

Graphene derivatives and electrocatalysts as composite electrodes for selective detection of catecholamine neurotransmitters

A thesis submitted in fulfilment of the requirements

for the degree of

DOCTOR OF PHILOSOPHY

of

RHODES UNIVERSITY

By

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November 2023

DEDICATION

Dedicated to my father, wife and sons

ACKNOWLEDGMENTS

First and foremost, I would like to thank the Almighty God for the gift of life and strength bestowed on me.

I sincerely express my heartfelt gratitude to my supervisor, Prof. Philani Mashazi, for accepting me into his group. During my tenure, you contributed to rewarding graduate experience by giving me intellectual freedom in my work, engaging me in new ideas, supporting me in attendance of conferences, and demanding a high quality of work in all my endeavours. I really cherished your guidance and direction in this scientific journey, I say thank you Prof. I am very much thankful for your valuable time you put in correcting, reviewing progress reports, manuscripts, and this thesis. Without your input this journey would have not been possible. Many thanks to the Chemistry Department, Institute of Nanotechnology Innovation for the equipment and Mzuzu University for the PhD study support.

Many thanks to my wife, three sons and my father for all the support and encouragement. I appreciate you all.

I am also thankful to the Rhodes University Chemistry staff and postgraduate students of the chemistry department for all the support.

I acknowledge our nanobiosensor research group for all the helpful discussions during and after presentations. This thesis is a product of those times, thank you.

ABSTRACT

In this thesis, we investigated the development of electrochemical sensing electrodes based on graphene derivatives and the electrocatalyst composites of metallophthalocyanines and metal oxides towards the selective and sensitive detection of catecholamine neurotransmitters (NTs). The graphene and its derivatives offer excellent electron conductivity and hence their use as base electrode materials. Metallophthalocyanines are N_4 -macrocyclic metal organic complexes that have structural modification flexible and $18-\pi$ electrons with excellent electrocatalytic properties. They can incorporate transition metal ions at the centre of the macrocycle ring, and this further enhances their electrocatalytic activity. Transition metal oxides are of interest as these also offer excellent redox properties and pH sensitivity. A pH sensitive electrochemical sensor based on aminated graphene quantum dots (AmGQDs) and cobalt tetra carboxyphenoxy phthalocyanine (CoTCPhOPc) covalent conjugates onto the pre-grafted isophthalic acid (IPA) gold electrode was fabricated. It displayed excellent analytical performance towards the detection of dopamine (DA), norepinephrine (NOR) and epinephrine (EP). The electrochemical sensor exhibited good ability to suppress the background current due to ascorbic acid (AA), a major interference in the detection catecholamine NTs. The carboxylic acid functional group of cobalt phthalocyanine induced the pH sensitivity of the thin film and electrostatic repulsion of the negatively charged ascorbate (AA^-) anion at physiological pH. In addition, the electrode could screen-off biological molecules in the newborn calf serum as a representative of a real sample. An electrochemically reduced graphene oxide-based cobalt phthalocyanine polymer (polyCoTAPc) composite electrochemical sensor was fabricated on glassy carbon electrode, GCE-ERGO/polyCoTAPc. The GCE-ERGO/polyCoTAPc could detect dopamine and paracetamol simultaneously as co-existing analytes in buffer and synthetic urine samples. We observed, (i) very strong absorption of the materials, (ii) increase in the electrode surface area, (iii) excellent conductivity, and (iv) electrocatalytic activity of the

ERGO/polyCoTAPc composite surface. Furthermore, a simple ultrasensitive electrochemical sensor based on nickel hydroxide thin film electrodeposited onto reduced graphene oxide-molybdenum disulfide (RGO/MoS₂) composite resulted in the electrochemical sensor that can selectively and simultaneously detect ascorbic acid, dopamine, and uric acid. A distinct peak potential separation was observed with excellent electrocatalytic oxidation currents. The electrochemical sensors developed in this work exhibited high sensitivity, selectivity, stability, and low detection limits suitable for the detection and determination of catecholamine NTs.

TABLE OF CONTENTS

DEDICATION	i
ACKNOWLEDGMENTS.....	ii
ABSTRACT	iii
TABLE OF CONTENTS	v
LIST OF ABBREVIATIONS	xi
CHAPTER 1	1
1.1 Justification.....	1
1.2 Research aims and objectives.	11
1.3 Thesis outline.....	12
1.4 References	14
CHAPTER 2	24
2 Literature review	24
2.1 Catecholamine neurotransmitters	24
2.1.1 Oxidation of catecholamine neurotransmitters.....	25
2.1.2 The effect of interferents on catecholamine neurotransmitters detection	27
2.1.3 Detection of catecholamine neurotransmitters in real samples	28
2.2 Graphene and its derivatives as electrode modifiers	28
2.2.1 Graphene oxide (GO) as graphene derivative	29
2.2.2 Graphene quantum dots (GQDs)	32
2.2.3 Aminated graphene quantum dots (AmGQDs)	35
2.3 Graphene and its derivative composites for neurotransmitters detection.....	36

2.3.1 Graphene-metallo phthalocyanine composites.....	37
2.3.2 Graphene- metal oxide composite thin films.....	40
2.4 Basic principle of electrochemical sensors.....	41
2.5 Voltammetric methods of electrochemical measurements.....	42
2.6 Fabrication of graphene and its derivative composite electrodes.....	46
2.6.1 Electrografting with diazonium salts.....	46
2.6.2 Carbodiimide coupling.....	51
2.6.3 Drop-dry.....	53
2.6.4 Electrodeposition.....	53
2.6.5 Electropolymerization.....	56
2.7 Characterization of chemically modified electrodes.....	59
2.8 References.....	62
CHAPTER 3.....	80
3. In-sera selective detection of catecholamine neurotransmitters.....	80
3.1 Introduction.....	81
3.2 Experimental.....	83
3.2.1 Materials and methods.....	83
3.2.2 Apparatus.....	84
3.2.3 Synthesis of amino-functionalized GQDs (AmGQDs), Scheme 3.1.....	85
3.2.4 Electrode preparation.....	87
3.2.5 Modification of gold electrode surface, Scheme 3.2.....	87

3.2.6 Real samples preparation and analysis	88
3.3 Results and discussion	89
3.3.1 Synthesis and characterization of GQDs and AmGQDs	89
3.3.2 Electrografting of 5-DIPA onto gold electrode	92
3.3.3 Fabrication of Au-IPA-AmGQD-CoTCPhOPc, Scheme 3.2	93
3.3.4 Electrochemical characterization of surface modification	94
3.3.5 Blocking characteristics of Au-IPA-AmGQDs-CoTCPhOPc	97
3.3.6 The effect of solution pH at Au-IPA-AmGQD-CoTCPhOPc	100
3.3.7 Electrocatalysis of DA, EP and NOR at Au-IPA-AmGQDs-CoTCPhOPc	103
3.3.8 Detection of DA, EP, and NOR at Au-IPA-AmGQDs-CoTCPhOPc	107
3.3.9 Real sample analysis	111
3.3.10 The effect of interference by Ascorbic Acid	114
3.3.11 Repeatability and stability of the Au-IPA-AmGQDs-CoTCPhOPc	117
3.4 Conclusions	118
3.5 References	118
CHAPTER 4	126
4 Simultaneous detection of dopamine and paracetamol on composite electrode	126
4.1 Introduction	127
4.2 Experimental	130
4.2.1 Chemicals and reagents	130
4.2.2 Apparatus	131

4.2.3 Preparation of graphene oxide (GO)	131
4.2.4 Electrode fabrication to form GCE-ERGO/polyCoTAPc	132
4.2.5 Electrochemical analysis	133
4.3. Results and discussion	133
4.3.1 Electrodeposition of reduced GO and CoTAPc polymer onto GCE	133
4.3.2 FT-IR ITO Surface characterization.....	136
4.3.3 Electrochemical characterization of the GCE, GCE-ERGO and GCE- ERGO/polyCoTAPc.....	137
4.3.4 Electrocatalytic detection of DA and PA at GCE-ERGO/polyCoTAPc	141
4.3.5 Effect of solution pH and scan rates	143
4.3.6 Electroanalysis of DA and PA at GCE-ERGO/polyCoTAPc	147
4.3.7 Screening of ascorbic acid and uric acid at GCE-ERGO/polyCoTAPc.....	149
4.3.8 Stability and repeatability	152
4.3.9 Real sample analytical application of GCE-ERGO/polyCoTAPc	153
4.4 Conclusions	154
4.5 References	155
CHAPTER 5	161
5 Electrocatalysis of conducting nickel hydroxide on graphene and molybdenum composites	161
5.1 Introduction	162
5.2 Experimental.....	164
5.2.1 Materials and methods.....	164

5.2.2 Equipment.....	165
5.2.3 Preparation of RGO/MoS ₂ composites.....	165
5.2.4. Electrode modification to form GCE-RGO/MoS ₂ /Ni(OH) ₂	166
5.2.5. Electrochemical measurements	167
5.3 Results and discussion	167
5. 3.1 SEM, FTIR and Raman characterization of the RGO/MoS ₂ composites.....	167
5.3.2 Preparation of the GCE-RGO/MoS ₂ /Ni(OH) ₂ modified electrode	169
5.3.3 XPS characterization of GCE-RGO/MoS ₂ /Ni(OH) ₂	170
5.3.4 Electrochemical characterization of electrode modification	173
5.3.5 Electrocatalysis of AA, DA and UA at GCE-RGO/MoS ₂ /Ni(OH) ₂	175
5.3.6 Effect of deposition potential and time of Ni(OH) ₂ on the AA, DA and UA signals	178
5.3.7 Effect of pH and scan rate on the AA, DA and UA electrocatalysis.....	179
5.3.8 Detection of AA, DA and UA at GCE-RGO/MoS ₂ /Ni(OH) ₂	183
5.3.9 Reproducibility and stability of the GCE-RGO/MoS ₂ /Ni(OH) ₂	188
5.3.10 Analytical application.....	189
5.4 Conclusions	191
5.5 References	192
CHAPTER 6	199
6 Conclusions and future perspectives	199
6.1 Conclusions	199
6.2 Future Perspectives.....	201

7 APPENDICES.....	203
7.1 Supplementary figures from chapter 4	203
7.2 Supplementary figures from chapter 5	204
7.3 References	207

LIST OF ABBREVIATIONS

5-DIPA	5-Diazonium Isophthalic Acid
ABS	Acetate buffer solution
ACN	Acetonitrile
Ag	Silver
AA	Ascorbic acid
AmGQDs	Aminated graphene quantum dots
Au	Gold Electrode
CA	Citric acid
CoTAPc	Cobalt (II) tetra amino phthalocyanine
CoTCPhOPc	Cobalt (II) tetracarboxyphenoxy phthalocyanine
CE	Counter Electrode
CV	Cyclic Voltammetry
DA	Dopamine
DMF	Dimethylformamide
DPV	Differential pulse voltammetry
EIS	Electrochemical Impedance Spectroscopy
ERGO	Electrochemically reduced graphene oxide
EP	Epinephrine
FTIR	Fourier Transform Infrared
GCE	Glassy Carbon Electrode
GQDs	Graphene quantum dots
IPA	Isophthalic Acid
LOD	Limit of Detection
LOQ	Limit of Quantification

MoS ₂	Molybdenum disulfide
Ni(OH) ₂	Nickel hydroxide
NOR	Norepinephrine
PA	Paracetamol
PBS	Phosphate Buffer Solution
Pt	Platinum
RE	Reference Electrode
RGO	Reduced graphene oxide
RSD	Relative standard deviation
SEM	Scanning electron microscopy
SWV	Square wave voltammetry
TBABF ₄	Tetrabutylammoniumtetrafluoroborate
UA	Uric acid
UV-vis	Ultraviolet-Visible Spectroscopy
WE	Working Electrode
XPS	X-ray Photoelectron Spectroscopy

LIST OF FIGURES

Figure 2.1: (a) Neurotransmitters released in the nervous system, (b) an action potential (yellow lighting) causes NTs to be released and travels across the gap to activate receptors on the receiving neuron [1].	25
Figure 2.2: (a) Schematic illustration for chemical oxidation graphite flake to graphene oxide [12] and (b) Chemical structure of an isolated sheet of graphene oxide (GO).	31
Figure 2.3: Molecular structure of unmetalated (metal free) phthalocyanine (H ₂ Pc, 1) and metalated (metal containing) phthalocyanine (MPc, 2). Both showing α - and β - positions and their numerical notations.	38
Figure 2.4: A schematic representation of three-electrode electrochemical set-up for an electrochemical measurement.	42
Figure 2.5: Potential-time excitation signal for cyclic voltammetric experiment.	43
Figure 2.6: Typical cyclic voltammogram for a reversible $O \rightleftharpoons R$ redox process.	44
Figure 2.7: (a) Nyquist plot and (b) Equivalent circuit representing the observed impedance data.	61
Figure 3.1: (a) UV-vis spectra, (b) fluorescence spectra, (c) FT-IR spectra, and (d) Raman spectra of (i) GQDs and (ii) AmGQDs.	91
Figure 3.2: CVs for the electrografting of 2.50 mmol.L ⁻¹ of 5-DIPA in 3% HCl solution of acetonitrile for gold electrode.	93
Figure 3.3: (a) Cyclic voltammograms and (b) EIS spectra of (i) bare Au, (ii) Au-IPA, (iii) Au-IPA-AmGQDs and (iv) Au-IPA-AmGQDs-CoTCPhOPc measured in 2.0 mmol.L ⁻¹ [Fe(CN) ₆] ^{3-/4-} containing 0.10 M KCl. Scan rate = 50 mV.s ⁻¹	96
Figure 3.4: CVs of (i) bare Au, (ii) Au-IPA, (iii) Au-IPA-AmGQDs and (iv) Au-IPA-AmGQDs-CoTCPhOPc measured in (a) 0.010 M KOH solution, and (b) 1.0 mM CuSO ₄ in 0.50 M H ₂ SO ₄ solution. Scan rate of 50 mV.s ⁻¹	99

Figure 3.5: CVs, EIS and plot of R_{CT} or ΔE_p vs. pH at different pH values between 3 – 9 in 2.0 mM of (a) $[\text{Fe}(\text{CN})_6]^{3-/4-}$ and (b) $[\text{Ru}(\text{NH}_3)_6]^{2+/3+}$	101
Figure 3.6: CVs of Au-IPA-AmGQDs-CoTCPhOPc in the (i) absence and (ii) presence of 1.0 mM (a) DA, (b) EP, and (c) NOR in PBS buffer (pH 7.4). Scan rate = 50 mV.s^{-1}	104
Figure 3.7: CVs and the calibration plots of Au-IPA-AmGQDs-CoTCPhOPc at different scan rates ranging from 25 mV.s^{-1} to 125 mV.s^{-1} in pH 7.4 phosphate buffer solution containing 1.0 mM (a) DA, (b) EP and (c) NOR.....	106
Figure 3.8: Differential pulse voltammograms (DPVs) and the calibration curves of Au-IPA-AmGQDS-CoTCPhOPc modified electrode with varying concentrations from 1.0 μM to 50 μM for (a) DA, (b) EP, and (c) NOR in PBS (pH 7.4). Scan rate = 50 mV.s^{-1}	109
Figure 3.9: DPV and corresponding calibration curves for Au-IPA-AmGQDs-CoTCPhOPc from (i) 10 μM – (vii) 60 μM of (a) DA, (b) EP and (c) NOR in newborn calf serum (in-sera) samples.....	112
Figure 3.10: Square wave voltammograms (SWV) of Au-IPA-AmGQDs-CoTCPhOPc in the presence of 50 μM each of (i) AA, (ii) (a) DA, (b) EP, (c) NOR and (iii) mixture of (a) DA + AA, (b) EP + AA, (c) NOR + AA in PBS (pH 7.4).....	116
Figure 3.11: A bar chart showing variation of peak current of individual DPVs of 20 μM DA in 0.10 M PBS (pH 7.4) at Au-IPA-AmGQDs-CoTCPhOPc modified electrode.....	117
Figure 4.1: CVs of (a) the electrodeposition of 2.0 mg.mL^{-1} GO in PBS (0.10 M, pH 7.4) on a GCE, scan rate: 25 mV.s^{-1} (b) GCE-ERGO in dry DMF solution containing CoTAPc (2.0 mg, 3.0 μmol) and TBABF ₄ (0.160 g, 0.50 mmol), scan rate = 25 mV.s^{-1} , and (c) electropolymerization of CoTAPc on GCE-ERGO, scan rate = 100 mV.s^{-1}	135
Figure 4.2: FT-IR spectra ITO with (i) electrodeposited ERGO and (ii) electropolymerized	137

Figure 4.3: (a) CVs and (b) Impedance spectra (Nyquist plots) of (i) GCE, (ii) GCE-ERGO, (iii) GCE-polyCoTAPc and (iv) GCE-ERGO/polyCoTAPc in (1:1) 2.0 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ containing 0.10 M KCl. Scan rate of 50 mV.s^{-1}	138
Figure 4.4: (a) CVs at increasing scan rates from 25 - 350 mV.s^{-1} in a 0.10 M PBS (pH 7.4) at GCE-ERGO/polyCoTAPc, and (b) the plot of I_{pc} vs scan rate.	141
Figure 4.5: CVs of GCE-ERGO/polyCoTAPc in (a) 0.10 M PBS (pH 7.4), and with (b) 1.0 mM DA, (c) 1.0 mM PA, (d) 1.0 mM DA + 1.0 mM PA. Scan rate: 50 mV.s^{-1} . All solutions were purged with N_2 gas for 10 minutes prior to electrochemical measurement.	143
Figure 4.6: CVs of (a, a') 0.10 mM DA and (b, b') 0.10 mM PA and corresponding linear plots in 0.10 M PBS of pH values ranging from 2 to 9. All solutions were purged with N_2 gas for 10 minutes prior to measurement.....	145
Figure 4.7: CVs and corresponding linear plots of GCE-ERGO/polyCoTAPc in 0.10 M PBS (pH 7.4) containing 0.10 mM (a, a') DA and (b, b') PA. Scan rates 25 - 350 mV.s^{-1} . All solutions were purged with N_2 gas for 10 minutes prior to measurements.	146
Figure 4.8: DPVs of GCE-ERGO/polyCoTAPc in 0.10 M PBS (pH 7.4) containing increasing concentrations of (a) DA (2.0 – 100 μM) and (b) PA (7.0 – 90 μM), and corresponding calibration curves. All solutions were deaerated with N_2 gas for 10 minutes prior to DPV measurements.....	148
Figure 4.9: DPVs for GCE-ERGO/polyCoTAPc in 20 μM (a) DA and (b) PA in 0.10 M PBS (pH 7.4) (i) before and (ii) after adding 0.10 mM AA.....	150
Figure 4.10: DPVs for GCE-ERGO/polyCoTAPc in 50 μM (a) DA and (b) PA in 0.10 M PBS (pH 7.4) (i) before and (ii) after adding 0.20 mM UA.....	151
Figure 4.11: DPVs for GCE-ERGO/polyCoTAPc in a mixture of DA and PA (50 μM each) in 0.10 M PBS (pH 7.4) (i) before and (ii) after adding (a) 1.0 mM AA and (b) 0.20 mM UA.	152

Figure 4.12: DPVs of 20 μM of (a) DA and (b) PA at the GCE-ERGO/polyCOTAPc at different times and the corresponding bar graphs.....	153
Figure 5.1: (a) SEM, (b) FT-IR spectra and (c) Raman spectra of (i) RGO, (ii) MoS ₂ and (iii) RGO/MoS ₂ composites.....	169
Figure 5.2: CV of GCE-RGO/MoS ₂ /Ni(OH) ₂ in a 0.10 mol L ⁻¹ NaOH solution at a scan rate of 50 mV.s ⁻¹	170
Figure 5.3: (a) XPS survey spectra of the GCE-RGO/MoS ₂ /Ni(OH) ₂ and high resolution of (b) C 1s, (c) Mo 3d, (d) S 2p, (e) Ni 2p and (f) O 1s of the GCE-RGO/MoS ₂ /Ni(OH) ₂	172
Figure 5.4: (a) CVs and (b) EIS (Nyquist plot representation) for (i) GCE, (ii) GCE-RGO/MoS ₂ and (iii) GCE-RGO/MoS ₂ /Ni(OH) ₂ in (1:1) 2.0 mM Fe(CN) ₆ ³⁻ /Fe(CN) ₆ ⁴⁻ containing 0.10 M KCl. Scan rate = 50 mV.s ⁻¹	174
Figure 5.5: CVs of GCE-RGO/MoS ₂ /Ni(OH) ₂ in (i) 0.10 M PBS (pH 5.0) and (ii) containing (a) 1.0 mM AA, (b) 0.30 mM DA, (c) 0.50 mM UA, and (d) the mixture of 1.0 mM AA + 0.30 mM DA + 0.50 mM UA. Scan rate: 50 mV.s ⁻¹	176
Figure 5.6: Effect of (a) deposition potential and (b) deposition time of Ni(OH) ₂ on the electrocatalytic current of 1.0 mM AA, 0.30 mM DA, and 0.50 mM UA at GCE-RGO/MoS ₂ /Ni(OH) ₂ in 0.10 M PBS (pH 5.0).....	179
Figure 5.7: CVs recorded in pH 4.0 – 10.0 PBS containing 300 μM AA, 20 μM , and 30 μM UA. (b) Plots of peak current versus the pH value. (c) Plots of peak potential versus the pH value. (n = 3).....	180
Figure 5.8: CVs and the calibration plots of GCE-RGO/MoS ₂ /Ni(OH) ₂ at different scan rates ranging from 25 to 175 mV.s ⁻¹ of (a) 2.0 mM AA , (b) 0.050 mM DA and (c) 0.70 mM UA in 0.10 M PBS (pH 5.0) solution.	182

Figure 5.9: DPVs and the corresponding calibration curves of the GCE-RGO/MoS ₂ /Ni(OH) ₂ at different concentrations in PBS (pH 5.0) for (a) AA (20 - 1000 μM) (b) DA (1 - 50 μM), and (c) UA (5.0 - 150 μM). (n = 3).....	184
Figure 5.10: DPVs and corresponding calibration curves of GCE-RGO/MoS ₂ /Ni(OH) ₂ in 0.10 M PBS (pH 5.0) for the simultaneous detection of (a) AA (0.10 - 3.0 mM) in the presence of 5.0 μM DA and 100 μM UA, (b) DA (1.0 - 50 μM) in the presence of 1.0 mM AA and 200 μM UA , and (c) UA (5.0 - 200 μM) in the presence of 500 μM AA and 5 μM DA. Scan rate: 20 mV.s ⁻¹ , pulse interval 200, pulse amplitude 25 mV, pulse width 50 ms. (n = 3)	187
Figure 5.11: Shows (a) ten repetitive CV measurements in 0.10 M PBS (pH 5.0) containing 1.0 mM AA, 0.30 mM DA and 0.50 mM UA at GCE-RGO/MoS ₂ /Ni(OH) ₂ , and (b) the stability response of the GCE-RGO/MoS ₂ /Ni(OH) ₂ toward 1.0 mM AA, 0.30 mM DA and 0.50 mM UA measured weekly for three weeks.	189
Figure 5.12: DPVs of GCE-RGO/MoS ₂ /Ni(OH) ₂ in 1 % newborn serum in PBS (0.10 M, pH 5.0) (i) alone and (ii) containing (a) 0.40 mM AA, (b) 20 μM DA, and (c) 75 μM UA.	190

LIST OF TABLES

Table 1.1: Showing the concentrations of the catecholamine NTs for optimal and healthy body function.	3
Table 3.1: Comparison of the electrocatalytic activity of the Au-IPA-AmGQDs-CoTCPhOPc for detection of catecholamine neurotransmitters with some of the reported modified electrodes.	110
Table 3.2: Detection of DA, EP and NOR in newborn calf serum sample using percentage recovery method and calibration curve in Fig. 3.9 and the results were conducted in triplicates (n = 3).....	113
Table 4.1: Comparison of GCE-ERGO/polyCoTAPc toward the detections of DA and PA with other reported modified electrodes using DPV method.....	149
Table 4.2: Detection of 20 μ M, 40 μ M and 60 μ M DA and PA concentrations in 10% urine samples using the GCE-ERGO/polyCoTAPc.....	154
Table 5.1: Comparison of electrocatalysis of GCE, GCE-RGO/MoS ₂ and GCE-RGO/MoS ₂ /Ni(OH) ₂ towards the detection of AA, DA, UA, and a mixture of AA+DA+UA.	177
Table 5.2: Summary of electrochemical analytical parameters for the detection of AA, DA and UA at GCE-RGO/MoS ₂ /Ni(OH) ₂ compared with reported electrodes.....	185
Table 5.3: Summary of the detection of AA, DA, and UA in newborn calf serum (n = 3) for the following concentration for AA (0.40 mM, 0.80 mM, and 1.0 mM), DA (10 μ M, 15 μ M and 20 μ M) and for UA (50 μ M, 75 μ M and 0.10 mM).	191

LIST OF SCHEMES

Scheme 2.1: (a) Mechanism of the cutting of graphene sheets into GQDs (b) Models of the GQDs in acidic (right) and alkali (left) media [48].	34
Scheme 2.2: Schematic illustration of (a) the proposed formation mechanism for AmGQDs, and (b) nucleophilic substitution of epoxide with ammonia [54, 60].	36
Scheme 2.3: Illustration of the aryl diazonium electrografting of para-substituted aryl diazonium salt, (i) electrochemical reduction step leading to the generation of the aryl radical, followed by (ii) spontaneous chemisorption of the aryl radical onto the electrode surface.	47
Scheme 2.4: (a) Electrografting which results in multilayer film, and (b) electrografting that result in monolayer formation using ortho substituted aryl diazonium salts.	50
Scheme 2.5: Mechanism of carbodiimide-mediated carboxyl activation and amide bond formation.	52
Scheme 2.6: (a) Structure of metal (II)-4, 9, 16, 23-tetraaminophthalocyanine and (b) corresponding electropolymerization process [130].	58
Scheme 3.1: Synthesis of graphene quantum dots (GQDs) and amino functionalized graphene quantum dots (AmGQDs) [15].	90
Scheme 3.2: Fabrication of Au-IPA-AmGQDs-CoTCPhOPc via electrochemical grafting of IPA and covalent immobilization of AmGQDs and CoTCPhOPc using carbodiimide coupling reaction.	94

CHAPTER 1

1.1 Justification

Neurotransmitters (NTs) are endogenous chemical messengers that transmit, enhance, and convert specific signals between neurons and other cells across chemical synapses exchanging information throughout brain and body [1–4]. This transmission proceeds by secretion of the NTs from one neuron to the next. They do this by binding to the specific receptor located on the membrane of the target cell [5]. In the body, NTs are produced by glands such as the pituitary, pineal and adrenal glands [4]. Since the discovery of the first neurotransmitter in 1921, more than one hundred chemical messengers have been identified, which are involved in synaptic transmission. They play a key role in the functioning of the brain and control various behavioural and physiological conditions that affect our daily life, such as learning, memory, sleeping, consciousness, mood and the regulation of muscle tone, heart rate and blood pressure [6,7]. There are three main classes of NT molecules and they are based on their chemical structure: these are (i) amino acids (glutamic acid and tyrosine), (ii) catecholamines (dopamine, epinephrine, norepinephrine and serotonin), and (iii) soluble gases (nitric oxide and hydrogen sulfide) [8,9]. Among these NTs, catecholamines are one of the most important groups of biochemical molecules in the central nervous system (CNS) [9–11]. They play an important role in the functioning of cardiovascular, renal, and hormonal systems [12–14]. Catecholamines have important pharmacological functions such as the treatment of myocardial infarction, hypertension, and organic diseases [15]. Catecholamine NTs are of major interest.

Dopamine (DA) was discovered in 1950s and has a variety of functions such as human movement, memory, behaviour, sleep, and cognition [16,17]. It is generated by the brain,

specifically in the dopaminergic neuron. DA in the frontal lobe is known to control the flow of information from other areas of the brain which is important for attention, problem solving and memory [9]. DA is also termed as the “reward chemical” since it is released in the course of rewarding experiences such as eating food, engaging in sexual intercourse, and other stimulating experiences [9]. Lack or abnormal levels of DA is strongly associated with many body disorders such as sleeping and eating disorders, addictive behaviours due to drug abuse, Parkinson’s disease (PD) and schizophrenia [18,19]. In patients with Parkinson’s disease, loss of dopaminergic neurons leads to dopamine depletion in the brain and drastically reduces voluntary movement [7]. Clinically, most patients with PD disorder suffer from slowness of movement, rest tremor, rigidity, and disturbances in balance [20]. According to world health organization (WHO), the prevalence of PD has doubled in the past 25 years with global estimates in 2019 showing over 8.5 million individuals living with PD globally.

Epinephrine (EP), also called adrenaline, is an important catecholamine NT in mammalian CNS [8,9,21]. EP is produced by the adrenal glands, and it prepares the body for the fight or flight reaction [8,22]. It boosts the supply of oxygen and glucose to the brain and muscles in the emergency situations [9]. Many diseases (neurological, psychiatric, and cardiovascular) are related to changes of EP concentrations in the human body [23]. It is also prescribed as a drug in the treatment of asthma, sepsis, severe allergic reactions, cardiac arrest, and anaphylaxis [24,25].

Norepinephrine neurotransmitter is also present in the CNS and is secreted by the adrenal medulla [9,15,26]. NOR is involved in emotional arousal, blood pressure regulation and mood disorders in the CNS [8]. However, abnormal concentration levels of NOR in the human body can lead to many diseases, such as ganglia neuroblastoma, Parkinson’s diseases, coronary

heart disease, multiple sclerosis, psychosocial stress, anxiety, depression, Alzheimer's disease and paraganglioma [26,27].

The normal concentration levels of the above-mentioned catecholamine neurotransmitters in biological fluids in a healthy body are summarized in **Table 1.1**. **Table 1.1** shows the concentration levels in different samples or bodily fluids [2]. There is a close correlation between DA and NOR in plasma and in blood this is because plasma is a subcomponent of blood. Concentration of NTs in urine is much higher than in plasma and blood.

Table 1.1: Showing the concentrations of the catecholamine NTs for optimal and healthy body function.

Catecholamine NTs	Plasma	Urine/24 hrs	Blood
DA	0.06 to 20 ng/mL	51 to 645 µg	10 to 480 pM
EP	0.02 to 0.46 nM	2.5 to 20 µg	20 to 460 pM
NOR	0.45 to 2.49 nM	15 to 80 µg	0.45 to 2.49 nM

Variation (decrease or increase) in the concentrations of catecholamine NTs in biological fluids is crucial for the disease or disorder diagnosis. Therefore, it is very necessary to develop fast, accurate and sensitive methods for the determination of catecholamine NTs in various physiological media such as urine, plasma, and cerebral fluids. The detection and quantification of catecholamine NTs is thus of great importance for medical diagnosis and treatment, as well as for the discovery of new drugs [8,28]. A wide variety of methods have been developed for the detection of catecholamine NTs in biological samples. These include chromatography [29], spectrophotometry [17], capillary electrophoresis [30] and chemiluminescence [31] just to name a few. Chromatography method involves separation of the individual catecholamine NTs

for excellent sensitivity, selectivity, and low detection limits. The separation of individual catecholamine NTs has many drawbacks, such as cost of analysis, long analysis time and complicated samples pretreatment. In addition, there is a demand of highly skilled personnel for a routine analysis [32]. Spectrophotometric methods are often used in analytical laboratories for the detection of catecholamine NTs. The principal reasons for their popularity are easy availability of photometers, colorimeters, or single beam spectrophotometers. Most of these methods involves molecular absorption spectroscopy in the ultraviolet and visible region, infrared spectroscopy with Fourier transformation and nuclear magnetic resonance. The disadvantages of spectroscopic methods are time-consuming and complicated sample preparation. Spectroscopy methods also require preconcentration step before analysis to improve sensitivity. Electrochemical techniques have attracted attention toward detection of catecholamine NTs. This is due to their inherent advantages such as simplicity, rapidity, cheap/affordable, selective, have high sensitivity, can be of miniaturized and also integrated in portable devices [33,34]. Catecholamine NTs are electroactive and can undergo two electron reduction or oxidation (redox) reaction on the catechol ring. Electrochemical methods can also allow for the direct detection of catecholamine NTs in biological fluids [8]. They represent useful analytical tools for real time and online clinical applications.

Electrochemical detection of catecholamine NTs at bare electrodes such as gold (Au), platinum (Pt) and glassy carbon (GC) results in signal interference from other chemicals that coexists in similar biological media. These are the challenges that unmodified electrodes suffer. Catecholamine NTs coexist with other interfering molecules such as ascorbic (AA), uric acid (UA) and paracetamol (PA) in human body fluids. This is a serious issue in the electrochemical detection of catecholamine NTs [35]. Catecholamine NTs and coexisting molecules usually oxidize at similar oxidation potentials with poor selectivity at bare or unmodified electrodes

[36,37]. Therefore, selective detection of catecholamine NTs in the presence of interfering agents cannot be achieved at the bare electrodes [38]. Bare or unmodified electrodes also suffer from passivation due to the adsorption of products that form during the oxidation or reduction of catecholamine NTs [18]. Passivation leads to decrease in the electrocatalytic signal.

To overcome these challenges, several forms of chemically modified electrodes (CMEs) have been constructed for the detection of catecholamine NTs. CMEs are formed by binding the mono or multilayer molecules on the surface of the electrode [39]. In this case the sensitivity and selectivity of the electrode surface is improved. A wide variety of electrode modifying materials, such as metal nanoparticles, metal alloys, metal oxides, conducting polymers and carbonaceous materials, have been employed for fabrication of CMEs. CMEs do not easily get passivated and can be used several times without losing the catalytic activity. Carbon-based nanomaterials have attracted scientific attention for the development of CMEs and the detection of catecholamine NTs due to their remarkable physico-chemical properties.

Carbon materials have excellent electrical conductivity, high effective surface area, high porosity, and can be obtained from non-toxic, low-cost environmentally friendly methods [40]. Furthermore, carbon-based materials are used as electrode modifiers due to their antifouling properties and also large adsorption ability toward various analytes [41,42]. Among these materials, graphite, graphene, carbon nanotubes, carbon dots, carbon black and bio char have been used for fabrication of conducting electrode materials. More importantly, these carbon-based materials have specific molecular structures and can be easily combined with inorganic/organic moieties to form composite materials. Composite materials have been shown to exhibit an increase in the electrochemical sensitivity towards the target analytes [43]. Graphene (GR) and carbon nanotubes (CNTs) have been widely used for the construction of

electrochemical sensors over the past decade [42]. These materials are used for electrode modification or as components of hybrid electrode materials [44, 45]. The electrodes modified with CNTs and GR exhibit significantly larger electroactive surface area and excellent electrocatalytic properties [44,45]. More importantly CNTs and GR are biocompatible materials, as such they provide suitable conditions for electron transfer between biomolecules and the electrode. GR is an emerging carbon-based nanomaterial for electrochemical electrode modification due to its carbon sp^2 structure. GR has numerous advantages such as unique electronic, mechanical, high surface area, biocompatibility, high conductivity, and optical properties [44]. Graphene is an inexpensive, green, and non-toxic material unlike the carbon nanotubes (CNTs) which have toxic effects due to existence of metallic impurities and biomolecules coils around them [46]. GR modified electrodes have shown better electrocatalytic performance compared to CNT electrodes [47]. In addition, GR is the basic building block for graphitic materials of all dimensions. Therefore, studying GR is expected to provide a fundamental insight into all carbon materials. Most of the graphene used in electrochemistry is produced through chemical, electrochemical and thermal reduction of graphite oxide (GO) [44].

GR sheets are hydrophobic in nature and tend to form aggregates which limit their applications. Aggregation of GR can be reduced by attaching small molecules or polymers. So far graphene and its derivatives have been used to form composites with metallo phthalocyanine, metal and/or metal oxides on the electrodes. The graphene-based composite electrodes show synergistic effects with unique properties of individual components [48]. As a result, the electrocatalytic activity and stability of the composited modified electrode is further enhanced.

Metallo phthalocyanines (MPcs) are fascinating materials with various sites for structural modifications. As electrode materials, MPcs can be used to enhance sensor applications [49, 50]. MPcs are often used in the development of sensors due to their facile preparation methods, extensive redox behaviour, excellent electrocatalytic activity and ease of surface modification [51]. Therefore, MPc modified electrodes can perform qualitative and quantitative analyses of many substances at trace levels. The electrochemical properties of MPcs strongly depend on the central metal ion at the centre of the macrocycle ring [52]. Also, MPcs can bear substituents on the peripheral and non-peripheral positions. The MPcs containing Co, Mn and Fe as the central metal are the best for electrode modification. Their ability to complex metal ions at the centre of the macrocycle ring allows for redox reaction to occur. Paramagnetic metal ions (Fe, Co, and Mn) have half-filled d-orbitals and can accept and donate electrons. Their interaction with analytes is via the central metal ions. However, MPcs alone have low conductivity when they are employed as electrode materials and thus slow electron transfer [48, 53]. Therefore, alone would be catalytic but they require composition with conducting properties [18]. To overcome the low conductivity of MPcs, they are combined with novel nanostructures such as GR and its derivatives as well as carbon nanotubes [48,54]. This forms a composite material with excellent conductivity and electrocatalytic properties. Here, MPcs can increase sensor sensitivity due their rich electrocatalytic properties.

Besides, MPcs, noble metals nanoparticles (Pt, Au, Pd and Ag) supported on graphene and its derivatives have also been greatly employed as electrode materials for the determination of catecholamine NTs. Noble metals have unique structures, high electrical conductivity, good electrocatalytic conductivity and good biocompatibility. However, noble metals are very expensive, scarce, and susceptible to fouling [55, 56]. This has resulted in low researcher interests for their use to commercialize electrochemical sensors for practical applications.

Transition metals and their oxides have been found to be alternatives to replace noble metal nanoparticles because they are cheap, abundant, and highly electroactive. Among the low-cost metal/metal oxides, transition metal oxides (TMOs) have been employed as electrode materials for electrochemical sensing of catecholamine NTs. TMOs have flexible valence oxidation states and enable reversible faradaic reactions at an electrode surface [55,57]. Among the TMOs, nickel oxide/hydroxide (NiO/Ni(OH)_2) have been extensively used in electrode modification. NiO/Ni(OH)_2 have inherent electrocatalytic activity for oxidation reactions, which is based on redox reaction of $\text{Ni}^{\text{II}}/\text{Ni}^{\text{III}}$ on the electrode surface [58]. Nickel/nickel oxide nanoparticles have been used to fabricate electrodes because of their good stability, mechanical strength, rapid electron transport and low cost [59]. However, Ni-based electrodes alone have poor electrical conductivity when applied as electrode material. Nickel/nickel oxides have been combined with graphene and its derivatives in order to improve their electrochemical sensing performance. For the past decade, layer-structured transition-metal chalcogenides (TMDs), such as tungsten disulphide (WS_2) and molybdenum disulphide (MoS_2), have attracted a lot of attention due to their unique chemical and physical properties. Specifically, MoS_2 has gained research attention in fields such as electrochemical sensors, hydrogen storage, catalysis, and capacitors. MoS_2 is analogous to graphene as it form a sheet-like structure but it is composed of three atom layers. A molybdenum layer is sandwiched between two sulphur layers and the triple layers are stacked and held together by weak van der Waals interactions [60]. Graphene based materials and metal or metal oxides when integrated with MoS_2 , form composites with excellent electrocatalytic properties due to synergistic effects from various materials [61].

Research into electrochemical sensors involving graphene and its derivatives as composites still continues to attract attention toward electrochemistry and related technologies. The benefits of using graphene based composite materials as electrode modifiers continues to attract

attention of researchers. Novel strategies for integrating these materials are continue to be explored. The understanding of graphene and its derivatives as electrode modifiers was of interest to this research.

Detection of catecholamine NTs in real samples such as biological fluids is a challenge and this is due to complex matrix effect of real samples and interference from ascorbic acid and uric acid [2]. The electrochemical response signal of catecholamine NTs increases or decreases due to interferences or background due to matrix effect. Therefore, the reported amounts of catecholamine NTs in real samples are not accurately represented. The challenges due to matrix effect in real samples can be overcome by modifying electrodes with novel materials that induce selectivity towards the analyte of interest. The materials used as electrode modifiers also introduce or impart their character onto the electrode. Two main approaches are employed (a) the attachment of some functional groups or chemical moieties to the modified electrode surface which selectively bind the analyte of interest, (b) modifying the electrode surface with some functional groups or ion-exchange resins which can exclusively repel all the interferences [2]. In this regard, nafion and chitosan have been largely employed for repelling interferences due to their anionic nature [62]. Furthermore, the challenges due to matrix effect in real sample can be overcome by diluting the real samples to several folds and also extraction of analyte or interferences from sample solution before analysis [2]. This elongates the analysis process and is undesirable (unfavourable) for point-of-care or online analysis.

The sensitivity of an electrochemical method for the detection of catecholamine NTs can be enhanced by accelerating electron transfer at the electrode surface. This can be achieved through accumulation of the analyte molecules as they interact with the functional groups or chemical moieties at the electrode surface. Analyte molecules can also accumulate on the

electrode surface via physical adsorption [2]. Increasing surface area of a working electrode is another important factor to improve sensitivity of an electrochemical sensor. At the enhanced surface area, more functional groups or chemical moieties are exposed to the target analytes, thereby enhancing the sensitivity of an electrochemical sensor.

In order to evaluate the applicability of an electrochemical sensor the detection of catecholamine NTs is usually conducted in biological matrices such as sweat, urine, and serum and cell culture media. Herein, real samples are spiked with known concentrations of the catecholamine NTs. Standard addition method is employed to determine the recovery concentrations of the target analyte.

This thesis investigates the engineering of various electrode surfaces with different functional groups for the detection of dopamine in the presence of different interfering species with increased sensitivity and selectivity. Epinephrine and norepinephrine catecholamine NTs were also detected using the engineered surfaces. The suppression of the interfering species such as ascorbic acid and uric acid was evaluated using pH sensitive surface. Another very important aspect was increasing the sensitivity by adding onto the electrode materials with excellent conductivity. These were also combined with electrocatalysts to form novel composite materials. The electrochemical detection was also studied in real samples for evaluating the matrix effect and the modified electrode performance.

1.2 Research aims and objectives.

The overarching aim of this study was developing affordable, sensitive, selective electrochemical methods based on graphene and its derivatives as composite electrodes for detection of catecholamine neurotransmitters.

The following objectives were set-out to achieve this aim:

- (a) Fabrication and characterization of a (i) covalent composite cobalt (II) tetracarboxyphenoxy phthalocyanine and aminated graphene quantum dots gold electrode, (ii) electroreduced graphene oxide-cobalt(II) tetra amino phthalocyanine polymer composite electrode, and (iii) nickel hydroxide electrodecoated molybdenum disulfide-reduced graphene electrode.
- (b) Detection of catecholamine neurotransmitters selectively using composite of cobalt (II) tetracarboxyphenoxy phthalocyanine and aminated graphene quantum dots gold electrode.
- (c) Enhancing sensitivity of catecholamine neurotransmitter detection using aminated graphene quantum dots.
- (d) Screening of ascorbic acid as a strong interferent to the detection of catecholamine neurotransmitters using pH effect.
- (e) Detection of catecholamine neurotransmitters in phosphate buffer solution (PBS) and buffer diluted newborn calf serum samples.
- (f) Simultaneous detection of dopamine and paracetamol simultaneously at the electroreduced graphene oxide - cobalt (II) tetra amino phthalocyanine nanocomposite polymer electrode in phosphate buffer solutions and synthetic urine samples.

- (g) Synthesis and characterization of molybdenum disulfide -graphene oxide materials.
- (h) Simultaneous detection of dopamine at nickel hydroxide electrodeposited molybdenum disulfide - reduced graphene oxide electrode in the presence of ascorbic acid and uric acid.

1.3 Thesis outline

Chapter 1: Justification

This chapter describes the importance of electrochemical sensing of catecholamine neurotransmitters as biomolecular analytes and their relevance to clinical and monitoring of diseases. The chapter also highlights the current challenges faced by electrochemical methods towards detection of catecholamine neurotransmitters. This chapter proposes the use of conducting thin films composited with graphene-based materials that can enhance sensitivity, reduce overpotential, improve selectivity and minimize the interferences towards detection of catecholamine NTs in biological fluids. Lastly, the chapter highlights the aims and objectives of the study and the thesis outline.

Chapter 2: Literature review

Chapter two provides the background for the detection and monitoring of catecholamine NTs. It highlights the current trends in the development of different electrochemical sensing methods based on graphene and its derivatives as composite materials for the detection of catecholamine NTs. The chapter highlights problems faced during simultaneous detection of catecholamine NTs in the presence of coexisting biomolecules and possible solutions.

Chapter 3: In-sera selective detection of catecholamine neurotransmitters

Method development for the detection of catecholamine NTs using stable electrografting method, conjugated aminated graphene, and acid functionalized cobalt phthalocyanine for selective screening of interferents. A covalent composite of cobalt tetra-(3-carboxyphenoxy) phthalocyanine (CoTCPhOPc) onto aminated graphene quantum dots (AmGQDs) coupled onto the isophthalic acid electrografted gold electrode (AuE-IPA). The modified AuE-AmGQDs-CoTCPhOPc was employed for selective detection of DA, EP and NOR using square wave voltammetry in phosphate buffer solution (PBS, pH 7.4). The modified sensor exhibited excellent electrocatalytic activity towards the oxidation of DA, EP and NOR with enhanced current responses. The method was further employed for the analysis of DA, EP and NOR in newborn calf serum samples validating the sensor applicability in real time sample analysis. The modified sensor was further employed for the detection of DA, EP and NOR in the presence of large concentration of ascorbic acid. The pH sensitive electrode successfully screened the strong interference from ascorbic acid.

