electrode surface. In **Figure 2B**, the blockage of the gold electrode surface was confirmed with the reduction in the cathodic peak at 0.9132 V for both the electrografting and the modified surfaces. The cobalt oxidation peak of the CoOcPhOPc on the gold electrode surface was shown at 1.1037 V.

3.3. Surface analysis of modified gold electrode

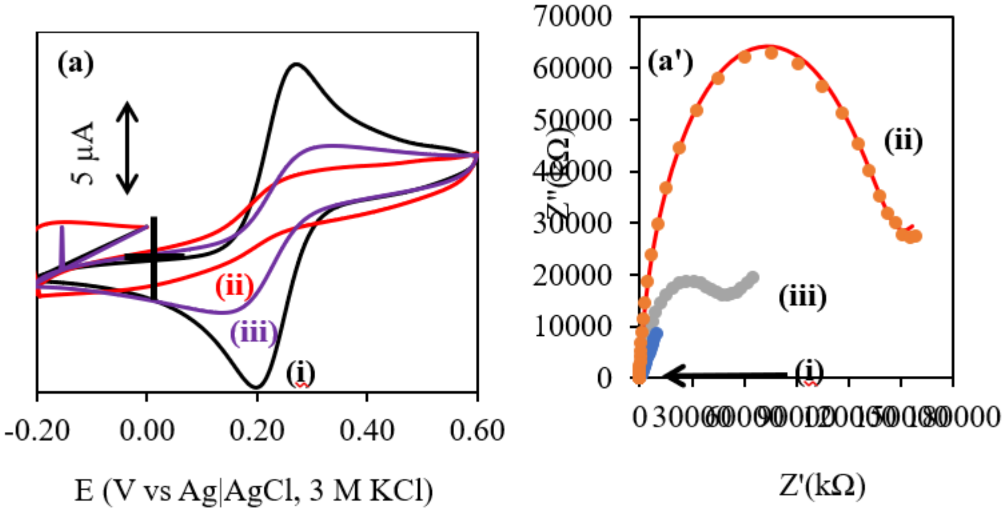
The surface analysis gave information about the performance and effectiveness of gold electrode and of the thin film layer of the electrocatalyst on its surface which helps to improve the performance of the electrode. The surface roughness area of the electrode was calculated using the Randles-Sevcik equation for reversible electrochemistry reaction:

$$\mathbf{I_p = (2.69\ x105)\ n^{3/2}AD^{1/2}\ Cv^{1/2.}}$$

Where n is the number of electrons involved in $Fe(CN)_6^{3-/4-}$ system, C is the concentration of $Fe(CN)_6^{3-/4-}$ (2.0×10^{-6} mol cm^{-3}), D is the diffusion coefficient of $Fe(CN)_6^{3-}$ (7.6×10^{-6} cm^2/s), A is the geometric surface area of the gold electrode (0.0201 cm^2) and ν is the scan rate of the reaction (0.050 V/s). The I_p calculated gave 2.0×10^{-5} A, with roughness factor (ratio of real surface and geometrical surface area) (r) of 1.05 and a real surface area of 0.022 cm^2. The surface coverage (Γ) was calculated to be 6.497×10^{-9} mole/cm^2 using the CV of the electrode in 0.5M H_2SO_4 with the equation ($\Gamma = Q/nFA$), where Q is the charge of the anodic wave (1.26×10^{-5}) and F is the Faraday constant (96485.33C mol^{-1}). The surf. ce coverage value confirmed the upright position of the complex on the electrode surface being higher than the theoretical value for a flat orientation of molecule on electrode surface (1×10^{-10} mol/cm$^{2)}$. The ion barrier factor $\Gamma_{ibf} = (Q_{mod..} / Q_{bare\ surface})$ was calculated to be 1.84 ± 0.05 indicating that the PA-CoOcPhOPc was strongly adsorbed and formed compact thin film layer on the gold electrode. The movement of ion from the solution to the electrode surface was prevented by Au-PA-CoOcPhOPc. Therefore, the flow of electron from the bare electrode surface to the bulk solution was reduced. This is further confirmed with EIS analysis in **Figure 3** and **Table 1,** where the charge transfer resistance in the electrografting surface is more than the charge transfer resistance in bare electrode due to the ion barrier of 1, 4-phenylene diamine dihydrochloride. The R_{ct} of the modified electrode surface was not noticeable due to the electroactive complex used for the modification.