Chapter 4: Simultaneous detection of dopamine and paracetamol on a polymer composite electrode

Graphene oxide (GO) was successfully electroreduced and deposited onto a glassy carbon electrode (GCE) forming highly conducting electrochemically reduced graphene oxide (GCE-ERGO). On this electrode, cobalt tetra amine phthalocyanine (CoTAPc) was electropolymerized to yield an excellent electrocatalytic surface. The modified surface was used to detect dopamine in the presence of paracetamol (PA) in phosphate buffer solution and synthetic urine samples as way of validating the sensor applicability in real life situation. The

modified sensor was finally used for the detection of DA and PA in the presence of major interference of ascorbic acid and uric acid.

Chapter 5: Electrocatalysis of conducting graphene, metal oxide, and sulfide composites

In this chapter, a glassy carbon electrode (GCE) modified with molybdenum-disulfide (MoS_2) reduced graphene oxide (RGO)-nickel hydroxide ($\text{Ni}(\text{OH})_2$) was prepared. RGO/ MoS_2 composite was prepared ex-situ, characterized using materials techniques, and drop-casted on the GCE and dried in air for overnight to form GCE-RGO/ MoS_2 . On this electrode the electrodeposition of nickel hydroxide thin film resulted in a GCE-RGO/ MoS_2 / $\text{Ni}(\text{OH})_2$. The modified electrode was successfully used for the simultaneous electrochemical detection of DA, AA, and UA in phosphate buffer solutions and in diluted newborn calf serum samples (representing real sample analysis).

Chapter 6: Conclusions and future perspectives

This chapter summarized the conclusions of the research conducted in this work. The future perspectives and work that needs to be realized for real applications was highlighted. This chapter further highlighted the applications of these systems.

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CHAPTER 2

2 Literature review

This chapter provides the background of the development electrochemical sensors based on graphene derivatives and electrocatalytic materials as composite electrodes for the detection of catecholamine neurotransmitters. The chapter highlights the problems that are faced with simultaneous detection of catecholamine NTs in the presence of coexisting biomolecules and possible solutions.

2.1 Catecholamine neurotransmitters

Neurotransmitters (NTs) are the body's chemical messengers. NTs are the molecules used by the nervous system to transmit messages between the neurons, or from neurons to muscles. Most NTs are small amine molecules, amino acids (catecholamines), and/or neuropeptide. Communication between two neurons happens in the synaptic cleft (the small gap between the synapses of neurons) as shown in **Fig. 2.1**, where, electrical signals are briefly converted into chemical signals through the release of neurotransmitters, causing a specific response in the receiving neuron. Neurons produce electrical events called action potentials, which are also known as impulses or spikes. They allow neurons to communicate with each other, computation to be performed, and information to be processed. When a neuron spikes it releases a NT, a chemical that travels a tiny distance across a synapse before reaching the other neurons.

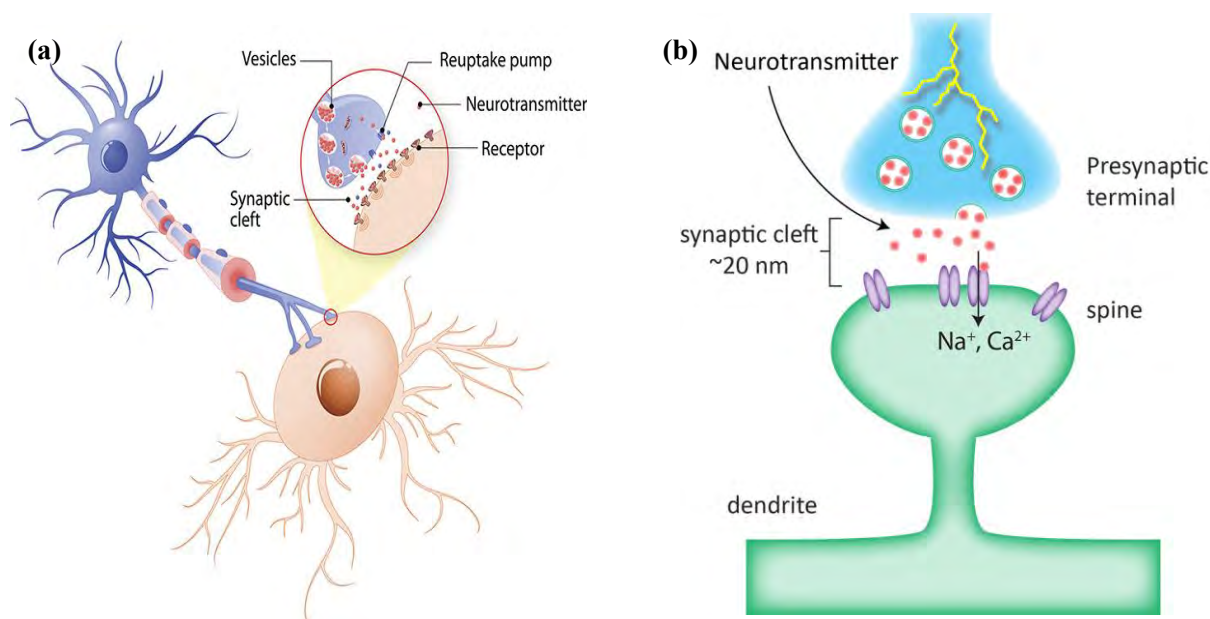
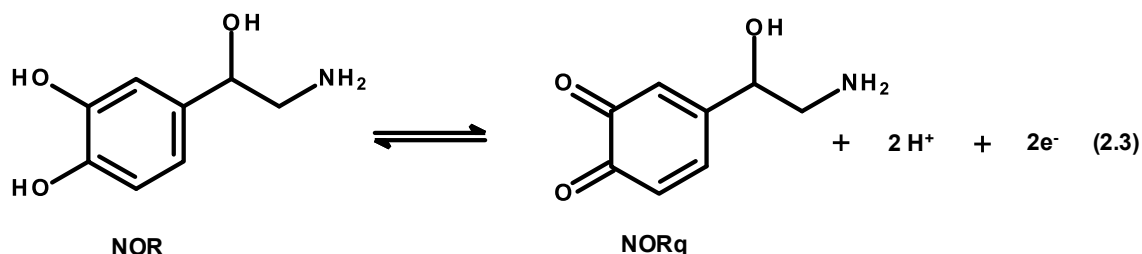
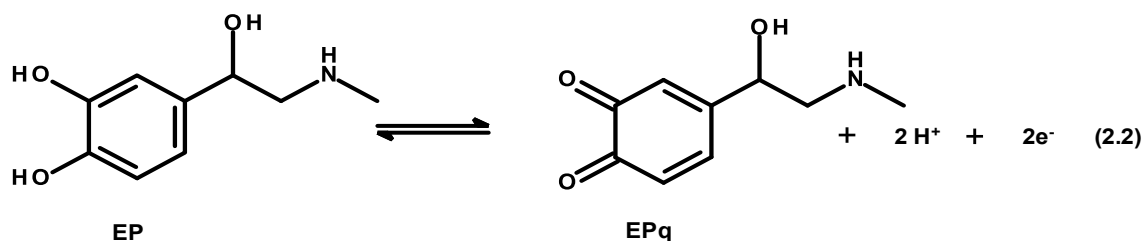
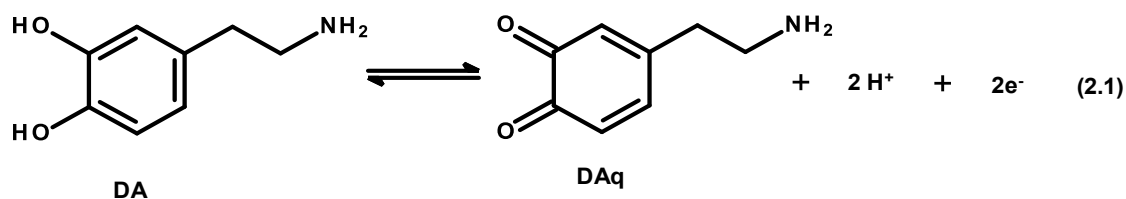


Figure 2.1: (a) Neurotransmitters released in the nervous system, (b) an action potential (yellow lighting) causes NTs to be released and travels across the gap to activate receptors on the receiving neuron [1].

2.1.1 Oxidation of catecholamine neurotransmitters

Dopamine (DA), epinephrine (EP), and norepinephrine (NOR) are very important catecholamine NTs in the mammalian central nervous system. They play an essential role in controlling bodily operations [1,2]. Understanding the oxidation chemistry of dopamine, epinephrine and norepinephrine is necessary because this enables researchers to develop suitable electroanalytical methods for detection of these compounds [3]. This will in turn aid in diagnosis of some serious and incurable diseases. The electrochemical oxidation reactions are proposed to occur via the following half-reactions for dopamine (Equation 2.1), epinephrine (Equation 2.2) and norepinephrine (Equation 2.3).



Dopamine, epinephrine, and norepinephrine are oxidized to form open chain quinones (DAq, EPq and NORq) in Equations (2.1 - 2.3), respectively. These open-chain quinones undergo intramolecular cyclization by 1,4-micheal addition from quinone derivatives (dopamine-o-quinone, epinephrinequinone and norepinephrinequinone) to form leucochromes (dopaminechrome, epinephrinechrome, and norepinephrinechrome) [3-5]. Leucochromes get easily oxidized to form oxidized cyclization products that are pH dependent. In low pH or acidic aqueous solutions, the open chain quinones are protonated and the cyclization product is not favoured. In high pH or alkaline aqueous solutions, non-protonated open chain quinones are present and cyclization products are favoured.

2.1.2 The effect of interferents on catecholamine neurotransmitters detection

Intensive efforts have been devoted to in vitro and in vivo electrochemical detection of catecholamine NTs. However, a major problem in the detection is the coexistence of interfering molecules such as ascorbic acid (AA), uric acid (UA), and paracetamol in the human body. AA is a water-soluble vitamin C found mostly in citrus fruits and green leafy vegetables. It is the most chemically labile one amongst all vitamins. AA is found in the plasma, it is excreted in the urine, and it plays an important role in redox reactions in the human body. It is the most important antioxidant substance which is found in extracellular fluids. Ascorbic acid concentration is much higher than the concentrations of catecholamines. At most solid or unmodified electrodes, AA is oxidized at potentials close to that of the catecholamines, resulting in overlapping electrochemical responses [6]. Also, in the case of electrochemical oxidation of dopamine, the oxidized product dopamine-o-quinone (DAq) is reduced back to dopamine in the presence of ascorbic acid, resulting in an enhanced anodic current [6].

Uric acid is another common antioxidant with similar oxidation potential to catecholamine neurotransmitters. UA is an end product of purine metabolism and is excreted with the urine in humans [7]. UA is a strong hydrophilic antioxidant in extracellular fluids. It can remove free radicals such as hydroxyl radical, singlet oxygen, and peroxynitrites. UA is also present at extracellular concentration several orders of magnitude higher than the catecholamine NTs in the brain [8].

2.1.3 Detection of catecholamine neurotransmitters in real samples

Apart from major interferences in terms of UA and AA, there are numerous other factors that either significantly increase or decrease the electrochemical response of catecholamines. Electrochemical analysis of catecholamine neurotransmitters in real samples such as urine or blood is challenging due to unknown matrix effect [9]. Therefore, the reported amounts of catecholamines in real samples do not represent the actual amounts. Recently, electrochemical methods have been validated by addition of few steps to sample preparation to reduce matrix effect of real samples. As such challenges due to matrix effect in real samples can be overcome by opting following steps:

- (i) Inducing selectivity of electrode surfaces by modifying with novel materials.
- (ii) Diluting real samples to several folds.
- (iii) Extracting analytes or interferents from sample solution before analysis.

For blood samples, centrifugation is the first step to remove red blood cells and followed by dilution. Urine samples are only diluted before measurement or analysis. The quantification in these samples is usually done by spiking with a standard of catecholamines and applying the standard addition method.

2.2 Graphene and its derivatives as electrode modifiers

Graphene has been a material of choice for electrode modification due to its highly conjugated structure and excellent conducting properties. It is an excellent material for supporting variety of inorganic compounds/complexes and organic compounds on the electrode surface. It has superior physico-chemical properties for the applications in electrochemical studies.

2.2.1 Graphene oxide (GO) as a graphene derivative

Carbon based nanostructures have been studied extensively due to their excellent properties and numerous applications. Amongst the various carbon materials, graphene is one of the most interesting in the present era due to its remarkable properties like high surface area, tunable band gap, and excellent electrical, thermal and conducting properties [10]. Structurally, graphene is a one-atom-thick planar sheet of sp^2 bonded carbon atoms, two dimensional and simple layered structure with the honeycomb-like structure [11, 12]. The several methods that have been used for the preparation of graphene materials, are micromechanical exfoliation [13], chemical vapour deposition [14], epitaxial growth [15], arch discharge [16], unzipping of carbon nanotubes [17], electrochemical [18] and chemical methods [19]. Amongst them, chemical methods have been widely employed for the preparation of graphene and result in high yields [20]. Chemical methods can either be top-down or bottom-up. The top-down method involves strong oxidation of graphite flakes to graphite oxide and subsequent exfoliation to graphene oxide as shown in **Fig. 2.2**.

The graphene oxide prepared following a top-down method is obtained through chemical reduction from graphite oxide following a reported Hummers method [21]. The Hummers method has attracted a large attention because of its high efficiency and satisfying reaction safety [22]. Here graphite oxide is usually prepared through the oxidation of graphite using oxidants such as concentrated sulphuric acid, nitric acid, and potassium permanganate [21]. Graphite oxide has a similar layered structure as that of graphite, but the plane of carbon atoms in graphite oxide is heavily decorated by oxygen-containing groups [22]. The oxygen functionalities expand the interlayer distance and also make the atomic-thick layers hydrophilic [22, 23]. Graphene oxide (GO) is obtained by exfoliation of graphite oxide in water by

ultrasonication [24, 25]. The GO product can be purified by centrifugation and dialysis to remove aggregates and various inorganic impurities such as metal ions and acids [26]. GO is most commonly prepared using chemical methods. The chemically prepared GO consists of layered GO sheets that contain many oxygen-containing functional groups such as carboxyl (COOH), hydroxyl (-OH), epoxy (C-O-C), and carbonyl (C=O) groups [24, 27]. Therefore, with oxygenated functionalities, GO sheets can be dispersed in water or polar organic solvents [28]. The presence of these oxygen-containing functional groups affects the planar sp^2 carbons in the graphitic structure and converts it to sp^3 carbons [29]. As a result, the properties of GO change when compared to pure graphene. For instance, the electrical conductivity of GO is significantly decreased [10,29]. The electrical conductivity can be recovered by restoring the π -network through removal of some oxygen functionalities through reduction of GO. The reduced GO is often referred to as reduced graphene oxide (RGO).

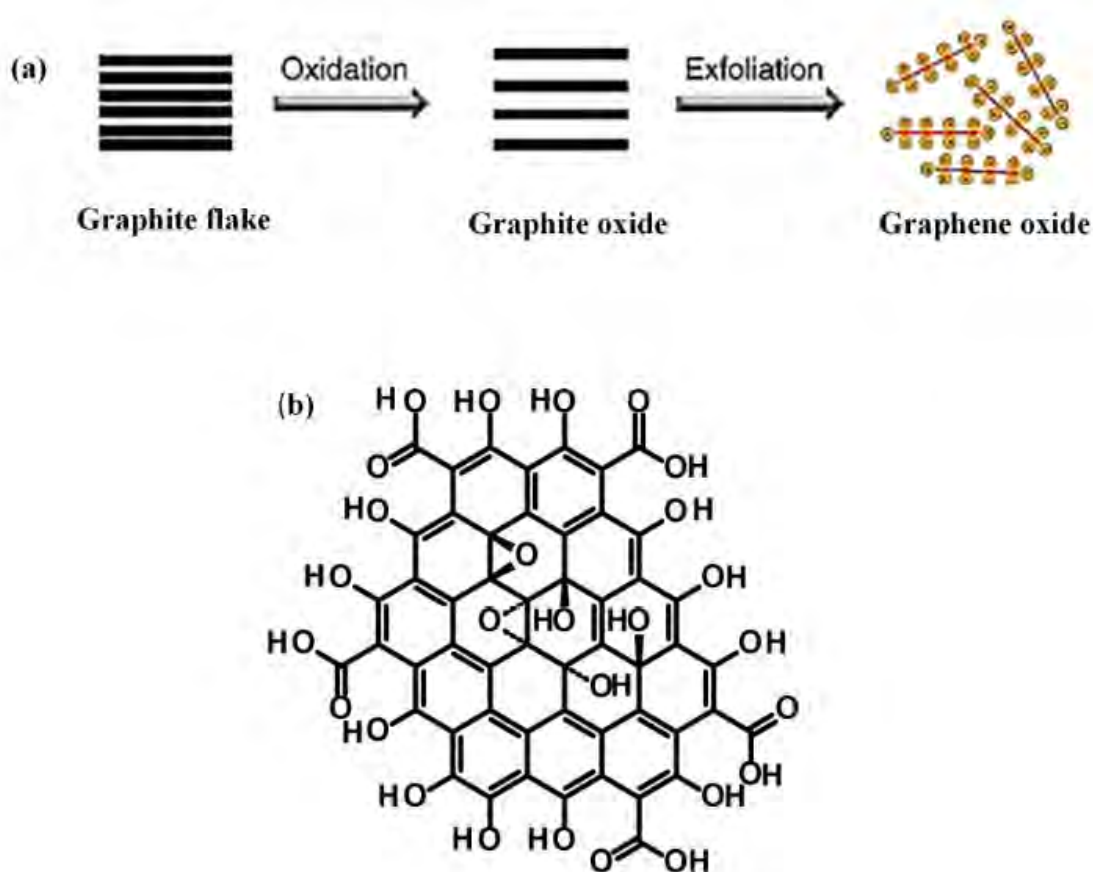


Figure 2.2: (a) Schematic illustration for chemical oxidation graphite flake to graphene oxide [12] and (b) chemical structure of an isolated sheet of graphene oxide (GO).

In electrochemical sensing, the RGO is frequently used instead of pristine graphene (GR). The extensive use of RGO in electrochemical sensing is due to its facile and cost-effective preparation, easy functionalization, and ease of handling. RGO is usually prepared by reducing GO in a solid or dispersing state [28]. The chemical reduction is the most widely used technique to convert GO to RGO. A variety of inorganic salts or complexes or organic compounds such as hydrazine monohydrate, sodium borohydrate, hydroquinone and vitamin C have been used as reducing agents [30]. Most of the reducing agents described above are toxic or difficult to handle for large scale production [28, 31]. GO can also be converted to RGO by electrochemical, thermal, microwave assisted thermal, hydrothermal or solvothermal reduction methods [32-35]. These techniques have been widely used to produce highly conductive RGO

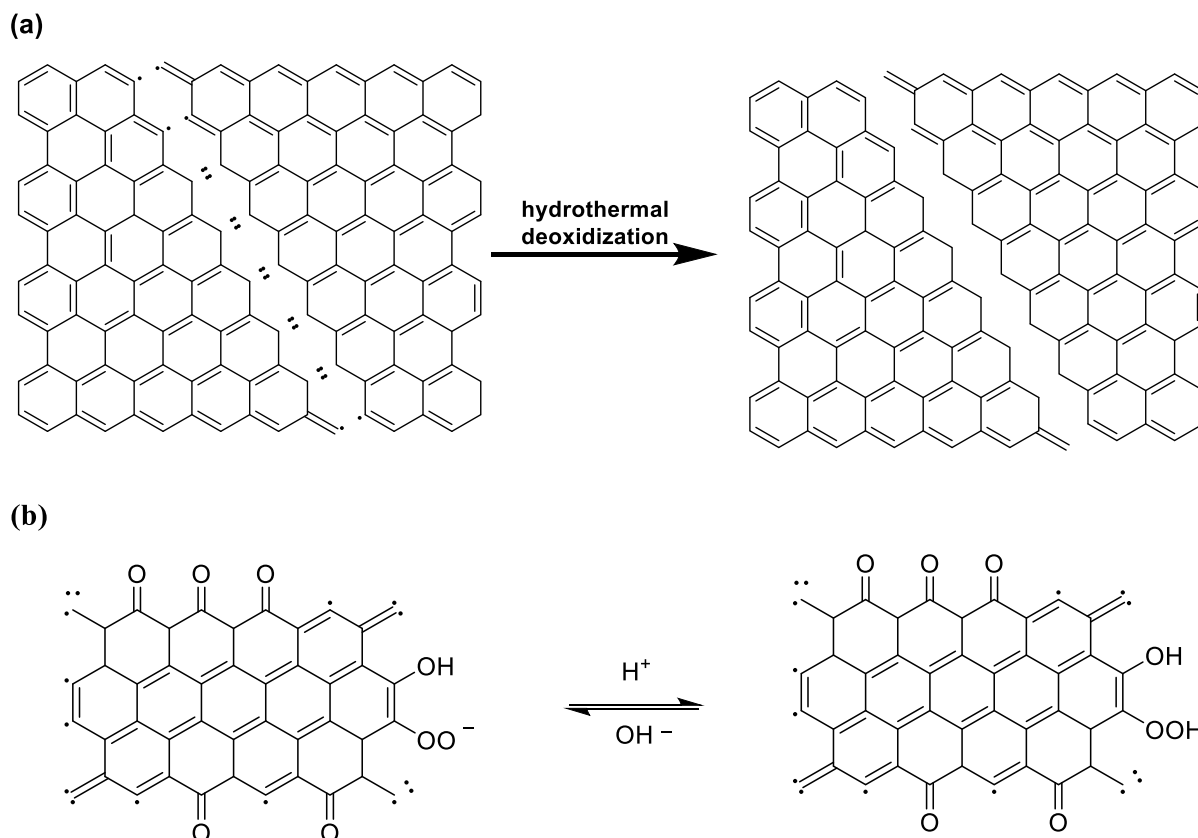
with few residual oxygenated groups on a large scale [28]. Electrochemical reduction of GO is an effective and clean route to form RGO [36]. Particularly, it provides unique controllable method for depositing thin RGO films with desired shapes and patterns [28]. Moreover, the electrical conductivity and electrochemical performance of the electrochemically reduced graphene oxide (ERGO) are much better than those of chemically or thermally RGO materials [25, 36, 37]. Graphene oxide can be electrochemically reduced and deposited onto the electrode surface by applying the constant or sweeping potential for regulated period of times. Studies have shown that the electroreduction of graphene oxide improve the electron transfer kinetics of the electrode surface for electrooxidation of the biomolecules [38, 39]. More importantly, the potential controlled partial reduction of graphene oxide also helps to improve the sensitivity and specifically selectivity for the certain biomolecules [40]. Guo and co-workers prepared high quality graphene nanosheets through electrochemical reduction of exfoliated GO precursor on a surface of GCE [36]. The produced ERGO nanosheets were carefully characterized with both spectroscopic and electrochemical techniques. Particularly, the FTIR spectra indicated that a variety of the oxygen-containing functional groups were thoroughly removed from the GO plane via electrochemical reduction in comparison to the chemically reduced GO. More importantly, the ERGO accelerated the electron transfer rate in DA electrochemistry. The real electrocatalytic mechanism of DA on graphene modified electrode is not yet clear [36]. The peak-to-peak separation was as small as 44 mV which was much smaller than that on a bare GCE.

2.2.2 Graphene quantum dots (GQDs)

Graphene quantum dots (GQDs) have been considered as the most interesting materials in studying carbon nanomaterial applications [41]. Some studies have reported the development

of electroanalytical methods employing GQDs as electrode modifiers and for electrochemical sensing [42, 43]. Graphene quantum dots have excellent opto-electrical properties, extremely low cytotoxicity, and good biocompatibility [44,45]. GQDs are widely used as a suitable electrode material in the field of electrochemical sensors due to their novel properties such as controllable surface functionalities, quantum confinement and edge effects [44,45]. They expand contact area with the analyte, which increases the electrochemical active surface area to interact with some electroactive analytes [44]. The presence of oxygenated functional groups enables its interaction with several analytes including metals, dyes, proteins, hormones, and pesticides. This enhances their use in electrochemical analysis.

GQDs are prepared through simple and low-cost methodologies that can be grouped into top-down and bottom-up methods. In the top-down method, large graphene sheets, carbon nanotubes, carbon fibers or graphite are cut down into smaller pieces of graphene quantum dots [46]. While in bottom-up methods involve the use of small molecules such as citric acid or glucose as starting materials to build the GQDs via thermal treatment [43]. The bottom-up growth process is widely used due to its simple operation, low environmental pollution, better purity, and aqueous dispersion of the formed GQDs [47]. Pan and co-workers prepared water-soluble blue luminescent GQDs of diameter 5-13 nm, using a hydrothermal cutting method from bulk graphene source (**Scheme 2.1**) [48]. The method involved several steps, namely, thermal reduction of graphene oxide to graphene sheets, oxidation of graphene sheets in acid media and hydrothermal reduction to afford the GQDs. The oxidation step, in acidic conditions, creates epoxy groups that tend to form in a line along a carbon lattice causing a rupture of the C–C bonds [42]. The epoxy chain is then further oxidized into epoxy pairs that are converted to more stable carbonyl pairs. The carbonyl groups make the GQDs dispersible in water.



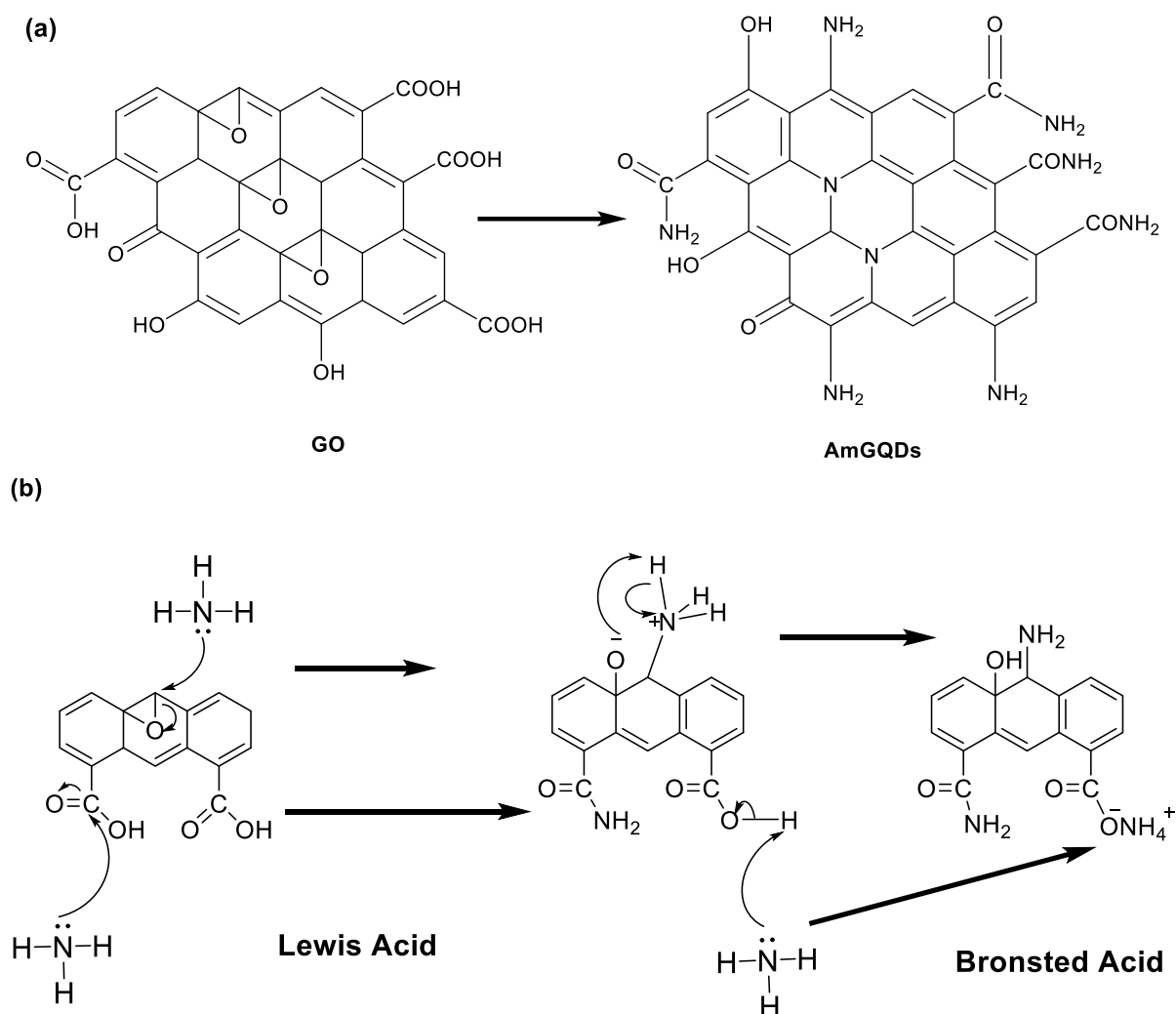
Scheme 2.1: (a) Mechanism of the cutting of graphene sheets into GQDs (b) Models of the GQDs in alkali (left)) and acidic (right media [48].

GQDs can be functionalized with surface groups such as carboxyl and hydroxyl [49,50]. These surface functional groups improved the optical properties of GQDs and made it easy to disperse in aqueous medium. More importantly, these functional groups at the margin of GQDs allow for further functionalization with a wide range of inorganic, organic, polymeric, and biological molecules. Pang and co-workers developed a novel electrochemical sensor for sensitive and selective detection of DA based on GQDs and Nafion composite modified glassy carbon electrode (GCE) [51]. GQDs were synthesized by a hydrothermal approach through cutting graphene sheets into GQDs. The carboxyl groups on the surface of GQDs provided good stability and also enabled interaction with amine functional groups in DA through electrostatic interaction. The GQDs-Nafion composite exhibited a good linear range of 5 nM to 100 μ M and

a limit of detection (LOD) of 0.45 nM for the detection of DA. Moreover, the proposed electrochemical sensor also displayed good selectivity and high stability. Tashkhourian and co-workers synthesized chitosan GQD carbon paste composite electrode using an easy bottom-up procedure via carbonization of citric acid (CA) [52]. The presence of carboxyl groups in the GQDs result in strong electrostatic interactions with amine functional groups of the chitosan polymer. An excellent electrochemical platform for epinephrine (EP) detection was realised due to strong electrostatic interactions and conjugated π - π bond in the chitosan-GQD composite. Furthermore, the modified electrode gave a good linear relationship between the peak current and concentration of EP over a wide concentration range 0.36 to 380 μ M with a limit of detection of 0.30 nM.

2.2.3 Aminated graphene quantum dots (AmGQDs)

Recently, the functionalization of GQDs with amine groups has gained interest in tuning the electronic and optical properties of GQDs. Nitrogen doping on GQDs results in a strong covalent interaction between the nitrogen atoms and the graphene network [53]. The amine-functionalized GQDs is prepared by hydrothermal method using ammonia [54-56] or urea [57] as nitrogen containing compounds. Typically, amine-functionalized graphene quantum dots (AmGQDs) are prepared through the amine-mediated bond-scission using a nucleophilic substitution reaction [58-60]. The oxygen functional groups on oxidized graphene sheets (OGSs) reacts with ammonia under low-temperature amine-hydrothermal conditions as shown in **Scheme 2.2(a)**. Ammonia reacts with epoxy groups to form primary, secondary and tertiary amines and primary alcohols thus enabling simultaneous extraction of the GQD from the OGS. The ring opening of the epoxide is shown in **Scheme 2.2(b)**. The produced AmGQDs are doped with nitrogen, edge-terminated with primary amines ($-\text{NH}_2$), and terminal amides ($-\text{CONH}_2$).



Scheme 2.2: Schematic illustration of (a) the proposed formation mechanism for AmGQDs, and (b) nucleophilic substitution of epoxide with ammonia [54, 60].

2.3 Graphene and its derivatives as composites for neurotransmitters detection

The detection and quantification of catecholamines NTs in vitro or in vivo is a subject of great importance in clinical medical research. A variety of graphene and its derivative composites have been studied as sensing materials for these biomolecules. A major goal of using graphene or its derivatives is to improve the performance of bare electrodes. Spanning through literature, the composites of metallophthalocyanines (MPcs), transition metal dichalcogenides (TMDs) and transition metal hydroxides with graphene and/or its derivatives have been sparingly used

especially for the detection of catecholamine NTs. This is despite the synergistic effects which can be realized from individual components if used as composites. Therefore, the work in this thesis is focussed on the development of electrochemical platforms based on graphene and its derivatives as composites with MPcs, TMDs and metal oxides/hydroxides for detection of catecholamine NTs.

2.3.1 Graphene-metallo phthalocyanine composites

Metallophthalocyanines (MPcs) are electron rich compounds with interesting physico-chemical properties. They are known to possess good chemical and thermal stability and also good spectral properties. These properties have seen MPcs being widely used in electrocatalysis, electrochemical sensors, electrochromism, photodynamic therapy, photovoltaic cells, photosensitizer, liquid crystal materials, and as catalysts [61]. Metals at the centre of MPcs can bind a variety of analytes in an indifferent manner, that is (i) via the coordination, (ii) hydrogen bonds, and (iii) van der Waals forces [62]. MPcs are highly suitable for incorporation into electrochemical sensors [61]. Phthalocyanines occur in two different forms as an unmetalated (H_2Pc) and metal containing (MPc). For metal containing MPcs, there are two methods of their preparation, that is (i) direct metalation and (ii) substituting hydrogens on the H_2Pc . Other than substituting H_2Pc to form MPc, interesting compound can also be substituted at two positions, which is at α -positions or β -positions. The α -positions are also referred to as non-peripheral positions whilst β -positions are called peripheral positions. **Figure 2.3** shows the chemical structure of H_2Pc (**1**) and MPc (**2**). The non-peripheral (α) substituents are found at positions 1,4,8,11,15,18,22, 25, whereas the peripheral (β) substituents are found at positions 2,3,9,10,16,17,23, 24.

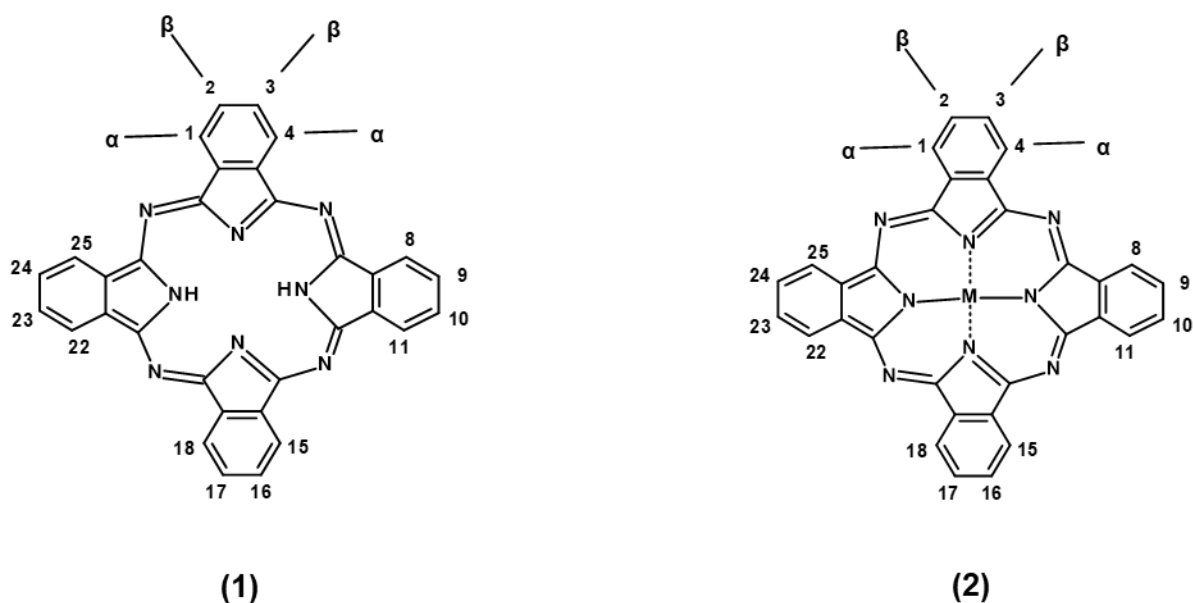


Figure 2.3: Molecular structure of unmetallated (metal free) phthalocyanine (H_2Pc , **1**) and metalated (metal containing) phthalocyanine (MPc, **2**). Both showing α - and β - positions and their numerical notations.

Recently, MPcs have been extensively studied as electrocatalysts for many reactions with the aim of developing sensitive, selective, and stable electrochemical sensors. The use of MPcs is due to their several advantages over noble metal electrocatalysts and can be obtained at relatively low cost [63]. The reactive centres MPc are clearly identified and can be modulated by choice of the central metal and/or functional group or substituent on the ring [63]. When immobilized onto the electrode surfaces, MPcs act as electrocatalysts and can increase peak currents and lower redox potential. Electrocatalysis of MPcs is due to their ability to change oxidation states of the central metal and also of the ring structure. Amongst the studies of MPcs, cobalt phthalocyanine (CoPc) is one of the well-researched electrocatalysts for oxidation or reduction reactions of biomolecules [64]. CoPc has rich redox chemistry with high electron-transfer ability and has been widely used in sensor fabrication because of its excellent electrocatalytic activity [65, 66]. The electrocatalytic activity of MPcs is however hindered

when deposited alone on the electrode surface and this is due to the formation of aggregates [67]. And also, the method of modifying electrodes with MPcs plays a role. The most widely used drop-drying method for depositing MPcs on the electrode surface results in the MPc materials peeling off from the electrode surfaces. When this occurs under the physiological conditions, it affects the electrochemical activity and stability [68]. Hence, it is of paramount importance to seek for a substrate material, which can not only provide a suitable microenvironment for retaining the electroactive molecules but also enhance direct electron transfer between the electroactive species and underlying electrodes [69]. Studies have shown that graphene, a novel carbon material with high surface area, many functional groups, and defects on the surface has been widely used as a good “anchor” for the MPcs [70]. The resulting graphene-MPcs composite displayed extraordinary electrochemical catalytic responses towards various biomolecules. Ndebele and co-workers prepared CoPcs π - π stacked onto nitrogen-doped graphene quantum dots for electrochemical oxidation of dopamine [71]. An improvement in the sensitivity and low limit of detection was observed. Yang et al, fabricated a cobalt phthalocyanine-graphene (CoPc-Gr) complex through π - π interaction of each component [72]. The CoPc-Gr modified glassy carbon electrode showed excellent response to the electrooxidation of dopamine and ascorbic acid, much better than those of CoPc, graphene oxide or graphene modified electrode alone. Xu and co-workers prepared a novel electrochemical sensing platform based on nitrogen-doped graphene (N-G)/nickel tetra sulfonated phthalocyanine (NiTsPc) through π - π interaction [69]. The proposed modified presented superior catalytic efficiency for dopamine in the presence of ascorbic acid and uric acid owing to the synergistic effect of N-G and NiTsPc. The perm-selective layer of negatively charged polymer complexes NiTsPc with negative SO_3^- repelled negatively charged species such as ascorbate anion from the electrode surface. Thus, the electrooxidation of the negatively charged species was prevented on the electrode surface without the loss of sensitivity.

2.3.2 Graphene – metal oxide composite thin films

Graphene is an excellent material when used as an electrocatalyst support, however, has some disadvantages such as slow electron transfer due to agglomeration of the graphene layers and their transformation into graphite [70]. Agglomeration of the graphene is mainly caused by van der Waals forces, which reduces the active surface area. The number of electrocatalytically active sites as such the electrocatalytic performance is decreased. It is possible to avoid restacking of graphene by forming composites with metal sulfides, such as molybdenum disulfide (MoS_2). MoS_2 is a two-dimensional transition metal dichalcogenide (2D-TMDs) that has gained scientific attention due to its unique properties similar to those of graphene [73]. Molybdenum disulfide nanosheets can be sandwiched between two layers RGO [74]. This will result into well-ordered RGO layers rather than undesirable stacking of RGO multilayers. Combination of the MoS_2 and RGO has the capacity to improve the sensor sensitivity and selectivity [75]. Xing and Ma developed a modified GCE sensor based on a hybrid nanocomposite of MoS_2 and RGO for the electrocatalysis of ascorbic acid (AA), dopamine (DA), and uric acid (UA), with enhanced sensitivities of 0.12, 4.11 and $1.59 \mu\text{A } \mu\text{M}^{-1} \text{cm}^{-2}$, respectively [76]. The oxidation potentials were well separated for AA-DA (232 mV), DA-UA (152 mV) and AA-UA (384 mV). The electrochemical catalytic properties of MoS_2/RGO nanocomposites can be further enhanced by the introduction of foreign transition metal hydroxides. Metals such as Fe, Co, and Ni and their oxides/hydroxides in the MoS_2/RGO lattice can engineer the electronic and surface structures [77]. Amongst these metals and their oxides, nickel oxides/hydroxides are widely used due to their relative low cost and toxicity, strong redox activity, and stable electrochemical response [78]. Geng and co-workers successfully

fabricated a Ni-doped MoS₂ /rGO composite for the detection of glucose in human blood. The composite shows improved electrocatalytic activity for glucose oxidation [79].

2.4 Basic principle of electrochemical sensors

An electrochemical sensor is a device that has the ability to detect chemical reactions that happen at the surface of the electrode through measuring certain electrical signals, such as current (amperometric, voltammetry), potential or capacitance. Electrochemical sensors are made up of three essential components: a **receptor** that binds the analyte, the **sample** or **analytes**, and a **transducer** to convert the reaction between a receptor and analyte into a measurable electrical signal. In the case of electrochemical sensors, the electrode acts as the transducer. In most electrochemical sensors, an electrode surface is used as the working electrode and site of the chemical reaction. The electrode will either oxidize or reduce the analyte of interest. In electrochemical sensors, a potentiostat controls the potential which is applied between the working electrode (WE) and reference electrodes (RE) [80,81] and the current is measured between the working electrode (WE) – counter electrode (CE). Current is measured as a function of applied potential. A potentiostat is as an active element whose function is to apply the potential overtime (excitation signal) and the required current through the WE is measured. **Figure 2.4** shows a three-electrode electrochemical set-up for an electrochemical measurement.

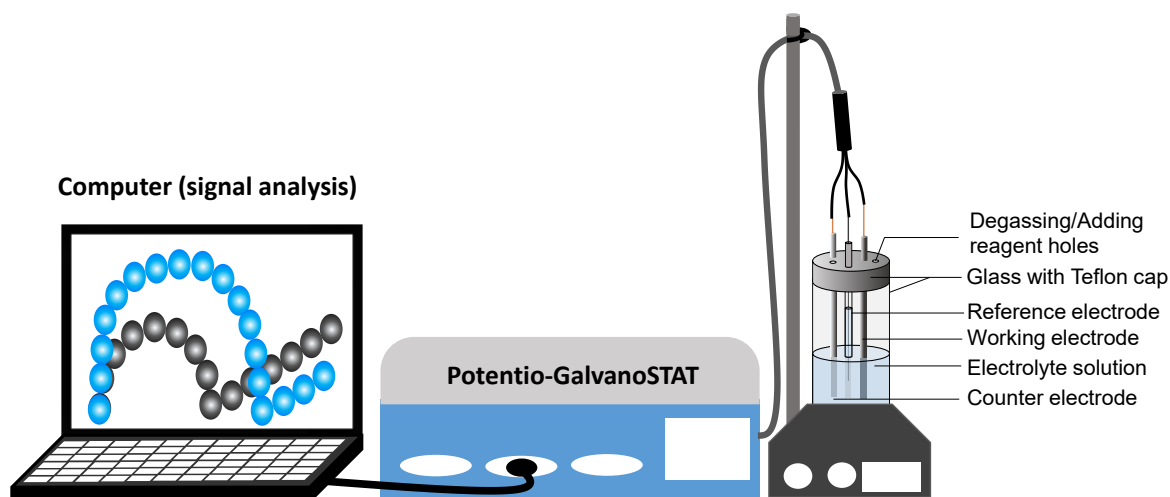


Figure 2.4: A schematic representation of three-electrode electrochemical set-up for an electrochemical measurement.

2.5 Voltammetric methods of electrochemical measurements

Voltammetry is a type of an electrochemical sensor in which a potential is varied and then a current is measured. The peak potential position is the formal potential of the compound detected, and thus different analytes have different voltammograms. The applied potential can take all different shapes called excitation signals, for example constant potential for amperometric measurement and stepping potentials for differential pulse and square wave voltammetry. Also, by ramping the potential linearly for techniques such as cyclic voltammetry or linear sweep voltammetry. Cyclic voltammetry is popular because it provides more information than amperometry and provides some selectivity to discriminate different compounds using their formal potential.

Cyclic voltammetry (CV) is the most widely used technique for acquiring qualitative information about electrochemical reactions [82]. CV provides considerable information on the

thermodynamics of redox processes and the kinetics of heterogeneous electron transfer reactions [82]. It offers a rapid location of redox potentials of the electroactive species, and convenient for evaluating the effect of media on the redox process [82]. Cyclic voltammetry consists of scanning linearly the potential of a stationary working electrode (in an unstirred solution) and this results in a triangular potential waveform also called excitation signal as shown in **Fig. 2.5**.