Cyclic voltammetry and electrochemical impedance spectroscopy studies

Figure 3 shows the cyclic voltammograms (CV) and the electrochemical impedance spectroscopy (EIS) of the **(i)** bare Au, **(ii)** Au-PA and **(iii)** Au-PA-CoOcPhOPc in **(a)** 2.0 mmol.L^{-1} $K_3/K_4[Fe(CN)_6]$ and **(b)** 2 mmol.L^{-1} $[Ru(NH_3)_6]$ containing 0.10 mol.L^{-1} KCl at a scan rate of 50 mV.s^{-1}. The CV of the bare Au electrode in $K_3/K_4[Fe(CN)_6]$ in **Figure 3 (a) (i)** showed Fe^{II}/Fe^{II} reversible redox couple of $[Fe(CN)_6]^{3-/4-}$ with a peak-to-peak separation (ΔE) of 73 ± 4.4 mV. The CV of the Au-PA electrode in **Figure 3 (ii)** showed a decrease in peak current when compared to the bare Au electrode. The cathodic peak shifted to higher potential and the anodic peak was not observed. This showed that the PA-layer passivates the Au surface and blocks electron transfer process. The CV of the Au-PA-CoOcPhOPc showed restoration (to some extent) of the Fe^{II}/Fe^{II} reversible redox couple of $[Fe(CN)_6]^{3-/4-}$. At Au-PA-CoOCPhOPc, the ΔE was 154 ± 4.53 mv. The restoration of the redox process at the Au-PA-CoOcPhOPc surface was attributed to the electrocatalytic properties of CoOcPhOPc towards $[Fe(CN)_6]^{3-/4-}$ redox couple. The anodic and cathodic current at the Au-PA-CoOcPhOPc were very small compared to the bare Au surface but much higher compared to Au-PA surface.



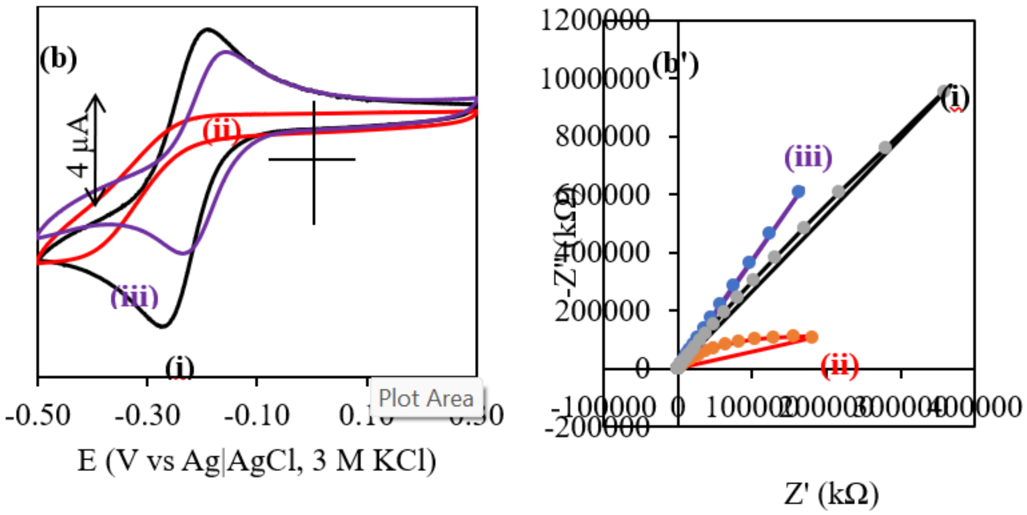


Figure 3: The cyclic voltammograms (CV) (a)(b) and the electrochemical impedance spectroscopy (EIS) (a')(b') of the (i) bare Au, (ii) Au-PA and (iii) Au-PA-CoOcPhOPc in (a) 2.0 mmol.L^{-1} $K_3/K_4[Fe(CN)_6]$ and (b) 2 mmol.L^{-1} $[Ru(NH_3)_6]$ containing 0.10 mol.L^{-1} KCl at a scan rate of 50 mV.s$^{-1.}$

The impedance analysis is used to measure the ability at which a circuit can resists the flow of electrical current. In this work, the impedance analyses were carried out in $K_3[Fe(CN)_6/K_4[Fe(CN)_6]$ – KCl, being a fast electron transfer redox couple. In **Figure 3,** the blocking capacity from electrografting and the immobilization process was confirmed using this redox probe. The electronic communication between gold electrode surface and $Fe(CN)_6^{3-/4-}$ was reduced by the thin film layer of Au-PA-CoOcPhOPc. The signals from the electrode surface gave information of the electron transfer process, the charge transfer, (R_{ct}) the solution resistance (R_s) and conductance (C) shown in the Nyquist plot and Bode plots (displayed the frequency). The equivalent circuit inserted in **Figure 3a'** was used to model the diffusion process in the medium.

Nyquist plot is an imaginary Z' (Y- axis) against the real part of the impedance Z" (X- axis). It comprises of the arc or semi-circle and the straight line. The arc appeared at the left-hand side where there are high frequency data. The arc gives information about the solution resistance and charge transfer resulting from the electrical circuit. The arc shows the reaction of the electrode surface in the redox probe, confirming that the complex did not fully block the $Fe(CN)_6^{3-/4-}$ from the electrode surface as in the CV in **Figure 3**. At the right-hand side of the circuit is the low frequency region which is a straight line called a Warburg line, which confirmed the diffusion process in the medium.

In **Figure 3(a'),** the bare electrode shown a small arc at the high frequency region and a Warburg line at the low frequency region. The arc after electrografting increases due to the high conductivity of 1,4-phenylene diamine dihydrochloride which brought about fast electron diffusion into the gold electrode surface. The metal ions also diffused into the electrolyte and thereby charge is being transferred. Immobilization of the CoOcPhOPc drastically decrease the arc being electroactive which confirmed the electrocatalytic properties of the complex in the redox process.

Table 1: Electrochemical impedance spectroscopy results on electrode modification process.

Circuit Element	**Bare Electrode surface**	**Electrografted electrode surface**	**Modified electrode surface**
R_{ct} (kΩ)	**3.56**	**15.6**	**-**
C (µF)	**0.73**	**0.82**	**3.01**
R_s (Ω)	**354**	**377**	**274**
W $_{(Yo)}$ (µMho)	**90.1**	**38.7**	**32.5**

The results from the analysis in **Table 1**, shows an increase in the charge transfer resistance from the bare electrode (3.56 kΩ) to the electrografted surface (15.6 kΩ) confirming the electrografting of the 1,4 phenylenediamine dihydrochloride, while in the modified, the charge transfer (**R_{ct}**) are insignificant. The Warburg (Yo) also decreased from clean bare electrode (90.1 µMho) to the modified surface (32.5 µMho), confirming reduction in mass transfer and strong adsorption of the thin film of the Au-PA-CoOcPhOPc on the electrode surface. The capacitance and solution resistance of bare surface increase with Au-PA while the solution resistance decrease after the immobilization of CoOcPhOPc which confirmed the blocking capacity of the cobalt octacarboxyphenoxy phthalocyanines on the gold electrode.

The outer sphere electron transfer reaction and kinetic properties of the complex on the electrode and its interaction with the redox probe $(Fe(CN)_6{}^{3-/4-})$ in solution was calculated to be 1.32 X 10^{-5} using equation:

$$Kapp' = k^0 = \frac{RT}{n^2F^2AR_{CTC}}$$

The low value obtained confirmed a strong electronic coupling reaction between $[Fe(CN)_6]^{3-}$ and $[Fe(CN)_6]^{4-}$, therefore, inner sphere electron transfer occurred.

Modified electrode surface at different pH

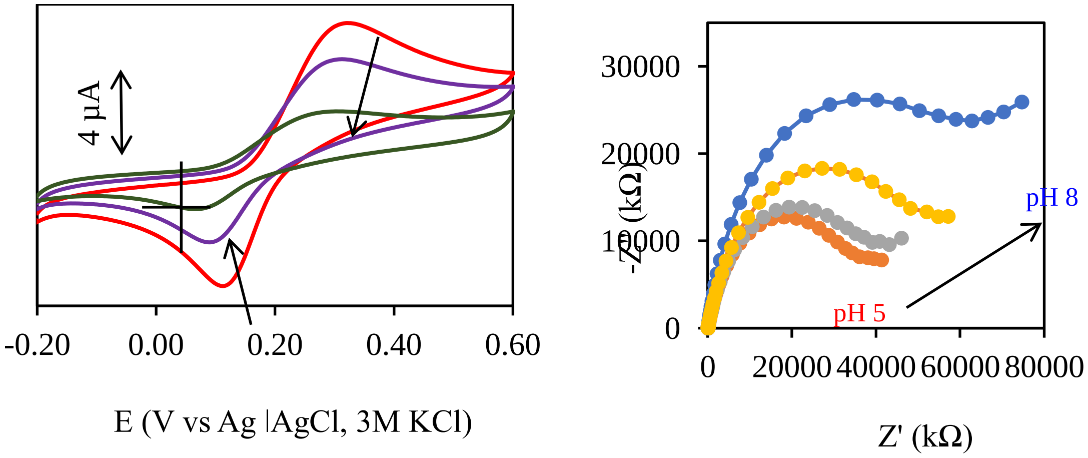


Figure 4: Cyclic voltammograms and impedance spectroscopy (Nyquist plot) for (a) Au-PA-CoOcPhOPc, in 2.0 mmol.L^{-1} $K_3/K_4[Fe(CN)]_6$ containing 0.10 mol.L^{-1} KCl at varied pH conditions.