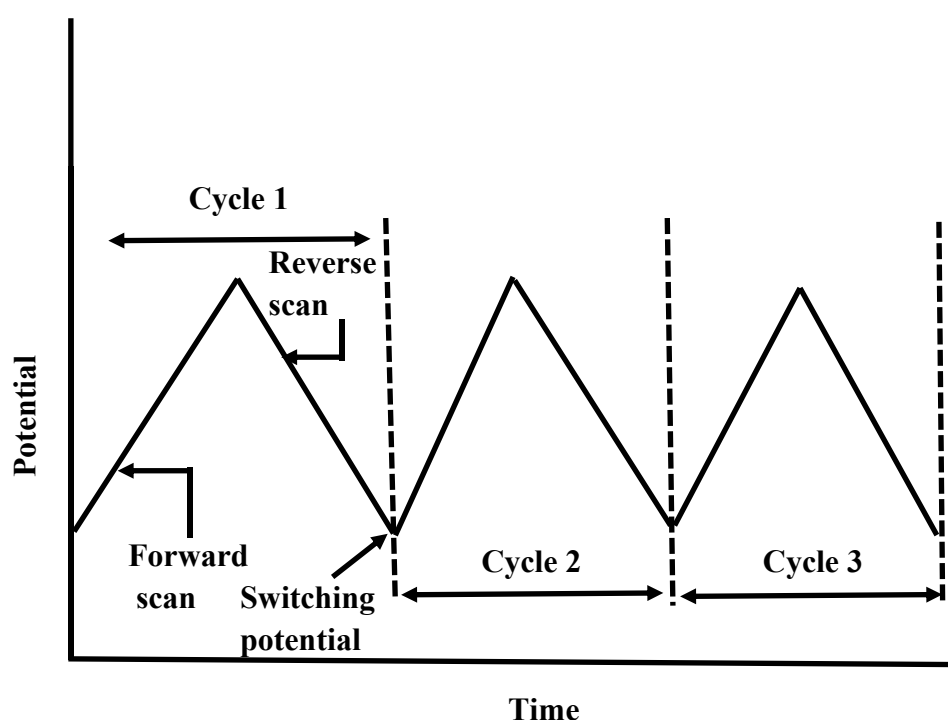


Figure 2.5: Potential-time excitation signal for cyclic voltammetric experiment.

During the potential sweep, the potentiostat measures the current resulting from the applied potential. The resulting current–potential plot is termed a cyclic voltammogram and useful for identifying analytes. **Figure 2.6** illustrates the expected response of a reversible redox couple during a single potential cycle. It is assumed that only the oxidized form O is present initially. Thus, a negative-going potential scan is chosen for the first half-cycle, starting from a value

where no reduction occurs. As the applied potential approaches the characteristic standard potential (E^0) for the redox process, a cathodic current begins to increase (onset potential), until a peak is reached. After traversing the potential region in which the reduction process takes place, the direction of the potential sweep is reversed. During the reverse scan, R (reduced) molecules (generated in the forward half-cycle and accumulated near the surface) are reoxidized back to O, resulting in an anodic peak.

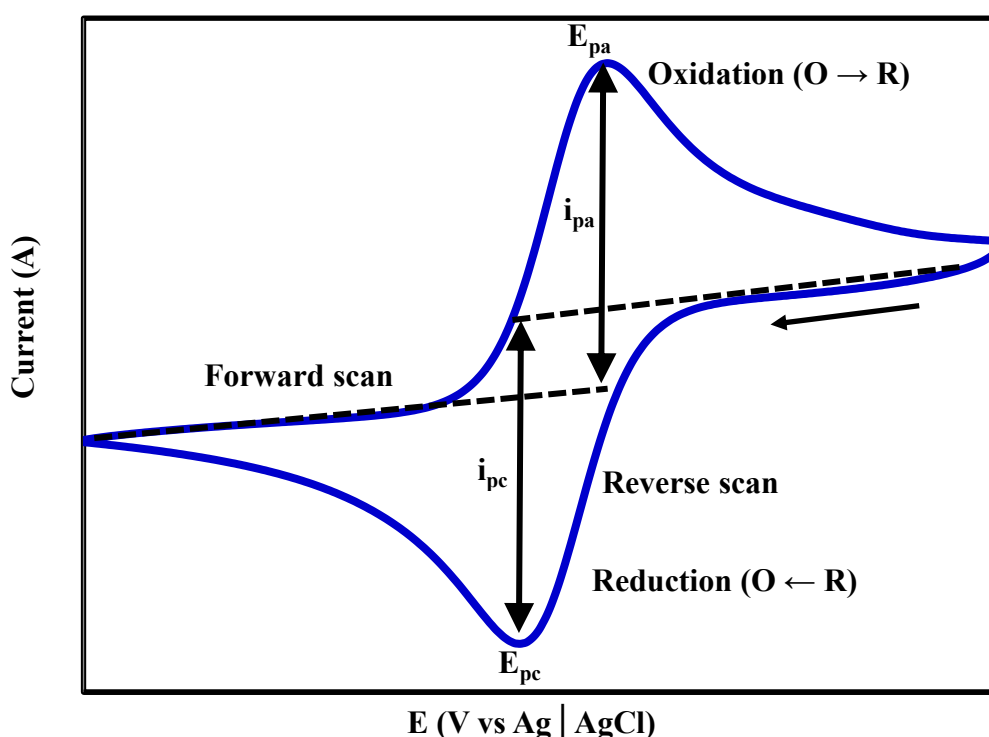


Figure 2.6: Typical cyclic voltammogram for a reversible $O \rightleftharpoons R$ redox process.

The cyclic voltammogram is characterized by several important parameters. Four of these are the two peak currents and two peak potentials. They provide the basis for analyzing the cyclic voltammetric response. The position of the peaks on the potential axis (E_p) is related to the formal potential of the redox process. The formal potential for a reversible couple is centred between anodic peak potential (E_{pa}) and cathodic peak potential (E_{pc}) as given by Eq. (2.4):

$$E^o = \frac{E_{pa} + E_{pc}}{2} \quad (2.4)$$

The separation between the peak potentials (for a reversible couple) is given by Eq. (2.5):

$$\Delta E = E_{pa} - E_{pc} = \frac{0.0592}{n} V \quad (2.5)$$

Thus, the peak separation (ΔE_p) can be used to determine the number of electrons transferred (n), and as a criterion for a Nernstian behavior about electrochemical reversibility for a redox couple. Accordingly, a fast one-electron process exhibits a ΔE_p of about 59.2 mV. The ratio between the anodic peak current (I_{pa}) and the cathodic peak current (I_{pc}) is another important parameter that relates the electrochemical reversibility of an electrode reaction [83]. For a simple reversible reaction, this ratio is equal to unity (1).

Differential pulse voltammetry (DPV) is one of the first electrochemical techniques used for detecting catecholamine NTs [84]. In DPV, a small amplitude, short pulses are superimposed on a linear ramp. Current is measured before the application of the pulse and at the end of each pulse, and the difference between the currents is calculated [80]. This procedure effectively reduces the background current due to the direct current (DC) ramp, and thus results in a Faradaic current free of most capacitive or background current. The major advantage of DPV is low capacitive current, which leads to high sensitivity. The small step sizes in DPV also lead to narrower voltammetry peaks and thus DPV is often used to discriminate analytes that have similar oxidation potentials [85].

Square wave voltammetry (SWV) is one of the most frequently used pulse voltammetry techniques, as it provides fast and highly accurate analysis [86]. SWV uses a combined square wave and staircase potential applied to a working electrode [80]. It offers high sensitivity in trace determination of analytes with pre-accumulation process.

2.6 Fabrication of graphene and its derivative composite electrodes

In order to establish high performance electrochemical sensors, modification to electrode surface is of major importance. For example, modification by electrocatalytic materials has been proposed to attain high sensitivity. Graphene and its derivatives form composites that are the preferred electrode materials. To attain high sensitivity and selectivity for the detection catecholamine NTs, graphene and its derivatives are fabricated as composite electrodes with the following characteristics:

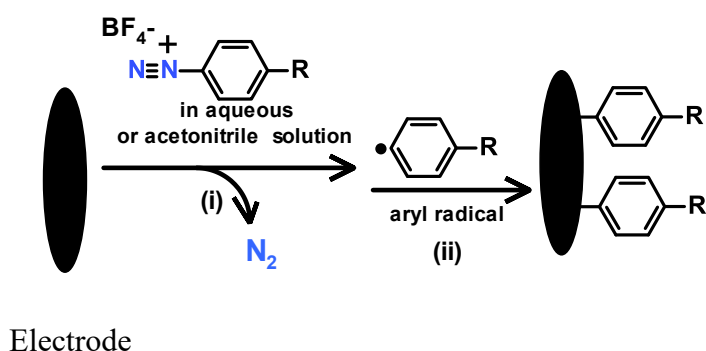
- (i) The fabricated surface should not be affected or interfere with the biological matrix in real complex samples.
- (ii) The developed electrochemical sensor should have excellent reproducibility and the repeatability.
- (iii) The developed electrochemical sensor should have a long shelf life and reusable.
- (iv) The material for electrochemical sensor fabrication process should be cost-effective.

The methods of modifying electrodes with graphene and its derivatives to make novel composite materials are drop-dry, polymerization, electro grafting, and electrodeposition just to name a few. Each method has its own merits, and these are discussed below.

2.6.1 Electrografting with diazonium salts

Electrografting is the term given to the covalent linkage of diazonium salts to a conductive surface by electrochemical reduction [87-89]. Electrochemical reduction of aryl diazonium salts

bearing different functional groups has been extensively used for electrografting various electrode surfaces due to its simplicity [90]. Electrografting has been defined as the electrochemical process that allows for the attachment of organic molecules onto the conducting solid surfaces [91]. The electrografted electrode usually serves as a platform for the fabrication of electrochemical sensors. Electrografting relies on the in-situ generation of the diazonium radical [92]. The aromatic amine can be transformed into a diazonium by a standard diazotization procedure [93], and that resulting solution can be used to modify carbon [94] or gold [95] through electrode electrochemical reduction. The thin electrografted layer is formed from the covalent bonding of the aryl group to the electrode surface [96, 97]. Electrografting of diazonium involves two steps: (i) Step 1 involves the electrochemical reduction of diazonium resulting in the removal of N_2 to form the aryl radicals, (ii) Step 2 is the grafting or attachment of the aryl radical by the formation of covalent bond with the electrode surface as shown in **Scheme 2.3** [98]. Electrochemical grafting is usually performed using 1.0 mmol/L or higher concentrations of aryl diazonium salt in acetonitrile (ACN) or sulphuric acid [99].



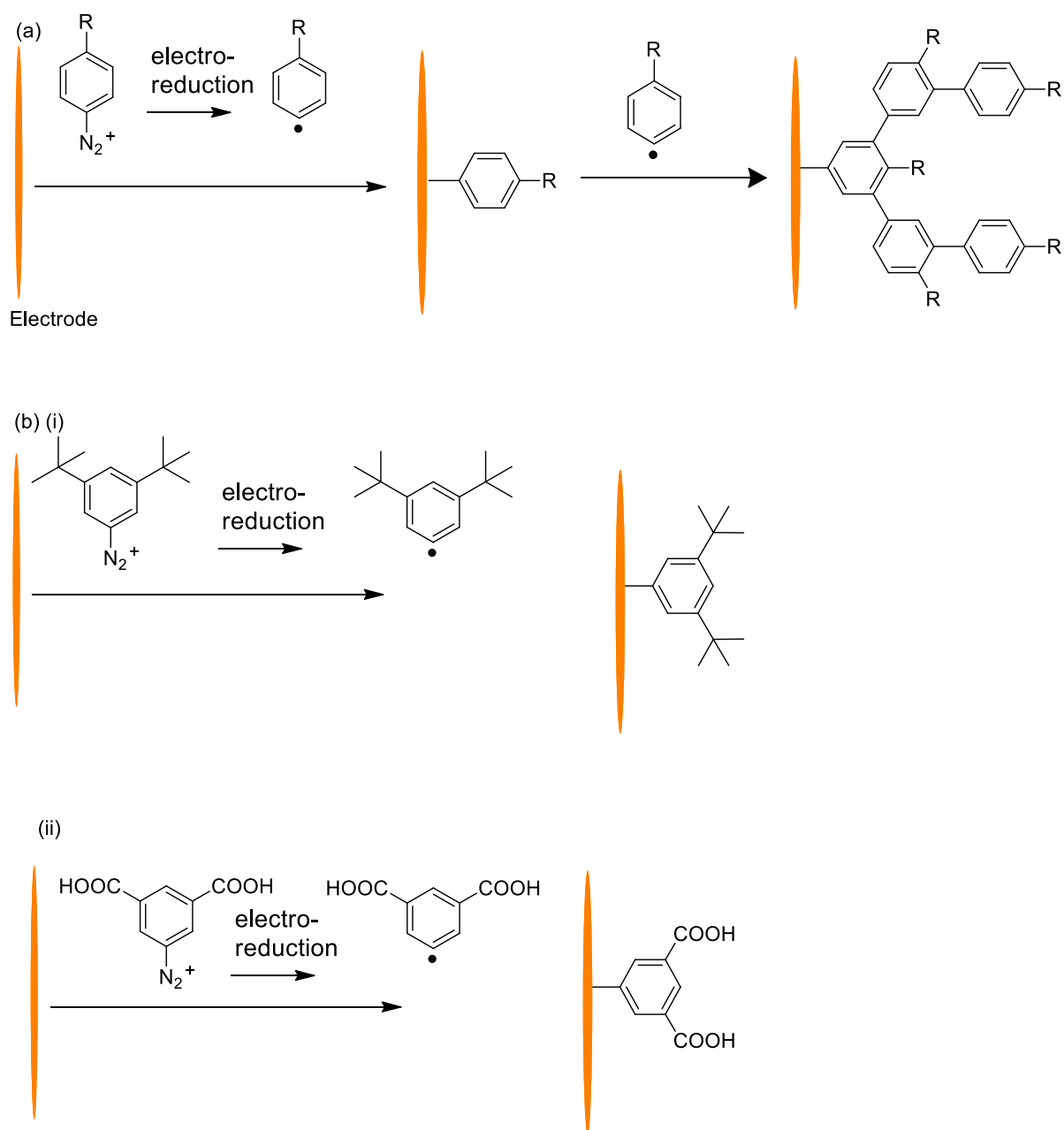
Scheme 2.3: Illustration of the aryl diazonium electrografting of para-substituted aryl diazonium salt, (i) electrochemical reduction step leading to the generation of the aryl radical, followed by (ii) spontaneous chemisorption of the aryl radical onto the electrode surface.

Electrografting was first reported and pioneered by Pinson research group [100]. Since the discovery, several research groups have explored the method for electrochemical modification. The method on glassy carbon electrode results in calculated C-C bond energy of 105 kcal.mol⁻¹ [101]. Also, coinage metal electrode surfaces have shown to result in M-C (carbide) bond with bond energy of 317 kJ. mol⁻¹ [101]. The method is simple and has received tremendous attention. Electrografting method addressed the disadvantages of modifying metal electrode using self-assembled monolayer (SAM). SAM method utilized thiol and amine functionalized compounds to form self-assembly via chemisorption. When thiolated compounds are used, a thiolate (M-S) bond forms with a bond energy of 184 kJ.mol⁻¹[102]. The challenge with M-S bond, is susceptible to oxidative and reductive desorption, thus limiting the solution pH and potential for electrochemical analysis [103]. In alkaline condition reductive desorption occurs at potential < -0.60 V vs Ag|AgCl. Here a self-assembly monolayer for instance of alkyl thiols on a gold surface (Au-SR) can be removed by applying a negative potential. The thiolated group (RS⁻) is electrochemically desorbed from the gold surface by following a one electron reduction in an alkaline solution, Eq. (2.6):



Furthermore, SAM on coinage metal suffers from mobility. All these critical issues hinder the electrochemical sensing applications involving SAMs on substrate electrodes where stable monolayers are required. Electrografting addressed the challenges of SAM and also form thin films with different functional groups which can further be used to immobilize electrocatalysts and biomolecules [104,105]. The grafting can be done in a short time (1-5 min), and the resulting layer is stable over a wide potential window as well withstands sonication [103,106]. During electrografting, stable organic thin layers are attached to the electrode surface through covalent bonding of the aryl group [88]. The organic layers attached to solid conducting substrates are to withstand the strong ultrasonic waves produced in the medium during

sonication. Controlling the film thickness of the electrografted layer is also important. Researchers have explored the formation of thin film by controlling the concentration of the diazonium salt. Usually, the concentration of 1.0 mM is used. Also, the parasubstituted diazonium salt are used for electrografting. The electrografted layer results in the formation of multilayers. The electrogenerated aryl radical species have the ability to attack not only the surface but also already attached aryl layers [107,108]. **Scheme 2.4 (a)** shows the formation of multilayers using parasubstituted diazonium salt. The radical generated by the electrochemical reduction result in an ortho substitution. Blocking ortho positions allows for thin monolayer formation. Combella and co-workers reported the use of 3, 5-bis- *tert*-butyl benzenediazonium salt resulted in a thin monolayer [101]. The blocking of the ortho position was also exploited by Mwanza et al [109] and the monolayer of isophthalic acid for antibody binding and sensor development. The mechanism of ortho blocking is shown in **Scheme 2.4(b)**.

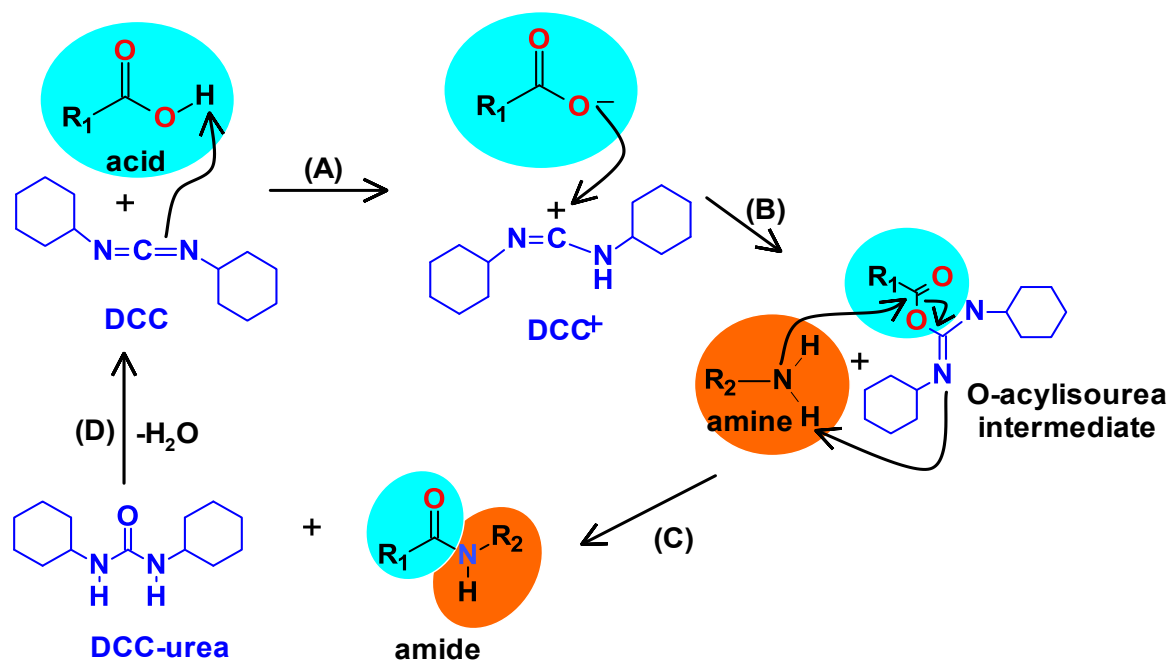


Scheme 2.4: (a) Electrografting which results in multilayer film, and (b) electrografting that result in monolayer formation using ortho substituted aryl diazonium salts [101,109].

2.6.2 Carbodiimide coupling.

Carbodiimides are also known as zero-length cross-linkers and are mainly used in the formation of amide linkages between amines and carboxylates. *N,N'*-dicyclohexyl carbodiimide (DCC) is one of the most frequently used coupling agents, especially in organic synthesis applications [90]. DCC is insoluble in water, and it is largely used in organic solvents such as dimethylformamide (DMF) for coupling reactions of small molecules. DCC has also been used to prepare active esters of carboxylate-containing compounds together with *N*-hydroxysuccimide (NHS). An amine nucleophile can attack at the primary position of the carbonyl group of an activated ester, and as a result the NHS group rapidly leaves, creating a stable amide linkage [90,110]. The mechanism of carbodiimide-mediated carboxyl activation and amide bond formation involves the following steps (**Scheme 2.5**):

- (A) the first step involves, the proton abstraction of the to-be-activated carboxylic acid derivative (**acid**) by carbodiimide (**DCC**) and the consequent formation of deprotonated carboxyl acid derivative (**-COO⁻**).
- (B) The deprotonated carboxyl derivative (**-COO⁻**) initiates subsequent nucleophilic attack on (**DCC⁺**) and gives rise to the formation of activate O-acylisourea intermediate,
- (C) O-acylisourea intermediate undergoes nucleophilic addition from the incoming primary amine to form the target product (**amide**). The O-acylisourea intermediate undergoes rearrangement and proton abstraction from the primary amine to form DCC-urea as by-product.
- (D) Finally, DCC-urea by-product undergoes dehydration to regenerate DCC.



Scheme 2.5: Mechanism of carbodiimide-mediated carboxyl activation and amide bond formation.

Tshenkeng et al. used carbodiimide (DCC and NHS) chemistry to covalently immobilize cobalt (II) tetra-(3-carboxyphenoxy) phthalocyanine (CoTCPhOPc) on the pre-electrografted phenylethylamine gold electrode (Au-PEA) for the determination of catecholamine NTs [111]. The amide coupling of CoTCPhOPc onto Au-PEA surface resulted in the formation of Au-PEA-CoTCPhOPc thin monolayer film. The NHS and DCC were used to activate carboxylic acid to form succinimide ester which reacted easily with amine functional group on the Au-PEA. Iphithuli et al. also covalently immobilized cobalt (II) octa acyl chloride phthalocyanines (CoOAClPc) onto phenylethylamine pre-grafted gold via spontaneous amidation for the determination of catecholamine NTs [112].

2.6.3 Drop-dry

Drop dry or drop casting is a non-covalent method of electrode modification, where simple intermolecular forces such as electrostatic attraction and π - π interactions are used to hold a thin layer of modifying species on a surface [113]. This technique is particularly useful for modifying carbon surfaces through the use of π - π interactions. In this method, a dispersion of the electrocatalytic composite dissolved into solution is dropped onto an electrode surface and the solvent allowed to evaporate in air or oven. A major advantage of this method is that the electrocatalyst coverage is immediately known from the original solution concentration and droplet volume. The method is simple and fast. There are mainly physical interactions between the electrode surface and the modifier materials. The drawback of this method is that the deposited composite easily leaches or peels off from the electrode surface. In addition, the material is adsorbed in a random manner which cannot be controlled.

2.6.4 Electrodeposition

Electrodeposition is a simple, fast, flexible, green, and cost-effective method for modifying electrode surfaces with electrocatalytic materials [38, 75, 114]. This route enables the tailoring of electrode properties by simply adjusting the deposition parameters thereby achieving optimal electrochemical response [114]. No binders are required, which would decrease the overall electrochemical performance. There are two modes of electrochemical deposition:

(i) cathodic electrodeposition also called electrochemical reduction. Here a negative current is applied at the working electrode (WE). The WE is negatively polarized and positive ions are attracted to it.

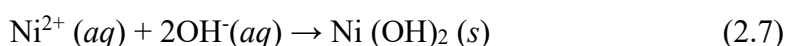
(ii) Anodic electrodeposition also referred to as electrochemical oxidation. In electrochemical oxidation, the positive current is applied to the WE, making the electrons flow to the WE. Here the WE is positively polarized and attract negatively charged species.

Graphene and its derivatives can be electrodeposited onto the carbon electrode surface. Two materials of graphene origin can be electrodeposited as reduced graphene oxide (RGO) via reduction of GO. Further oxidation of graphene to form graphene oxide (GO). The attachment of the graphene derivative result in an electron conducting material. Reduced graphene oxide (RGO) thin films have been electrodeposited on the electrode surfaces via one or two step route. A one step route involves direct electrochemical reduction of GO in a suspension onto the electrode surface [75]. A two-step route requires pre-deposition of GO onto the substrate electrode prior to electrochemical reduction process [37]. The electrochemical reduction process can be performed with cyclic voltammetry (CV), linear sweep voltammetry (LSV) or chronoamperometry at a constant potential mode in a standard three-electrode electrochemical system at room temperature [37]. CV technique is often used for electrodeposition of GO as compared to the constant reduction potential technique. The main advantage of CV technique is that it provides information about redox potential and reversibility of the reaction [115,116]. The electrochemical reduction is believed to take place on the GO sheets adjacent to the electrode surface. The insoluble electrochemically reduced graphene oxides (ERGOs) then attaches directly onto the electrode surface. According to Liu et al., the electro-deposition is driven by the difference in solubility between the GO and the resulting ERGO in the aqueous electrolyte [115]. In CV technique, the electrochemical reduction of GO from aqueous suspension is carried out in the potential range of 0 V to -1.5 V and a scan rate ranging from 20 – 100 mV.s⁻¹ [115,117,118]. It has been reported that the electrochemically reduced GO substantially improve the electron transfer kinetics of the electrode surface for the

electrooxidation of the biomolecules [38,39,119]. For example, Chen et al., prepared a graphene-based hybrid composite film by electrodeposition of RGO and overoxidized electropolymerized polypyrrole onto a GCE (OPPy/ERGO/GCE) using cyclic voltammetry [120]. The modified GCE displayed excellent electrocatalytic activity for DA and UA, but electrostatically repelled ascorbate anion under physiological pH conditions.

Transition metallic oxides/hydroxides have also been extensively electrodeposited onto electrode surfaces for electrocatalytic applications [121]. Among many possible transition metal oxides/hydroxides, nickel hydroxide has been the subject of much investigation due to its rich redox properties, abundance, and is a low-cost raw material. Previous studies of nickel hydroxide electrodeposition have focussed on two approaches:

(i) the electrochemical generation of a base in the presence of Ni^{2+} to form nickel hydroxide directly [122] as shown in Equation 2.7:



(ii) an indirect approach in which nickel is first electrodeposited, following Equation (2.8)



and the nickel is reduced oxidized by potential cycling in basic media [123,124]. In some cases, it is also possible to form mixtures of nickel hydroxide and metallic nickel due to both processes described in eqs (2.7) and (2.8) occurring during electrodeposition. Awad and co-workers electrochemically fabricated a nickel oxide onto a gold electrode to form nano-NiOx/Au for

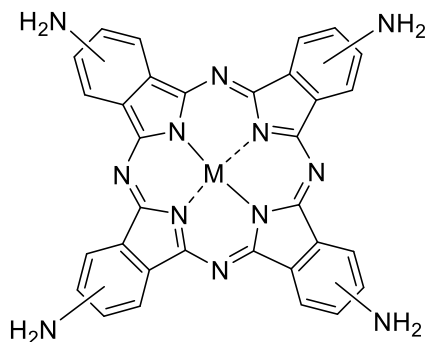
the determination of DA and UA [125]. The effect of loading level of nickel was examined by changing the number of potential cycles for the deposition of nickel nanoparticles. The nano-NiOx/Au electrode prepared by 2 potential cycles displayed high electrocatalytic activity and it separated the two oxidation peaks of DA and AA. The modified sensor demonstrated linear concentration ranges of 20 to 200 μM for DA and 50 to 900 μM for AA with LOD of 65 nM for DA and 0.23 μM for AA. Using Ni Oxide on gold electrode resulted in the separation of potential for the detection of DA and AA. Furthermore, the determination could be achieved using Au/NiOx electrode. This is the advantage of metal oxide electrode. Interference of AA in the determination of NTs is a huge challenge and metal oxides can suppress its oxidation.

2.6.5 Electropolymerization

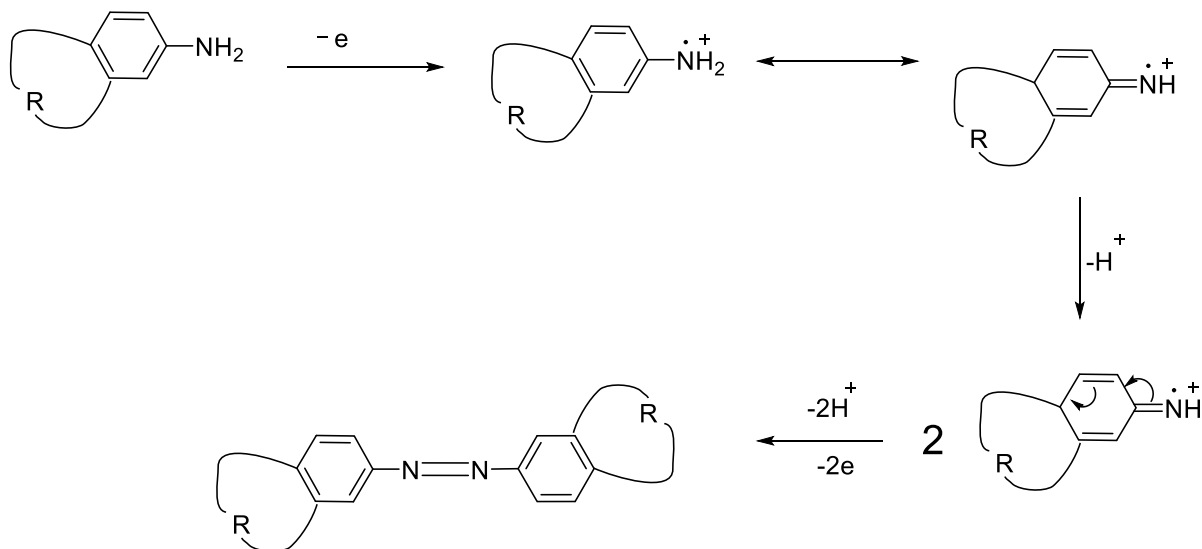
Electropolymerization is an elegant, attractive, and easy strategy for the deposition of metal complexes on electrode surfaces [126,127]. The principle is based on the electrochemical oxidation (or reduction) of a suitably designed monomer to form a polymeric film incorporating the metal complex [63,128]. The formed metal polymeric films are stable and possess desirable electrochemical properties [129]. More importantly, modifying an electrode surface using electropolymerization helps tailoring an electrode surface for specific applications [130]. Electropolymerization require a specific polymerizable functional groups. Polymeric films containing MPcs can be formed on different conductive substrates by electropolymerizing a functionalized monomeric phthalocyanine directly onto an electrode surface or coating the electrode with a preformed polymer [63]. One of the most widely used methods in developing polymeric films on various electrode surfaces is electropolymerization via cyclic voltammetry [131]. The growth of the polymeric films (or the polymer film thickness) can be easily controlled by monitoring CV conditions such as potential window, scan rate and number of

cycles. Electropolymerization can be performed when compounds have substituents such as amino-[132], hydroxyl-groups [133] and pyrrole [134]. For metallo phthalocyanine, and due to their functional flexibility, they can be synthesized bearing amino, hydroxy, and pyrrole functional groups. The only requirement is that the compound contains a polymerizable group. Electropolymerization of amino, hydroxyl and pyrrole functional groups proceed by oxidation involving the lone pair of electrons on the heteroatom or pyrrole ring. The first step in the oxidative electropolymerization of thin films and electrooxidative formation of a radical cation from the chosen monomer [128,135] shown in **Scheme 2.6 (a)** showing tetra amino phthalocyanines. This oxidation reaction is followed by a dimerization process, followed by further oxidation, and coupling reactions. This leads to the formation of oligomers and polymers on the electrode surface. For compounds such as metal (II) tetraaminophthalocyanine (M (II) TAPc) multiple amine sites allow polymerization to continue as shown in **Scheme 2.6 (b)**, and film thickness to increase with greater crosslinking [130].

(a)



(b)



Scheme 2.6: (a) Structure of metal (II)-4,9,16,23-tetraaminophthalocyanine and (b) corresponding electropolymerization process [130].

Polymeric metal tetraaminophthalocyanine on electrode surfaces have been shown to possess excellent electrocatalytic properties towards organic and inorganic species [136]. Li and Gurr first reported on the electrochemical polymerization of cobalt tetra aminophthalocyanines (CoTAPc) and the films obtained were highly electronically conductive and stable under a variety of condition [129]. Goux et al, fabricated a novel electrode material through

electropolymerization of nickel tetra aminophthalocyanines (NiTAPc) for electrocatalysis towards DA [137]. The film exhibited a much larger electrocatalytic behaviour for DA. This novel poly-NiTAPc electrode material is suitable for highly sensitive detection of other neurotransmitters. Combination of polymer with Ni was attractive for the detection of neurotransmitters.

2.7 Characterization of chemically modified electrodes

Chemically modified electrodes have been characterized using a variety of electrochemical, spectroscopic, and microscopic techniques. The most notably electrochemical characterization tools are cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). CV is the first and most widely used technique for acquiring qualitative information about electrochemical reactions that occur on modified electrodes [82]. In this regard, CV is often performed to evaluate the surface chemistry of the modified electrodes. The effect of the material used to modify an electrode can have insulating or conducting properties. Insulating properties results in the blocking effect on the electrode surface [89]. Nyokong and Khene studied the blocking effect of electrodes surfaces modified with metallo phthalocyanine nanomaterial hybrids [113]. This blocking effect can thus be used to determine whether the layer is pinhole free, or if there is any defects in the coverage using CV. Ferri/ferrocyanide $[\text{Fe}(\text{CN})_6]^{3-/4-}$ is often employed as redox probe to show blocking effects of modifier materials on the electrode surfaces. The efficiency of the layers at blocking redox probes is highly dependent on the structure of the molecules making up the layer. This determines the uniformity of the surface coverage, whether the layer is pinhole and defect free, and whether the layer is tightly packed.

Where Nernstian behaviour is observed, Eqs. (2.9) or (2.10) can be used to calculate surface coverage [80,82].

$$I_p = \frac{n^2 F^2 \Gamma A v}{4RT} \quad (2.9)$$

$$Q = nFA\Gamma \quad (2.10)$$

where I_p = the peak current, Q = total charge, n = number of electrons transferred, F = Faraday's constant, A = real surface area, v = scan rate, R = gas constant, Γ = surface coverage and T = temperature. The real surface area can be calculated using a redox active probe ($[\text{Fe}(\text{CN})_6]^{3-/4-}$) with a known diffusion constant (D) and the Randles-Sevcik equation (2.11):

$$I_p = (2.69 \times 10^5) n^{\frac{3}{2}} A C D^{\frac{1}{2}} v^{\frac{1}{2}} \quad (2.11)$$

The parameters are as described above, and C is the concentration of a redox active probe.

Electrochemical impedance spectroscopy (EIS) is a non-destructive method of analysis that is used to study electrode interface phenomena [130,138]. It provides useful information for each step and also probing the changes of the surface modification. Impedance is measured by applying sinusoidal potential, $V(t)$ of small amplitude to an electrochemical cell and measuring resultant sinusoidal current, $I(t)$, through the cell. The measurements are done over a suitable frequency range and the results can be related to the physical and chemical properties of the material. The complex notation of impedance is shown in equation (2.12).

$$Z = Z' + jZ'' = Z_{real} + jZ_{imaginary} \quad (2.12)$$

where Z' and Z'' are the real and imaginary parts of the impedance respectively and j is a complex number.

The impedance data are fitted to an equivalent circuit called the Nyquist plot, which is imaginary impedance ($-Z''$, y-axis) versus the real impedance (Z' , x-axis) as shown in **Fig.2.7**

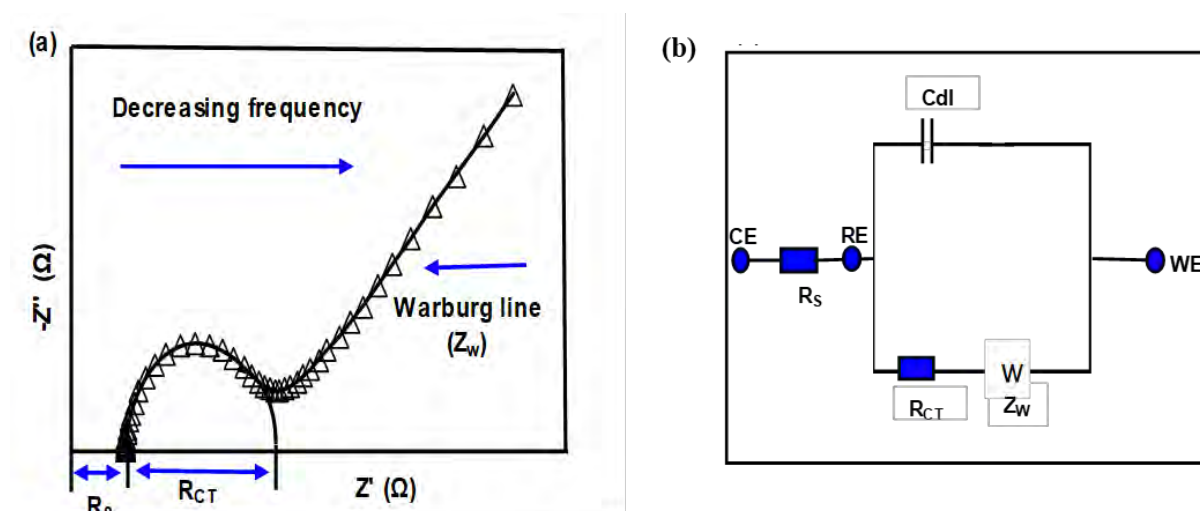


Figure 2.7: (a) Nyquist plot and (b) Equivalent circuit representing the observed impedance data.

The Nyquist plot consists of semicircular part at higher frequency region corresponding to the electron transfer limited process and its diameter is equal to the electron transfer resistance or charge transfer resistance (R_{CT}). The R_{CT} controls the electron transfer kinetics of the redox probe at the electrode surface. The linear part at lower frequencies corresponds to the diffusion process conductivity of the electrolyte.

Spectroscopic techniques are of importance as they reveal the chemical composition and oxidation states of various materials. X-ray Photoelectron spectroscopy (XPS) is the widely used spectroscopic technique. It is also useful as a quantitative technique. In addition, Raman spectroscopy is used for the confirmation of functional groups on the material used to modify

electrodes. Microscopic techniques are useful for surface characterization and scanning electron microscopy (SEM) is used for morphological and crystal structure identification.

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LIST OF PUBLICATIONS

Here are a list of papers published or submitted for publication that form part of this thesis and will not be cited further:

1. **Charles Luhana**, Iphithuli Moyo, Keamogetse Tshekeng, Philani Mashazi. In-situ selectivity detection of catecholamine neurotransmitters using covalent composite of cobalt phthalocyanine and aminated graphene quantum dots. *Microchemical Journal* 180 (2022) 107605.

Charles contribution: Conceptualization, method development, data collection and analysis, writing of original manuscript.

2. **Charles Luhana**, Philani Mashazi. Simultaneous detection of dopamine and paracetamol on electroreduced graphene oxide-cobalt phthalocyanine polymer nanocomposite electrode. *Electrocatalysis* 14 (2023) 406-417.

Charles contribution: Conceptualization, method development, data collection and analysis, writing of original manuscript.

3. **Charles Luhana**, Philani Mashazi. Conducting nickel hydroxide thin film on molybdenum disulfide-reduced graphene oxide electrocatalysts for selective detection and determination of ascorbic acid, dopamine, and uric acid. (under review).

Charles contribution: Conceptualization, method development, data collection and analysis, writing of original manuscript.

CHAPTER 3

3. In-sera selective detection of catecholamine neurotransmitters

Abstract

Herein we report the construction an electrochemical sensor for the detection of catecholamine neurotransmitters based on covalent composite of cobalt tetra-(3-carboxyphenoxy) phthalocyanine (CoTCPhOPc) with aminated graphene quantum dots (AmGQDs). The electrochemical behaviour of the covalent composite modified gold surface was studied using cyclic voltammetry, differential pulse voltammetry, square wave voltammetry and electrochemical impedance spectroscopy. The electrochemical sensor fabricated using gold surface with AmGQDs-CoTCPhOPc exhibited enhanced electrical conductivity. The fabricated electrochemical sensor displayed excellent analytical performance towards the detection of dopamine (DA), norepinephrine (NOR) and epinephrine (EP). The analytical parameters were linear within the concentration range of 1.0 to 50 μM with detection limits of 0.20 μM (DA), 0.45 μM (NOR), and 0.23 μM (EP) using signal to noise ratio (S/N) of 3. Furthermore, the fabricated electrochemical sensor exhibited good ability to suppress the background current due to ascorbic acid (AA), a major interferent during detection of DA, EP and NOR analytes. More importantly, the developed covalent electrochemical sensor was successfully utilized for the detection catecholamine neurotransmitters in newborn calf serum samples with high recovery percentages.

3.1 Introduction

Phthalocyanines (Pcs) are N_4 -macrocyclic compounds that have 4 isoindole units connected via an abridge and are $18-\pi$ conjugated system [1]. Pcs can be substituted on the benzene ring of the isoindole for increased solubility. The centre of the Pc can also be substituted with metal ions to form metallophthalocyanines (MPcs). MPcs show very good thermal, chemical and electrochemical properties due to the π -electron conjugation system. If the central metal ions are paramagnetic, they can undergo oxidation or reductions. MPcs containing transition metal ions at the centre of the ring have been shown to possess excellent electrocatalytic properties towards sensing of various analytes [2]. Tshenkeng et. al, recently fabricated a cobalt(II) tetra-(3-carboxyphenoxy) phthalocyanine gold electrode (Au-PEA-CoTCPhOPc) surface for the detection of dopamine (DA), epinephrine (EP), and norepinephrine (NOR) at physiological pH environment with low detection limits in micro molar concentrations [3]. Moreover, MPc-based electrodes have also shown to be excellent redox mediators for enzyme-based glucose sensors [4]. However, MPcs on their own suffer from low conductivity, hence the need to combine these with materials showing excellent electrocatalytic and conducting activities [5]. For the past decade, researchers have demonstrated that carbon nanomaterials when combined with MPcs form a more stable nanocomposite due to the strong π - π interaction [6–8]. More importantly, when MPcs contain active substituents, this provides for an effective way of coupling with carbon nanomaterials through non-covalent and covalent modification.

Hybrid materials based on graphene and its derivatives have shown to enhance electrochemical reactivity at electrode surfaces [9]. Graphene quantum dots (GQDs) are a class of carbon nanomaterial with zero-dimension that have attracted great attention to the scientific research community. This is due to the unique chemical stability, unique catalytic activity, and feasible

chemical functionalization opportunities [10,11]. In addition, GQDs possess high conductivity and large surface area [12] for further modification. MPcs π -conjugated macrocyclic structure is similar to GQDs structure, thus allowing for their π - π stacking or covalent attachment via the functional groups at the peripheral and non-peripheral positions. The composite material from MPcs and GQDs will exhibit synergistic effect by harnessing excellent structural interactions, good conductivity and excellent electrocatalytic activity from each of the material [13].

In this chapter, the electrochemical sensor was constructed based on the excellent synergistic effect of both MPc and GQDs. The covalent method of gold electrode modification was followed, and it involved electrografting a thin film (layer) of isophthalic acid to yield Au-IPA. Au-IPA resulted in the stable covalent thin film with terminal carboxylic acid functional groups. The carboxylic acid functionalized thin film was used to covalently immobilize amine functionalize GQDs and subsequently CoTCPhOPc monolayers for enhanced stability of the electrochemical sensor, Au-IPA-AmGQDs-CoTCPhOPc. The electrochemical sensor was used to detect dopamine (DA), norepinephrine (NOR) and epinephrine (EP) in physiological PBS (pH 7.4). The detection of catecholamine neurotransmitters was also investigated in the serum (in-sera) to further demonstrate the real-life applications of the designed electrochemical sensor. The method of electrochemical sensor fabrication, in-sera electrochemical detection of catecholamine neurotransmitters, and screening of ascorbic acid as strong interference was reported here.

This chapter was set out to achieve the following objectives of the thesis:

- (a) Fabrication and characterization of a covalent composite cobalt (II) tetracarboxyphenoxy phthalocyanine and aminated graphene quantum dots gold electrode.
- (b) Detection of catecholamine neurotransmitters selectively using composite using cobalt (II) tetracarboxyphenoxy phthalocyanine and aminated graphene quantum dots gold electrode.
- (c) Enhanced sensitivity of catecholamine neurotransmitter detection using aminated quantum dots.
- (d) Screening of ascorbic acid as a strong interferent to catecholamine neurotransmitter detection using pH sensitive of the carboxylic acid functional groups on the CoTCPhOPc thin films.
- (e) Comparison of detection of neurotransmitters both in buffer (PBS) and serum samples.

3.2 Experimental

3.2.1 Materials and methods

Acetonitrile, tetrabutylammonium tetrafluoroborate (TBABF₄), potassium ferricyanide, potassium hexacyanoferrate (II) trihydrate, dopamine hydrochloride (DA), (-)-epinephrine (EP), (-)-norepinephrine (NOR), l-ascorbic acid (AA), N,N'-dicyclohexyl carbodiimide (DCC) and N-hydroxy succinimide (NHS) were purchased from Sigma-Aldrich. 32% Hydrochloric acid (HCl), potassium chloride (KCl) and sodium chloride (NaCl) were purchased from Spellbound Laboratory Solutions. Sodium hydroxide (NaOH) was purchased from B & M

Scientific. Dimethylformamide (DMF), potassium dihydrogen orthophosphate (KH_2PO_4) and disodium hydrogen orthophosphate (Na_2HPO_4) were purchased from Associated Chemical Enterprises (ACE). Sodium hydroxide (NaOH , 1.0 M) and hydrochloric acid (HCl , 1.0 M) were used to adjust the pH of the PBS solution to appropriate values. All aqueous solutions were prepared using ultra-pure water obtained from purification through a Milli-Q Water System (Millipore Corp. Bedford, MA, USA). With the exception of DMF, all chemicals were of analytical reagent grade and were utilized as received from the suppliers without any further purification. Cobalt tetra-(3-carboxyphenoxy) phthalocyanine (CoTCPhOPc) [3] and 5-diazoniumisophthalic acid (5-DIPA, stored at $-20\text{ }^\circ\text{C}$) [14] were prepared as reported elsewhere by our group. Graphene quantum dots (GQDs) were synthesized following a reported method [10]. Amino functionalized GQDs (AmGQDs) was prepared according to the procedure previously reported by Tetsuka's group with some modification [15].

3.2.2 Apparatus

Fourier transform infrared (FT-IR) spectra were obtained from a Perkin-Elmer Universal ATR Sampling accessory spectrum 100 FT-IR spectrometer. Ultraviolet-visible (UV-vis) electronic absorption spectrum was obtained on a Thermo Scientific Multiskan Sky Microplate spectrophotometer. Photoluminescence (PL) spectra were recorded on a Shimadzu RF-5301 PC luminescence spectrometer. The synthesized GQDs and AmGQDs were dispersed in de-ionized water to final pH of 7. Then GQDs and AmGQDs in aqueous solution were placed in a quartz cuvette and excited at 350 nm at $25\text{ }^\circ\text{C}$. The Raman data was collected using the Bruker Vertex 70-Ram II Raman spectrometer (equipped with a 1064 nm Nd: YAG laser and liquid nitrogen cooled germanium detector). Cyclic voltammetry (CV), differential pulse voltammetry (DPV), square wave voltammetry (SWV) and electrochemical impedance

spectroscopy (EIS) experiments were performed using an AUTOLAB PGSTAT302N potentiostat/ galvanostat workstation interfaced to a Proline desktop computer equipped with a 1.10 version NOVA software. The EIS experiments were recorded in the frequency of range between 10 kHz to 100 MHz at a formal potential of $E_{1/2}$ of the $[\text{Fe}(\text{CN})_6]^{3-/4-}$ and $[\text{Ru}(\text{NH}_3)]^{2+/3+}$ redox couples for a bare gold electrode and with an amplitude 5 mV rms sinusoidal modulation. Measurements of pH were conducted using a Metrohm 827 pH lab, 230 V, EU with Primatrode. An electrochemical cell containing an analytical solution and three electrodes consisting of a working electrode (WE, gold electrode 1.6 mm, 0.0201 cm^2), a counter electrode (CE, Pt wire), and silver|silver chloride reference electrode immersed in a 3.0 M KCl (RE, Ag|AgCl, 3.0 M KCl), was used for all the electrochemical measurements. All reactions were carried out under a N_2 atmosphere in order to prevent the interference of the oxygen reaction at the electrode surface.

3.2.3 Synthesis of amino-functionalized GQDs (AmGQDs), Scheme 3.1

Graphene quantum dots (GQDs) were synthesized following a reported bottom-up method [16]. Briefly, citric acid (CA, 2.0 g, 10.4 mmol) was added into a 5 mL beaker and heated to 200°C using a heating mantle until citric acid become a colourless liquid. As the reaction continued the colourless liquid changed to pale yellow, and finally orange (in 30 min) signifying the formation of GQDs. The obtained orange liquid was added dropwise into 100 mL of 0.25 M NaOH solution. The pH of the GQDs solution was adjusted to 7 using 0.10 M NaOH solution. The obtained pale-yellow GQDs solution was centrifuged at 10000 rpm for 5 min to remove insoluble solids. Then the extracted supernatant was dialyzed for 48 h using a dialysis tube (MW 3.5 kDa). To further purify the dialyzed pale-yellow solution containing GQDs, ethanol was added with vigorous stirring in the volume ratio 1:3 (GQDs solution:

ethanol). Ethanol was evaporated from the obtained solution at room temperature to get the yellow GQDs solid. Finally, the GQDs solid was then dried in a vacuum oven at 80°C for 24 h. The obtained solid GQDs were re-dissolved in deionized water and kept at 4°C in refrigerator for further use.

UV-Vis (λ , nm): 300 (π - π^*), 359 (n - π^*). FT-IR (ν , cm^{-1}): 3300 (OH), 1564 (C=C), 1373 (C-O-C). Raman (Raman shift, cm^{-1}): 1371 (D band), 1600 (G-band).

As mentioned previously, amino functionalized GQDs (AmGQDs) was prepared according to the procedure reported by Tetsuka's group with some modification [15, 17]. Briefly, 5.0 mL of the dispersed GQDs solution was mixed with 2.16 mL ammonia solution (25 wt% in water) and 7 mL deionized water in a round bottom flask. The mixture was stirred for 30 min and refluxed at 170°C for 10 h. After cooling to room temperature, the precipitated insoluble large fragments were filtered out. The desired AmGQDs present in the supernatant was heated to 100°C for 1 h to remove excess ammonia. The pale-yellow supernatant was further dialyzed for 48 h using a dialysis tube (MW 3.5 kDa). The dialysis membrane/tube was determined through a careful and thorough tracing of the dialysis process. Here no fluorescence was noticed in the dialysis waters, and no carbonaceous solids upon filtering the resultant supernatant. The GQDs fully remained inside the dialysis membrane/tube. The AmGQDs were further purified by adding ethanol to the resultant pale-yellow solution under vigorous stirring in the volume ratio 1:3 (AmGQDs solution: ethanol). The pale-yellow mixture was then dried in vacuum oven at 60°C to obtain solid AmGQDs. Finally, the obtained AmGQDs solid was re-dissolved in deionized water and kept at 4°C in refrigerator for further use.

UV-Vis (λ , nm): 300 (π - π^*), 359 cm^{-1} (n - π^*). FT-IR (ν , cm^{-1}): 3300 (OH), 1636 (C=O), 2914 (N-H), 1094 (C-N), 968 (NH_2). Raman (Raman shift, cm^{-1}): 1292 (D band), 1593 (G-band).

3.2.4 Electrode preparation

Prior to the electrode fabrication, the gold electrode (Au) was polished to a mirror-finish on a Beuhler felt pad using an aqueous slurry of aluminium oxide (1.0 μm , 0.30 μm and 0.050 μm). The polished gold electrode was then chemically treated by etching for about 2 minutes in a freshly prepared “Piranha” solution [3:1 (v/v) concentrated (32%) sulphuric acid (H_2SO_4) and 30% hydrogen peroxide (H_2O_2)]. The etched Au electrode was then rinsed with copious amounts of deionized water. The Au electrode was then further electrochemically cleaned by immersing it in 0.50 M H_2SO_4 and scanning multiple times in the potential window between -0.20 V and 1.50 V (vs Ag|AgCl in 3.0 M KCl) at a scan rate of 100 mV.s^{-1} until reproducible scans were obtained. Finally, the cleaned Au electrode rinsed with high purity water from the Milli-Q system and dried in a continuous stream of nitrogen. The gold electrode can be reused several times between multiple runs of the experiment by simply performing cyclic voltammetry in 0.50 M H_2SO_4 solution for ten cycles.

3.2.5 Modification of gold electrode surface, Scheme 3.2

The clean gold electrode was first modified with 5-diazoniumisophthalic acid (5-DIPA) via electrografting to yield an isophthalic acid monolayer denoted as Au-IPA. The electrografting was done by cycling the potential window between 0.60 to -0.20 V in an acidified ACN solution containing 2.5 mM 5-DIPA and 50 mM TBABF₄. The scan rate was 50 mV.s^{-1} . Several scans were used for electrografting until reproducible scans were obtained. After the electrografting, isophthalic acid (IPA) was attached on the Au electrode surface successfully. The Au-IPA electrode was rinsed with acetonitrile to remove physically adsorbed 5-DIPA. Then Au-IPA electrode was immersed in 1.0 mL DMF solution containing DCC (25.5 mg, 0.124 mmol) and

NHS (21.7 mg, 0.189 mmol) for 2 hrs. Thereafter, the succinimide activated Au-IPA electrode was immersed in 1.0 mL DMF solution of AmGQDs (1.0 mg mL^{-1}) overnight, to yield Au-IPA-AmGQDs. Finally, the Au-IPA-AmGQDs electrode was immersed in 1.0 mL DMF solution of CoTCPhOPc (1.0 mg mL^{-1}) containing DCC (25.5 mg, 0.124 mmol) and NHS (21.7 mg, 0.189 mmol) which had been left for 2 hrs to react under stirring. After 4 hrs, the Au-IPA-AmGQDs-CoTCPhOPc electrode resulted and was rinsed with DMF to remove any unreacted physically adsorbed CoTCPhOPc. Au-IPA-AmGQDs-CoTCPhOPc was then finally rinsed with 0.10 M PBS (pH 7.4). The electrode was stored in the fridge at 6°C before use.

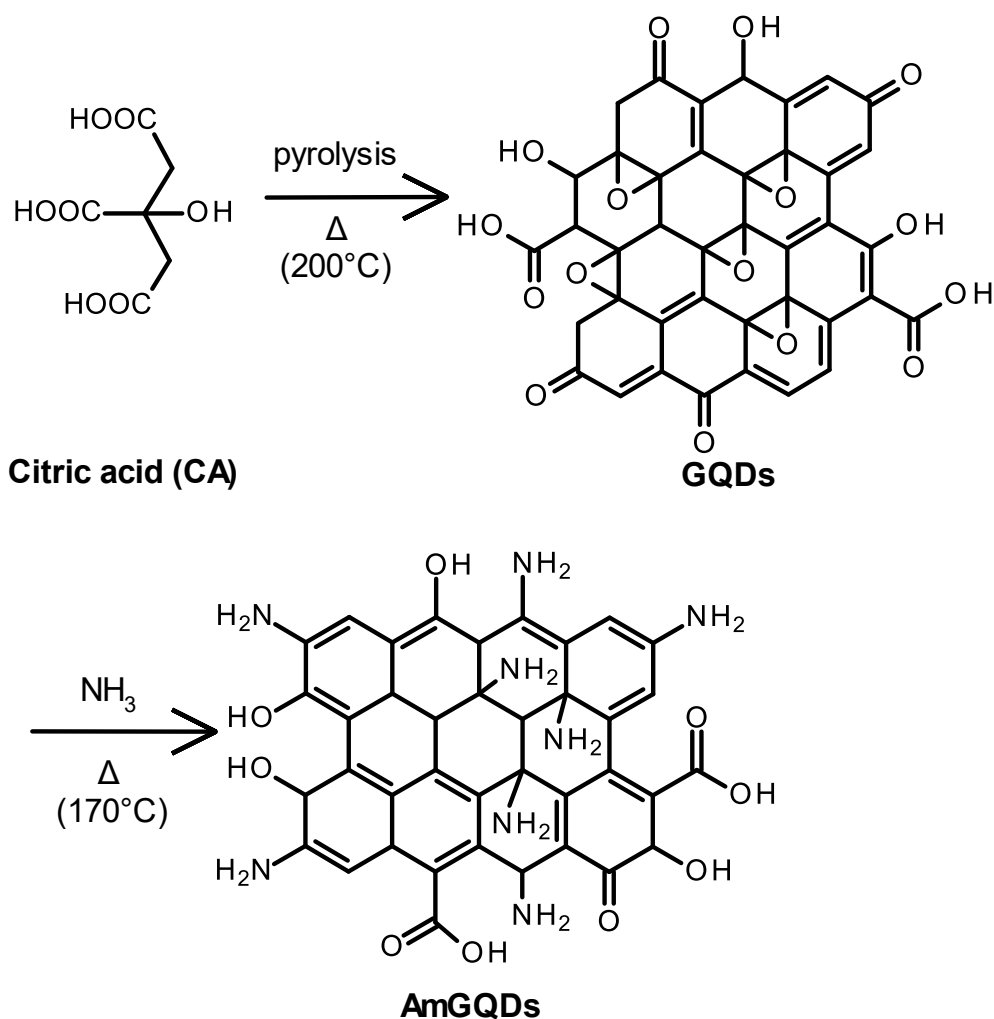
3.2.6 Real samples preparation and analysis

For the real sample analysis, briefly, 1.0 mL of the newborn calf serum sample was diluted to 100-fold with 0.10 M PBS (pH 7.4) solution. Then 5.0 mL of the diluted newborn calf serum sample was transferred into an electrochemical cell. The samples were then ready for DPV analysis. The recovery studies for DA, EP and NOR in newborn calf serum were done by standard addition method. Firstly, a calibration curve was obtained from the standard solutions of known concentration of DA, EP and NOR ranging from 10 – 60 μM in newborn calf serum buffer solution. The recovery method was measured following this procedure, the diluted newborn serum samples were spiked to a final concentration of 20, 30 or 40 μM for DA, EP or NOR.

3.3 Results and discussion

3.3.1 Synthesis and characterization of GQDs and AmGQDs

The synthesis scheme of GQDs and AmGQDs is shown in **Scheme 3.1**. Citric acid (CA) is decomposed at its melting temperature and aromatic clusters are formed via aldol condensation and cycloaddition [16]. These aromatic clusters reach a critical supersaturation point, and a burst nucleation takes place to form GQDs [16]. Amino-functionalized graphene quantum dots (AmGQDs) were synthesized via a hydrothermal method using ammonia as the source for nitrogen. The ammonia reacts with epoxy groups and phenolic oxygens to form a primary amine by nucleophilic substitution. This enables ring opening of the epoxide and simultaneous direct bonding of a primary amine on the graphene edge of the GQDs to form amino-graphene quantum dots (AmGQDs) [15,17-19].



Scheme 3.1: Synthesis of graphene quantum dots (GQDs) and amino functionalized graphene quantum dots (AmGQDs) [15].

The UV-vis absorption spectra of (i) GQDs and (ii) AmGQDs are shown in **Fig. 3.1(a)**. GQDs and AmGQDs showed a typical strong absorption peak below 300 nm assigned to the π - π^* transitions of aromatic sp^2 hybridized carbon core domains [20,21]. The GQDs UV-vis spectrum showed a broad absorption band at 359 nm which can be attributed to the n - π^* transitions of the C-O bond [22]. The C-O bond disappeared upon the formation of AmGQDs in **Fig. 3.1(a)(ii)**. Fluorescence properties of (i) GQDs and (ii) AmGQDs was performed at the excitation wavelength of 380 nm and are shown in **Fig. 3.1(b)**. AmGQDs are blue fluorescent

and show maximum emission when excited at about 400 nm [23]. Photoluminescence (PL) results showed that the amino functionalization affected the oxygen functional groups on the surface of GQDs. This inhibited the nonradiative recombination process of electron-hole pairs and facilitated radiative transition which led to the $n-\pi^*$ transition. There was an increase in fluorescence intensity also after the formation of AmGQDs [24].

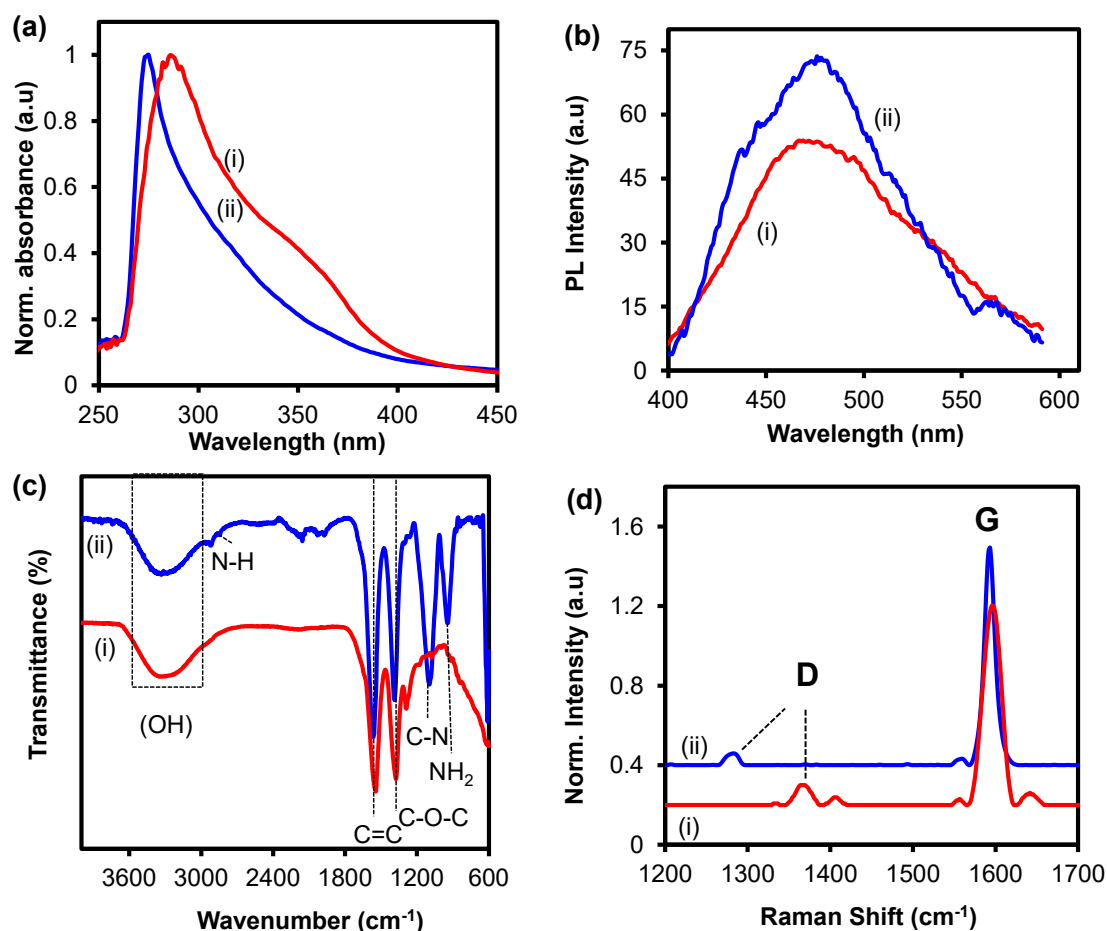


Figure 3.1: (a) UV-vis spectra, (b) fluorescence spectra, (c) FT-IR spectra, and (d) Raman spectra of (i) GQDs and (ii) AmGQDs.

The FTIR spectrum of GQDs was observed with characteristic broad peaks at 3300 cm⁻¹ due to O-H stretching band of the alcohol in **Fig. 3.1(c)(i)**. A stretching band at 1564 cm⁻¹ was

observed due to C=C stretching. At 1373 cm^{-1} , a stretching band due to epoxide (C-O-C) was observed which confirmed the synthesis of GQDs. The observed peaks and their interpretation or assignments are similar to those reported in literature [15, 25, 26]. The presence of C-O-C and -OH functional terminating edge groups of GQDs play an important role in the functionalization of amine functional groups [27]. The FT-IR spectrum of AmGQDs in **Fig. 3.1(c) (ii)** showed the presence of a peak at 1636 cm^{-1} due to C=O stretch. Also, a presence of a broad peak at 3300 cm^{-1} due to -OH was observed. A shoulder peak at 2914 cm^{-1} due to N-H stretching was observed and indicative of amino functionalization of GQDs [27] to form AmGQDs. The presence of C-N bending peak at 1094 cm^{-1} and -NH₂ stretching peak at 968 cm^{-1} confirmed the doping of NH₂ on GQDs to form AmGQDs. **Figure 3.1(d)** shows the Raman spectra of (i) GQDs and (ii) AmGQDs with characteristic D and G bands. For GQDs, D and G bands were observed at 1371 and 1600 cm^{-1} respectively. After amine functionalization of GQDs, G and D bands shifted to 1593 cm^{-1} and 1292 cm^{-1} respectively. The D band shifted from 1371 cm^{-1} for GQDs to 1292 cm^{-1} for AmGQDs confirming the amination. The presence of D band was related to the presence of sp³ defects of oxygen functional groups in the carbon network, and the crystalline (G) band related to the in-plane vibration of sp² carbon [24].

3.3.2 Electrografting of 5-DIPA onto gold electrode

Figure 3.2 shows the cyclic voltammograms for the electrografting of 5-DIPA onto gold electrode. There is a broad peak around $+0.243\text{ V}$ signifying the electrografting of isophthalic acid (IPA) onto the gold surface. Upon successive scanning, the reduction peak decreased in current density, until it became constant. This confirmed the formation of the IPA layer on the gold surface and was denoted as Au-IPA. The electrografting of IPA thin monolayer film took

7 scans. The reduction peak was still observed even after the thin film has formed. The peak is due to the reduction of 5-DIPA to form IPA radicals. The thin monolayer film could still see the diazonium in solution hence the peak after 7th scan. The grafting however did not occur due to the bulky carboxylic acid groups at positions 3 and 5 blocking the formation of a thick film which would passivate the electrodes as has been reported [28].

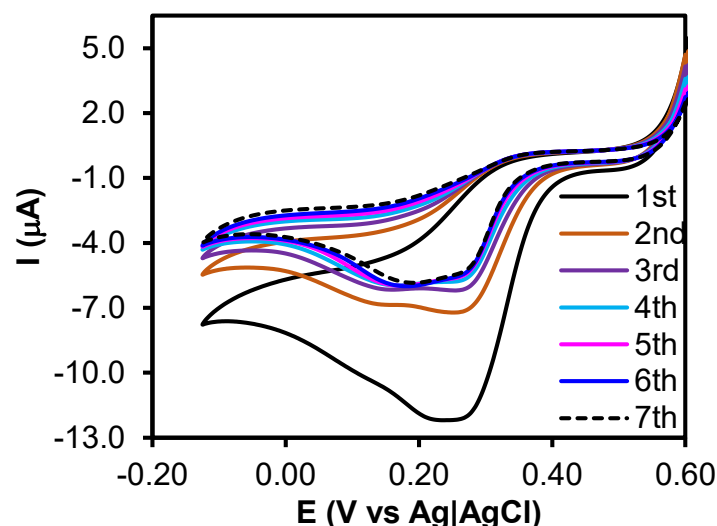
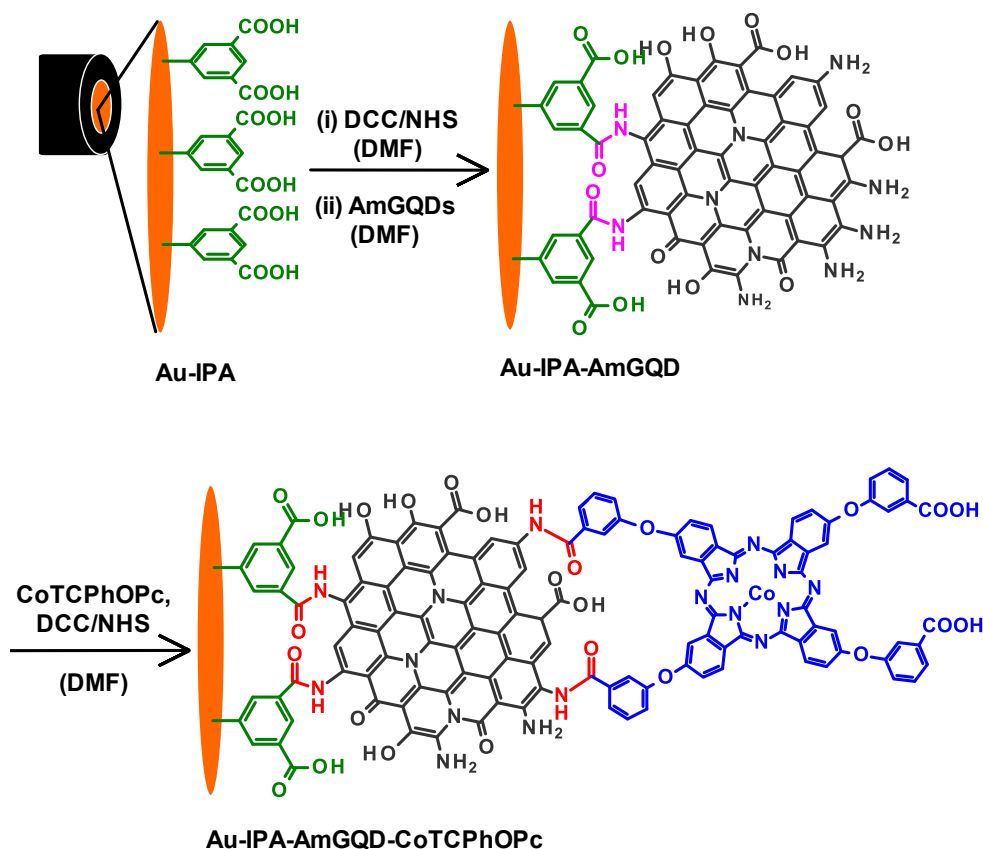


Figure 3.2: CVs for the electrografting of 2.50 mmol.L⁻¹ of 5-DIPA in 3% HCl solution of acetonitrile for gold electrode.

3.3.3 Fabrication of Au-IPA-AmGQDs-CoTCPhOPc, Scheme 3.2

The immobilization of AmGQDs and CoTCPhOPc onto the gold electrode is shown in **Scheme 3.2**. The presence of amine group on the edge of the amine functionalized AmGQD acted as site for binding on the di-basic carboxylic acid [27] of Au-IPA after activating with EDC and NHS (carbodiimide amide coupling). The amine functional groups of AmGQDs were further used to immobilize CoTCPhOPc using carbodiimide chemistry. The CoTCPhOPc carboxylic acid functional groups were activated using EDC and NHS to form amine reactive ester which reacted with amine functionalized groups of AmGQDs to form amide bonds.



Scheme 3.2: Fabrication of Au-IPA-AmGQDs-CoTCPhOPc via electrochemical grafting of IPA and covalent immobilization of AmGQDs and CoTCPhOPc using carbodiimide coupling reaction.

3.3.4 Electrochemical characterization of surface modification

Figure 3.3 shows (a) cyclic voltammograms (CV) and (b) corresponding impedance spectroscopy (EIS) of (i) bare Au, (ii) Au-IPA, (iii) Au-IPA-AmGQDs and (iv) Au-IPA-AmGQDs-CoTCPhOPc measured in 2.0 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.10 M KCl solution at a scan rate of 50 $\text{mV}\cdot\text{s}^{-1}$. The CV of bare Au in $[\text{Fe}(\text{CN})_6]^{3-/4-}$, **Fig. 3.3(a)(i)**, shows a redox peak at $E_{1/2}$ of 0.124 V with a peak-to-peak separation (ΔE) equals to 0.071 ± 0.0045 V and a ratio of anodic to cathodic peak currents ($I_{\text{pa}}/I_{\text{pc}}$) of 1.01. The value of 0.071 ± 0.0045 V was within experimental error close to the ideal redox peak of 0.0592 V from Nernst equation for one

electron reversible redox peak. The peak current ratio was equal to unity, indicating that a reversible redox couple on Au electrodes. After electrografting with IPA in **Fig. 3.3(a)(ii)**, the redox peak current intensities remained almost the same as that of the bare Au for the $[\text{Fe}(\text{CN})_6]^{3-}/[\text{Fe}(\text{CN})_6]^{4-}$ redox reaction. However, the peak potential slightly increased in ΔE to 0.076 ± 0.0065 mV and also shift of redox peak to more positive potentials. The IPA thin film did not fully block the $[\text{Fe}(\text{CN})_6]^{3-/4-}$ redox peak and this was due to a very thin film of IPA [29]. However the slight shift of redox peak to positive potential was due to repulsive interactions between the deprotonated $-\text{COO}^-$ groups on the Au-IPA electrode surface and $[\text{Fe}(\text{CN})_6]^{3-/4-}$ redox probe molecules. The COOH functional groups at positions 3 and 5 prevented the formation of multilayers from forming in **Fig. 3.2**. Upon coupling AmGQDs onto the Au-IPA to form Au-IPA-AmGQDs, the currents of redox peaks slightly decreased with a negative shift in peak potentials in **Fig. 3.3(a)(iii)**. The ΔE for the CV for AmGQDs was 0.073 ± 0.0034 V. The negative shift in peak potentials was attributed to the improved electron transfer of the Au-IPA-AmGQDs thin film on the gold electrode surface. The slight decrease in redox peak currents was due to repulsive interactions between the residual $-\text{COO}^-$ groups on the AmGQDs and $[\text{Fe}(\text{CN})_6]^{3-/4-}$ redox probe molecules. When CoTCPhOPc was immobilized onto Au-IPA-AmGQDs to form Au-IPA-AmGQDs-CoTCPhOPc in **Fig. 3.3(a)(iv)**, the peak potentials shifted, and redox peak currents decreased drastically. The ΔE increased from 0.073 ± 0.0034 V (for Au-IPA-AmGQDs) to 0.141 V for Au-IPA-AmGQDs-CoTCPhOPc. The increase in ΔE was attributed to the negatively charged COO^- at pH 7.4 above the pK_a of COOH (3.4) which results in the electrostatic repulsion of $[\text{Fe}(\text{CN})_6]^{3-/4-}$. The conductivity of the graphene material was not compromised upon anchoring CoTCPhOPc molecules onto the AmGQDs via the amide bond [30]. The changes in the CVs confirmed the electrode was successfully fabricated as outlined in **Scheme 3.2**.

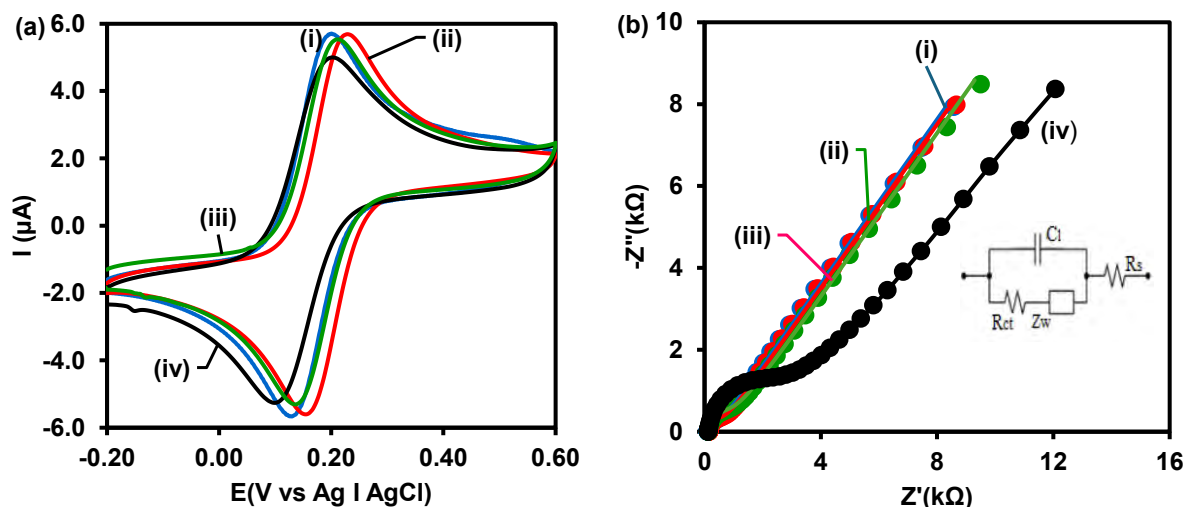


Figure 3.3: (a) Cyclic voltammograms and (b) EIS spectra of (i) bare Au, (ii) Au-IPA, (iii) Au-IPA-AmGQDs and (iv) Au-IPA-AmGQDs-CoTCPhOPc measured in 2.0 mmol.L^{-1} $[\text{Fe}(\text{CN})_6]^{3-/4-}$ containing 0.10 M KCl . Scan rate = 50 mV.s^{-1} .

The EIS was used to study electron transfer properties of bare and modified Au surface as is an effective technique for the characterization and examining interfacial properties of modified electrodes [31, 32]. Electrochemical impedance spectra were obtained at the applied half-wave potential ($E_{1/2}$) of bare Au (0.172 V) in order to observe the impedance variation at the electrode interface. Nyquist plot representation was used for EIS data analysis. **Figure 3.3(b)** shows Nyquist plots of (i) bare Au, (ii) Au-IPA, (iii) Au-IPA-AmGQDs and (iv) Au-IPA-AmGQDs-CoTCPhOPc in (1:1) of 2.0 mmol.L^{-1} $\text{K}_3[\text{Fe}(\text{CN})_6]:\text{K}_4[\text{Fe}(\text{CN})_6]$ in 0.10 mol.L^{-1} KCl solution. The Randles-Sevcik equivalent circuit, was then used to fit and analyze the Nyquist plot ($-Z''$ and Z'). The Randles circuit offers information about the effect of the double layer charging (C_{dl}), solution resistance (R_s) and charge transfer resistance (R_{CT}). Upon fitting, the Nyquist plot of bare Au in **Fig. 3.3(b)(i)** showed a small semicircle with charge-transfer resistance (R_{CT}) of about $0.470 \text{ k}\Omega$. R_{CT} is directly related to the electron transfer process of the redox

couple at the interface [32]. The R_{CT} increased by 9 Ω to 0.479 k Ω at the Au-IPA thin film electrode in **Fig. 3.3(b)(ii)**. The Au-IPA displayed a typical Nyquist plot that shows a semicircle portion at high frequency region corresponding to the electron transfer-limited process and a linear part at the low frequency range representing diffusion-limited process [32]. Thus, the functionalization of the bare Au with IPA slightly increased the surface resistance. At the Au-IPA-AmGQDs in **Fig. 3.3(b)(iii)**, the R_{CT} increased to 0.774 k Ω . At Au-IPA-AmGQDs-CoTCPhOPc in **Fig. 3.3(b)(iv)**, a marked increase of R_{CT} to 9.07 k Ω confirmed the success of the electrode modification with CoTCPhOPc. The greater R_{CT} value at Au-IPA-AmGQDs-CoTCPhOPc can be explained by the anionic charge repulsion between the deprotonated $-\text{COO}^-$ groups on the modified electrode surface and $[\text{Fe}(\text{CN})_6]^{3-/4-}$ anions. The changes in R_{CT} confirmed modification and were in-line with the changes in the CV. The presence of $-\text{COO}^-$ groups on the modified surface will positively interact with catecholamine neurotransmitters.

3.3.5 Blocking characteristics of Au-IPA-AmGQDs-CoTCPhOPc

It is expected that when thin films are immobilized onto the bare Au electrode Faradaic processes should be blocked on the modified electrode surface [33]. When bare Au, Au-IPA, Au-IPA-AmGQDs and Au-IPA-AmGQDs-CoTCPhOPc were immersed in 0.010 M KOH, all displayed oxidation and reduction peaks with varying current densities. From these results the oxidation peaks are ascribed to gold oxidation and gold oxide stripping peaks [34]. **Figure 3.4(a)** show the CVs of cycling the (i) bare gold (Au), (ii) Au- IPA, (iii) Au-IPA-AmGQDs and (iv) Au-IPA-AmGQDs-CoTCPhOPc electrodes in 0.010 M KOH solution. The CV of a bare gold electrode exhibited a broad oxidation peak at about 0.470 V and a reduction (stripping) peak at about 0.050 V, **Fig. 3.4(a)(i)**. While on the Au-IPA, **Fig. 3.4(a)(ii)**, an

oxidation peak occurred at around 0.530 V and the corresponding (stripping) reduction peak at about 0.020 V. The redox peak currents decreased when the bare Au electrode was modified with IPA. The reduction in the oxidation and reduction peak currents at Au-IPA indicates that the ions in the electrolyte were being blocked by the thin layer of IPA. There was a further decrease in peak currents upon coupling the Au-IPA with AmGQDs to form Au-IPA-AmGQDs in **Fig. 3.4(a)(iii)**. The oxidation and reduction peak currents at Au-IPA-AmGQDs modified electrode occurred at about 0.060 V and 0.030 V respectively. Whereas at the Au-IPA-AmGQDs-CoTCPhOPc, the small oxidation peak current occurred at around 0.150 V without a noticeable reduction peak, **Fig. 3.4(a)(iv)**. It is clearly evident that the redox peak currents were strongly suppressed after coating the Au-IPA-AmGQDs modified electrode with CoTCPhOPc. Then, the film ion barrier factor (Γ_{ibf}) was evaluated according to Eq. (3.1) [33].

$$\Gamma_{ibf} = 1 - \frac{Q_{thinfilm}}{Q_{bare-Au}} \quad (3.1)$$

where $Q_{thin\ film}$ and $Q_{bare-Au}$ are the charges under the gold oxide stripping peaks for Au-IPA, Au-IPA-AmGQDs, Au-IPA-AmGQDs-CoTCPhOPc surface and bare gold electrode. The $Q_{thin\ film}$ and Q_{Bare} values were obtained by integrating the charge under the reduction (stripping) peaks. The Γ_{ibf} was obtained to be 0.10 for Au-IPA, 0.62 for Au-IPA-AmGQDs and 0.73 for Au-IPA-AmGQDs-CoTCPhOPc. The 0.73 value for Au-IPA-AmGQDs-CoTCPhOPc is close to unity and confirms that the Au-IPA-AmGQDs-CoTCPhOPc thin film acted as a barrier against the permeation of electrolyte species [33].

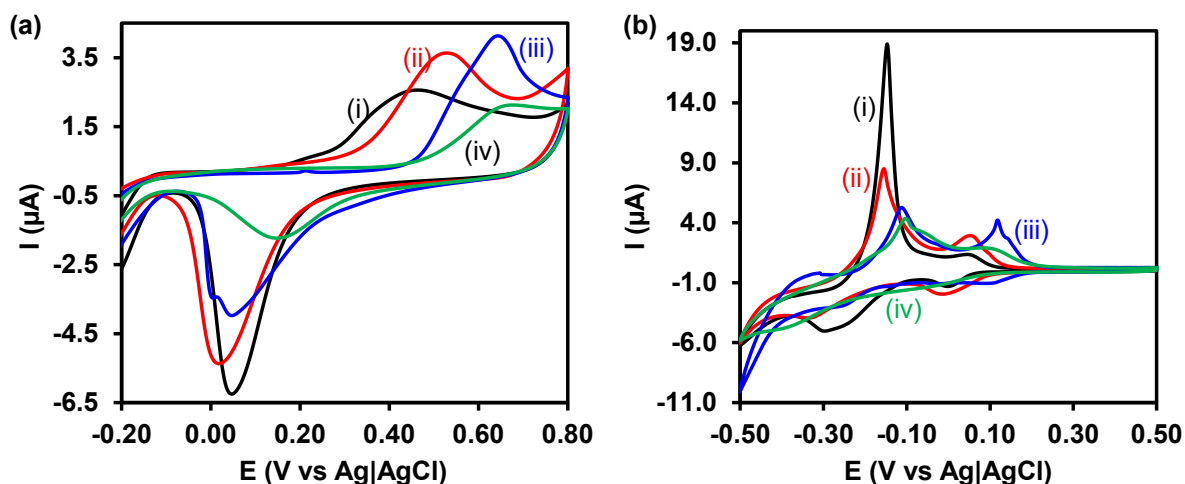


Figure 3.4: CVs of (i) bare Au, (ii) Au-IPA, (iii) Au-IPA-AmGQDs and (iv) Au-IPA-AmGQDs-CoTCPhOPc measured in (a) 0.010 M KOH solution, and (b) 1.0 mM CuSO₄ in 0.50 M H₂SO₄ solution. Scan rate of 50 mV.s⁻¹.

Under-potential deposition (UPD) of a metal such as copper is another method used to characterize the formation of thin films onto the gold electrode [35]. **Figure 3.4(b)** are the CVs of (i) bare gold (Au) electrode, (ii) Au-IPA, (iii) Au-IPA-AmGQDs and Au-IPA-AmGQDs-CoTCPhOPc in 1.0 mM CuSO₄ in 0.050 M H₂SO₄. The CVs demonstrated that the effect of the thin films coated onto gold electrode on Faradaic processes by inhibition of Cu metal through deposition. However, literature has shown that the size and functional groups of the modifying thin films onto gold electrode tend to affect the inhibition process and different results were obtained [35]. **Figure 3.4(b)(i)** shows the reduction peaks at about 0.107 V and 0.260 V on the bare gold electrode. These peaks are attributed to the Cu^{II}/Cu^I and Cu^I/Cu⁰ redox peaks, resulting in the formation of copper metallic thin film that is deposited onto gold electrode. On the return scan two oxidation peaks at about -0.108 V and 0.122 mV are observed. The oxidation peak at about -0.108 V corresponds to the complete stripping of the adsorbed Cu⁰ metallic layer to Cu^I ions which was further oxidized to Cu^{II} at 0.122 V and dissolve into the solution from the gold surface. From **Fig. 3.4(b)**, these peaks are suppressed upon

modifying the gold electrode with (ii) IPA to form (Au-IPA) and coupling of AmGQDs to form (iii) Au-IPA-AmGQDs and further amide coupling of CoTCPhOPc to (iv) Au-IPA-AmGQDs-CoTCPhOPc.

3.3.6 The effect of solution pH at Au-IPA-AmGQDs-CoTCPhOPc

The effect of solution pH at Au-IPA-AmGQDs-CoTCPhOPc modified electrode surface was investigated by CV and EIS at varying pH of 2.0 mmol.L⁻¹ [Fe(CN)₆]^{3-/4-} and 2.0 mmol.L⁻¹ [Ru(NH₃)₆]^{2+/3+} redox probes both containing 0.10 mol.L⁻¹ KCl. CoTCPhOPcs and AmGQDs contain pH sensitive carboxylic acid (COOH) groups which undergoes protonation or deprotonation under varying pH conditions. **Figure 3.5(a)** shows the cyclic voltammograms at Au-IPA-AmGQDs-CoTCPhOPc in phosphate buffer solutions at different pH values between 3 to 9 in the presence of 2.0 mmol.L⁻¹ [Fe(CN)₆]^{3-/4-} as a negatively charged redox couple. At pH < 8, the response at the modified Au electrode was found to be pH dependent.

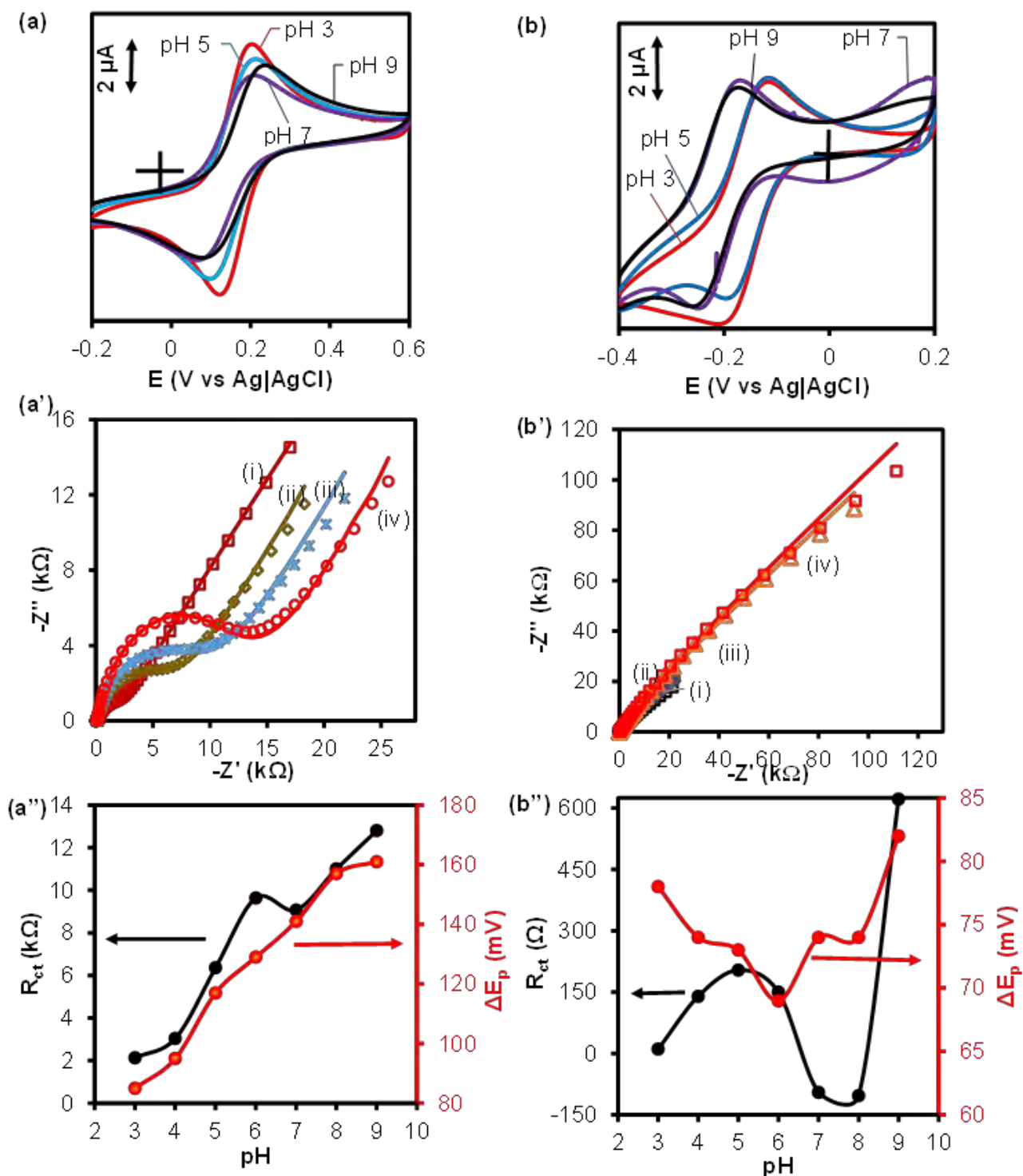


Figure 3.5: CVs, EIS and plot of R_{ct} or ΔE_p vs. pH at different pH values between 3 – 9 in 2.0 mM of (a) $[\text{Fe}(\text{CN})_6]^{3-/4-}$ and (b) $[\text{Ru}(\text{NH}_3)_6]^{2+/3+}$.