3.3. Electrochemical oxidation of epinephrine on modified gold electrode

3.3.1. Cyclic voltammetry analysis

Figure 5 shows the electrochemical response of the modified electrode on epinephrine was observed showing the CV redox peak current at 0.25 V. The anodic peak current increased with increasing concentration of epinephrine giving a linear graph that confirmed the process to be diffusion controlled. The linear plot gave a correlation coefficient of 0.9 with linear regression of 6.0 X 10^{-8}C (µM) + 4.0 X 10^{-6} confirming the strong positive linear response between the concentration and the current. The LOD and LOQ were calculated to be 0.27 nM and 0.90 nM respectively. The sensitivity derived with cyclic voltammetry was 6.0 nA nM^{-1}.

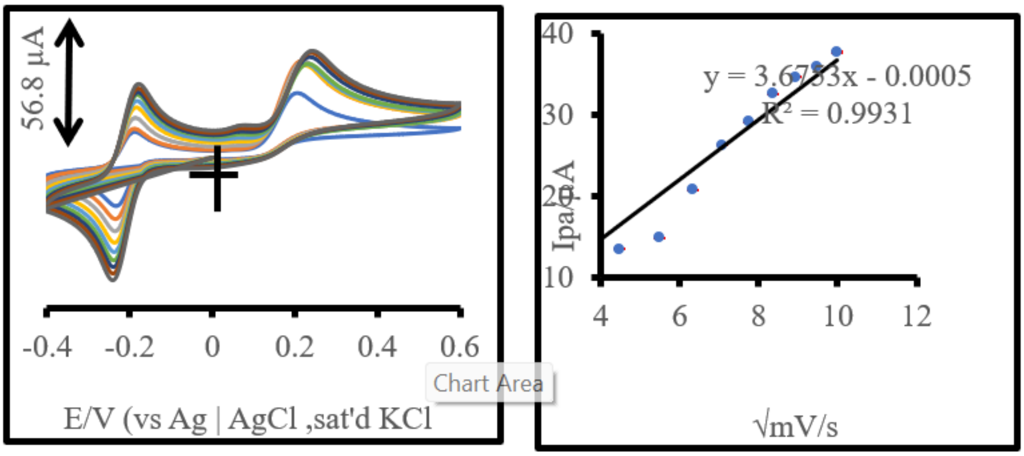


Figure 5: (A) Cyclic voltammetry of epinephrine at different concentration on Au-PA-CoOcPhOPc.

Linear sweep voltammetry

The electron transfers kinetic and transport properties of Au-PA-CoOcPhOPc was further studied using LSV as shown in **Figure 6.** At fixed potential range, the voltage was scanned from the lower limit to upper limit. The chemical reactivity of the electroactive species shown increase in the current at increased concentration of epinephrine. The sensitivity derived with LSV method towards epinephrine detection is 5.0 nA nM^{-1}. The linearity of the calibration plot confirmed the process to be diffusion controlled. The correlation coefficient obtained was +0.98, establishing the strong and positive relationship between the current and potential.

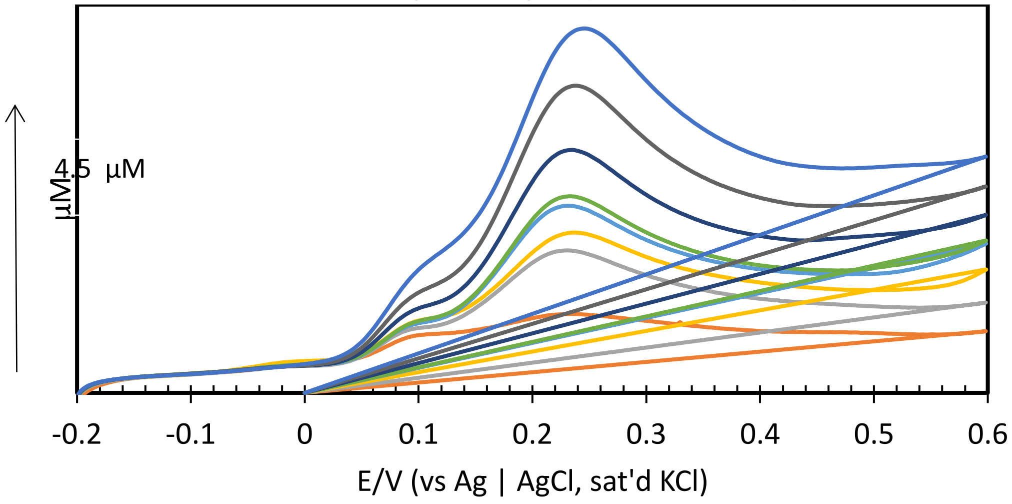


Figure 6: Linear sweep voltammetry of Epinephrine on Au-PA-CoOcPhOPc at different concentrations.

Differential Pulse voltammetry

The Differential Pulse voltammetry was also used as shown in **Figure 7**, confirming the sensitivity of the modified electrode in the detection of epinephrine.

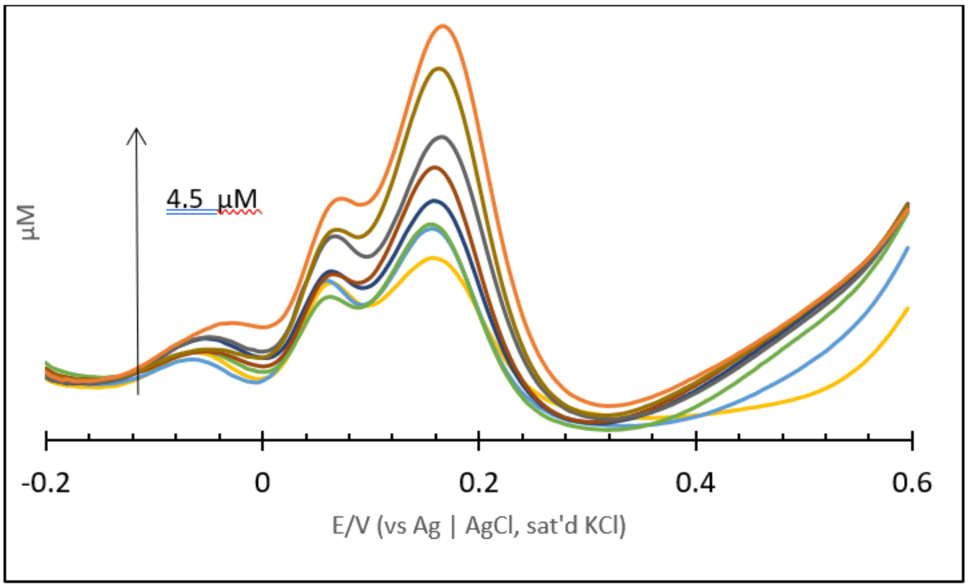


Figure 7: Differential Pulse voltammetry of epinephrine at different concentration on Au-PA-CoOcPhOPc.

Electrocatalysis and analysis of epinephrine from interference

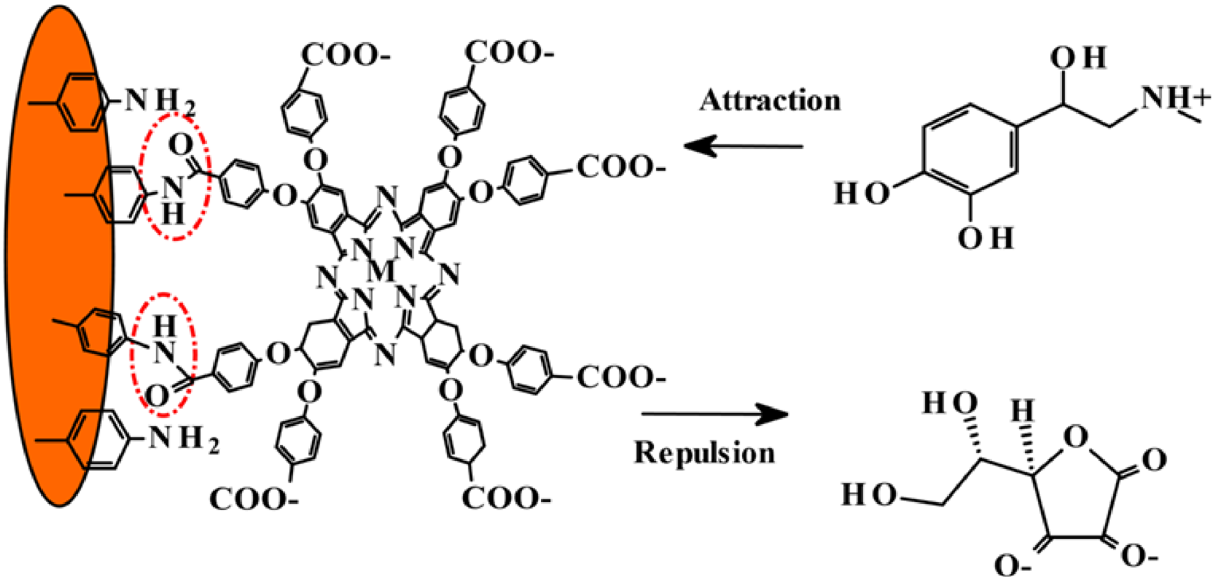
3.4. Selective electrocatalytic detection of epinephrine from interference

CV, LSV and Chronoamperometry analyses were carried out with the modified electrode to confirm the functionalization of the CoOcPhOPc catalyst on the electrode surface and its effectiveness in screening off the interference from epinephrine.