The $[\text{Fe}(\text{CN})_6]^{3-/4-}$ redox peak current decreased as the pH increased and due to slow or inhibited electron transfer. $[\text{Fe}(\text{CN})_6]^{3-/4-}$ redox couple was electrostatically repelled by the

deprotonated carboxylic acid ($-\text{COO}^-$) on the Au-IPA-AmGQDs-CoTCPhOPc. The observations are in line with literature, which showed that the ferri/ferrocyanide redox couple was very sensitive to the electrode surface charges [36,37,38]. The impedance measurement in **Fig. 3.5(a')**, Nyquist plot representation showed an increase in the R_{CT} as the pH increased from pH 3 (2.13 k Ω) to pH 9 (12.8 k Ω). Additionally, the peak potential separation (ΔE_p) drastically increased from pH 3 (0.081 V) to pH 9 (0.159 V) in **Fig. 3.5(a'')**. **Figure 3.5(b)** shows Au-IPA-AmGQDs-CoTCPhOPc investigated at different pH values (3 to 9) containing 2.0 mM $[\text{Ru}(\text{NH}_3)_6]^{3+/2+}$, a positively charged redox species. The redox peaks at the modified electrode were clearly defined, indicating that they are without inhibition [38]. The redox peak currents slightly increased from pH 3 to pH 5 and then decreased with increasing pH of the solution. ΔE_p drastically decreased from pH 3 to pH 6 and then increased above pH 6 in **Fig. 3.5(b'')**. The CV responses for $[\text{Ru}(\text{NH}_3)_6]^{2+/3+}$ redox couple were independent of increasing pH of the solution. Usually the $[\text{Ru}(\text{NH}_3)_6]^{2+/3+}$ redox couple undergoes a simple charge transfer process, independent of the nature and concentration of the supporting electrolyte [39]. Furthermore, the impedance properties at Au-IPA-AmGQDs-CoTCPhOPc modified electrode for various pH levels (3 to 9) for $[\text{Ru}(\text{NH}_3)_6]^{2+/3+}$ redox systems in **Fig. 3.5(b')**. A variation in the R_{CT} was observed at the modified electrode for EIS studies with varying pH of solution for the $[\text{Ru}(\text{NH}_3)_6]^{2+/3+}$ redox system, **Fig. 3.5(b')**. The variation of pH of the electrolyte solution plays a significant role in the interaction of Au-IPA-AmGQDs-CoTCPhOPc with redox probes. At low pH ranges, there is repulsion for $[\text{Fe}(\text{CN})_6]^{3-/4-}$ and attraction for $[\text{Ru}(\text{NH}_3)_6]^{2+/3+}$ at the negatively charged layer. However, the AmGQDs has COOH and NH_2 functional groups which are pH sensitive, for example as pH increases above pK_a of COOH , the negatively charged COO^- forms. The NH_2 functional groups are positively charged $-\text{NH}_3^+$ at low pH due to high pK_a of NH_2 . The CoTCPhOPc also has COOH functional groups which deprotonates to $-\text{COO}^-$ at high pH above the $\text{pK}_a = 3.4$. The pH effect on the fabricated electrochemical

sensor in **Fig. 3.5** showed the suppression of the signal due to negatively charged redox, $[\text{Fe}(\text{CN})_6]^{3-/4-}$, and no suppression of the signal was observed towards the positively charged $[\text{Ru}(\text{NH}_3)_6]^{2+/3+}$. These preliminary results confirmed the intended study in that the Au-IPA-AmGQDs-CoTCPhOPc will screen-off negatively charged analytes whilst catalyzing the positive and neutral analytes.

3.3.7 Electrocatalysis of DA, EP and NOR at Au-IPA-AmGQDs-CoTCPhOPc

Figure 3.6 shows CVs of 1.0 mM of (a) DA, (b) EP and (c) NOR at Au-IPA-AmGQDs-CoTCPhOPc in 0.10 M PBS (pH = 7.4) at a scan rate of 50 mV.s^{-1} . In 0.10 M PBS (pH = 7.4) alone there was no peak observed in **Fig. 3.6 (i)**. When 1.0 mM DA, EP or NOR was added, a well-defined electrocatalytic oxidation peaks were observed. For DA in **Fig. 3.6(a)(ii)**, the electrochemical detection showed an electrocatalytic oxidation peak at 310 mV and a reduction peak at 27 mV. The Au-IPA-AmGQDs-CoTCPhOPc was immersed in 1.0 mM EP in **Fig. 3.6(b)(ii)** and NOR in **Fig. 3.6(c)(ii)**, both displayed an electrocatalytic oxidation peak at 483 mV. The presence of the electrocatalytic oxidation peak was due to Au-IPA-AmGQDs-CoTCPhOPc catalysing the oxidation of catecholamine neurotransmitters. A shift of oxidation peaks of EP and NOR to high oxidation potential allow for selective simultaneous determination of DA and EP or NOR in PBS buffer. The electrocatalytic peak potential for EP and NOR was the same, therefore cannot be selectively detected. The electrocatalytic peak potential for DA was 310 mV and for EP or NOR was 483 mV and this gave a difference of 173 mV. DA can be selectively determined.

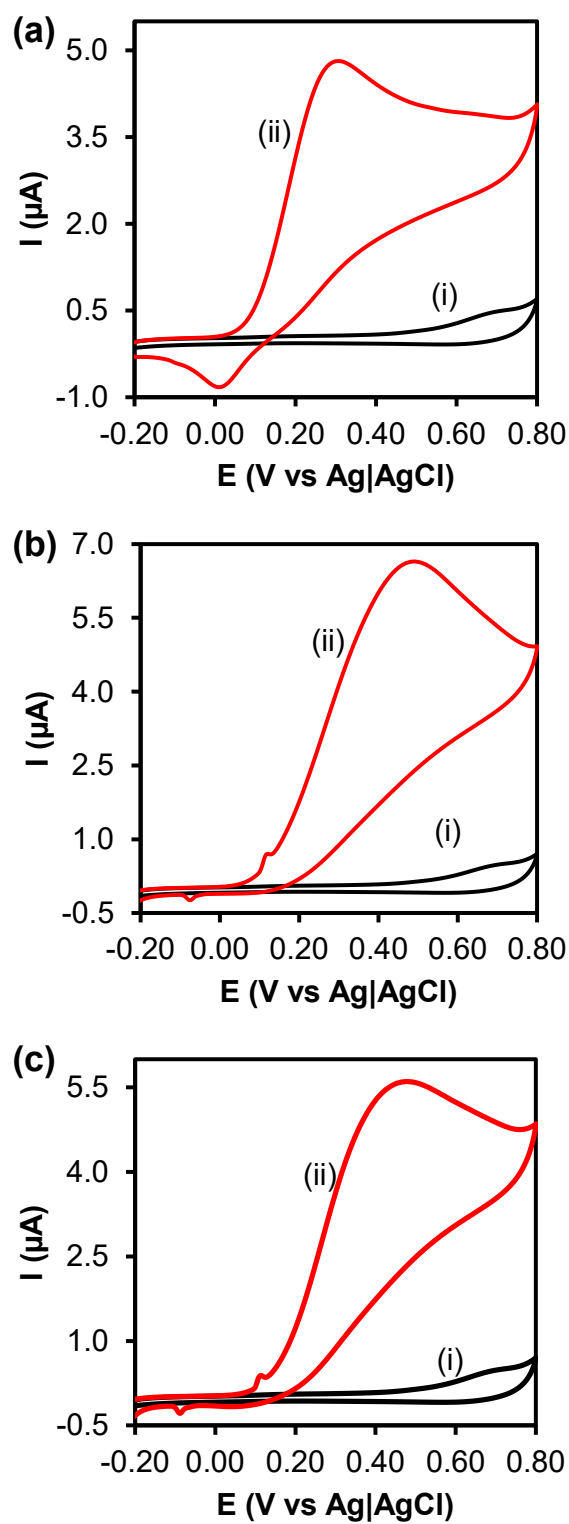


Figure 3.6: CVs of Au-IPA-AmGQDs-CoTCPhOPc in the (i) absence and (ii) presence of 1.0 mM (a) DA, (b) EP, and (c) NOR in PBS buffer (pH 7.4). Scan rate = 50 mV.s^{-1} .

Simultaneously, we also studied the effects of the scan rate on the electrocatalytic peak potentials and currents of DA, EP and NOR at Au-IPA-AmGQDs-CoTCPhOPc. **Figure 3.7** shows the effect of scan rate on the electrocatalytic oxidation peak current and potential. The electrocatalytic oxidation peak currents increased as the scan rate increased for Au-IPA-AmGQDs-CoTCPhOPc towards DA, EP and NOR indicative of diffusion-controlled processes [27, 40]. The plot of peak currents against the scan rate was linear confirming the diffusion controlled electrocatalytic oxidation of DA, EP and NOR. The electrocatalytic peak potential remained the same confirming that no adsorption of the analyte or its oxidized or reduced products of DA, EP and NOR.

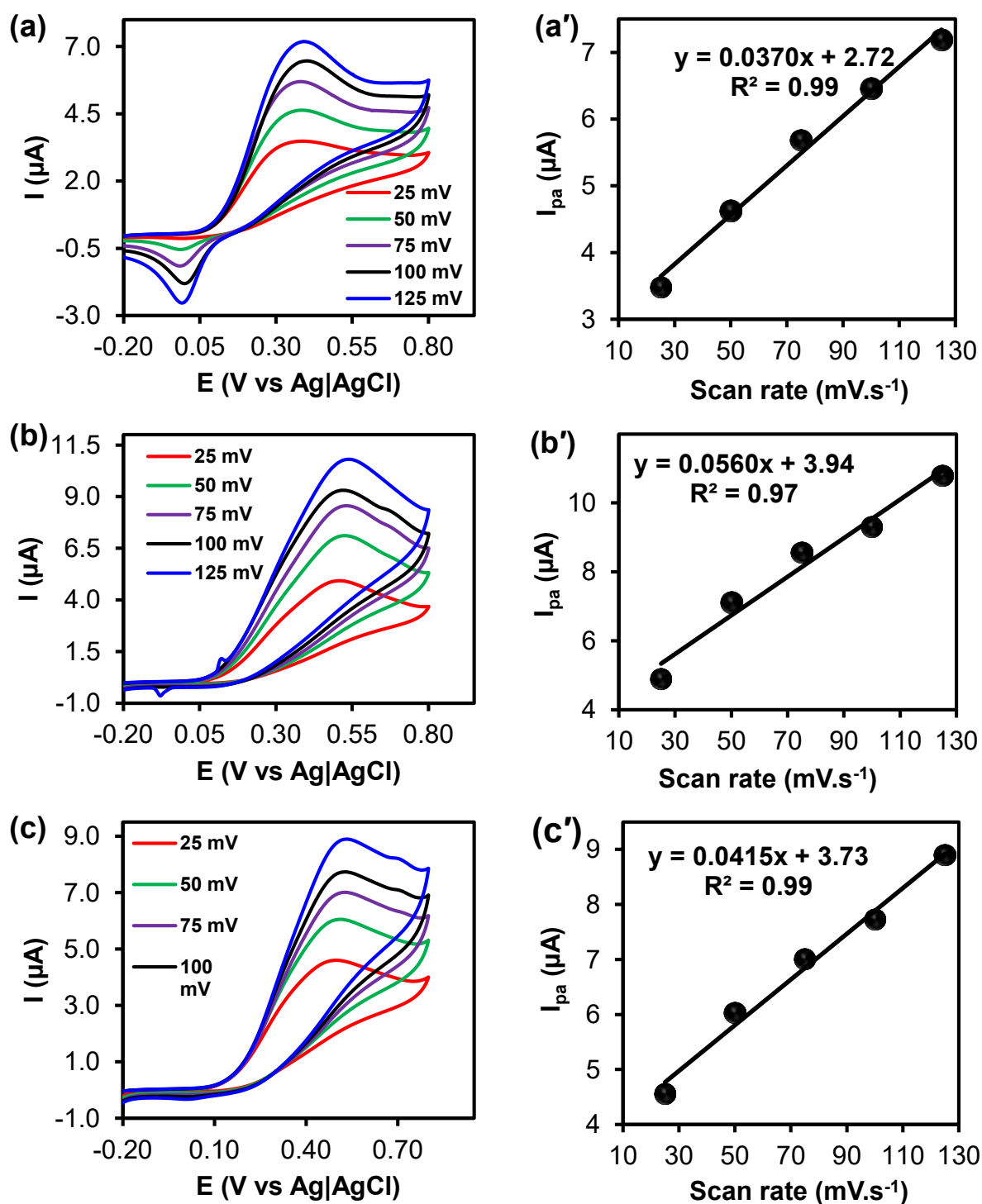


Figure 3.7: CVs and the calibration plots of Au-IPA-AmGQDs-CoTCPhOPc at different scan rates ranging from 25 mV.s⁻¹ to 125 mV.s⁻¹ in pH 7.4 phosphate buffer solution containing 1.0 mM (a) DA, (b) EP and (c) NOR.

3.3.8 Detection of DA, EP, and NOR at Au-IPA-AmGQDs-CoTCPhOPc

The detection of the DA, EP and NOR at physiological pH was conducted using differential pulse voltammetry (DPV). DPV is a more sensitive electroanalytical technique compared to the cyclic voltammetry [29]. **Figure 3.8** shows DPV of Au-IPA-AmGQDs-CoTCPhOPc towards DA, EP and NOR at different concentrations of 1.0 μM – 50 μM , in PBS (pH 7.4) at amplitude of 50 mV. The Au-IPA-AmGQDs-CoTCPhOPc exhibited an increase in peak currents as the concentration of the analyte increased. There was slight shift in peak positions from 0.037 V to 0.042 V, 0.062 V to 0.107 V and 0.082 V to 0.087 V for DA, EP and NOR, respectively. This was due to some repulsive interactions between the adsorbed products from the oxidation processes and DA, EP and NOR. Calibration graphs corresponding to the DPVs are shown in **Fig. 3.8** and exhibit a linear dependence of oxidation peak currents vs (a') [DA], (b') [EP] and (c') [NOR].

The linear regression equation for the changes in DA concentrations was (3.2):

$$I (\mu\text{A}) = 0.0766 [\text{DA}] + 1.55 (R^2 = 0.98) \quad (3.2)$$

for EP the linear regression equation was (3.3)

$$I (\mu\text{A}) = 0.0190 [\text{EP}] + 1.76 (R^2 = 0.97) \quad (3.3)$$

and for NOR, the linear regression equation was (3.4)

$$I (\mu\text{A}) = 0.0596 [\text{NOR}] + 0.827 (R^2 = 0.98) \quad (3.4)$$

The limit of detection (LoD) for DA, EP and NOR were calculated to be 0.20 μM , 0.45 μM and 0.23 μM , respectively. The LoD was calculated using 3 Sb/m (where Sb is the standard

deviation of the blank signals ($n = 10$) and m is the slope of the calibration curve). The sensitivity of Au-IPA-AmGQDs-CoTCPhOPc towards the DA was found to be $4.84 \mu\text{A} \cdot \mu\text{M}^{-1} \cdot \text{cm}^{-2}$. For EP the sensitivity was $1.20 \mu\text{A} \cdot \mu\text{M}^{-1} \cdot \text{cm}^{-2}$ and for NOR it was $3.76 \mu\text{A} \cdot \mu\text{M}^{-1} \cdot \text{cm}^{-2}$. The sensitivity was calculated from the slope of the calibration curve divided by area of the electrode.

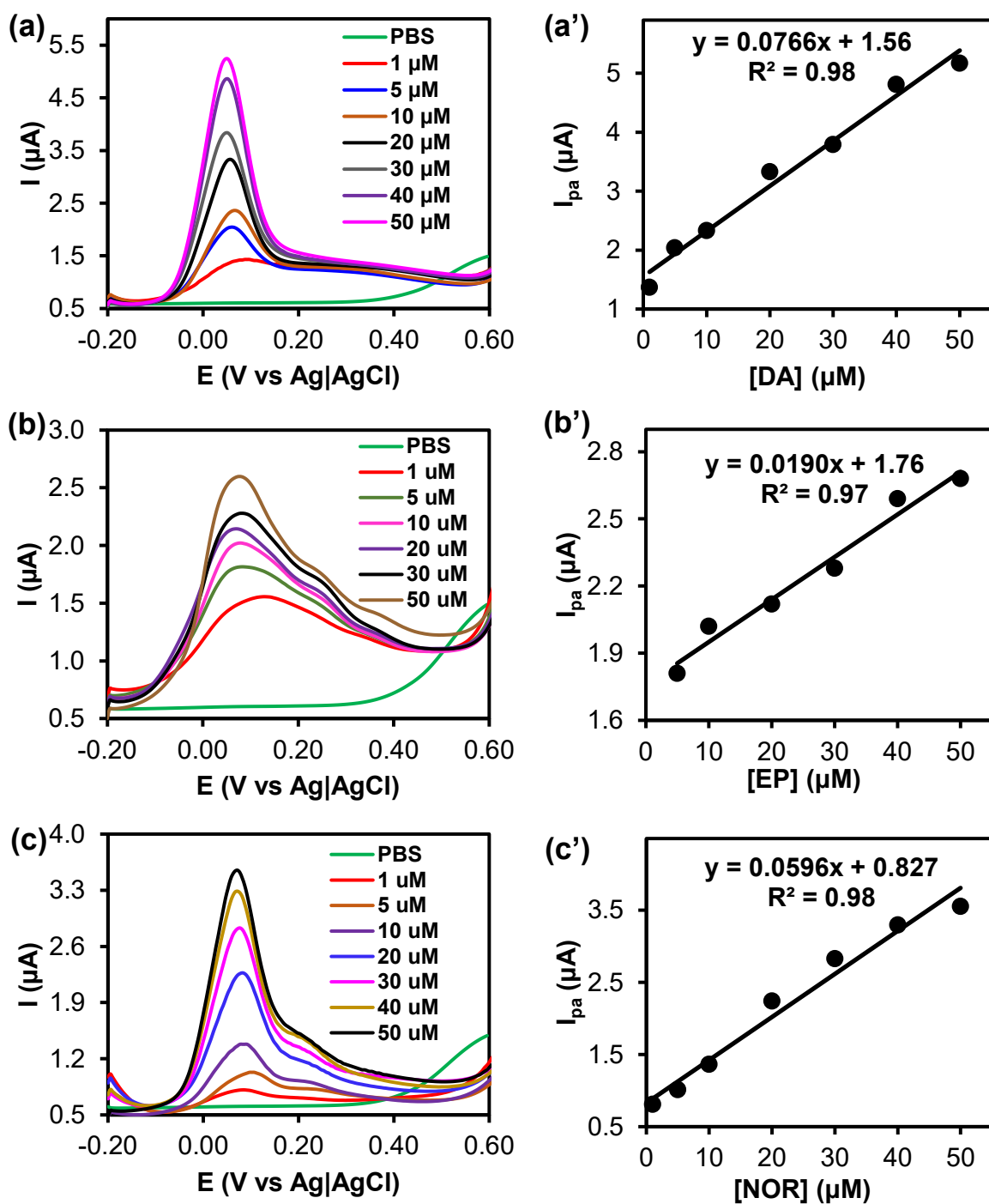


Figure 3.8: Differential pulse voltammograms (DPVs) and the calibration curves of Au-IPA-AmGQDS-CoTCPhOPc modified electrode with varying concentrations from 1.0 μM to 50 μM for (a) DA, (b) EP, and (c) NOR in PBS (pH 7.4). Scan rate = 50 mV.s^{-1} .

Table 3.1 shows comparison of electrodes reported for the determination of neurotransmitters. The results indicated that Au-IPA-AmGQDs-CoTCPhOPc gave comparable results in terms of

linear concentration range (LCR), limit of detection (LoD) and sensitivity for the detection and determination of DA, EP and NOR. According to **Table 3.1**, the obtained LoDs for DA are lower than some modified electrodes [3,41,42]. For EP and NOR, LoDs are also lower than the previously reported modified electrodes [3 43].

Table 3.1: Comparison of the electrocatalytic activity of the Au-IPA-AmGQDs-CoTCPhOPc for detection of catecholamine neurotransmitters with some of the reported modified electrodes.

Electrode	Analyte	LCR (μM)	Sensitivity ($\mu\text{A}.\mu\text{M}^{-1}.\text{cm}^{-2}$)	LoD (μM)	Ref.
Au-PEA-CoTCPhOPc	DA	5.0 – 100	0.49	1.32	[3]
	EP		0.22	3.08	
	NOR		0.31	2.11	
CoTGPC/GCE	DA	2.0 – 10	2.54 (CV) 2.91 (DPV) 0.83 (CA)	0.03	[41]
GCE/MWCNTs/PdTAPc	DA	2.0 – 16	1.93 $\text{mA}.\mu\text{M}^{-1}.\text{cm}^{-2}$	0.60	[42]
GCE-MWCNT/MO/Pc	EP	7.5 – 56	0.048	4.6	[43]
	NOR	7.5 – 48	-	1.7	
PAH/FePc/AgNP/ITO	DA	2.0 – 275	-	0.86	[44]
Au-IPA-AmGQDs-CoTCPhOPc	DA (PBS)	1.0 – 50	4.83	0.20	TW
	EP (PBS)		1.20	0.45	
	NOR(PBS)		3.76	0.23	
	DA (in-sera)	0.0 – 60	9.08	1.31	TW
	EP (in-sera)		5.06	2.20	
	NOR (in-sera)		8.95	0.82	

TW = this work, PEA = phenylethylamine, CoTCPhOPc = cobalt (II) tetra-(3-carboxyphenoxy)-phthalocyanine, MWCNT = Multiwalled carbon nanotube, MO = metal oxides, Pc = phthalocyanine, CoTGPC = Tetra ganaclovir cobalt (II) phthalocyanine, PdTAPc = palladium (II) tetra aminophthalocyanine, PAH = poly(allylamine hydrochloride), FePc = iron phthalocyanine, AgNP = Silver nanoparticles, ITO = Indium tin oxide, IPA = Isophthalic acid, AmGQDs = Aminated graphene quantum dots

3.3.9 Real sample analysis

The applicability of the proposed electrochemical sensor (Au-IPA-AmGQDs-CoTCPhOPc) was investigated for real sample analysis by detecting DA, EP and NOR in newborn calf serum (NCS). The NCS samples were diluted 100 times with PBS (pH 7.4) and the known concentration of DA, EP and NOR were added. No further pretreatment of the samples was performed. **Figure 3.9** shows the increase in the DPV peak currents and corresponding calibration curves of increasing concentrations from 10 μM - 60 μM of (a) DA, (b) EP and (c) NOR in newborn calf sera samples. The calibration curves for the detection of catecholamine neurotransmitters exhibited linearity.

For DA, the linear regression equation was (3.5):

$$I (\mu\text{A}) = 0.144 [\text{DA}] + 3.29 (R^2 = 0.99) \quad (3.5)$$

For EP, the linear regression equation was (3.6)

$$I (\mu\text{A}) = 0.0803 [\text{EP}] + 2.84 (R^2 = 0.98) \quad (3.6)$$

and for NOR, the linear regression equation was (3.7)

$$I (\mu\text{A}) = 0.142 [\text{NOR}] + 4.28 (R^2 = 0.98) \quad (3.7)$$

Au-IPA-AmGQDs-CoTCPhOPc showed excellent electrocatalysis and electroanalysis of catecholamine neurotransmitters for in-sera (CNS) samples. The detection limits were determined to be 1.31 μM , 2.20 μM and 0.82 μM for DA, EP and NOR respectively. When comparing the LoDs for in-sera analysis with PBS, the LoDs for in-sera analysis were higher than the PBS. The sensitivity of Au-IPA-AmGQDs-CoTCPhOPc towards DA was found to be

9.08 $\mu\text{A} \cdot \mu\text{M}^{-1} \cdot \text{cm}^{-2}$. For EP, the sensitivity was found to be 5.06 $\mu\text{A} \cdot \mu\text{M}^{-1} \cdot \text{cm}^{-2}$ and for NOR, it was found to be 8.95 $\mu\text{A} \cdot \mu\text{M}^{-1} \cdot \text{cm}^{-2}$. The LoD, linear concentration range (LCR) and sensitivity for the detection of DA, EP and NOR in-sera are summarized in **Table 3.1** and compared to the results in PBS.

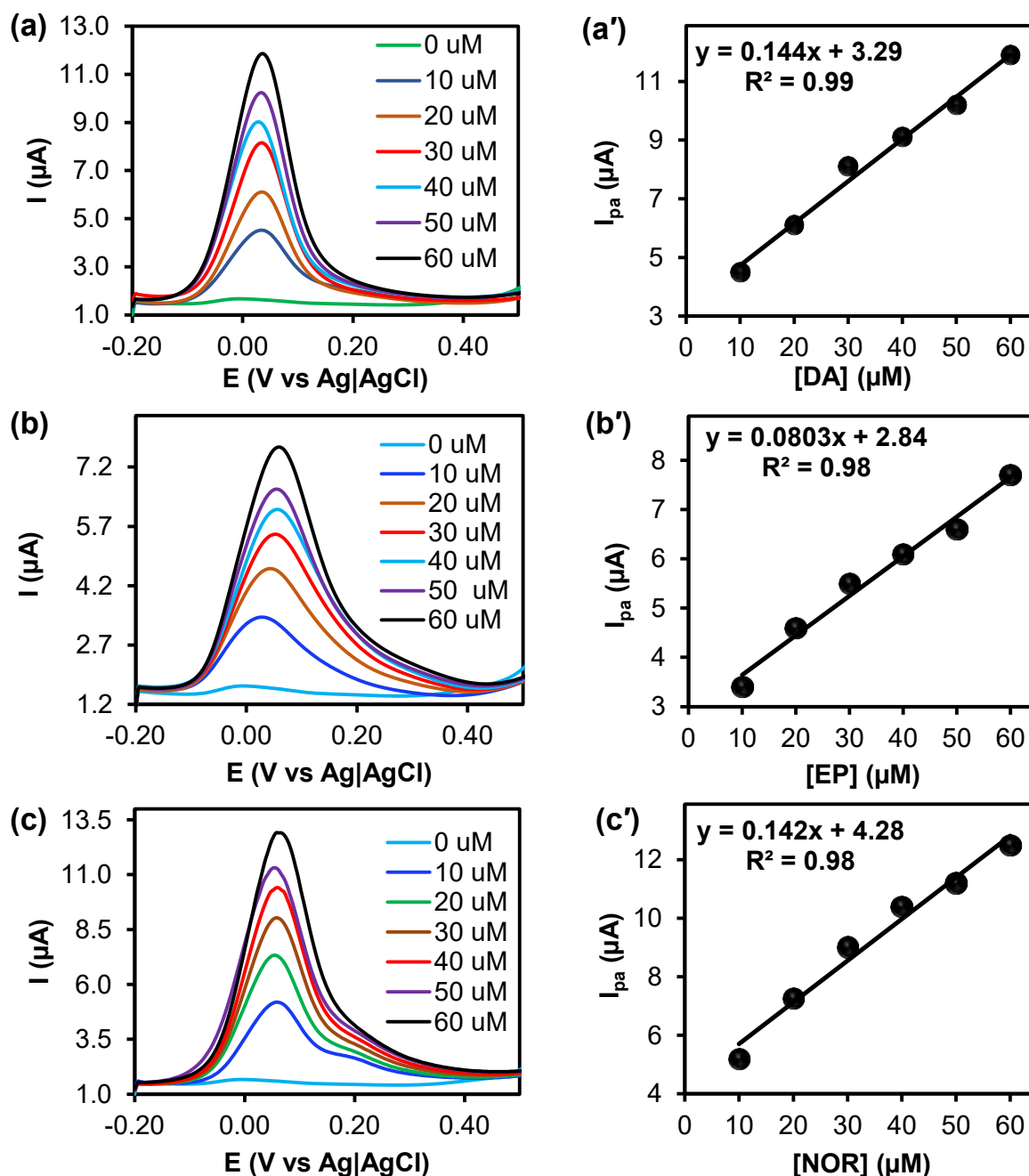


Figure 3.9: DPV and corresponding calibration curves for Au-IPA-AmGQDs-CoTPhOPc of varying concentrations from 10 μM to 60 μM of (a) DA, (b) EP and (c) NOR in newborn calf serum (in-sera) samples.

For both PBS and in-sera, the Au-IPA-AmGQDs-CoTCPhOPc modified electrode gave lowest detection limits with high sensitivity to DA as compared to EP and NOR. The decrease in sensitivity for EP and NOR might be due to slow electron transfer processes during the oxidation of EP and NOR [44]. In addition, high sensitivity and LoDs were obtained in-sera as compared to the PBS for the detection of DA, EP and NOR. The high sensitivity does not necessary equate to low LoDs, but this might be due to a decrease in matrix interference and high background noise [43].

The recovery rates are shown in **Table 3.2**. The percentage recoveries were in the range of 101 - 106% for DA, 89 – 92% for EP and 85 – 102% for NOR. The percentage relative standard deviation (%RSD) (n = 3) were 8% or less for the detection of catecholamine neurotransmitters. These results indicated that the proposed sensor (Au-IPA-AmGQDs-CoTCPhOPc) can potentially be applied for the determination of DA, EP, and NOR in real sample (newborn calf serum) within experimental conditions.

Table 3.2: Detection of DA, EP and NOR in newborn calf serum sample using percentage recovery method and calibration curve in **Fig. 3.9** and the results were conducted in triplicates (n = 3).

Analyte	In-sera sample	Added (μM)	Found (μM)	Recovery (%)	%RSD
DA	1	20	20.3	101	7.0
	2	30	32.0	106	8.0
	3	40	40.4	101	5.0
EP	1	20	18.5	92	1.3
	2	30	26.8	89	8.0
	3	40	36.9	92	3.0
NOR	1	20	20.4	102	7.0
	2	30	29.7	99	2.0
	3	40	34.0	85	8.0

3.3.10 The effect of interference by Ascorbic Acid

The detection of DA, EP and NOR are commonly affected by the presence of some interfering species such as small amino acids, common cations ions, proteins, and ascorbic acid (AA) just to mention a few. Among the interfering species, ascorbic acid is the most serious interference during the detection of the DA, EP and NOR. AA has similar oxidation mechanism as DA, EP and NOR. Ascorbic acid usually coexist with catecholamine neurotransmitters in biological samples [45-47]. In this study, the effect of AA interference to DA, EP and NOR on the Au-IPA-AmGQDs-CoTPhOPc electrode was studied by square wave voltammetry (SWV). SWV was employed because it offers very high sensitivity in trace determination of analytes. **Figure 3.10 (i)** shows SWVs for the detection of 50 μM (i) AA, (ii) (a) DA, (b) EP and (c) NOR, (iii) mixed solutions of AA with NTs in 0.10 M PBS (pH 7.4). No peak was observed for AA alone. This was attributed to CoTPhOPc bearing COOH functional groups which are deprotonated at pH 7.4 which is higher than the pKa (~ 5) of COOH. The negatively charged carboxylic acid (COO^-) functional groups repels the ascorbate anion at pH 7.4. The DA, EP and NOR showed electrocatalytic oxidation peaks at the Au-IPA-AmGQDs-CoTPhOPc in **Fig. 3.10(ii)**. In the presence of mixture of 50 μM AA + 50 μM DA in **Fig. 3.10(a)(iii)** two broad peaks were observed. The oxidation peak at high potential was due to the adduct (AA-DA) [47, 48] as result of DA interacting with AA. The mixture of 50 μM EP + 50 μM AA showed similar oxidation potential as for EP alone in **Fig. 3.10(b)(iii)** indicating no interference from AA. The same was observed for a mixture of 50 μM NOR + 50 μM AA in **Fig. 3.10(c)(iii)**. The presence of AA in the same solution with EP and NOR showed no interference. The high potential oxidation peak for a mixture of DA and AA was due to an adduct that resulted from electrostatic interactions between DA and AA. A given concentration of DA will generate a broad electrooxidation peak current which shifts to positive potential in the presence of ascorbic acid.

AA reduce the oxidation product of DA, dopamine-o-quinone back to DA and become available one more for electrochemical oxidation at the electrode surface. Therefore, the interference by AA is not largely avoided especially when DA is directly oxidized on the electrode surface [49, 50].

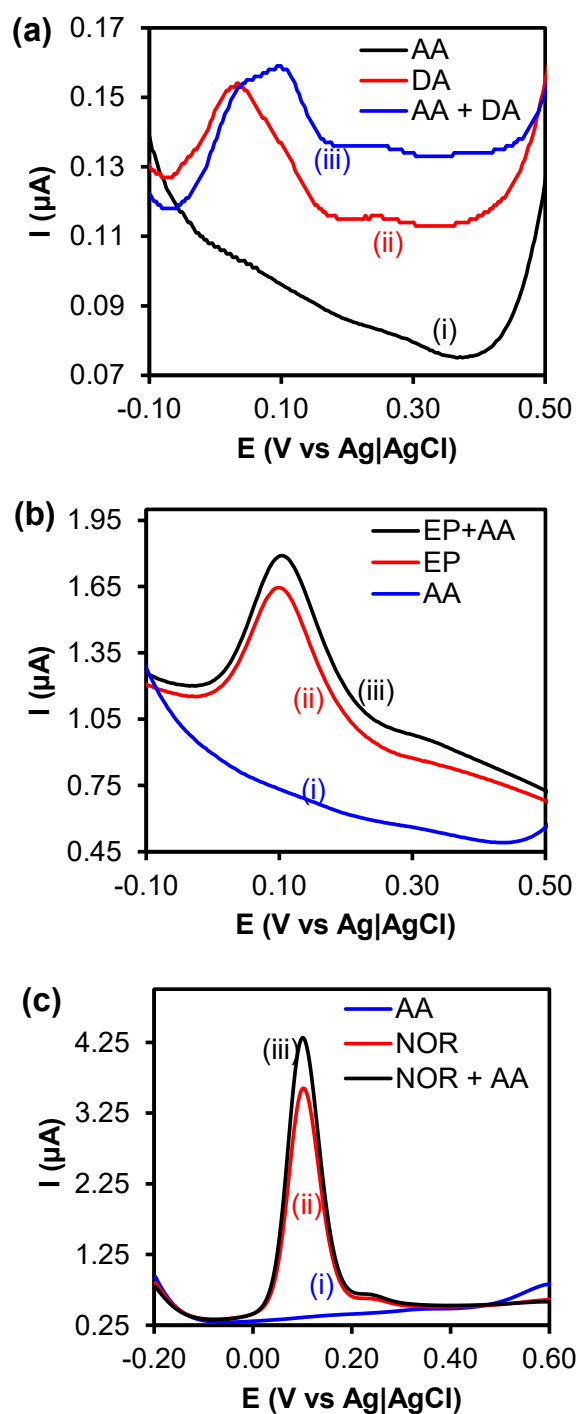


Figure 3.10: Square wave voltammograms (SWV) of Au-IPA-AmGQDs-CoTCPhOPc in the presence of 50 μM each of (i) AA, (ii) (a) DA, (b) EP, (c) NOR and (iii) mixture of (a) DA + AA, (b) EP + AA, (c) NOR + AA in PBS (pH 7.4).

3.3.11 Repeatability and stability of the Au-IPA-AmGQDs-CoTCPhOPc

The repeatability and stability of the Au-IPA-AmGQDs-CoTCPhOPc were evaluated in PBS containing 20 μM of DA. Ten measurements were performed on the same Au-IPA-AmGQDs-CoTCPhOPc in 0.10 M PBS of pH 7.4 containing 20 μM DA, **Fig. 3.10**. The results showed that the modified electrode has satisfactory repeatability with a percentage relative standard deviation of 4.5%. Similarly, the stability of modified sensor (Au-IPA-AmGQDs-CoTCPhOPc) was evaluated for 30 days storage in the dark and rinsed with PBS (pH 7.4). Before each use, the Au-IPA-AmGQDs-CoTCPhOPc was measured in blank buffer, PBS (pH 7.4) and then in the freshly prepared buffer containing 2.0 μM DA (as a representative of catecholamine neurotransmitters). The oxidation peak current decreased to 97 % of the original curve within the 30 days of evaluation, indicating that the modified electrode had excellent storage stability.

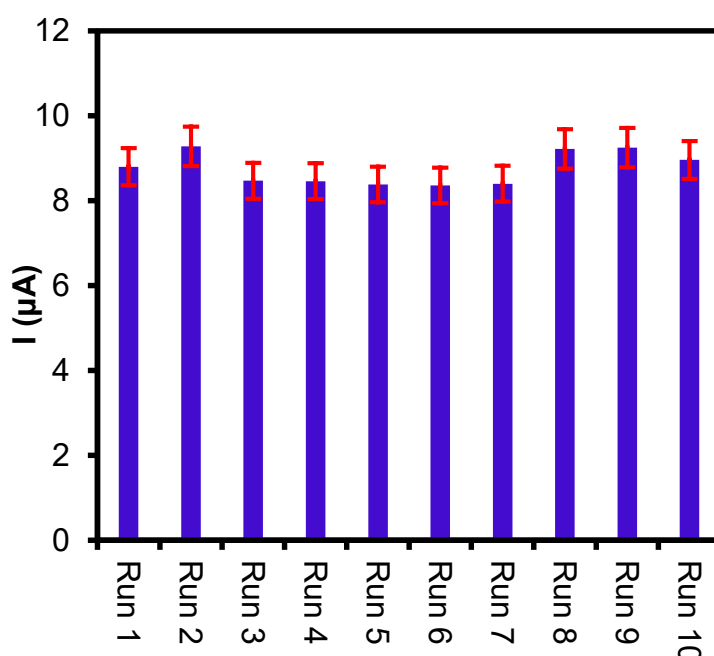


Figure 3.11: A bar chart showing variation of peak current of individual DPVs of 20 μM DA in 0.10 M PBS (pH 7.4) at Au-IPA-AmGQDs-CoTCPhOPc modified electrode.

3.4 Conclusions

In this chapter, a sensitive electrochemical sensor based on Au-IPA-AmGQDs-CoTCPhOPc was constructed. Cobalt (II) tetra (3-carboxyphenoxy) phthalocyanines (CoTCPhOPc) was successfully anchored onto the surface of amino graphene quantum dots (AmGQDs) through covalent functionalization to the isophthalic electrografted gold electrode (Au-IPA). The electrochemical behaviour of the AmGQDs with CoTCPhOPc was studied by CV and EIS. The fabricated sensor showed an enhanced electrochemical performance towards dopamine, epinephrine, and norepinephrine. The sensor displayed a wide linear range in PBS of (1.0 – 50 μ M) and in-sera (0.0 – 60 μ M) with low detection limit in the μ M range, enhanced detection, and good reproducibility. The interference from AA on DA, EP and NOR detection was greatly reduced. An excellent recovery from 85 – 106 % was achieved when DA, EP and NOR were spiked in newborn calf serum samples.

3.5 References

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CHAPTER 4

4 Simultaneous detection of dopamine and paracetamol on a composite electrode

Abstract

The fabrication of sensitive, fast, cost-effective, and eco-friendly electrochemical sensors is essential for monitoring analytes of biomedical, environmental, and pharmaceutical interests. In this chapter, we report the simultaneous electroreduction and deposition of graphene oxide (GO) to form electrochemically reduced graphene oxide (ERGO) onto a glassy carbon electrode (GCE) represented as GCE-ERGO. Onto the GCE-ERGO, cobalt (II) tetra-amino phthalocyanine was electropolymerized to form a stable GCE-ERGO/polyCoTAPc. The sensing surface, GCE-ERGO/polyCoTAPc, was characterized using cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) techniques to ascertain its electron transfer and conducting properties. The electrode surface functional groups and composition were confirmed using Raman spectroscopy and energy-dispersive X-ray spectroscopy. The prepared sensing electrode displayed enhanced electrocatalytic activity towards ferri/ferrocyanide $[\text{Fe}(\text{CN})_6]^{3-/4-}$, as a redox probe. GCE-ERGO/polyCoTAPc was further used for ultrasensitive simultaneous detection and determination of dopamine (DA) and paracetamol (PA). The electrocatalytic peak currents for DA and PA were greatly enhanced with an oxidation potential difference of 0.264 V and wide enough for simultaneous determination. The electrocatalytic oxidation peak currents of DA and PA at GCE-ERGO/polyCoTAPc showed linear dependence with the changes in concentrations up to 100 μM for DA and up to 90 μM for PA using differential pulse voltammetry (DPV). The limits of detection (LoD) values were 0.095 μM and 0.10 μM using a signal-to-noise (S/N) ratio of 3 for DA and PA, respectively. The GCE-

ERGO/polyCoTAPc displayed excellent sensitivity of $8.39 \mu\text{A} \cdot \mu\text{M}^{-1} \cdot \text{cm}^{-2}$ for DA and $1.32 \mu\text{A} \cdot \mu\text{M}^{-1} \cdot \text{cm}^{-2}$ for PA. The fabricated ultrasensitive electrochemical sensor was successfully used for the determination of DA and PA in synthetic urine samples with excellent percentage recoveries.

4.1 Introduction

Dopamine (DA) is a naturally occurring biogenic compound which is considered to be one of the most important neurotransmitters in the brain. It is strongly associated with reward mechanisms like memory, locomotion, learning and behaviour of cognition [1]. Some diseases, such as epilepsy, schizophrenia, and Parkinson's disease, are caused by lacking proper DA concentrations in the human body [2]. Therefore, monitoring the concentration of DA in the bodily fluids can aid diagnosis of neurodegenerative diseases. On the other hand, paracetamol plays an important role in pharmacology. It is used as an analgesic and antipyretic drug. Paracetamol (PA) is often used to treat a myriad of health problems in the body like fever, muscular aches, chronic pain, migraine headache, relieve coughing and cold [3]. PA has some health side effects despite its numerous health benefits. It can cause kidney and liver damage if used excessively [4]. Prolonged use of PA also significantly reduces the concentration of DA in the body [5]. Dopamine and paracetamol co-exist in many biological fluids such as urine and they do interfere with each other during measurements. Hence accurate quantification of the concentration levels of these biomolecules is highly desirable. Therefore, development of a sensitive and simple analytical technique for simultaneous detection of DA and PA in clinical and pharmacological formulations is of paramount importance. Techniques such as chromatography [6] and spectrophotometry [7] have been widely used for the detection of DA and PA in real samples. However, these techniques are expensive, time consuming and require

highly skilled operators for sample processing and analysis. Recently, electrochemical techniques have been explored for the detection and determination of DA and PA due to their high selectivity, low cost of analysis, ease of handling and less time consuming [1]. Electrochemical sensors have been developed for detection of these biological molecules in real samples with satisfactory results [8].

In this chapter, metallophthalocyanine polymers with reduced graphene oxide as nanocomposites were fabricated onto glassy carbon electrode to study their electrocatalytic activity for simultaneous determination of dopamine and paracetamol. Metallophthalocyanines (MPcs) are N_4 -macrocycles with metal atoms at the centre and possess unique electrochemical behaviour [9]. MPcs, due to their rich redox chemistry and extended conjugation have been widely used as suitable electrode materials for the detection of other important biological molecules [10]. Amongst the metallophthalocyanines, cobalt phthalocyanines (CoPcs) have been widely used as mediator/electrode modifiers [11]. Cobalt as a central metal ion in the phthalocyanines can switch oxidation states between Co^{III}/Co^{II} and Co^{II}/Co^I with ease, thus making this an interesting metal for electrochemical investigation. Cobalt metal ion binds with reactants reversibly during electrocatalysis and thus exhibiting excellent electrocatalytic activity [12]. Electropolymerization is a simple, fast, and effective approach of modifying MPcs on electrode surfaces. The deposition of electropolymerized MPcs can be simply controlled by adjusting the electrochemical parameters and stable thin films are formed [13, 14]. Therefore, electropolymerized MPc films have attracted great attention to research scientists for the development of electrochemical sensors. In order to improve adhesion and conductivity of MPcs on electrode surfaces suitable support materials are required [15]. Graphene based carbon nanomaterials have been widely used as support materials for MPcs on electrode surfaces. Graphene nanomaterials possess excellent physicochemical properties such

as high surface area, high conductivity, and superior biocompatibility [16]. Modification of reduced graphene oxide (RGO) onto electrode surface can be via electrochemical or chemical reduction method. Electrochemical reduction method is a more desirable method because it allows for the electroreduction whilst simultaneously depositing GO onto the glassy carbon electrode surface. This allows for controllable and low C/O ratio [17]. Electrochemical reduction of GO (ERGO) also does not involve the use of harsh chemicals and thus minimizes contamination. Currently, there are two routes for electroreduction of GO: (i) reduction of predeposited GO films on the electrode surface and (ii) electro reductive deposition of GO dispersions [18]. Recently, Mani et al. prepared stable pTACoPc/RGO composite through electrodeposition/electropolymerization onto glassy carbon electrode. The formed thin film was used for electrochemical sensing of cysteine and hydrazine [12]. In their work, RGO thin films were fabricated through reduction of pre-deposited GO on GCE. Due to the high hydrophilicity of GO sheets, pre-deposited GO films easily peeled off from the electrode surface. This affected the film thickness and stability of RGO on the electrode surface. Following the second method, RGO thin films are deposited on the electrode surface by direct electrochemical reduction of GO from its dispersed solutions. During the direct electrodeposition, insoluble RGO sheets are formed which eventually attach onto the glassy carbon electrode surface. This method affords good control over film thickness of the RGO layer. In this chapter, the fabrication of nanocomposite sensor by direct electrodeposition of RGO thin films was conducted from a GO solution. The electrode was further modified by electropolymerization of cobalt phthalocyanines on GCE/ERGO and used for the simultaneous determination of dopamine and paracetamol. The simultaneous detection of DA and PA was studied in phosphate buffer saline and synthetic urine samples at physiological conditions.

This chapter was therefore set out to achieve the following objectives of the thesis:

- (a) Fabrication and characterization of electroreduced graphene oxide - cobalt (II) tetra amino phthalocyanine nanocomposite polymer electrode.
- (b) Detection of dopamine and paracetamol simultaneously at the electroreduced graphene oxide - cobalt (II) tetra amino phthalocyanine nanocomposite polymer electrode in phosphate buffer solutions.
- (c) Detection of dopamine and paracetamol selectively in synthetic urine samples.
- (d) Detection of dopamine selectively in the presence of large excess of ascorbic acid and uric acid.