Cyclic voltammetry selective detection of epinephrine

Electrocatalysis of Au-PA-MOcPhOPc towards ascorbic acid (AA)

In **Scheme 2,** the illustration of the reaction at the electrode surfaces was demonstrated in PBS pH 7.4. The carboxylate ions (COO$^-$) at the terminal end of the thin film of MOcPhOPc electrodes displayed an electrostatic attraction toward the cationic neurotransmitters. Therefore, neurotransmitters were detected at the Au-PA-MOcPhOPc modified electrode surfaces. The anionic ascorbic acid was repressed at the anionic COO$^-$ surface of the electrode due to the electrostatic repulsion thus the ascorbic acid was not detected. Therefore, the AuPA-MOcPhOPc modified electrode offered an advantage for accurate neurotransmitters detection.



Scheme 2: Illustration of the electrostatic reaction at the electrode surface at pH 7.4

The immobilization of CoOcPhOPc catalyst on the gold electrode improved the electrode performance in separating the oxidation potentials of epinephrine and ascorbic acid, interacted and attracted only the cationic epinephrine, thereby; screened off the anionic ascorbic acid in the phosphate buffer saline of pH 7.4, (the buffer solvent was used to stabilize the pH of the medium). Epinephrine can exist as cation, anion, neutral or as zwitter ions, depending on the pH of the medium. At pH 7.4, which is the physiological pH, epinephrine having the pka value of 8.66 protonated at the -NH group. The ascorbic acid with the pka value of 4.1 deprotonated and the carboxylate group on the terminal end of the cobalt octacarboxyphenoxy phthalocyanines (pka 4.20) deprotonated. There was electrostatic attraction coupled with the diffusion between the cationic epinephrine and anionic carboxylate ions on the electrode surface. The anionic ascorbic acid was repelled by the anionic carboxylate on the electrode surface. The selective and sensitive detection in the buffer medium was further confirmed with differential pulse voltammetry and linear Sweep Voltammetry [**20].**

In **Figure 8**, the CV signal of epinephrine shown the oxidation of epinephrine to epinephrine quinone at 0.262 V. It also shown a cathodic pea,k at -0.2046 V, confirming the reduction of epinephrine quinone to leucoadrenochrone and the oxidation of leucoadrenochrone to adrenochrone at -0.188 V. The potentials and the cathodic peaks of epinephrine and that of the equimolar mixture of the ascorbic acid and epinephrine shown insignificant difference compare to the potential and current from ascorbic acid which established the selective detection of epinephrine.

The cyclic voltammograms of 0.1 mM of (i) Epinephrine and (ii) ascorbic acid at Au-PA-CoOcPhOPc surface. In **Figure 8 (i)**, Epinephrine oxidation peak was observed at 5.20 V while the oxidation peak of ascorbic acid was observed at 0.289 V This clearly show that ascorbic acid was not detected on the AuPACoOcPhOPc surface, The oxidation process of ascorbic acid was inhibited and suppressed on the Au-PA-CoOcPhPc surface. Therefore, the modified surfaces could be employed for the neurotransmitter's detection without the problem of interference.