4.2 Experimental

4.2.1 Chemicals and reagents

The materials from the previous chapter were used. Additional materials and their suppliers not from the previous Chapter 3 are listed. Graphite flakes (<20 mm) were purchased from Sigma-Aldrich and used as received. Paracetamol tablets were purchased from Alpha Pharm Grahamstown Pharmacy (RSA). Sodium nitrate (NaNO_3), potassium permanganate (KMnO_4), sulfuric acid (H_2SO_4), hydrogen peroxide (H_2O_2) and nitric acid (HNO_3) were purchased from B&M Scientific Ltd. Cobalt tetraamino phthalocyanine (CoTAPc) was synthesized according to established literature procedure [19]. The 0.10 M pH 7.4 phosphate buffer saline (PBS) solution (1 L) was prepared by dissolving 0.24 g potassium dihydrogen orthophosphate (KH_2PO_4), 1.44 g di-sodium hydrogen orthophosphate (Na_2HPO_4), 8.0 g NaCl and 0.20 g KCl in Mill-Q water. Sodium hydroxide (NaOH , 1.0 M) and hydrochloric acid (HCl , 1.0 M) were used to adjust the pH of the PBS solution to appropriate values. Synthetic urine was used for real sample analysis and was prepared following a reported method [20]. Briefly, in 50 mL of

ultra-pure water, 60 mg of $\text{CaCl}_2 \cdot \text{H}_2\text{O}$, 80 mg of KCl , 70 mg of KH_2PO_4 , 0.15 g of NaCl , 0.11 g of Na_2SO_4 , 50 g of NH_4Cl and 1.25 g of urea were added and mixed well to make a synthetic urine. All other aqueous solutions were prepared using ultra-pure water obtained from a Milli-Q Water System (Millipore Corp. Bedford, MA, USA). Except for DMF, all chemicals were of analytical reagent grade and were utilized as received from the suppliers without any further purification.

4.2.2 Apparatus

The equipment used for characterization of materials and surfaces are listed in the previous chapter. The additional techniques are listed here. Scanning electron microscopy (SEM) images were obtained using an INCA PENTA FET coupled with VEGA TESCANA at 20 kV acceleration voltage. An electrochemical cell containing an analytical solution and three electrodes, consisting of a working electrode (WE, glassy carbon electrode or modified glassy carbon electrode), a counter electrode (CE, Pt), and a pseudo-reference electrode (RE, $\text{Ag}|\text{AgCl}$ wire), were used for all the electrochemical measurements.

4.2.3 Preparation of graphene oxide (GO)

Graphene oxide (GO) was synthesized following a top-down method from graphite flakes by modified Hummers method [21]. Firstly, NaNO_3 (0.050 g, 0.60 mmol) and 1.0 g graphite flakes were added into a 24 mL H_2SO_4 solution at 0°C under stirring. KMnO_4 (3.0 g, 19.0 mmol) was added slowly to keep the temperature of the suspension lower than 20°C . The reaction system was transferred to a 35°C water bath with stirring for 60 min and 46 mL of double-distilled water was added gradually into the above solution. The mixture was stirred further at 95°C for

15 min. After which 140 mL of double-distilled water was added and followed by 10 mL H₂O₂ (30%) to stop the oxidation reaction. The final product was centrifuged, washed with HCl (5%) and copious amount of de-ionized (DI) water until pH 7 was reached. The final product was dried in vacuum for 12 h at 40°C. The product graphite oxide (60 mg) was dispersed in 10 mL double-distilled water under ultrasonic homogenizer at ambient temperature for 1 h to exfoliate graphene oxide. IR spectrum (cm⁻¹): 2970 (-CH), 1740 (C=O) and 1091 (C-O).

4.2.4 Electrode fabrication to form GCE-ERGO/polyCoTAPc

GCE-ERGO/polyCoTAPc was fabricated as outlined in the reported literature [22] with some modification. Firstly, the bare GCE was polished to a mirror finish with 1.0 µm, 0.30 µm and 0.050 µm alumina aqueous slurry followed by ultrasonication in 50% HNO₃, ethanol (95%) and double distilled water for 5 min each. GO (20 mg) was dispersed in 10 mL of PBS (0.10 M, pH 7.4), ultrasonicated for 1 h to obtain GO concentration of 2.0 mg.mL⁻¹ and deaerated with N₂ gas under constant stirring for 10 min. Into this solution, a freshly cleaned GCE was placed, and electrodeposition of GO was carried out under cyclic voltammetry (CV) sweeping from 0.60 to -1.5 V at a scan rate of 25 mV.s⁻¹. Six cycles were used to afford an electrochemical reduction of graphene oxide (ERGO) and deposition to form GCE-ERGO. GCE-ERGO was washed and dried in vacuo. The GCE-ERGO was then immersed into a dry 5 mL DMF solution containing CoTAPc (2.0 mg, 3.0 µmol) and TBABF₄ (160 mg, 0.50 mmol) saturated with N₂ gas (5 min) and cycled for 25 scans between -2.6 V and +1.0 at 100 mV.s⁻¹. N₂ blanket was maintained over the solution during the entire experiment. The electrode was rinsed with dry DMF to remove physically adsorbed CoTAPc. The electrode was represented as GCE-ERGO/polyCoTAPc.

4.2.5 Electrochemical analysis

All the electrochemical experiments were performed using the CV, DPV and EIS techniques at room temperature in a 10 mL electrochemical cell. The CVs and DPVs potentials for all the experiments were against Ag|AgCl wire pseudo-reference electrode. Determination of DA and PA were carried in 10 mL of 0.10 M PBS (pH 7.4) saturated with N₂ gas. After adding the known analyte concentration to the electrochemical cell, the electrochemical measurement was performed. Each experiment was repeated three times ($n = 3$) to ensure reproducibility. For real samples analysis, 10% urine sample was prepared by diluting 1.0 mL of urine into 9.0 mL of PBS (pH 7.4, 0.10 M) and transferred into a 10 mL electrochemical cell for measurements. The 10% urine samples were spiked with a known amount of DA or PA and the samples were measured. The recovery rates of the spiked samples were determined also in triplicates ($n = 3$).

4.3. Results and discussion

4.3.1 Electrodeposition of reduced GO and CoTAPc polymer onto GCE

Figure 4.1(a) shows electrochemical reduction of GO (2.0 mg.mL⁻¹) by cyclic voltammetry between 0.6 and -1.5 V for 6 consecutive cycles at a scan rate of 25 mV.s⁻¹. In the cyclic voltammogram (CV), an anodic peak and two cathodic peaks were observed. The reduction peak (III) observed at the potential range between -1.0 V to -1.5 V is attributed to the irreversible reduction process of the oxygen-containing groups of GO and consistent with literature [23]. During the electrochemical reduction, the GO sheets in direct contact with an electrode surface are reduced [24] to form insoluble reduced graphene sheets (ERGO) which attaches onto the electrode surface. Furthermore, the irreversible reduction peak currents

persistently increased with repeated scanning confirming the ERGO electrodeposition on the GCE surface [24]. The reversible peak with (I) anodic and (II) cathodic peaks are due to electroactive oxygen functional groups on GO planes that are reduced [24]. Electrochemical deposition of ERGO was optimized, and the number of CV scans were monitored. After the sixth scan, the deposition signal started to decrease, and this was attributed to the thick ERGO film and loss of surface conductivity for further electrodeposition of ERGO. Therefore, six CV scans were used, and the resulted GCE-ERGO was used for electropolymerization of CoTAPc.

Electrochemical polymerization of CoTAPc resulted in the film thickness that can be easily controlled by varying the quantity of charge passed [25]. **Figure 4.1(b)** shows three important redox couples observed at formal potentials ($E'_{1/2}$) of (I) -1.74 V, (II) -0.79 V, and (III) -0.090 V due to $\text{Pc}^{2-}/\text{Pc}^{3-}$, $\text{Co}^{2+}/\text{Co}^+$ and $\text{Co}^{3+}/\text{Co}^{2+}$ respectively and in line with reported literature [14,26]. **Figure 4.1(c)** shows the growth of oxidation and reduction peak currents with increasing number of voltammetric scans from 1st scan to the 25th scan on GCE-ERGO to form GCE-ERGO/polyCoTAPc. The potential was scanned between 1.0 V to -2.8 V and at a scan rate of 100 mV.s⁻¹ in dry DMF containing CoTAPc (2.0 mg, 3.0 μmol) and TBABF₄ (0.160 g, 0.50 mmol). The gradual increase in redox peak currents confirms successful formation of electroactive polymer of polyCoTAPc [12,14]. The electropolymerization of CoTAPc mostly involves the oxidation of the amino groups to form radicals which then attack the benzene rings of the neighboring molecules [27] forming a polymer.

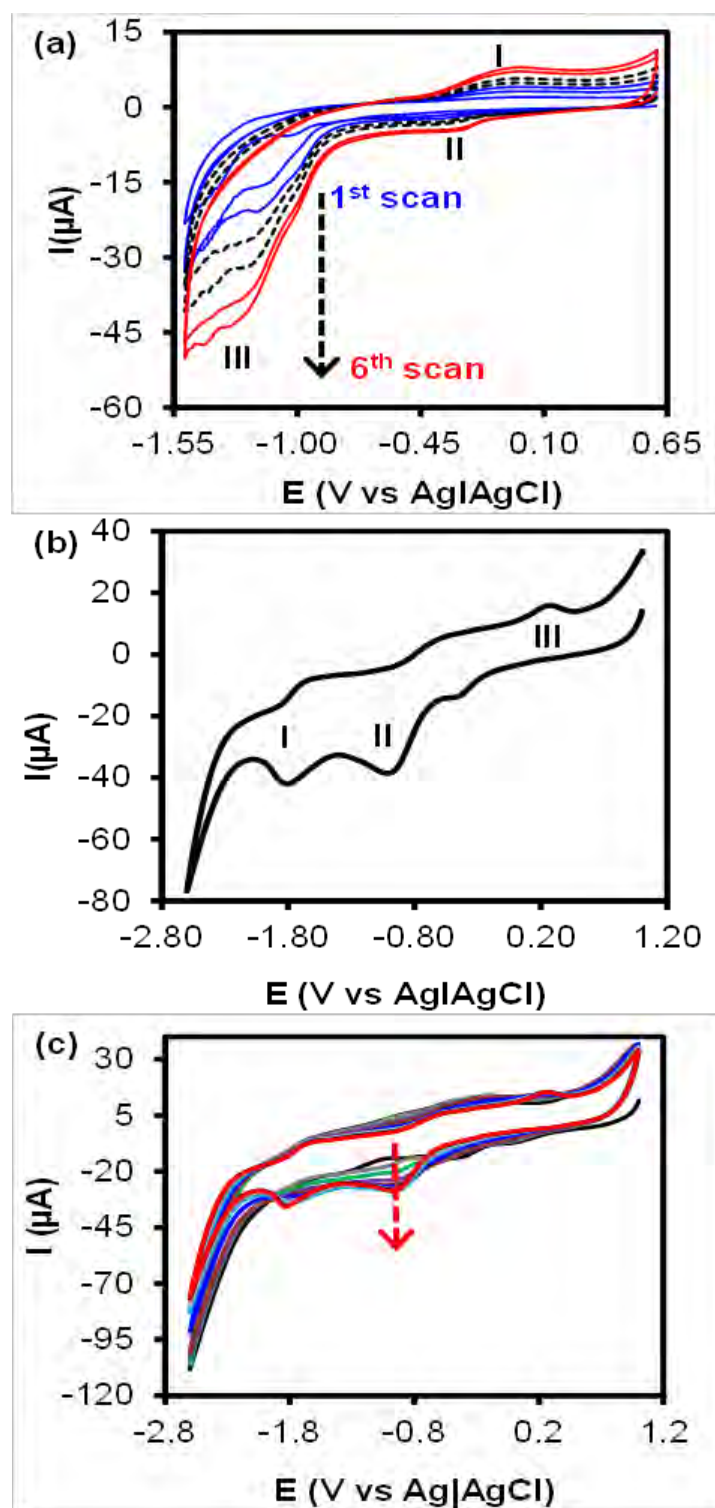


Figure 4.1: CVs of (a) the electrodeposition of 2.0 mg.mL⁻¹ GO in PBS (0.10 M, pH 7.4) on a GCE, scan rate: 25 mV.s⁻¹ (b) GCE-ERGO in dry DMF solution containing CoTAPc (2.0 mg, 3.0 μmol) and TBABF₄ (0.160 g, 0.50 mmol), scan rate = 25 mV.s⁻¹, and (c) electropolymerization of CoTAPc on GCE-ERGO, scan rate = 100 mV.s⁻¹.

The electrode modification steps were monitored and characterized by scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS) as shown in **Fig. A1**. The use of flat ITO conducting glass slide afforded the modification and characterization using SEM. The reduced graphene oxide electrodeposited surface yielded a wrinkle-like morphology, **Fig. A1(b)**, compared to smooth bare ITO in **Fig. A1(a)**. Upon electropolymerization of CoTAPc, **Fig. 1A(c)**, a thick film formed confirming the formation of ERGO/polyCoTAPc film. The EDS spectrum in **Fig. A1(d)** showed the presence of C K, O K, N K from ERGO/polyCoTAPc. The other elements, especially Sn M, In M, and O k were from the underlying ITO substrate.

4.3.2 FT-IR ITO Surface characterization

FT-IR in **Fig. 4.2**, the ERGO shows characteristic vibrational bands at 2970 cm^{-1} (C-H), 1740 cm^{-1} (C=O), and 1091 cm^{-1} (C-O). The vibrational spectrum of the ERGO/polyCoTAPc displays new peaks at 1042 cm^{-1} (C=N, N=N), 890 cm^{-1} , and 792 cm^{-1} (Co-N) which are attributed to the moieties of the cobalt phthalocyanine macrocycle. The C-N=C peak at 1092 cm^{-1} from the Pc isoindole appeared confirming the ring formation of polyCoTAPc.

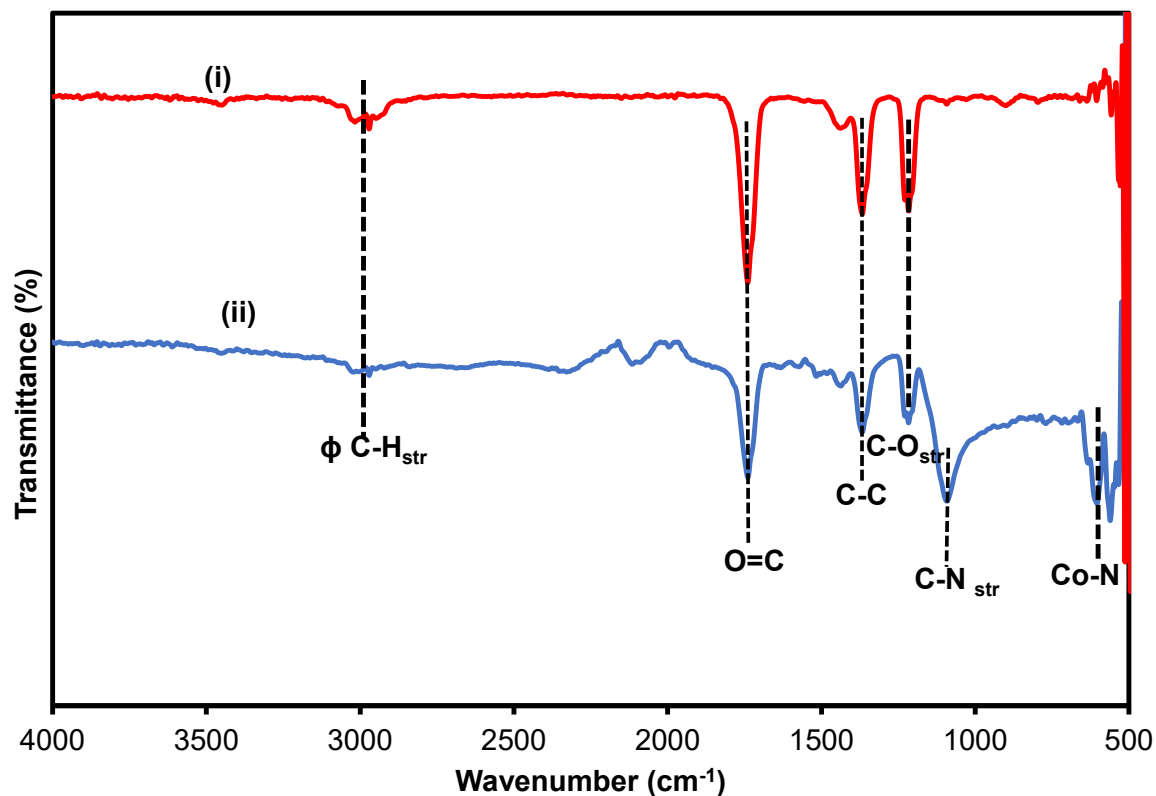


Figure 4.2: FT-IR spectra ITO with (i) electrodeposited ERGO and (ii) electropolymerized polyCoTAPc on ERGO (ERGO/polyCoTAPc).

4.3.3 Electrochemical characterization of the GCE, GCE-ERGO and GCE-ERGO/poly CoTAPc

The electrochemical behaviour of bare GCE, GCE-ERGO, GCE-polyCoTAPc and GCE-ERGO/polyCoTAPc in the presence of (1:1) 2.0 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ containing 0.10 M KCl. **Figure 4.3(a)(i)** shows a pair of reversible redox peaks at bare GCE. The peak potential separation (ΔE_p) is 109 mV and the ratio of oxidation peak current to reduction peak current (I_{pa}/I_{pc}) is 0.98 (≈ 1) demonstrating a good reversibility. At GCE-ERGO, **Fig. 4.3(a)(ii)**, the value of ΔE_p decreased to 90 mV. This can be attributed to the fact that

electrochemically reduced graphene oxide (ERGO) has a large surface area, and this resulted also in an increase in the peak currents. ERGO also has excellent electron conductivity properties which resulted in the decrease in ΔE_p to 90 mV from 109 mV for GCE. At the GCE-polyCoTAPc, **Fig. 4.3(a)(iii)**, the ΔE_p value was 93 mV and due to the conducting properties of polyCoTAPc. Finally, at GCE-ERGO/polyCoTAPc in **Fig. 4.3(a)(iv)** the value of ΔE_p decreased to 87 mV. The observed decrease in the ΔE_p values at GCE-ERGO/polyCoTAPc was due to excellent conductivity with an increased rate of electron transfer. The redox peak currents increased at GCE-ERGO/polyCoTAPc, further confirming the excellent conducting properties.

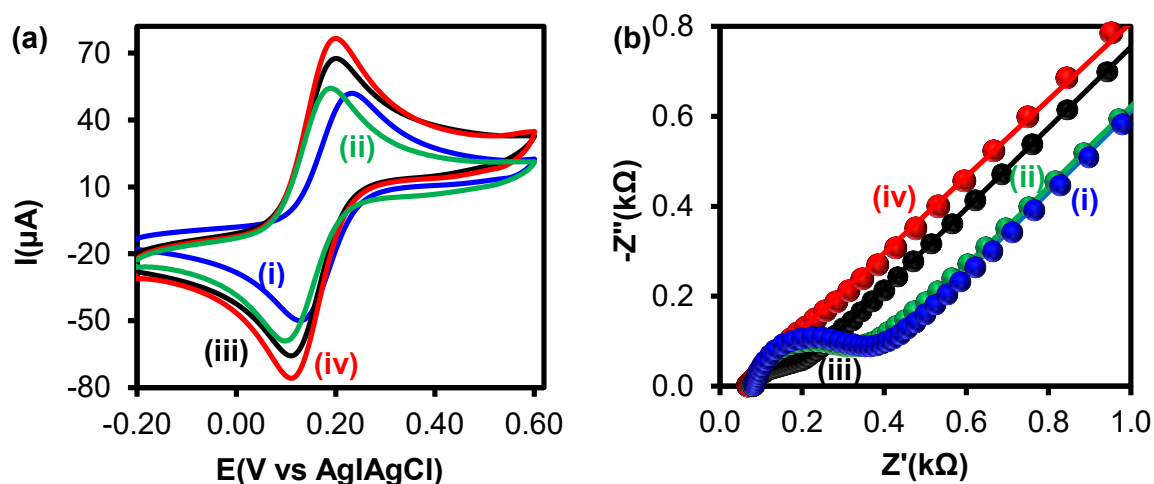


Figure 4.3: (a) CVs and (b) Impedance spectra (Nyquist plots) of (i) GCE, (ii) GCE-ERGO, (iii) GCE-polyCoTAPc and (iv) GCE-ERGO/polyCoTAPc in (1:1) 2.0 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ containing 0.10 M KCl. Scan rate of 50 $mV.s^{-1}$.

The electrochemically active surface areas of the bare GCE, GCE-ERGO, GCE-polyCoTAPc, and GCE-ERGO/polyCoTAPc were calculated using the general Randles-Sevcik Equation (4.1):

$$i_p = (2.69 \times 10^5)n^{3/2}ACD^{1/2}\nu^{1/2} \quad (4.1)$$

where A is the active surface area of the working electrode (cm^2), $n = 1$ is the number of electrons participating in the reaction, D ($7.6 \times 10^{-6} \text{ cm}^2.\text{s}^{-1}$) is the diffusion coefficient for $\text{K}_3[\text{Fe}(\text{CN})_6]$, C (2.0 mM) is the bulk concentration of the electroactive species in solution, i_p is the oxidation peak currents of each electrode surface from **Fig. 4.3(a)** and ν is the scan rate ($\text{V}.\text{s}^{-1}$). The effective surface areas were found to be 0.0780 cm^2 , 0.101 cm^2 , 0.0810 cm^2 and 0.120 cm^2 for the bare GCE, GCE-ERGO, GCE-polyCoTAPc and GCE-ERGO/polyCoTAPc, respectively. There was an increase in the effective surface area (0.0780 cm^2) of the GCE when compared with geometric area of GCE ($d = 1.6 \text{ mm}$, 0.0707 cm^2). The increase was due to mechanical treatment of the GCE surface by polishing with alumina slurry. The increase in effective area for GCE-ERGO, GCE-polyCoTAPc and GCE-ERGO/polyCoTAPc was due to electrodeposition of materials as proposed. Enhanced effective surface areas at the modified GCE surfaces is attributed to more conducting and electrocatalytic materials attached on the GCE such as ERGO, ERGO/polyCoTAPc, and polyCoTAPc.

The conductivity and electron transfer properties of different electrodes were examined by electrochemical impedance spectroscopy (EIS) in (1:1) 2.0 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ containing 0.10 M KCl. **Figure 4.3(b)** shows the Nyquist plots of (i) bare GCE, (ii) GCE-ERGO, (iii) GCE-polyCoTAPc, (iv) GCE-ERGO/polyCoTAPc. The Randles-Sevcik equivalent circuit was used to fit the raw data and quantitatively obtain the circuit parameters, such as charge-transfer resistance (R_{CT}), electrolyte/solution resistance (R_s), double-layer (Cdl) or constant phase element (CPE) capacitance. Amongst these, R_{CT} is a measure of the resistance to electron transfer. The R_{CT} value was $0.20 \text{ k}\Omega$ for the bare GCE, decreased at GCE-ERGO to $0.098 \text{ k}\Omega$. This is due to the conducting nature of ERGO which increased the charge transfer rate on the surface of modified surface. However, the R_{CT} value increased to $0.21 \text{ k}\Omega$ at GCE-polyCoTAPc

due to the electrodeposition of the polymer film. At GCE-ERGO/polyCoTAPc, the R_{CT} value was the lowest 0.061 k Ω due to an enhanced rate of charge transfer because of excellent conductivity. The smaller R_{CT} value for the GCE-ERGO/polyCoTAPc demonstrates synergistic effect of the combination of ERGO and polyCoTAPc.

Furthermore, the surface coverage (Γ) of the GCE-ERGO/polyCoTAPc was calculated using the Laviron's Equation (4.2) [28]:

$$I_p = \frac{n^2 F^2 A \Gamma v}{4RT} \quad (4.2)$$

where I_p is the peak current of the redox couple, $\text{Co}^{2+}/\text{Co}^+$. $n = 1$ is the number of electrons transferred. A is the geometric surface area of the bare GCE (0.0707 cm²), Γ is the film surface coverage in mol.cm⁻², v is the scan rate (V.s⁻¹), and other parameters have their usual meaning. The value of Γ for polyCoTAPc thin film on the GCE-ERGO was estimated from the background current (I_p) under the reductive $\text{Co}^{2+}/\text{Co}^+$ redox couple for $v = 25$ mV.s⁻¹, in **Fig. 4.4 (a)**. Γ value was 7.53×10^{-10} mol.cm⁻² and much higher than the 0.80×10^{-10} mol.cm⁻² reported for other metallo phthalocyanine macrocycles adsorbed as monolayers [29] confirming polymeric species formed. From **Fig. 4.4 (b)**, I_{pc} increased linearly as a function of scan rate. The gradient of the straight line was also used to calculate the Γ and the value was 6.83×10^{-9} mol.cm⁻² further confirming the polymer of CoTAPc.

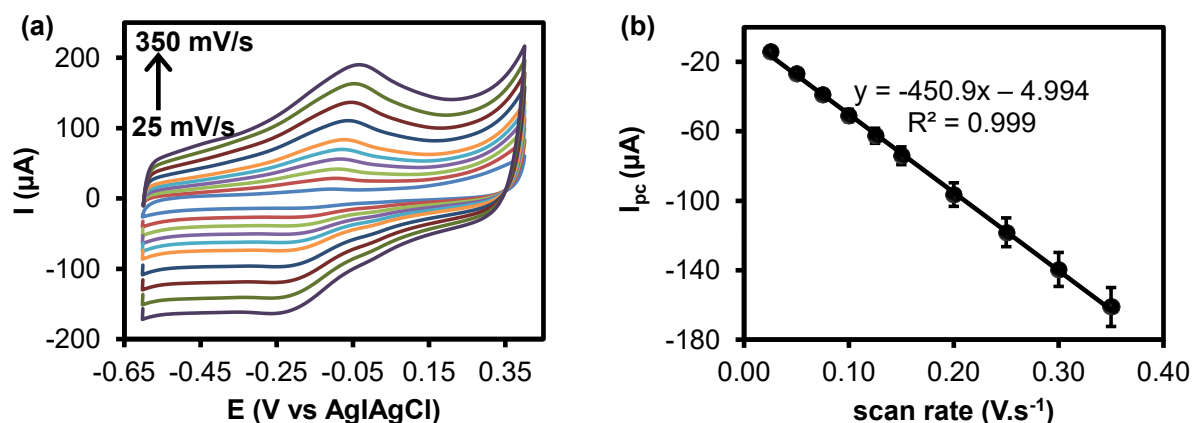
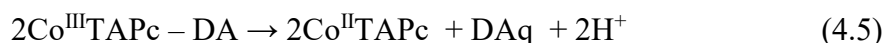
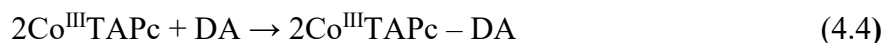
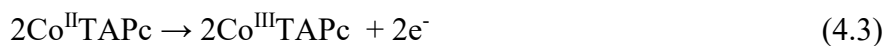


Figure 4.4: (a) CVs at increasing scan rates from 25 - 350 mV.s^{-1} in a 0.10 M PBS (pH 7.4) at GCE-ERGO/polyCoTAPc, and (b) the plot of I_{pc} vs scan rate.

4.3.4 Electrocatalytic detection of DA and PA at GCE-ERGO/polyCoTAPc

Figure 4.5 shows CVs of GCE-ERGO/polyCoTAPc in (a) 0.10 M PBS (pH 7.4), (b) 1.0 mM of DA, (c) 1.0 mM PA, and (d) 1.0 mM DA + 1.0 mM PA. In 0.10 M PBS (pH 7.4). In **Fig. 4.5(a)**, a small redox peak due to $\text{Co}^{\text{II}}/\text{Co}^{\text{III}}$ at 0.297 V was observed at GCE-ERGO/polyCoTAPc. In the presence of 1.0 mM DA in **Fig. 4.5(b)**, a sharp well-defined oxidation peak at 0.186 V and reduction peak at 0.071 V were observed. **Figure 4.5(c)** shows CV of GCE-ERGO/polyCoTAPc in 1.0 mM of PA solution in 0.10 M PBS (pH 7.4). A sharp well-defined oxidation and reduction peak at 0.373 V and 0.208 V potentials were observed. These results show that GCE-ERGO/polyCoTAPc enhances the electrocatalytic oxidation and reduction of DA and PA. The oxidation peak potentials of DA and PA analytes were in the proximity to the $\text{Co}^{\text{II}}/\text{Co}^{\text{III}}$ metal oxidation peak potential window at PBS (pH 7.4). Cobalt in the centre of CoTAPc is involved in the catalytic oxidation of DA and PA. The potential difference between the electrocatalytic oxidation peaks of the analytes was 0.264 V in **Fig. 4.5(d)** and it was wide enough for the selective and simultaneous detection of (I) DA and

(II) PA in PBS (pH 7.4). The electrocatalytic oxidation reaction mechanism for DA at GCE-ERGO/polyCoTAPc is described in Equations (4.3) – (4.5) and similar to the mechanism of PA electrocatalytic oxidation.



The first step is electrochemical oxidation of $\text{Co}^{\text{II}}\text{TAPc}$ to $\text{Co}^{\text{III}}\text{TAPc}$, Equation (4.3), followed by coordination of DA to $\text{Co}^{\text{III}}\text{TAPc}$ to form $\text{Co}^{\text{III}}\text{TAPc-DA}$ in Equation (4.4). DA is then oxidized to DAq (Dopamine-o-quinone) in Equation (4.5) and simultaneously $\text{Co}^{\text{III}}\text{TAPc}$ is reduced to $\text{Co}^{\text{II}}\text{TAPc}$ (regenerated) [30]. The net or overall mechanism for DA oxidation ($\text{DA} \leftrightarrow \text{DAq} + 2\text{e}^- + 2\text{H}^+$), $\text{Co}^{\text{II}}\text{TAPc}$ is a catalyst and is regenerated in the last step whilst $\text{Co}^{\text{III}}\text{TAPc}$ and $\text{Co}^{\text{III}}\text{TAPc-DA}$ are intermediate species. Similarly, paracetamol was oxidized to N-acetyl-p-benzoquinone imine (NAPQI) and the overall mechanism was ($\text{PA} \leftrightarrow \text{NAPQI} + 2\text{e}^- + 2\text{H}^+$). $\text{Co}^{\text{III}}\text{TAPc}$ was involved with the regeneration of $\text{Co}^{\text{II}}\text{TAPc}$.

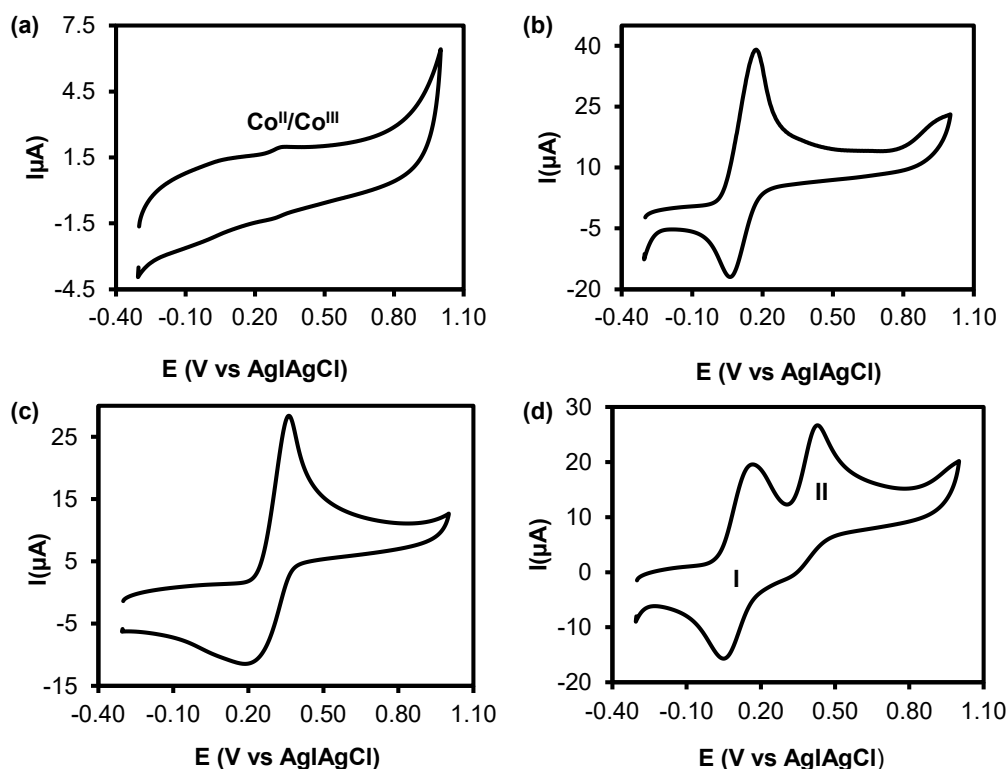


Figure 4.5: CVs of GCE-ERGO/polyCoTAPc in (a) 0.10 M PBS (pH 7.4), and with (b) 1.0 mM DA, (c) 1.0 mM PA, (d) 1.0 mM DA + 1.0 mM PA. Scan rate: 50 mV.s⁻¹. All solutions were purged with N₂ gas for 10 minutes prior to electrochemical measurement.

4.3.5 Effect of solution pH and scan rates

The structure of dopamine and paracetamol contain phenolic groups, therefore their electrochemical oxidation or reduction at the modified electrode are pH dependent [31]. The electrochemical behavior of DA and PA was investigated in 0.10 M phosphate buffer with various pH ranging from 2.0 to 9.0 using cyclic voltammetry. **Figure 4.6(a)** and **(b)** shows CVs of GCE-ERGO/polyCoTAPc 0.10 M PBS (pH 7.4) containing 0.10 mM of DA and PA in pH 2 - pH 9. The results showed a negative shift in electrocatalytic redox potentials for both DA and PA with increasing pH values. This is attributed to proton(s) participating in the redox processes [32]. The following is the suggested reaction mechanism for the oxidation process

of DA and PA. Suggested reaction mechanisms (a) for dopamine and (b) paracetamol at the prepared electrode.

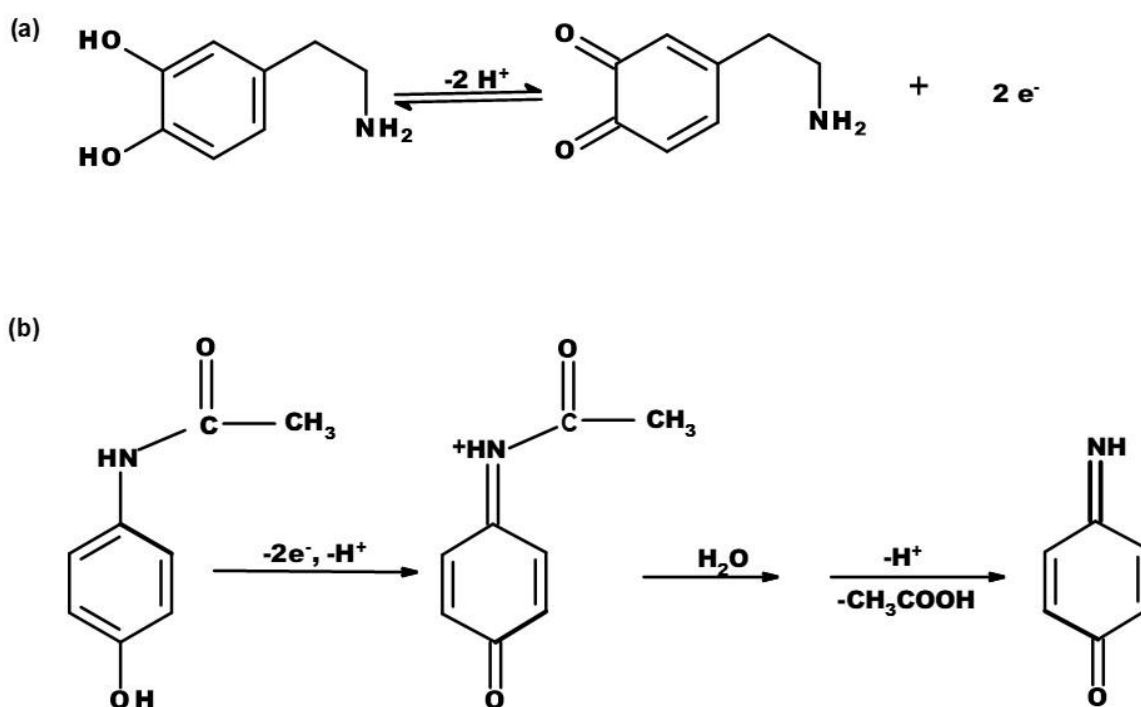


Figure 4.6(a') and **(b')** shows a linear relationship between the anodic peak potentials (E_{pa}) against pH. The linear dependence of E_{pa} on pH are represented in equation (4.6) and (4.7).

$$\text{DA: } E_{\text{pa}} (\text{V}) = -0.0460 \text{ pH} + 0.468 \quad (R^2 = 0.99) \quad (4.6)$$

$$\text{PA: } E_{\text{pa}} (\text{V}) = -0.0315 \text{ pH} + 0.624 \quad (R^2 = 0.98) \quad (4.7)$$

The slope of E_{pa} vs. pH for DA was 46 mV/pH and the slope for PA was 31.5 mV/pH. The slopes values indicate the involvement of two protons and two electrons in the electrocatalytic oxidation of DA and PA [33].

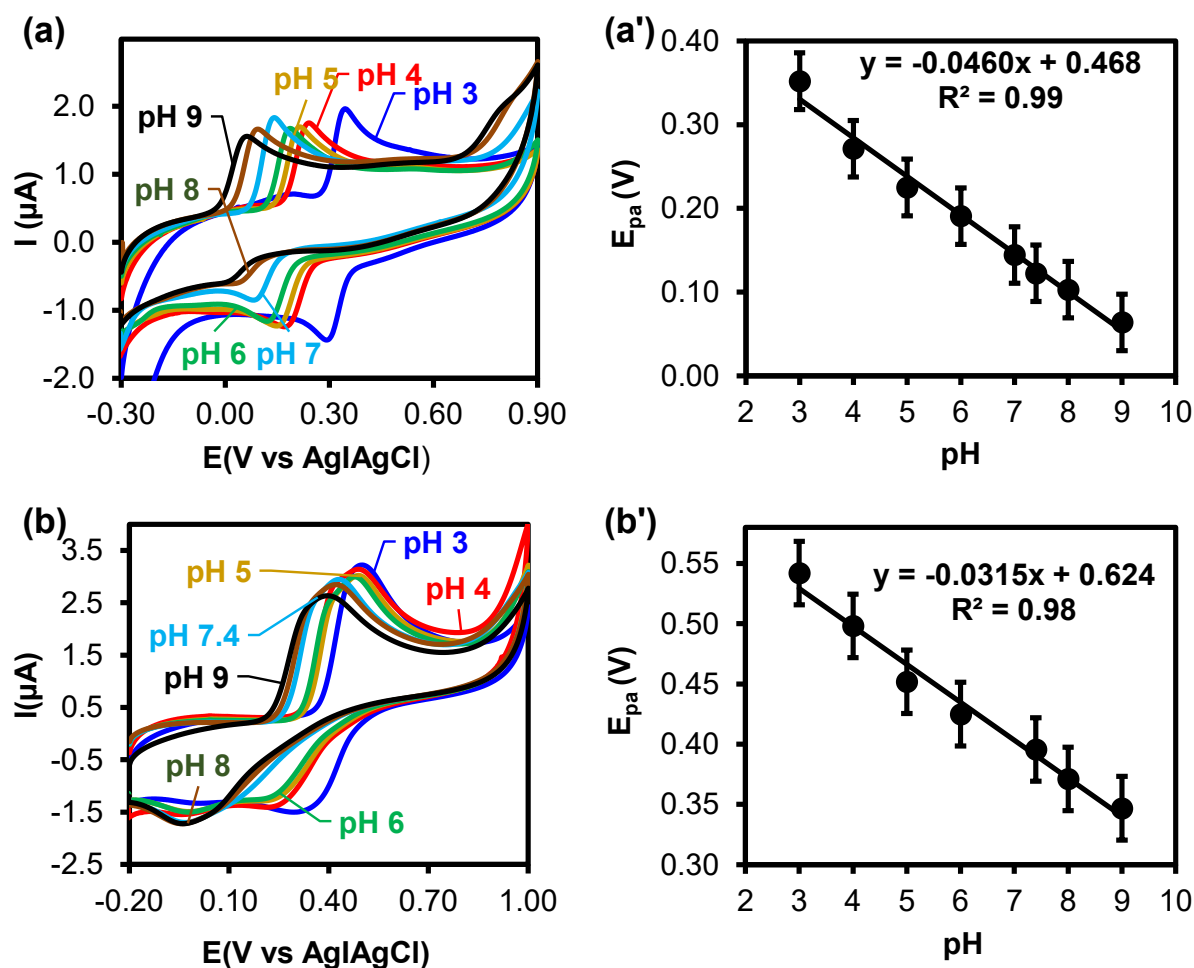


Figure 4.6: CVs of (a, a') 0.10 mM DA and (b, b') 0.10 mM PA and corresponding linear plots in 0.10 M PBS of pH values ranging from 2 to 9. All solutions were purged with N_2 gas for 10 minutes prior to measurement.

The effect of scan rate on the peak currents was investigated in the range from 25 to 350 mV.s^{-1} for 0.10 mM of (a) DA and (b) PA in 0.10 M PBS (pH 7.4) at the GCE-ERGO/polyCoTAPc in **Figure 4.7 (a)** and **(b)**. In both analytes the oxidation and reduction peak currents increased with increasing scan rate. Additionally, by increasing scan rate, very small shift for redox peaks were observed and therefore no kinetic limitation of the electrochemical reaction and no adsorption of the analytes [34]. **Figure 4.7(a')** show the plots of peak currents vs. square root of scan rates for DA. The linear regression equations for anodic peak currents and/or reduction

peak currents were $I_{pa} = 56.0 (v^{1/2}) - 5.24$ with a correlation coefficient of 0.999 and $I_{pc} = -38.5 (v^{1/2}) + 3.74$ with correlation coefficient of 0.990 for DA. **Figure 4.7(b')** show the plots of peak currents vs. square root of scan rates for PA. The linear regression equations for anodic peak currents and reduction peak currents were $I_{pa} = 31.6 (v)^{1/2} - 1.6$ with a correlation coefficient of 0.994 and $I_{pc} = -25.6 (v)^{1/2} + 2.09$ with a correlation coefficient of 0.998. The linear relationships of peak current (I) vs of scan rates (v) shows clearly that the electrode reactions are diffusion-controlled processes [34,35].

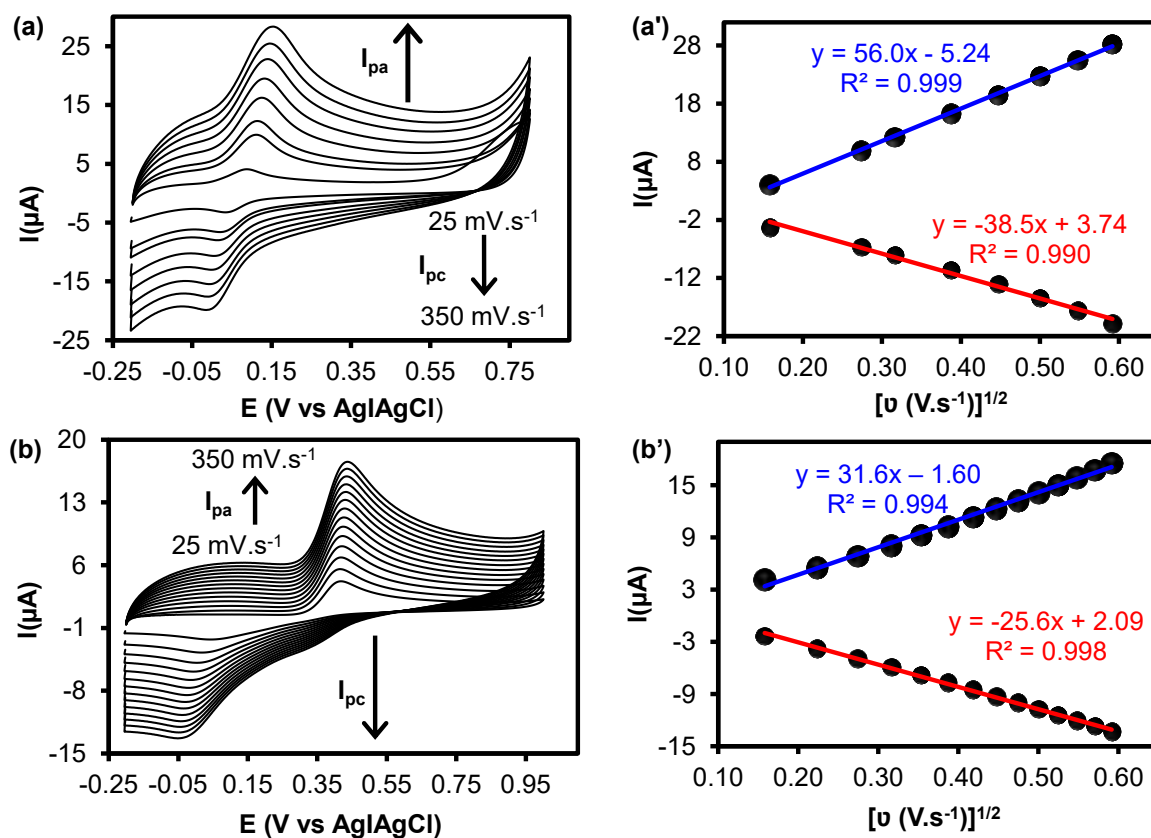


Figure 4.7: CVs and corresponding linear plots of GCE-ERGO/polyCoTAPc in 0.10 M PBS (pH 7.4) containing 0.10 mM (a, a') DA and (b, b') PA. Scan rates 25 - 350 $mV.s^{-1}$. All solutions were purged with N_2 gas for 10 minutes prior to measurements.