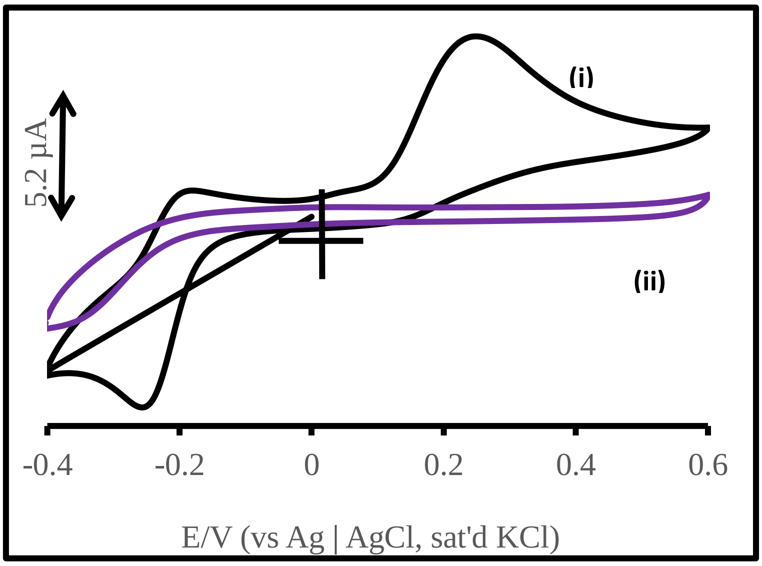


Figure 8: Cyclic voltammograms of 0.10 mM of (i) epinephrine, (ii) ascorbic acid at Au-PA-CoOcPhOPc surfaces.

Linear Sweep Voltammetry analysis (LSV)

LSV analysis, also confirmed the efficiency of Au-PA-CoOcPhOPc in the selective detection of epinephrine from ascorbic acid. In **Figure 9,** The linear sweep voltammetry obtained at CoOcPhOPc in PBS pH 7.4 measured the current while potential between the working and reference electrode was swept linearly with time. The response of the electrode towards the epinephrine in the equimolar concentration of the mixture of epinephrine and ascorbic acid, selectively detected epinephrine in the mixture at similar potential to epinephrine stock at 0.39 V in contrast to ascorbic acid behavior at the electrode.

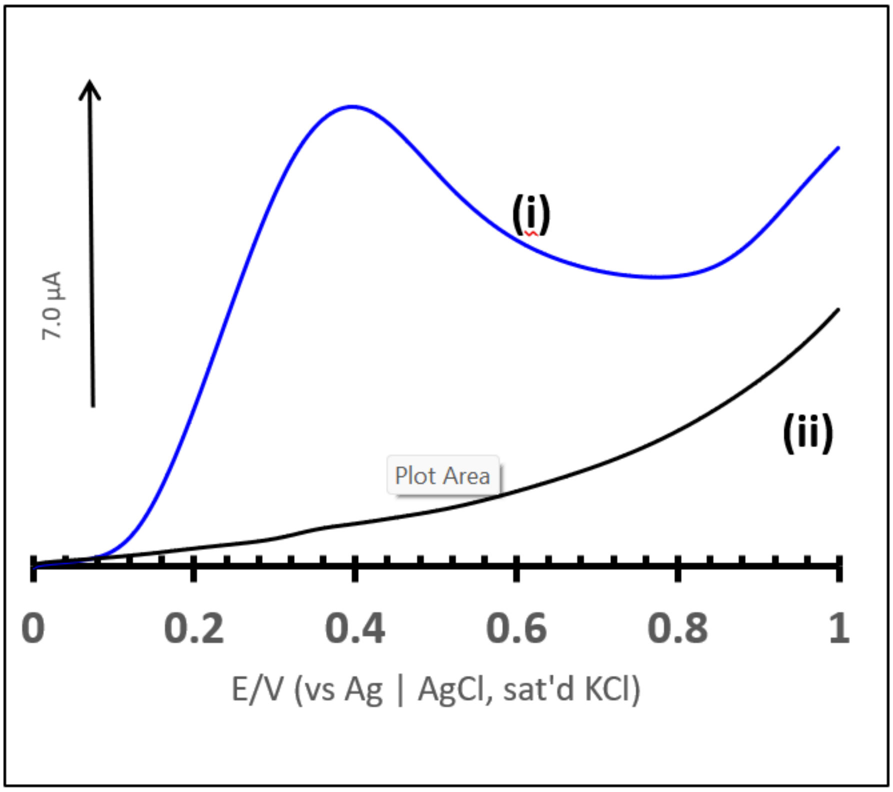


Figure 9: LSV analysis on epinephrine detection ((i) Epinephrine (ii) Ascorbic acid

Chronoamperometry Studies

In **Figure 10,** the response of the Au-PA-CoOcPhOPc sensor towards Epinephrine and ascorbic acid was further studied using chronoamperometry. Chronoamperometry method was used for more information on the detection of neurotransmitters and screening out of ascorbic acid using the modified electrode. Chronoamperometry method has a better signal-to-noise ratio than most techniques**.** In **Figure 10 (ii),** the response of the Au-PA-CoOcPhOPc towards the addition of each 20 µL epinephrine, measured at 50 seconds interval caused the potential at the working electrode stepped with the resulting current at a function of time. The current increased at the increasing concentration of the epinephrine. In **Figure 10 (i),** the Au-PA-CoOcPhOPc sensor on ascorbic acid using similar method was not stepped. The stepped confirmed the high response of the sensor towards epinephrine and the unstepped chronoamperometry confirmed the screen out of the ascorbic acid at the electrode surface.