4.3.6 Electroanalysis of DA and PA at GCE-ERGO/polyCoTAPc

The electroanalysis of DA and PA was carried out simultaneously using differential pulse voltammetry (DPV). DPV voltammograms were obtained first by changing the concentration of DA while keeping the concentration of PA constant. After each measurement, the electrode was removed from the electrochemical cell, washed thoroughly with double distilled water and dried using N₂ gas for the next measurement. The measurements were conducted from low to high concentrations of the analyte to avoid the electrode memory effect, which will result in enhanced electrocatalytic peak currents. **Figure 4.8 (a)** shows the increasing electrocatalytic oxidation peak currents due to the increase in DA concentrations from 2.0 – 100 µM. The concentration of PA was 30 µM and kept constant and its oxidation peak current and potential remained unaffected. A constant concentration of DA, 40 µM, was used whilst changing PA concentrations in **Figure 4.8 (b)** from 5.0 – 100 µM. The calibration curves of DA and PA at the GCE-ERGO/polyCoTAPc were obtained as shown in **Figure 4.8(a')** and **(b)'** respectively.

The linear regression equation (4.8) was for DA:

$$I_{pa} = 1.01 [DA] + 18.5 (R^2 = 0.999) \quad (4.8)$$

and the linear regression equation (4.9) was for PA:

$$I_{pa} = 0.158 [PA] + 9.08 (R^2 = 0.997) \quad (4.9)$$

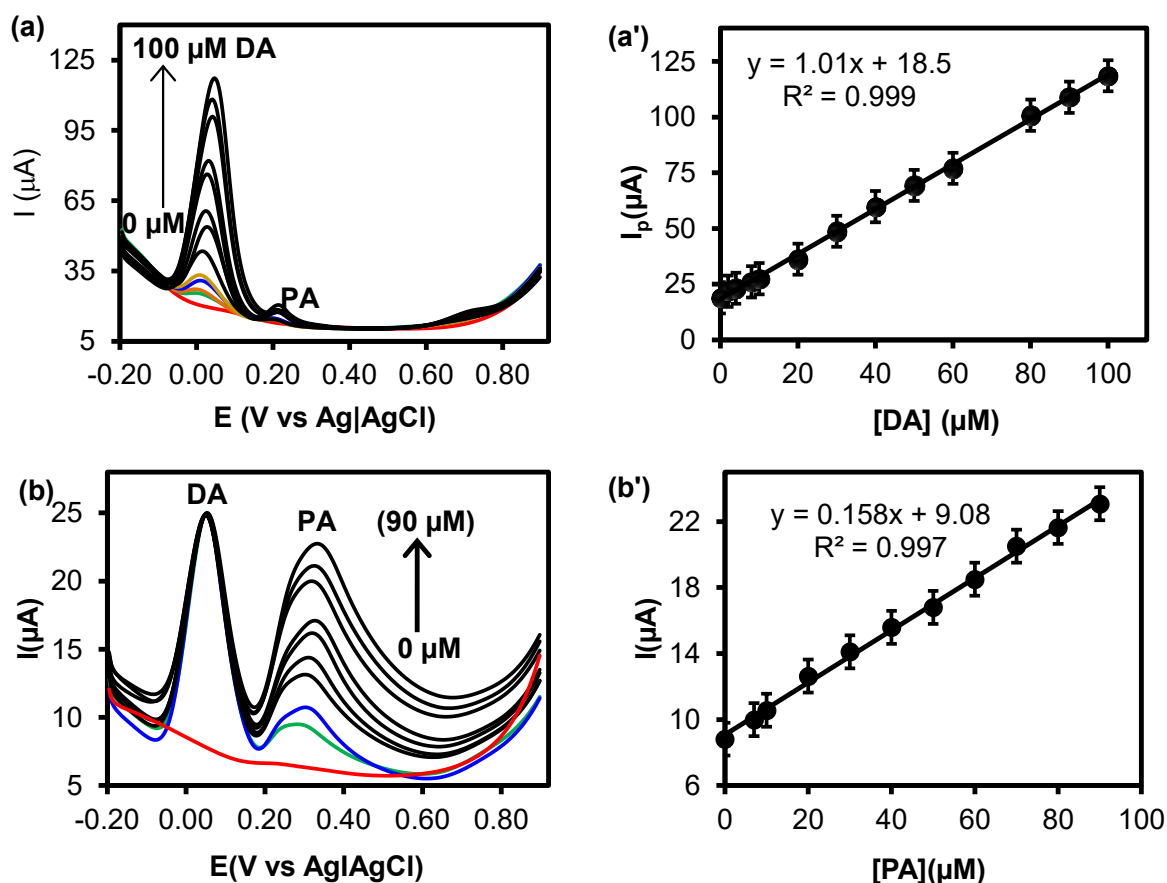


Figure 4.8: DPVs of GCE-ERGO/polyCoTAPc in 0.10 M PBS (pH 7.4) containing increasing concentrations of **(a)** DA (2.0 – 100 μ M) and **(b)** PA (7.0 – 90 μ M), and corresponding calibration curves. All solutions were deaerated with N₂ gas for 10 minutes prior to DPV measurements.

The limit of detection (LoD) for DA and PA were found to be 95 nM and 0.10 μ M, respectively. The sensitivity values for DA and PA were 8.39 μ A. μ M⁻¹.cm⁻² and 1.32 μ A. μ M⁻¹.cm⁻² for DA and PA, respectively. The proposed electrode exhibited comparable and lower LoDs when compared to reported electrodes as summarized in **Table 4.1**.

Table 4.1: Comparison of GCE-ERGO/polyCoTAPc toward the detections of DA and PA with other reported modified electrodes using DPV method.

Type of electrode	Linear range (μM)		LOD (μM)		Ref.
	DA	PA	DA	PA	
$\text{Fe}_3\text{O}_4@\text{Au-S-Fc/GS-Chitosan/GCE}$	0.50 – 50	0.40 - 32	0.10	0.050	[30]
SWCNT/CCE	0.40 -150	0.20 -100	0.22	0.12	[36]
Pyrolytic Carbon (PyC) film	18 – 270	15 - 225	2.30	1.40	[37]
Gr-FEPA-CS/GCE	2.0 -135	0.30 - 80	0.30	0.050	[38]
GC/CNT	3.0 – 200	3.0 - 300	0.80	0.60	[39]
GCE-ERGO/polyCoTAPc	2.0 – 100	7.0 - 90	0.095	0.104	TW

TW = this work, Fc = Ferrocene, G S = Graphene sheet, CCE = Carbon ceramic electrode, SWCNT = Single walled carbon nanotube, FEPA = (4-Ferrocenylethyne) Phenylamine, CNT = Carbon nanotube, and Gr = Graphene.

The physiological level for DA in the different bodily fluids and human body is shown in **Table 1.1** and is between 0.0010 and 1.0 μM [40]. For PA, the tolerable level in the blood of the human body is between 1.51 and 3.02 μM . With the obtained LoDs of 95 nM for DA and 0.104 μM for PA, the proposed sensor can be used for their detection.

4.3.7 Screening of ascorbic acid and uric acid at GCE-ERGO/polyCoTAPc

Ascorbic acid (AA) and uric acid (UA) are the most common interferents that coexist with DA and PA in the human body. DPV was recorded for a 20 μM of DA or PA in the absence and presence of 1.0 mM of AA or 0.20 mM UA in 0.10 M PBS (pH 7.4). **Figure 4.9** shows that

there was no significant evidence of interference in the DPVs from AA with concentration fifty times higher than the target analytes. The peak current variations of DA and PA from the recorded DPVs were found to be less than $\pm 1.3\%$. The ability of the GCE-ERGO/polyCoTAPc to screen out AA was due to the residual oxygenated functional groups on the reduced graphene oxide that bear negative charges. At the pH of 7.4, these negative charges repel the ascorbic acid, which carries a negative charge to form an ascorbate anion (AA^-). The results demonstrated that the interference effect of AA on both DA and PA species is negligible. The small broad peaks at about -0.10 V for DA and 0.10 V for PA were due to the catalytic oxidation of ascorbate anions by the oxidized products of dopamine and paracetamol.

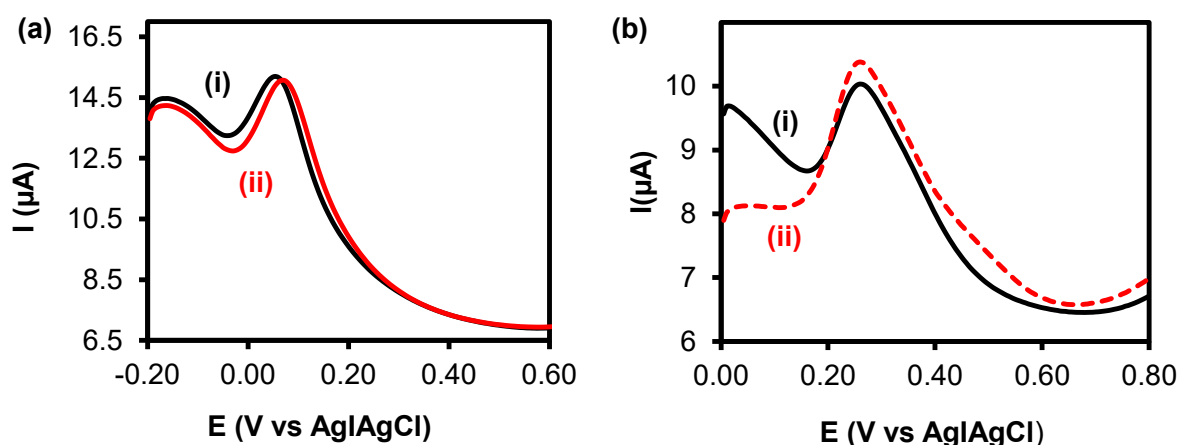


Figure 4.9: DPVs for GCE-ERGO/polyCoTAPc in $20\ \mu\text{M}$ (a) DA and (b) PA in $0.10\ \text{M}$ PBS (pH 7.4) (i) before and (ii) after adding $0.10\ \text{mM}$ AA.

Figure 4.10 shows DPV responses of (a) DA and (b) PA in the (i) absence and (ii) presence of $0.20\ \text{mM}$ UA at GCE-ERGO/polyCoTAPc. In **Fig. 4.10(a)(ii)**, there is a small shoulder peak at about $218\ \text{mV}$ due to UA oxidation, and the peak current is very small compared to the concentration of UA added which was $4\ \times$ higher than that of DA and PA. However, there is no obvious change in the oxidation peak potential of DA. Similarly, in **Fig. 4.10(b)(ii)**, there

is negligible change in the oxidation peak potential for PA in the presence of UA in the solution except for a broad oxidation peak. The peak shape, potential and current remained the same for PA even in the presence of UA in physiological (pH 7.4) conditions. UA has pKa value of 5.4 and therefore exist as an anion in pH 7.4. DA exists as a cation (protonated) due to their pKa values, that is, 8.87 for DA. PA remains neutral at pH 7.4. There are possible adducts (electrostatic interaction) of DA-UA that forms which therefore shows electrocatalytic oxidation especially in DA and UA solution as seen with a shoulder peak.

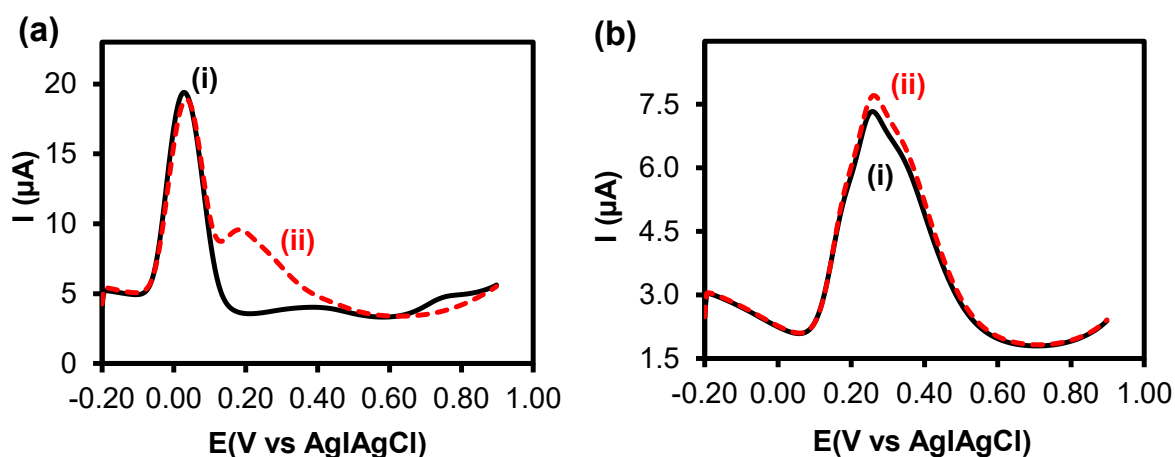


Figure 4.10: DPVs for GCE-ERGO/polyCoTAPc in 50 μM (a) DA and (b) PA in 0.10 M PBS (pH 7.4) (i) before and (ii) after adding 0.20 mM UA.

To further validate the applicability of the GCE-ERGO/polyCoTAPc, simultaneous detection of DA and PA was conducted in the presence of 1.0 mM AA and 0.20 mM UA. **Figure 4.11** shows (a) 50 μM DA + 50 μM PA in (i) absence and (ii) presence of 1.0 mM AA. In the presence of 1.0 mM AA, there was slight shift in the oxidation peak potentials for DA and for PA. No additional oxidation peak was observed due to AA. At pH 7.4, DA is protonated ($-\text{NH}_3^+$) and AA exists as an ascorbate anion (AA^-) this results in electrostatic interaction. This could be the reason for the shift in peak potential. The electrooxidation peak currents generated

for the DA and PA were within the experimental error similar before and after the presence of high concentrations (1.0 mM) AA. **Figure 4.11(b)** shows the electrooxidation of 50 μ M DA + 50 μ M PA (i) in the absence and (ii) presence of 0.20 mM uric acid. The peak potential of DA and PA remained the same. There was a slight increase in electrooxidation currents even at high concentration of 0.20 mM uric acid. No adduct (electrostatic interactions) formed and hence similar peak currents were obtained and no shift in potential.

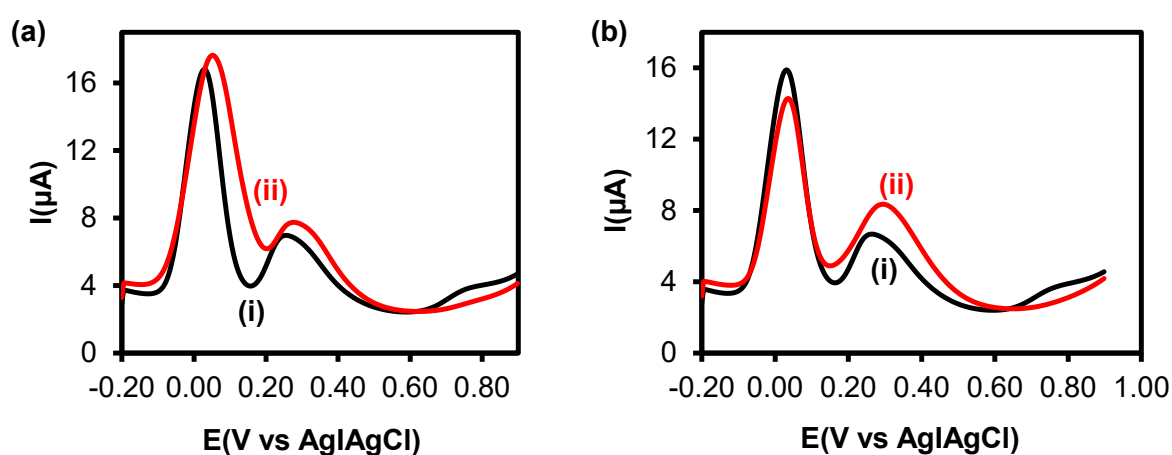


Figure 4.11: DPVs for GCE-ERGO/polyCoTAPc in a mixture of DA and PA (50 μ M each) in 0.10 M PBS (pH 7.4) (i) before and (ii) after adding (a) 1.0 mM AA and (b) 0.20 mM UA.

4.3.8 Stability and repeatability

The stability of GCE-ERGO/polyCoTAPc was investigated by recording DPVs of DA or PA in 0.10 M PBS (pH 7.4) every week for four weeks in **Figure 4.12(a)** and **(b)**. It was observed that the peak current response decreased to 87% for DA and 93.2% for PA, in four weeks confirming excellent stability of GCE-ERGO/polyCoTAPc. The repeatability for DA or PA detection was done by repetitive scans (five scans). The initial oxidation peak current response at the GCE-ERGO/polyCOTAPc was maintained by recycling the modified sensor ten times in PBS (pH 7.4) prior to every scan, indicating that the sensor had good repeatability.

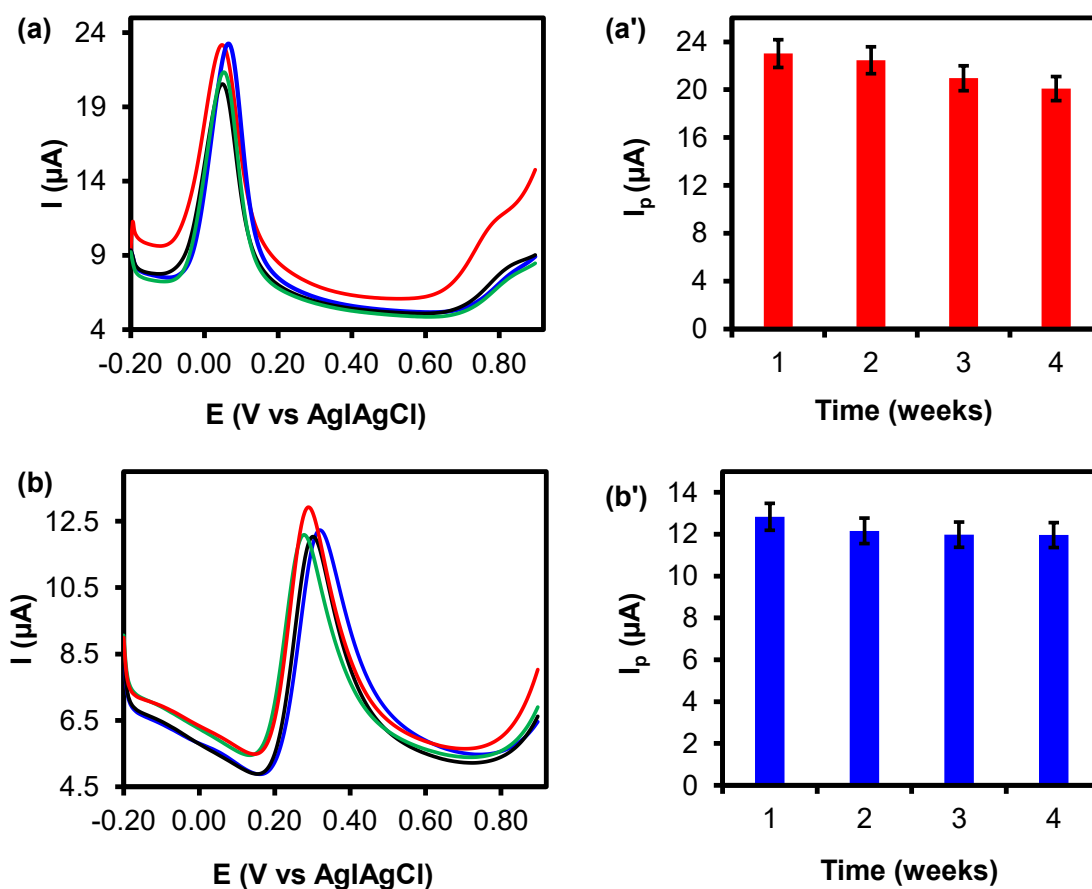


Figure 4.12: DPVs of 20 μ M of (a) DA and (b) PA at the GCE-ERGO/polyCOTAPc at different times and the corresponding bar graphs.

4.3.9 Real sample analytical application of GCE-ERGO/polyCoTAPc

In order to demonstrate the applicability of the developed sensor, the detection of DA and PA was conducted in spiked synthetic urine samples. The recovery values were estimated using a standard addition method, and the results are summarised in **Table 4.2**. The fabricated sensor has the ability to estimate the concentrations of DA and PA in a biological sample, with recovery rates ranging from 96.8% – 103% for DA and 96 – 101% for PA. The results showed excellent percentage recoveries and percentage relative standard deviation (%RSD) for the

analysis of DA and PA. This demonstrated that GCE-ERGO/polyCoTAPc sensor based on three measurements for each spiked concentration of the analyte ($n = 3$) can be used for the determination of dopamine and paracetamol in the synthetic urine samples representing real samples.

Table 4.2: Detection of 20 μM , 40 μM and 60 μM DA and PA concentrations in 10% urine samples using the GCE-ERGO/polyCoTAPc.

Sample	Added (μM)		Found (μM)		%Recovery		%RSD	
	DA	PA	DA	PA	DA	PA	DA	PA
1	20	40	21.6	38.3	103	96	3.6	5.1
2	40	60	38.7	59.3	96.8	99	3.9	3.0
3	60	80	59.7	81.2	99.5	101	1.0	2.3

4.4 Conclusions

In this chapter, a simple, electrochemical sensor was successfully constructed using electrochemical reduction and deposition of reduced graphene oxide and electropolymerization of cobalt tetra-amino phthalocyanine (CoTAPc). The modified electrode could simultaneously and selectively detect dopamine and paracetamol. The interaction of polyCoTAPc onto reduced graphene oxide surface remarkably enhanced the electrocatalytic activity of the electrochemical surface towards electrochemical oxidation of DA and PA due to the large surface area and rich redox properties of the CoTAPc in the composite electrode. The difference in electrooxidation potential between DA and PA was 264 mV and wide enough for the simultaneous determination. The GCE-ERGO/polyCoTAPc sensor achieved high sensitivity, and selectivity towards detection of DA and PA with linear concentration range, low and comparable LODs with other reported electrodes. The determination of DA and PA

was not significantly affected by the presence of high concentrations of ascorbic acid and uric acid. The applicability of the GCE-ERGO/polyCoTAPc sensor was demonstrated by analysis of DA and PA in synthetic urine samples with excellent recoveries ranging from 96.8 – 103 % for DA and 96- 101 % for PA. The fabricated nanocomposite was stable up to four weeks with 97 % current retained.

4.5 References

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CHAPTER 5

5 Electrocatalysis of conducting nickel hydroxide on graphene and molybdenum composites

Abstract

In this chapter, the fabrication of a simple electrochemical sensor using a composite of reduced graphene oxide - molybdenum disulphide (RGO/MoS₂) via drop dry deposition method onto glassy carbon electrode (GCE) was achieved. The GCE-RGO/MoS₂ was modified with nickel hydroxide thin film using electrodeposition to yield a GCE-RGO/MoS₂/Ni(OH)₂. The modified surface was used for selective and simultaneous detection of ascorbic acid (AA), dopamine (DA) and uric acid (UA). The morphological structure of RGO/MoS₂ composites were characterized fully using materials and thin films techniques. Nickel hydroxide thin film, Ni(OH)₂, was directly electrodeposited onto a GCE-RGO/MoS₂ modified electrode at constant potential of - 0.60 V to form GCE-RGO/MoS₂/Ni(OH)₂. The modified surfaces GCE-RGO/MoS₂ and GCE-RGO/MoS₂/Ni(OH)₂ were characterized by X-ray photoelectron spectroscopy (XPS). The GCE-RGO/MoS₂/Ni(OH)₂ showed excellent electrocatalytic potential separation for selective determination dopamine, uric acid, and ascorbic acid. The electrocatalytic oxidation peak potentials were at 3 mV for AA, 157 mV for DA and 303 mV for UA. The proposed electrode sensor was used for the detection of the mixture of AA, DA and UA without signal interference. The individual detection limits (S/N = 3) for AA, DA and UA were 1.17 μ M, 0.15 μ M and 1.15 μ M, respectively. Furthermore, the GCE-RGO/MoS₂/Ni(OH)₂ was applied for the detection of AA, DA and UA in spiked newborn calf serum samples with high percentage recoveries.

5.1 Introduction

Human metabolism processes govern a number of bodily functions by chemical and biomolecular interactions. Ascorbic acid (AA), dopamine (DA) and uric acid (UA) are important biological molecules in human metabolism processes [1]. AA is a vital vitamin in the mammalian body due to its antioxidant properties [2]. AA is used for prevention and treatment of disorders such as common cold, mental illness, diabetes, cancer, hepatic disease, infertility and acquired immunodeficiency syndrome (AIDS) [3]. In the human body, AA has a concentration of approximately $1.0\ \mu\text{M}$ [4] and is present along with other neurotransmitters. DA is a catecholamine neurotransmitter that facilitates the functioning of the central nervous system, renal, and hormonal systems in the human body [5]. In the central nervous system, DA is found in concentration of about $50\ \text{nM}$ [6,7]. Low concentrations of DA trigger neurological disorders such as Parkinson's disease [6,8]. UA is a primary end product of purine metabolism [2]. In the human blood plasma, UA is found in range of $140\text{--}420\ \mu\text{M}$ [7]. The abnormal levels of UA in the human body lead to diseases such as gout, kidney damage, cardiovascular diseases, and pneumonia [2]. AA, DA and UA usually coexist in body fluids such as blood serum and urine [5]. Amongst various methods for monitoring AA, DA, and UA, electrochemical methods have been developed for their quantification in body fluids due to their simplicity and low cost [9]. They are all electrochemically active biomolecules that is they can easily undergo oxidation and/or reduction at an electrode surfaces [10]. However, their simultaneous detection under physiological conditions still remains a challenge due to overlapping oxidation potentials at unmodified electrodes [8,11]. Therefore, development of an electrochemical sensor that can distinguish between AA, DA and UA signals in biological fluids is of paramount importance.

Noble metals and the alloys of Pt, Au, Ag and Pd have been reported as useful electrode platforms for the simultaneous detection of AA, DA and UA due their excellent electrocatalytic properties [1,12–15]. However, noble metals can be obtained at high cost. Transition metals (Ni, Cu and Co) and their oxides (ZnO, NiO) have been used as alternatives for the construction of electrochemical sensors and for the detection of AA, DA and UA [2,5,14,16–21]. Amongst these, nickel hydroxides (Ni(OH)_2) have been investigated for the detection of small organic molecules in alkaline conditions due to the rich redox properties and low cost of nickel [22]. The electrocatalytic effect of nickel arises from the partially filled d-orbitals which are available for coordinating with the analyte to induce its oxidation and/or reduction [23,24]. Despite many sensing applications, one major challenge of Ni(OH)_2 as an electrochemical sensor material is its low conductivity [25–27].

Carbon nanotubes, mesoporous carbon and graphene oxide are excellent supports for metal/metal oxides. Graphene oxide (GO) has been studied as a support material for metal oxides due to excellent properties such as high electrical conductivity, large surface area, high stability and low cost [26–29]. The addition of transition metal dichalcogenides (TMDs) such as molybdenum disulfide (MoS_2) helps to prevent agglomeration of GO sheets [30,31]. MoS_2 consists of a stack of hexagonal layers of Mo atoms sandwiched between two layers of S atoms [31,32]. The sheet-like morphology of MoS_2 can also act as a spacer in GO resulting in large surface area [33]. Besides, the three atomic layers of S-Mo-S which are stacked together provide additional electrochemically active sites which can accommodate elements such as metal/metal oxide or biorecognition agents such as antibodies, enzymes and aptamers [15,34]. Furthermore, the interconnected 3D material networks exhibit superior conductivity and excellent electrical contact between MoS_2 and GO for effective transportation of electrons between the electrode surface and target analytes [35].

In this chapter, $\text{Ni}(\text{OH})_2$ thin films were incorporated into MoS_2/RGO nanosheets and used for individual and simultaneous detection of AA, DA and UA in phosphate buffer solution (pH 5.0). The preparation of MoS_2/RGO nanosheets followed a one-step hydrothermal method in aqueous solution. $\text{Ni}(\text{OH})_2$ thin film was electrodeposited onto GCE-RGO/ MoS_2 to form GCE-RGO/ $\text{MoS}_2/\text{Ni}(\text{OH})_2$. We further investigated the electrocatalytic sensing and selective determination of ascorbic acid (AA), dopamine (DA), and uric acid (UA). We report the characterization and electrocatalytic sensing of NiOx thin films incorporated onto the GCE- MoS_2/RGO towards the simultaneous detection of AA, DA and UA.

This chapter was set out to achieve the following objectives of the thesis:

- (a) Synthesis and characterization of molybdenum disulfide-reduced graphene oxide materials.
- (b) Fabrication and characterization nickel hydroxide thin film electrodeposited molybdenum disulfide -reduced graphene oxide electrode.
- (b) Detection of dopamine at nickel hydroxide electrodeposited molybdenum disulfide - reduced graphene oxide electrode in the presence of ascorbic acid and uric acid.
- (c) Detection of dopamine, ascorbic acid, and uric acid in newborn serum samples.

5.2 Experimental

5.2.1 Materials and methods

The materials from the previous chapters were used and additional materials and their suppliers are listed here. Graphene oxide (GO) was prepared as discussed in Chapter 4. Nickel nitrate hexahydrate ($\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$), hexaammonium heptamolybdate tetrahydrate ($(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$) and thiourea ($\text{CS}(\text{NH}_2)_2$) were purchased from Sigma-Aldrich and used

as received. Phosphate buffer saline (PBS, 0.10 M, pH 7.4) solution (1L) was prepared as in Chapter 4. Sodium acetate (CH_3COONa) was purchased from Merck and acetic acid (CH_3COOH) was purchased from Spellbound Laboratory Solutions. Sodium hydroxide (NaOH , 1.0 M) and hydrochloric acid (HCl , 1.0 M) were used to adjust the pH of the PBS and acetate buffer solution (ABS) to appropriate values. All other aqueous solutions were prepared using ultra-pure water obtained from a Milli-Q Water System (Millipore Corp. Bedford, MA, USA). All chemicals were of analytical reagent grade and were utilized as received from the suppliers without any further purification.

5.2.2 Equipment

The equipment used for characterization of materials and surfaces are listed in the previous chapters. The additional techniques are listed here. Scanning electron microscopy (SEM) images were obtained using an INCA PENTA FET coupled with VEGA TESCANA at 20 kV acceleration voltage. X-ray photoelectron spectroscopy (XPS) measurements were conducted using an XPS from Kratos – Axis Ultra DLD, UK. The Vision Processing 2 software was used for the elemental composition and fitting of the high-resolution spectra.

5.2.3 Preparation of RGO/MoS₂ composites

Graphene oxide (GO) was synthesized from graphite flakes by the modified Hummer's method as discussed in Chapter 4 and section 4.2. The RGO/MoS₂ composites were synthesized using a modified hydrothermal method by Chang et. al [36]. Briefly, 20 mg GO was transferred into a 100 mL beaker with addition of 40 mL of DI water. Then 1.16 g (NH_4)₆Mo₇O₂₄·4H₂O was added to the GO suspension and sonicated for 30 min. Thereafter 2.28 g (NH_2)₂CS was added

with vigorous stirring for 1 h, followed by addition of 0.10 M NaOH until the pH value changed to 6.5. Then, the prepared solution was transferred into a Teflon-lined stainless steel autoclave vessel and allowed to react for 24 h at 220°C. After 24 h, the reaction mixture was allowed to cool to room temperature. The precipitate was collected, washed three times with DI water, and ethanol in order to remove impurities. It was further dried under vacuum oven at 60°C for overnight to obtain the RGO/MoS₂ composites in the powder form. IR spectrum (cm⁻¹) = 3675 (OH), 2971 (C-H), 1051 (C-O), 901, 893 (S-O) and 567, 520 (Mo-S). Raman (cm⁻¹) = 348 (in-plane E_{2g}^1), 414 (A_{1g}).

5.2.4. Electrode modification to form GCE-RGO/MoS₂/Ni(OH)₂

For electrode modification, first 1.0 mg of RGO/MoS₂ was dispersed in 10 mL of PBS (0.10 M, pH 7.4) ultrasonicated for 1 h to obtain a dispersion. Then 5.0 µL of the RGO/MoS₂ dispersion was drop-dried onto a clean GCE at room temperature overnight and the electrode was denoted as GCE-RGO/MoS₂. The GCE-RGO/MoS₂ was electrochemically treated in 0.10 M PBS (pH 7.4) by potential cycling between -0.50 and 1.2 V at 50 mV.s⁻¹ until reproducible cyclic voltammograms were obtained. Then *in situ* electrodeposition of Ni(OH)₂ thin films was carried out at GCE- RGO/MoS₂ in the presence of 4.0 mM nickel nitrate hexahydrate in 0.10 M acetate buffer solution under constant potential of -0.60 V for 10 s . The electrode was denoted as GCE-RGO/MoS₂/Ni(OH)₂. The GCE-RGO/MoS₂/Ni(OH)₂ was then dipped into 0.10 M NaOH aqueous solution and CV was run from -0.30 V to 0.70 V at 100 mV.s⁻¹ until a steady state cyclic voltammogram was obtained. This was done for the electro-dissolution and passivation of the GCE-RGO/MoS₂/Ni(OH)₂ with nickel oxide layer and growth of the nickel hydroxide thin film. Thereafter, GCE-RGO/MoS₂/Ni(OH)₂ electrode was rinsed thoroughly with DI water in order to remove residual nickel salt. Then GCE-RGO/MoS₂/Ni(OH)₂ was

dried under a stream of nitrogen gas. Before the voltammetric measurements, the modified electrode was cycled several times between -0.40 and 0.80 V at a scan rate of 50 mV.s⁻¹ in 0.10 M PBS (pH 7.4) to obtain a reproducible cyclic voltammograms.

5.2.5. Electrochemical measurements

All the electrochemical experiments, cyclic voltammetry (CV), differential pulse voltammetry (DPV) and electron impedance spectroscopy (EIS) were performed at room temperature in a 10 mL electrochemical cell. The potential values for CVs and DPVs were against Ag|AgCl (pseudo-reference) electrode. Stock solutions of AA, DA and UA were freshly prepared and used to prepare different concentrations. The detection of DA, AA and UA was carried out in 10 mL phosphate buffered solution (0.10 M, pH 5.0) saturated with N₂ gas. The cyclic voltammetry potential applied was from -0.40 to 0.60 V at a scan rate of 50 mV.s⁻¹. Electrochemical behaviour of bare GCE and modified electrodes towards DA, AA and UA was further investigated using (DPV) (recorded between -0.20 V and 0.60 V with amplitude of 50 mV and a pulse width of 0.20 s) in order to obtain better peak sensitivity and resolution.

5.3 Results and discussion

5.3.1 SEM, FTIR and Raman characterization of the RGO/MoS₂ composites

SEM in **Fig. 5.1(a)** showed the morphology of RGO, MoS₂ and RGO/MoS₂ composites. The SEM image in **Fig. 5.1(a)(i)** confirmed the RGO sheets with stacking arrangement. **Figure 5.1(a)(ii)** displays flake-like structures of the as-synthesized MoS₂. In **Fig. 5.1(a)(iii)**, the RGO/MoS₂ composites is consist of a disordered array of flakes with RGO and MoS₂

distinguishable from each other [37]. The growth of MoS₂ on the RGO sheets resulted into a rigid framework structure of the RGO/MoS₂ composite. The results demonstrate that anchoring of MoS₂ onto RGO prevented agglomeration of RGO or vice versa. **Figure 5.1(b)** is the FTIR spectra which shows the presence of functional groups of the RGO, MoS₂ and RGO/MoS₂ composites. From the spectra, absorption bands observed at 3675 cm⁻¹, 2971 cm⁻¹, and 1052 cm⁻¹ indicates the presence of O-H, C-H, and C-O stretching vibrations in all the samples. The peaks at about 567 cm⁻¹ and 901 cm⁻¹ are assigned to Mo-S and S-O stretching vibrations, respectively in the MoS₂ sample [38]. These peaks shifted to lower wavenumbers in the presence of RGO to 520 cm⁻¹ and 893 cm⁻¹ confirming the formation of RGO/MoS₂ composite material. This shift came from the contribution of overlapped bands and change of surface chemistry in RGO/MoS₂ composite. The absorption bands observed at about 1411 cm⁻¹ and 1541 cm⁻¹ reveal the COO⁻ and C=O stretching vibrations in RGO and RGO/MoS₂ composites [39]. The Raman spectra in **Fig. 5.1(c)** for MoS₂ and RGO/MoS₂ show two obvious peaks at about 348 cm⁻¹ and 414 cm⁻¹ which are attributed to the hexagonal MoS₂ well, connected with the in-plane E_{2g}^1 and A_{1g} vibration modes, respectively [40]. Peaks at about 833 cm⁻¹ and 959 cm⁻¹ are the characteristic bonds of Mo-C in the Raman spectra of RGO/MoS₂ [40]. The results confirms that the RGO is well anchored with MoS₂. Furthermore, there is a D peak at 1302 cm⁻¹ and G peak at 1595 cm⁻¹ in RGO, MoS₂ and RGO/MoS₂ spectra.

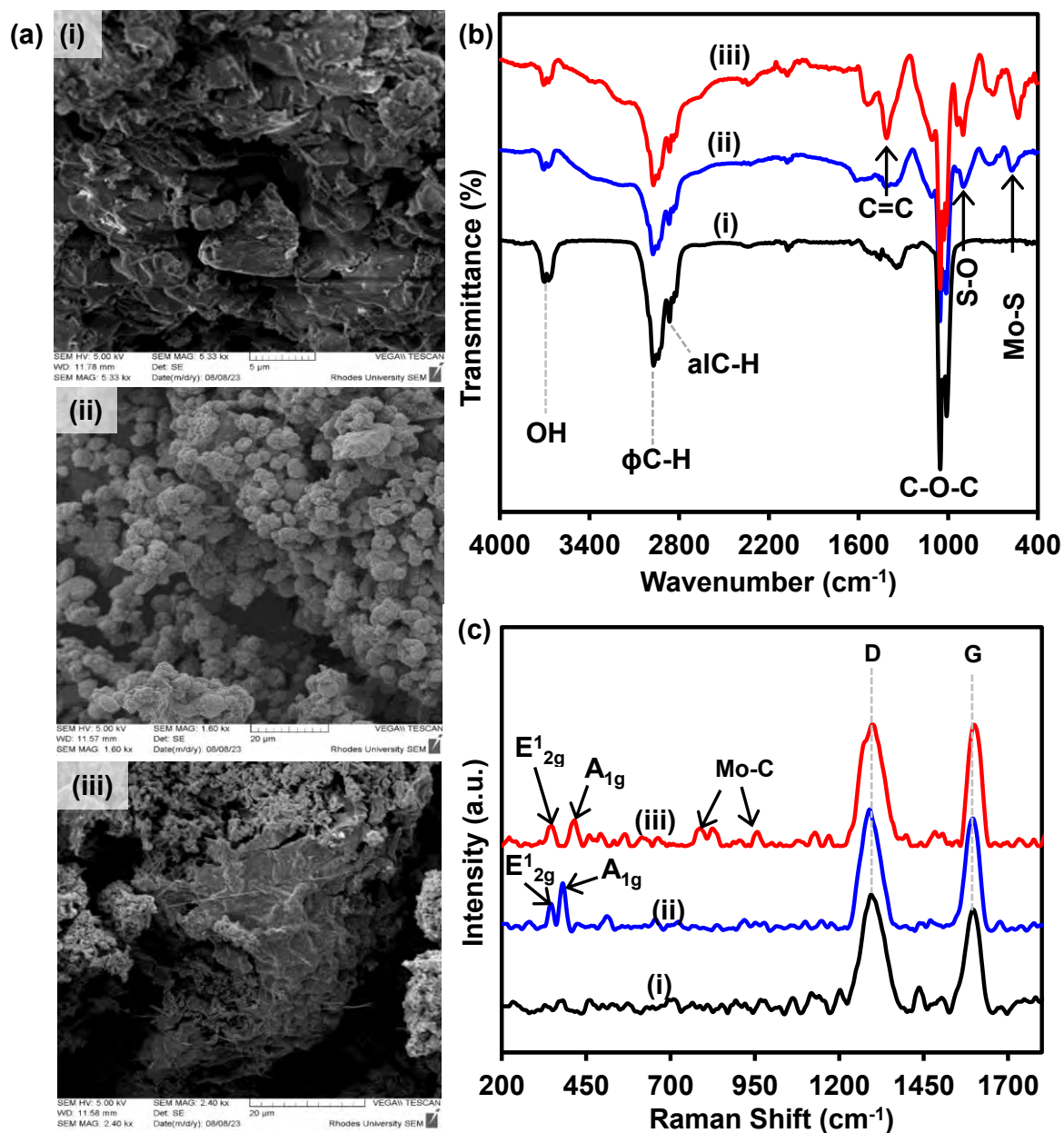


Figure 5.1: (a) SEM, (b) FT-IR spectra and (c) Raman spectra of (i) RGO, (ii) MoS₂ and (iii) RGO/MoS₂ composites.

5.3.2 Preparation of the GCE-RGO/MoS₂/Ni(OH)₂ modified electrode

A cathodic deposition technique was employed for the preparation of GCE-RGO/MoS₂/Ni(OH)₂ sensor at -0.60 V for 10 s in 4.0 mM aqueous nickel nitrate hexahydrate solution. The presence of nickel hydroxide, Ni(OH)₂, at the GCE-RGO/MoS₂/Ni(OH)₂ was

confirmed by using CV in 0.10 M NaOH solution at a scan rate of 50 mV.s⁻¹ in **Figure 5.2** which clearly shows a presence of the redox peak at E_{1/2} of 0.64 V vs Ag|AgCl for Ni^{II}/Ni^{III}. An increase in peak current was also observed due to electroactive Ni(OH)₂ thin film to form GCE-RGO/MoS₂/Ni(OH)₂. The mechanism for the redox reaction of nickel in alkaline solution is according to Eq. (5.1):

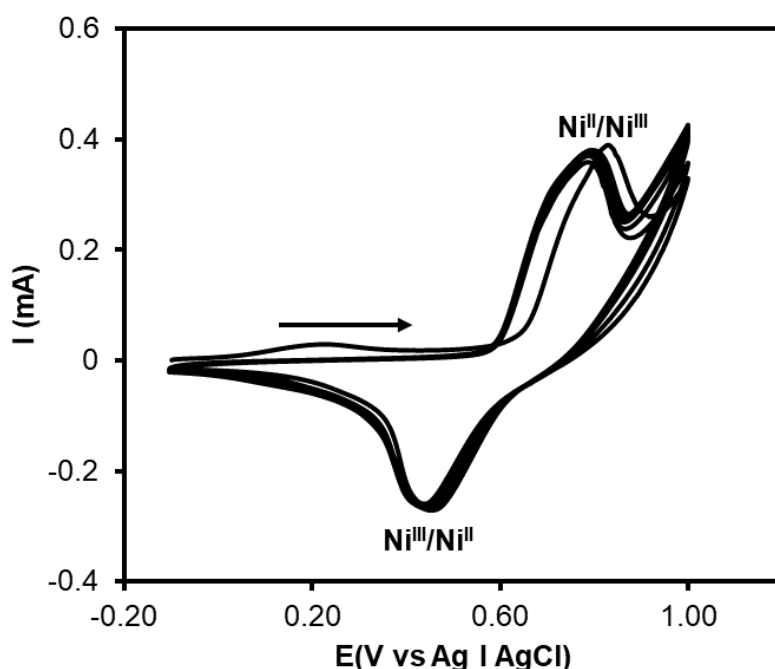
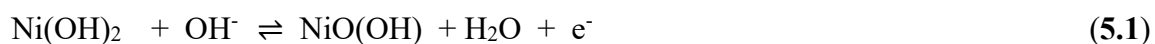


Figure 5.2: CV of GCE-RGO/MoS₂/Ni(OH)₂ in a 0.10 mol L⁻¹ NaOH solution at a scan rate of 50 mV.s⁻¹.

5.3.3 XPS characterization of GCE-RGO/MoS₂/Ni(OH)₂

The X-ray photoelectron spectroscopy (XPS) spectrum was used to analyse the surface chemistry of GCE-RGO/MoS₂/Ni(OH)₂. **Figure 5.3(a)** shows the survey spectra of (i) GCE-

RGO/MoS₂ and (ii) GCE-RGO/MoS₂/Ni(OH)₂. The survey spectra of (i) GCE-RGO/MoS₂ showed the presence of C 1s, Mo 3d, S 2p, and O 1s. A Ni 2p peak appeared in the survey spectrum of the (ii) GCE-RGO/MoS₂/Ni(OH)₂ confirming the successful electrodeposition of Ni(OH)₂ on the GCE-RGO/MoS₂ surface. As shown in **Fig. 5.3(b)**, the C 1s high-resolution spectrum of GCE-RGO/MoS₂/Ni(OH)₂ shows peaks of C-C, C=C at 284.9 eV, C-O at 285.8 eV, C=O at 286.8 eV, and O-C=O at 289.2 eV from the RGO [41,42]. The observed peaks in the XPS spectra confirmed the formation of GCE-RGO/MoS₂ in **Fig. A2 (a)**. The existence of C-O, C=O and O-C=O confirm the presence of the oxygen-containing groups at the GCE-RGO/MoS₂ and GCE-RGO/MoS₂/Ni(OH)₂.