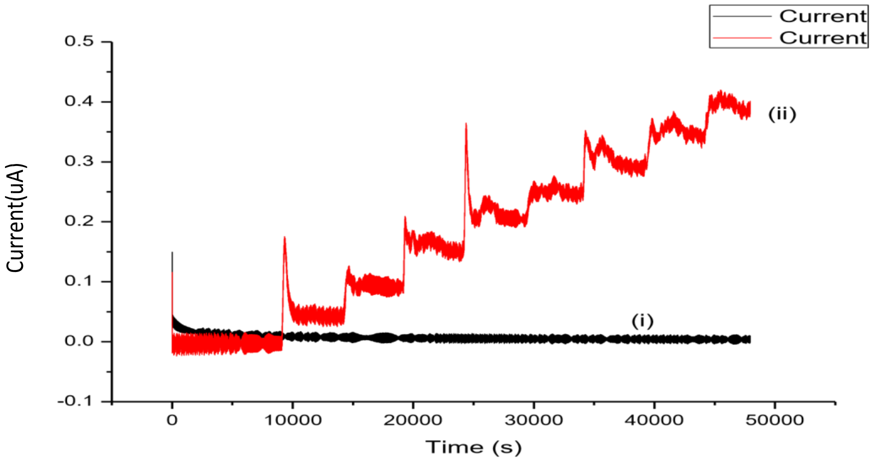


Figure 10: Chronoamperometry detection of (i) Ascorbic acid and (ii) Epinephrine on the Au-PA-CoOcPhOPc.

Stability and Reproducibility study

The stability of the cobalt octacarboxyphenoxy phthalocyanines on the electrode is an important factor in the detection process. The epinephrine of 10 µM was scan repeatedly at the scan rate of 50 mV/s to confirm the reusability and stability. The scan in figure 7, shown no significant increase at the anodic and cathodic peaks, confirming the stability with no change in both the potential peaks and the current response. The modified electrode was reused and kept for 72 days and the modification was discovered to be stable with reproducible electrochemical information.

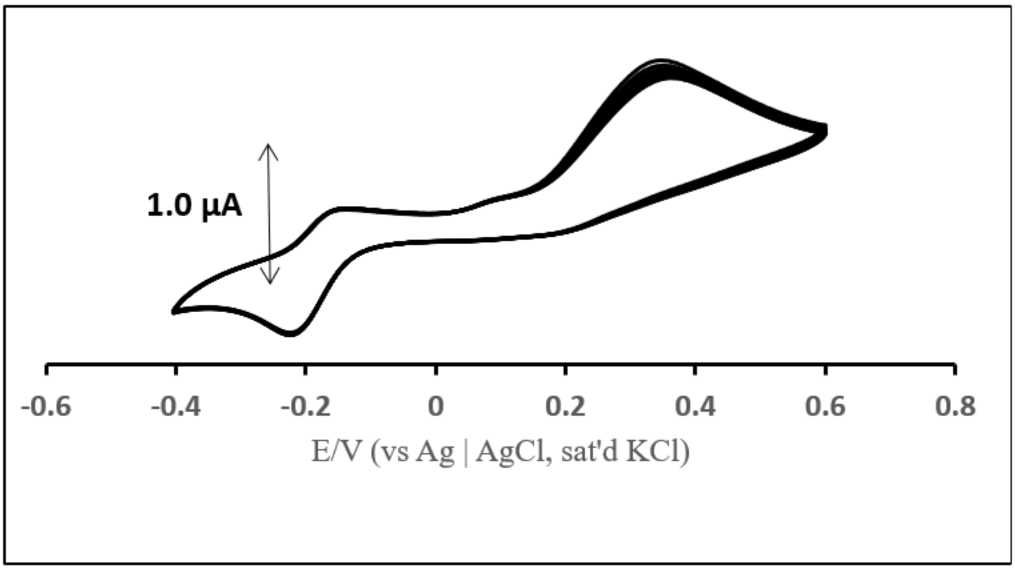


Figure 11: Cyclic voltammogram of repeated scans of epinephrine

4.0. CONCLUSION

In conclusion, the electrocatalyzed pH response modified gold electrode was highly efficient for the detection of epinephrine in the presence of ascorbic acid. The electrode was modified with cobalt octacarboxyphenoxy phthalocyanines resulting in high sensitivity of 0.6 ± 0.01 nA nM^{-1} toward the epinephrine detection with very low limit of detection of 0.27 nM and low limit of quantitation of 0.90 nM. The correlation coefficient from the calibration curves show very strong and positive linear relationship.

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