Different component compositions were observed for the surfaces, with the GCE-RGO/MoS₂/Ni(OH)₂ showing an increase from 54.7% to 66.0% in the C-C, C=C component due to the reduction on RGO. The oxygen containing carbon components showed a decreasing percent composition. C-O component decreased from 28.4% to 18.5%, C=O from 10.0% to 9.8%, and for O-C=O decreased from 6.9% to 5.7%. **Figure 5.3(c)** shows the high-resolution XPS spectra of Mo 3d of GCE-RGO/MoS₂/Ni(OH)₂ with peaks at 232.2 eV, 233.3 eV and 234.3 eV corresponding to Mo (IV), Mo (V) and Mo (VI), respectively [42,43]. The ammonium molybdate, (NH₄)₆Mo₇O₂₄·4H₂O, thermally decomposes and form MoS_x at about 200°C during the hydrothermal process [40]. In the presence of thiourea, the formation of MoS₂ resulted. The split peaks at 229.2 eV in **Fig. A2(b)** correspond to S 2s the bridging disulfides (S₂²⁻) of the GCE-RGO/MoS₂ [44]. **Figure 5.3(d)** shows the high-resolution spectra of S 2p of the GCE-RGO/MoS₂/Ni(OH)₂. The peaks at 162.1 eV, 164.0 eV and 168.7 eV are attributed to the Mo-S, C-S and SO₄, respectively as has been reported [45, 46].

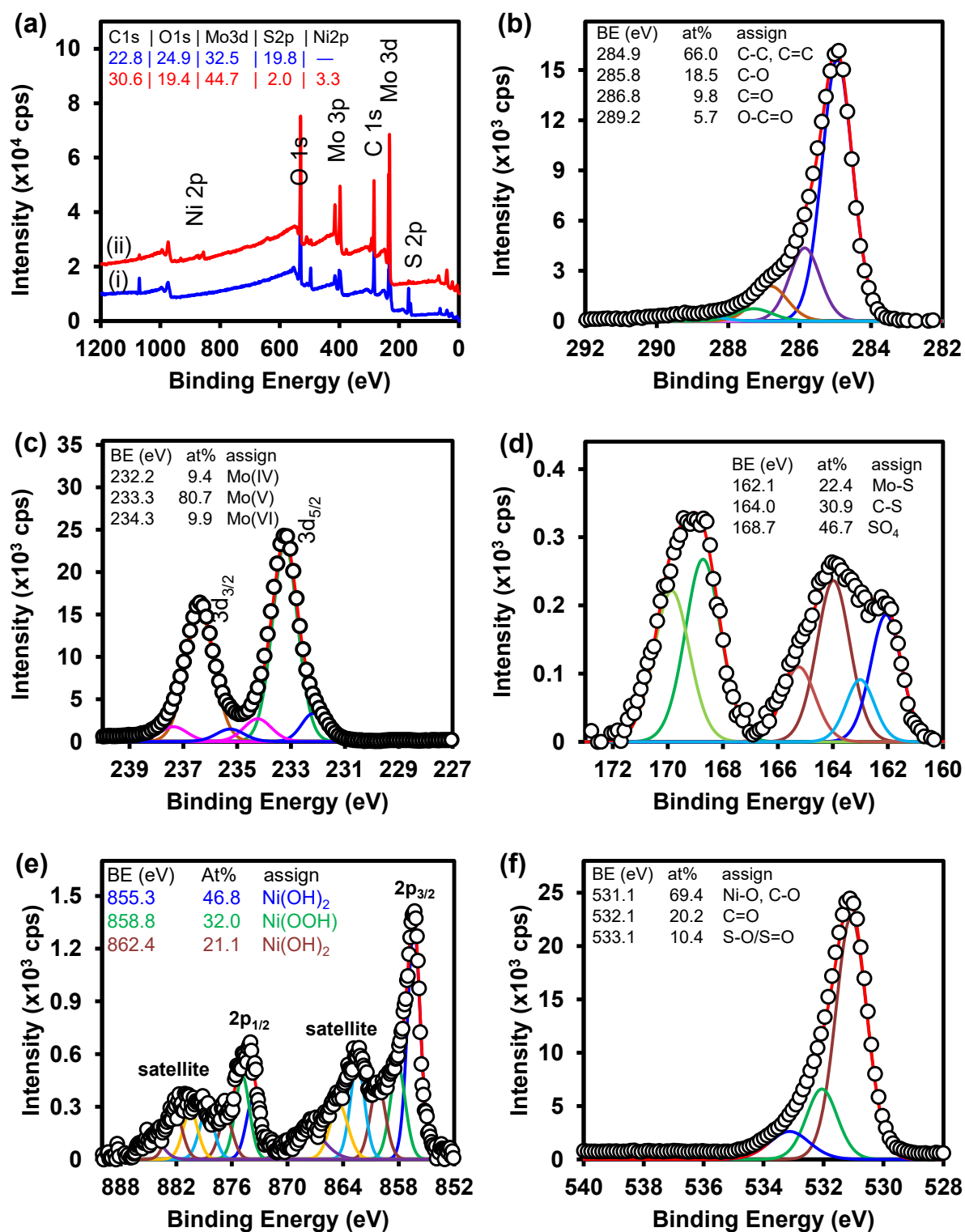


Figure 5.3: (a) XPS survey spectra of the GCE-RGO/MoS₂/Ni(OH)₂ and high resolution of (b) C 1s, (c) Mo 3d, (d) S 2p, (e) Ni 2p and (f) O 1s of the GCE-RGO/MoS₂/Ni(OH)₂.

The peaks also appeared in the spectrum of the GCE-RGO/MoS₂ in **Fig. A2(c)**, with lower intensities. The enhanced peaks of S 2p at GCE-RGO/MoS₂/Ni(OH)₂ indicate the presence S₂²⁻ species bonded to Ni^{II} [45]. **Figure 5.3(e)** shows the Ni 2p spectrum of the GCE-RGO/MoS₂/Ni(OH)₂. The peaks situated at 855.3 eV and 862.4 eV represent the Ni 2p_{3/2} and 2p_{1/2}, respectively [42]. The presence of the satellite peaks further confirms the Ni +2 oxidation state. **Figure 5.3(f)** shows the high-resolution of the O 1s with Ni-O, C-O component at 69.4% and C=O and S-O, S=O due to the organic matter on the surface. The increase from 17.8% in **Fig. A2(d)** to 69.4% in **Fig. 5.3(f)** for the C-O and Ni-O component further confirmed the formation of nickel thin film for GCE-RGO/MoS₂/Ni(OH)₂ surface.

5.3.4 Electrochemical characterization of electrode modification

The electrochemical behaviour of the bare GCE, GCE-RGO/MoS₂ and GCE-RGO/MoS₂/Ni(OH)₂ was investigated using a cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). **Figure 5.4** shows (a) CVs, and (b) EIS obtained for (i) GCE, (ii) GCE-RGO/MoS₂, and (iii) GCE-RGO/MoS₂/Ni(OH)₂ in (1:1) 2.0 mM [Fe(CN)₆³⁻]/[Fe(CN)₆⁴⁻] containing 0.10 M KCl, at a scan rate of 50 mV.s⁻¹. The bare GC electrode showed a reversible redox peak with peak-to-peak separation of 91 ± 4.5 mV. The ratio of oxidation to reduction peak currents (*i*_{pa}/*i*_{pc}) was 0.996 close to 1.0 for a reversible couple. An increase in the redox peak currents and capacitive or background current confirmed the electrode modification. At GCE-RGO/MoS₂, the *i*_{pa} increased by 41%, this is due to the large surface area and good electronic conductivity of RGO/MoS₂ nanosheets. While at GCE-RGO/MoS₂/Ni(OH)₂, the *i*_{pa} increased by 45%, due to the synergistic effects between RGO/MoS₂ nanosheets and Ni(OH)₂. The value of Δ*E*_p at the GCE-RGO/MoS₂ slightly decreased to 88 ± 5.5 mV. Whilst at GCE-RGO/MoS₂/Ni(OH)₂ the Δ*E*_p value decreased further

to 83 ± 4.5 mV. The lower ΔE_p at GCE-RGO/MoS₂/Ni(OH)₂ demonstrated higher conductivity of the RGO/MoS₂/Ni(OH)₂ composite material. The half-wave potential values ($E_{1/2}$) for GCE, GCE-RGO/MoS₂ and GCE-RGO/MoS₂/Ni(OH)₂ were determined to be 185 mV, 169 mV, and 183 mV, respectively. At GCE-RGO/MoS₂/Ni(OH)₂, the two redox peaks at a formal potential of 0.35 V were due to Ni^{II}/Ni^{III}. The redox peak confirmed the successful electrodeposition of Ni(OH)₂ thin film on the electrode surface. The EIS data in **Fig. 5.4(b)** shows a charge transfer resistance of (R_{CT}) 0.177 ± 0.001 k Ω at the (i) bare GCE which decreased considerably at (ii) GCE-RGO/MoS₂ and (iii) GCE-RGO/MoS₂/Ni(OH)₂. The decrease in the charge transfer resistance shows excellent conductivity of electrodes modified with RGO/MoS₂ and RGO/MoS₂/Ni(OH)₂. The CV experiment also confirmed excellent conductivity with increase in current densities and capacitance.

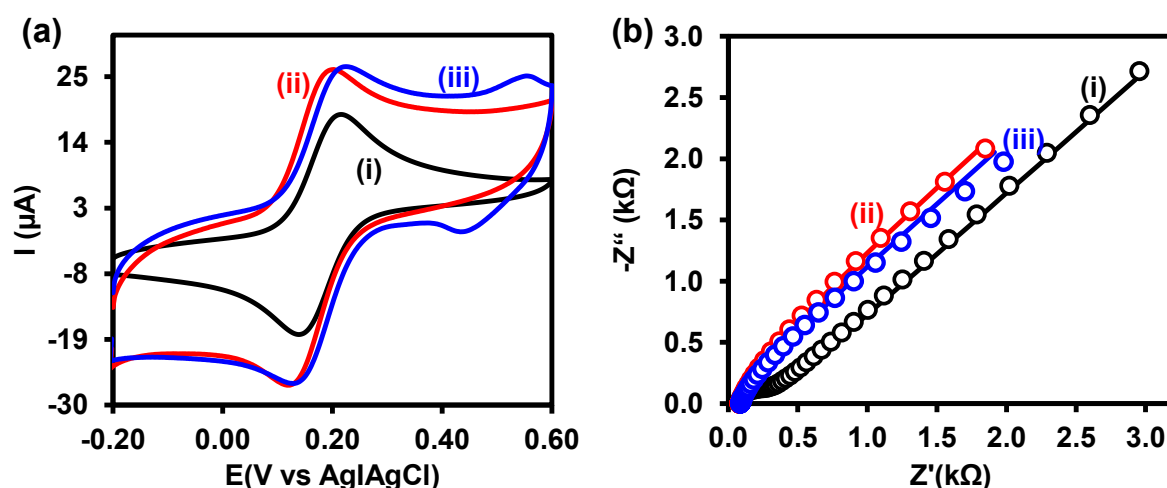


Figure 5.4: (a) CVs and (b) EIS (Nyquist plot representation) for (i) GCE, (ii) GCE-RGO/MoS₂ and (iii) GCE-RGO/MoS₂/Ni(OH)₂ in (1:1) 2.0 mM Fe(CN)₆³⁻/Fe(CN)₆⁴⁻ containing 0.10 M KCl. Scan rate = 50 mV.s⁻¹.

The effective surface areas of GCE, GCE-RGO/MoS₂ and GCE-RGO/MoS₂/Ni(OH)₂ were calculated using the Randles-Sevcik equation following reported method [7]. The diffusion coefficient of K₃[Fe(CN)₆] is 7.6×10^{-6} cm².s⁻¹. The effective surface areas of the GCE, GCE-

RGO/MoS₂ and GCE-RGO/MoS₂/Ni(OH)₂ were obtained as 0.0780 cm², 0.101 cm² and 0.104 cm², respectively. The results demonstrate that the GCE-RGO/MoS₂/Ni(OH)₂ has higher electrocatalytic surface area with more active sites for effective adsorption and electrocatalysis. Furthermore, it was also observed that the electrochemical response of the GCE-RGO/MoS₂/Ni(OH)₂ followed a perfect reversible system in the ferri/ferrocyanide probe system as discussed in **Section A3**.

5.3.5 Electrocatalysis of AA, DA and UA at GCE-RGO/MoS₂/Ni(OH)₂

The electrocatalysis of AA, DA, and UA at GCE, GCE-RGO/MoS₂, GCE-RGO/MoS₂/Ni(OH)₂ was investigated in 0.10 M PBS (pH 5.0) containing 1.0 mM AA, 0.30 mM DA, and 0.5 mM UA. **Fig. 5.5** shows the CVs at GCE-RGO/MoS₂/Ni(OH)₂ surface. **Figure 5.5(i)** shows the CV in pH 5.0 PBS solution and a broad redox peak due to Ni^{II}/Ni^{III} was observed. An enhanced oxidation peak current in **Fig. 5.8(a)(ii)** was observed for AA at 83 ± 0.45 mV which is 140 mV more negative than that at the bare GCE in **Fig. A4(a)** and 19 mV slightly positive than at the GCE-RGO/MoS₂ in **Fig. A5(a)**. The oxidation peak current densities at GCE-RGO/MoS₂/Ni(OH)₂ (216 µA.cm⁻²) is higher when compared to that of the bare GCE (182.7 µA.cm⁻²) and GCE-RGO/MoS₂ (132.7 µA.cm⁻²).

For DA in **Fig. 5.5(b)(ii)**, a well pronounced reversible peaks was observed at GCE-RGO/MoS₂/Ni(OH)₂ surface due to electrooxidation of DA to dopamine-o-quinone (DAq) and reduction of DAq back to DA in pH 5.0. The oxidation of DA at GCE-RGO/MoS₂/Ni(OH)₂ was observed at 193 ± 0.45 mV. There was enhanced redox peaks at GCE-RGO/MoS₂/Ni(OH)₂ when compared to the bare GCE in **Fig. A4(b)** and GCE-RGO/MoS₂ **Fig. A5(b)**. This remarkable electrocatalytic activity improvement for DA at GCE-RGO/MoS₂/Ni(OH)₂ is due

to: (i) excellent redox reaction properties of $\text{Ni}(\text{OH})_2$ thin films in the composite structure, and (ii) the electrostatic interaction between positively charged DA ($\text{pK}_a = 8.87$) and negatively charged GCE-RGO/ MoS_2 (remaining COOH) in the composite [47].

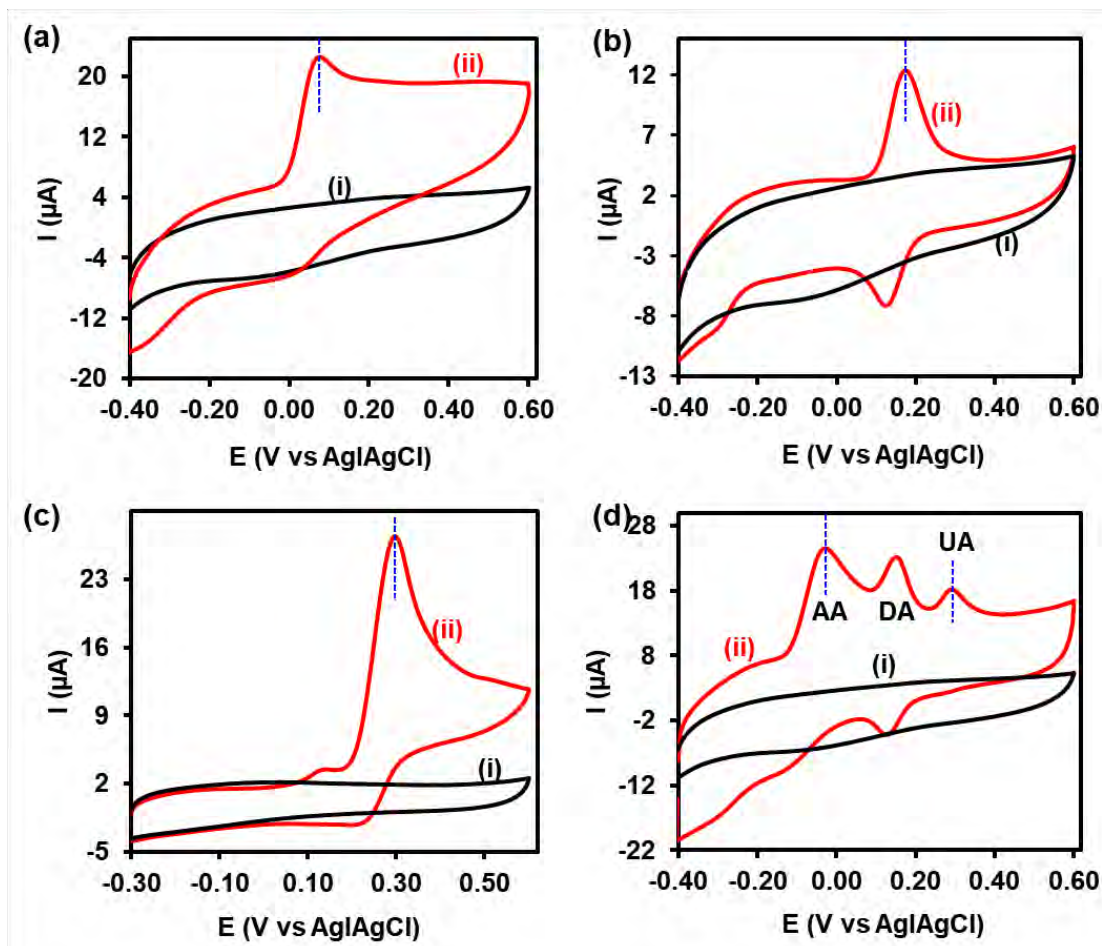


Figure 5.5: CVs of GCE-RGO/ MoS_2 / $\text{Ni}(\text{OH})_2$ in (i) 0.10 M PBS (pH 5.0) and (ii) containing (a) 1.0 mM AA, (b) 0.30 mM DA, (c) 0.50 mM UA, and (d) the mixture of 1.0 mM AA + 0.30 mM DA + 0.50 mM UA. Scan rate: $50 \text{ mV} \cdot \text{s}^{-1}$.

For UA in **Fig. 5.5(c)(ii)**, well resolved and enhanced oxidation peak appears at $310 \pm 0.45 \text{ mV}$. The peak current at GCE-RGO/ MoS_2 / $\text{Ni}(\text{OH})_2$ was enhanced about two-fold when compared to bare GCE in **Fig. A4(c)**. The oxidation peak potentials (E_{pa}) for UA shifted to lower potential by 49 mV at GCE-RGO/ MoS_2 / $\text{Ni}(\text{OH})_2$ as compared to GCE-RGO/ MoS_2 in **Fig. A5(c)**. The decrease in the oxidation peak potential (E_{pa}) at GCE-RGO/ MoS_2 / $\text{Ni}(\text{OH})_2$ was

due to excellent electrocatalytic activity of Ni(OH)₂ thin film coupled with a good electrical conductivity of RGO/MoS₂ nanosheets. The redox reaction of UA at GCE-RGO/MoS₂/Ni(OH)₂ show quasi-reversible process, suggesting that UA is first oxidized to its diamine intermediate and reduced back to uric acid.

The oxidation current densities and redox potentials for the oxidation of AA, DA and UA at GCE, GCE-RGO/MoS₂ and GCE-RGO/MoS₂/Ni(OH)₂ are summarized in **Table 5.1**.

Table 5.1: Comparison of electrocatalysis of GCE, GCE-RGO/MoS₂ and GCE-RGO/MoS₂/Ni(OH)₂ towards the detection of AA, DA, and UA.

Electrode	Analyte	Current Density ($\mu\text{A.cm}^{-2}$)	E _{ox} (mV)	E _{red} (mV)
GCE	AA	162	240	-
	DA	53	213	105
	UA	179	308	-
GCE-RGO/MoS ₂	AA	129	-0.01	-
	DA	60	179	64
	UA	244	359	-
GCE-RGO/MoS ₂ /Ni(OH) ₂	AA	213	91	-
	DA	115	188	159
	UA	260	308	-

The GCE-RGO/MoS₂/Ni(OH)₂ was subjected to the mixtures of AA (1.0 mM), DA (0.30 mM) and UA (0.50 mM) in PBS (pH 5.0). In **Fig. 5.5(d)(ii)**, three well-defined oxidation peak currents at about -7.0 ± 0.45 mV, 169 ± 0.45 mV, and 313 ± 0.45 mV were observed, corresponding to AA, DA and UA, respectively. The electrocatalytic peak potential differences between DA and AA was 97 mV, DA and UA was 120 mV, and UA and AA was 217 mV at GCE-RGO/MoS₂/Ni(OH)₂. The large separation of the peak potentials suggests that GCE-RGO/MoS₂/Ni(OH)₂ can be used for simultaneous determination of AA, DA, and UA. There

is a negative shift of about 22 mV in the oxidation peak potential during simultaneous detection compared to individual detection for DA. This suggests that there are no inter- or intra-molecular interactions between the oxidized product of AA with DA and UA molecules and also DA oxidized products with UA in the mixture. AA undergoes oxidation first before DA and UA with UA the last to undergo oxidation.

5.3.6 Effect of deposition potential and time of Ni(OH)₂ on the AA, DA and UA signals

The experimental parameters such as deposition potential and deposition time of Ni(OH)₂ onto GCE-RGO/MoS₂ were optimized. Their effects on the electrocatalytic activity of the GCE-RGO/MoS₂/Ni(OH)₂ sensor towards the detection of AA, DA and UA were investigated. The potential for the deposition of Ni(OH)₂ onto GCE-RGO/MoS₂ evaluated was -0.50 V, -0.60 V, -0.70 V, -0.80 V, -0.90 V and -1.0 V to establish the effect of applied potential. A deposition potential of -0.60 V gave high oxidation peak currents for all the three target analytes as shown in **Fig. 5.6(a)**. Furthermore, the effect of the deposition time of Ni(OH)₂ thin films on the electrocatalytic activity of 1.0 mM AA, 0.30 mM DA and 0.50 mM UA at GCE-RGO/MoS₂/Ni(OH)₂ were also investigated. **Figure 5.6(b)** shows the relationships between the oxidation peak currents of the AA, DA and UA with the deposition times of 2 s, 5 s, 10 s, 15 s, 20 s and 25 s of Ni(OH)₂ thin films onto the GCE-RGO/MoS₂. The results showed that the deposition time of 10 s gave high currents and hence chosen as the optimum deposition time. The low oxidation peak currents observed at short times < 10 s was due to the insufficient amount of electrocatalytic active sites of Ni(OH)₂ thin film at the GCE-RGO/MoS₂/Ni(OH)₂. Oxidation peak currents were also low at longer times > 10 s. The longer times results into thick Ni(OH)₂ films on the GCE-RGO/MoS₂/Ni(OH)₂ which reduces conductivity of the electrode leading into a decrease in surface area, hence a decrease in oxidation peak currents.

The optimum deposition potential of -0.60 V and deposition time of 10 s was used for applications.

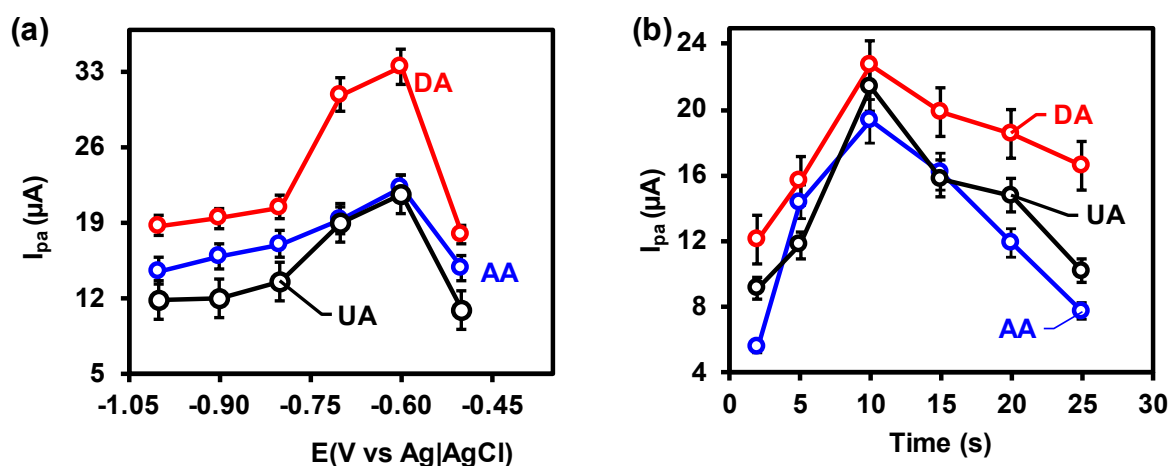


Figure 5.6: Effect of (a) deposition potential and (b) deposition time of $\text{Ni}(\text{OH})_2$ on the electrocatalytic current of 1.0 mM AA, 0.30 mM DA, and 0.50 mM UA at GCE-RGO/MoS₂/Ni(OH)₂ in 0.10 M PBS (pH 5.0).

5.3.7 Effect of pH and scan rate on the AA, DA and UA electrocatalysis

Figure 5.7(a) shows the effect of pH on the electrochemical behaviours of 0.30 μM AA, 20 μM DA and 30 μM UA solution at the GCE-RGO/MoS₂/Ni(OH)₂ at varying pH ranging from 4.0 to 10.0 in 0.10 M phosphate buffered solution as shown in the enlarged oxidation scans in **Fig. 5.7(a')**. The oxidation peak currents of AA, DA, and UA increased from pH 4.0 to 5.0 and then gradually decreased from pH 6.0 to 10.0 as shown in **Fig. 5.7(b)**. AA shows the largest decrease in anodic peak current with increase in pH. This is due to the pK_a value of 4.17 for AA [30]. There is electrostatic repulsion between the negatively charged AA molecules which with the carboxylate groups at the electrode surface in alkaline environments. We obtained good peak potential separation between the analytes at pH 4.0 and pH 5.0. High electrocatalytic oxidation currents were obtained at pH 5.0 in **Fig. 5.7(b)**. The detection of AA, DA and UA

was studied at pH 5.0 as the optimum pH condition. **Figure 5.7(c)** shows the plots of oxidation peak potentials (E_{pa}) vs pH for the GCE-RGO/MoS₂/Ni(OH)₂ in AA, DA, and UA, respectively. The oxidation peak potentials shifted negatively for AA, DA and UA as the pH values increased. The linear relationships between the peak potential (E_{pa}) vs pH are shown in Equations (5.2) to (5.4).

$$\text{For AA: } E_{pa} \text{ (V)} = -0.029 \text{ pH} + 0.241 \text{ (R}^2 = 0.964\text{)} \quad (5.2)$$

$$\text{For DA: } E_{pa} \text{ (V)} = -0.028 \text{ pH} + 0.359 \text{ (R}^2 = 0.969\text{)} \quad (5.3)$$

$$\text{For UA: } E_{pa} \text{ (V)} = -0.033 \text{ pH} + 0.542 \text{ (R}^2 = 0.964\text{)} \quad (5.4)$$

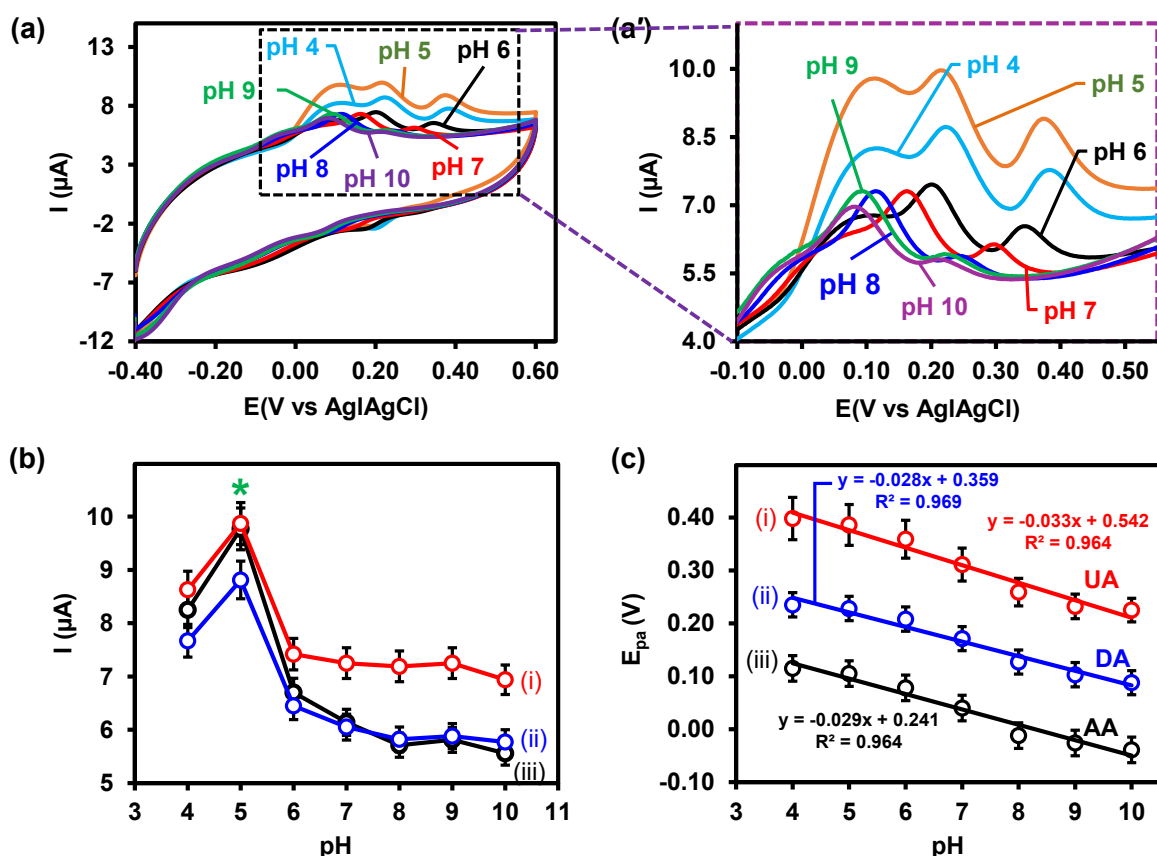


Figure 5.7: CVs recorded in pH 4.0 – 10.0 PBS containing 300 μM AA, 20 μM, and 30 μM UA. (b) Plots of peak current versus the pH value. (c) Plots of peak potential versus the pH value. (n = 3)

Using Nernst equation, the number of electrons and protons were calculated. The gradients of the E_{pa} vs pH were -0.029 V/pH for AA, -0.028 V/pH for DA, and -0.033 V/pH for UA. These values are half the Nernst gradient of -0.0592 V/pH. This means that there are 2 protons and 2 electrons involved in the electrocatalytic detection of AA, UA, and DA in line with literature [48].

Figure 5.8(a-c) shows the effect of scan rate on the oxidation peak currents of 2.0 mM AA, 0.050 mM DA and 0.70 mM UA in 0.10 M phosphate buffer solution (pH = 5.0) in the range from 25 to 175 mV.s⁻¹ at the GCE-RGO/MoS₂/Ni(OH)₂. The oxidation peak currents increased with increasing scan rates with very small shifts in the oxidation peak potentials to positive potentials. **Figure 5.8 (a'-c')** shows the plots of oxidation peak currents (I_{pa}) vs. scan rates (ν) for AA, DA and UA, respectively. The oxidation peak currents for AA, DA and UA were linear related to the scan rates and the regression Equations (5.5) - (5.7):

$$\text{For AA: } I_{pa} = 0.225 \nu - 15.070 \text{ (R}^2 = 0.987\text{)} \quad (5.5)$$

$$\text{For DA: } I_{pa} = 0.103 \nu - 2.177 \text{ (R}^2 = 0.995\text{)} \quad (5.6)$$

$$\text{For UA: } I_{pa} = 0.169 \nu - 15.980 \text{ (R}^2 = 0.992\text{)} \quad (5.7)$$

These results demonstrate that the catalytic oxidation reactions for AA, DA and UA at the GCE-RGO/MoS₂/Ni(OH)₂ were surface controlled processes.

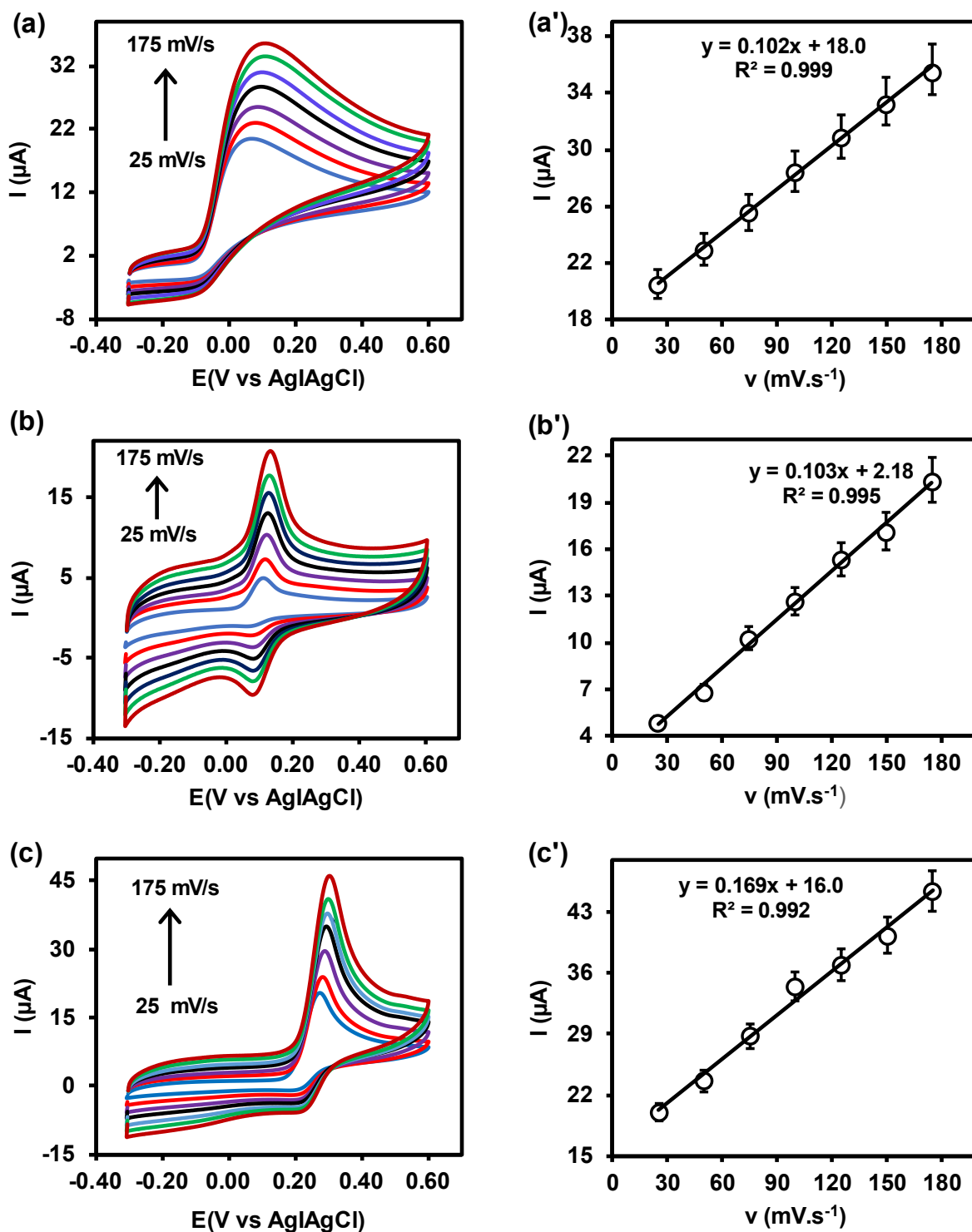


Figure 5.8: CVs and the calibration plots of GCE-RGO/MoS₂/Ni(OH)₂ at different scan rates ranging from 25 to 175 mV.s⁻¹ of (a) 2.0 mM AA , (b) 0.050 mM DA and (c) 0.70 mM UA in 0.10 M PBS (pH 5.0) solution.

5.3.8 Detection of AA, DA and UA at GCE-RGO/MoS₂/Ni(OH)₂

Under the optimized conditions, the determination of AA, DA and UA at GCE-RGO/MoS₂/Ni(OH)₂ was carried out by DPV in the potential range -0.20 to 0.60 V in 0.10 M PBS (pH 5.0). Firstly, the GCE-RGO/MoS₂/Ni(OH)₂ electrode was used for individual detection of (a) AA, (b) DA and (c) UA in **Fig. 5.9**. The oxidation peak currents for the corresponding concentrations for each analyte were analyzed and the linear relationship was obtained in **Fig. 5.9 (a') - (c')**. The linear regression Equations were (5.8) – (5.10):

$$I_{pa} (\mu A) = 0.0175 [AA](\mu M) + 12.0 (R^2 = 0.989) \quad (5.8)$$

$$I_{pa} (\mu A) = 0.372 [DA] (\mu M) + 24.9 (R^2 = 0.992) \quad (5.9)$$

$$I_{pa} (\mu A) = 0.128 [UA] (\mu M) + 20.7 (R^2 = 0.986) \quad (5.10)$$

Limit of detection (LoD) was calculated by using the equation of $LoD = 3 \sigma/m$, where σ is the standard deviation of the blank responses and m is the slope of the calibration plots. The LoDs were 1.17 μM for AA, 0.150 μM for DA and 1.15 μM for UA. The LoDs were comparable and even lower (especially for AA) than those previously reported modified electrodes as summarized **Table 5.2**.

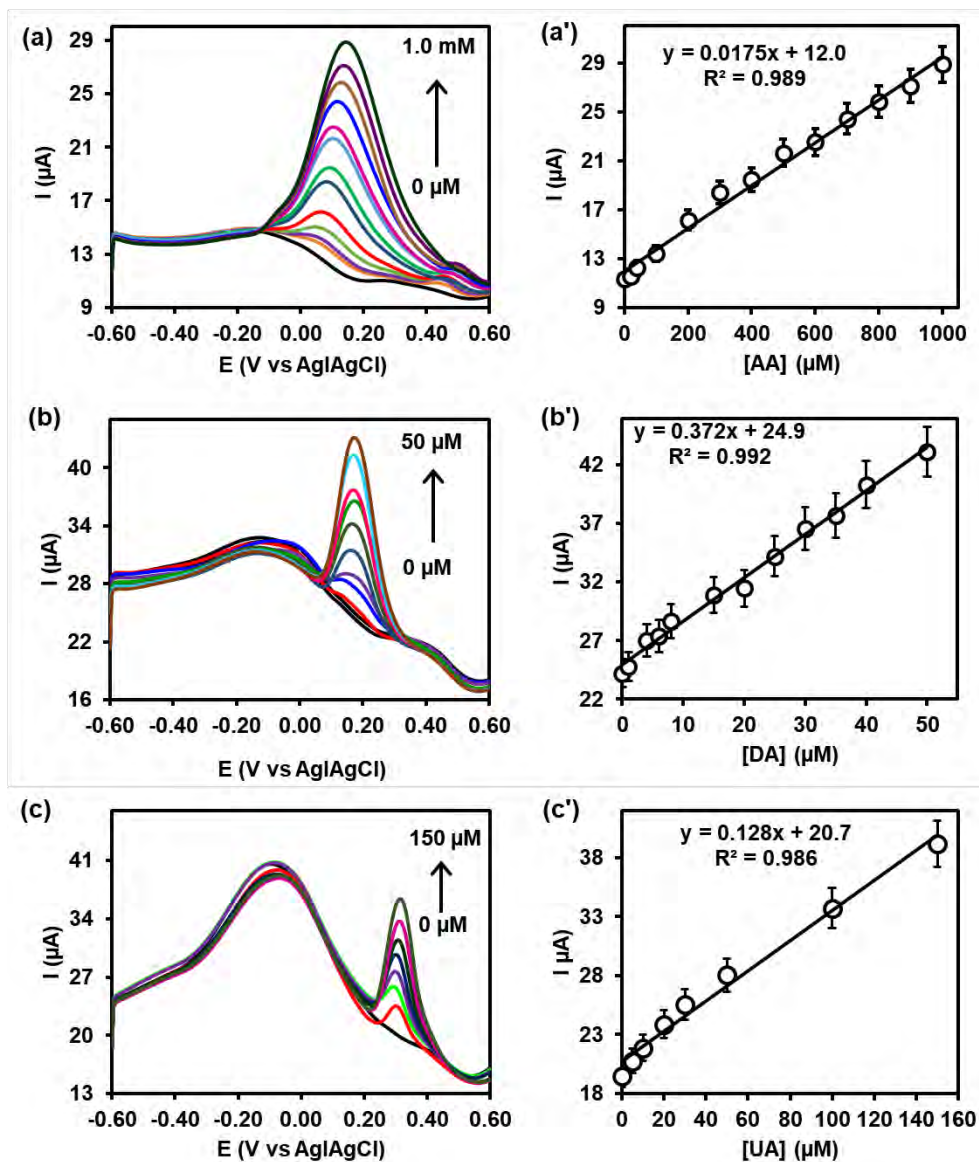


Figure 5.9: DPVs and the corresponding calibration curves of the GCE-RGO/MoS₂/Ni(OH)₂ at different concentrations in PBS (pH 5.0) for (a) AA (20 - 1000 μM) (b) DA (1 - 50 μM), and (c) UA (5.0 - 150 μM). (n = 3)

Table 5.2: Summary of electrochemical analytical parameters for the detection of AA, DA and UA at GCE-RGO/MoS₂/Ni(OH)₂ compared with reported electrodes.

Electrode	pH	LCR (μM)			LOD (μM)			Sensitivity (μA. μM ⁻¹ .cm ⁻²)			Ref.
		AA	DA	UA	AA	DA	UA	AA	DA	UA	
MoS ₂ /rGO/GCE	7.0	12-5402	5-545	25-2747	0.72	0.05	0.42	0.12	4.11	1.59	[11]
3D-MoS ₂ /rGO/Au@GCE	7.0	2-2400	0.3-198.3	5-2215	0.93	0.11	0.74	-	-	-	[15]
NiO/GO/GCE	4.7	40-700	2-60	2-120	5.5	0.10	0.14	-	-	-	[16]
[Ni(phen) ₂] ²⁺ /SWCNT/GCE	4.0	30-1546	1-780	1-1407	12	1.0	0.76	-	-	-	[19]
Ni/C/GCE	7.0	20-2400	1-55	5-180	5	0.05	0.10	0.352	45.571	11.873	[24]
Ni-sG/GCE	4.0	150-300	0.44-3.3	2-15	30	0.12	0.46	0.007 μA. μM ⁻¹	3.93 μA. μM ⁻¹	0.11 μA. μM ⁻¹	[30]
MoS ₂ /PEDOT/GCE	7.4	20-140	1-80	2-25	5.83	0.52	0.95	1.20 A. μM ⁻¹ m ⁻²	36.40 A. μM ⁻¹ .m ⁻²	105.17 A.μM ⁻¹ .m ⁻²	[47]
GCE-RGO/MoS ₂ /Ni(OH) ₂	5.0	20-1000	1-50	5-150	1.17	0.15	1.15	0.22	4.81	1.64	TW

TW = This work, MoS₂/rGO = Molybdenum disulfide- reduced graphene oxide, Au = Gold nanoparticles, Ni(phen)₂]²⁺/SWCNT = Nickel(II)-bis (1,10-phenanthroline)-single walled carbon nanotubes, NiO/GO = Nickel oxide –graphene oxide, PEDOT = poly(3,4-ethylenedioxythiophene, Ni-sG = Solar graphene-nickel hydroxide and Ni/C = Carbon-supported Ni nanoparticles.

The GCE-RGO/MoS₂/Ni(OH)₂ was also used for simultaneous detection of the analytes by addition of various concentrations of one analyte whilst concentrations of the other two compounds were kept constant. **Figure 5.10(a)** shows DPVs of increasing AA from 0 - 3.0 mM in the presence of 5.0 µM DA and 100 µM UA. The oxidation peak currents increased linearly with increasing AA concentrations in **Fig. 5.10 (a')**. The linear regression equation (5.11):

$$I_{pa} (\mu A) = 2.19 [AA] (mM) + 10.64 (R^2 = 0.996, (LoD = 22.4 \mu M)) \quad (5.11)$$

The LoD for the detection of AA was calculated and found to be 22.4 µM. Linear concentration range was observed up to 3.0 mM. **Figure 5.10 (b)** shows DPVs of increasing DA from 0 - 50.0 µM in the presence of 1.0 mM AA and 200 µM UA. The oxidation peak currents increased linearly with increasing DA concentrations in **Fig. 5.10 (b')**. The linear regression equation (5.12):

$$I_{pa} (mA) = 0.347 [DA] (\mu M) + 10.9 (R^2 = 0.994, (LOD = 0.030 \mu M)) \quad (5.12)$$

The LoD for the detection of DA was found to be 0.030 µM and the linear concentration range up to 50 µM. **Figure 5. 10 (c)** shows DPVs of increasing UA from 0 - 200 µM in the presence of 500 µM AA and 5 µM DA. The oxidation peak currents increased linearly with increasing UA concentrations in **Fig. 5.10 (c')**. The linear regression equation (5.13):

$$I_{pa} (\mu A) = 0.0750 [UA] (mM) + 8.66 (R^2 = 0.991, (LOD = 2.1 \mu M)) \quad (5.13)$$

The LoD was 2.1 µM and linear concentration range up to 200 µM.

There was no obvious interference from each of the analytes during the simultaneous detection except a broad oxidation peak of AA.

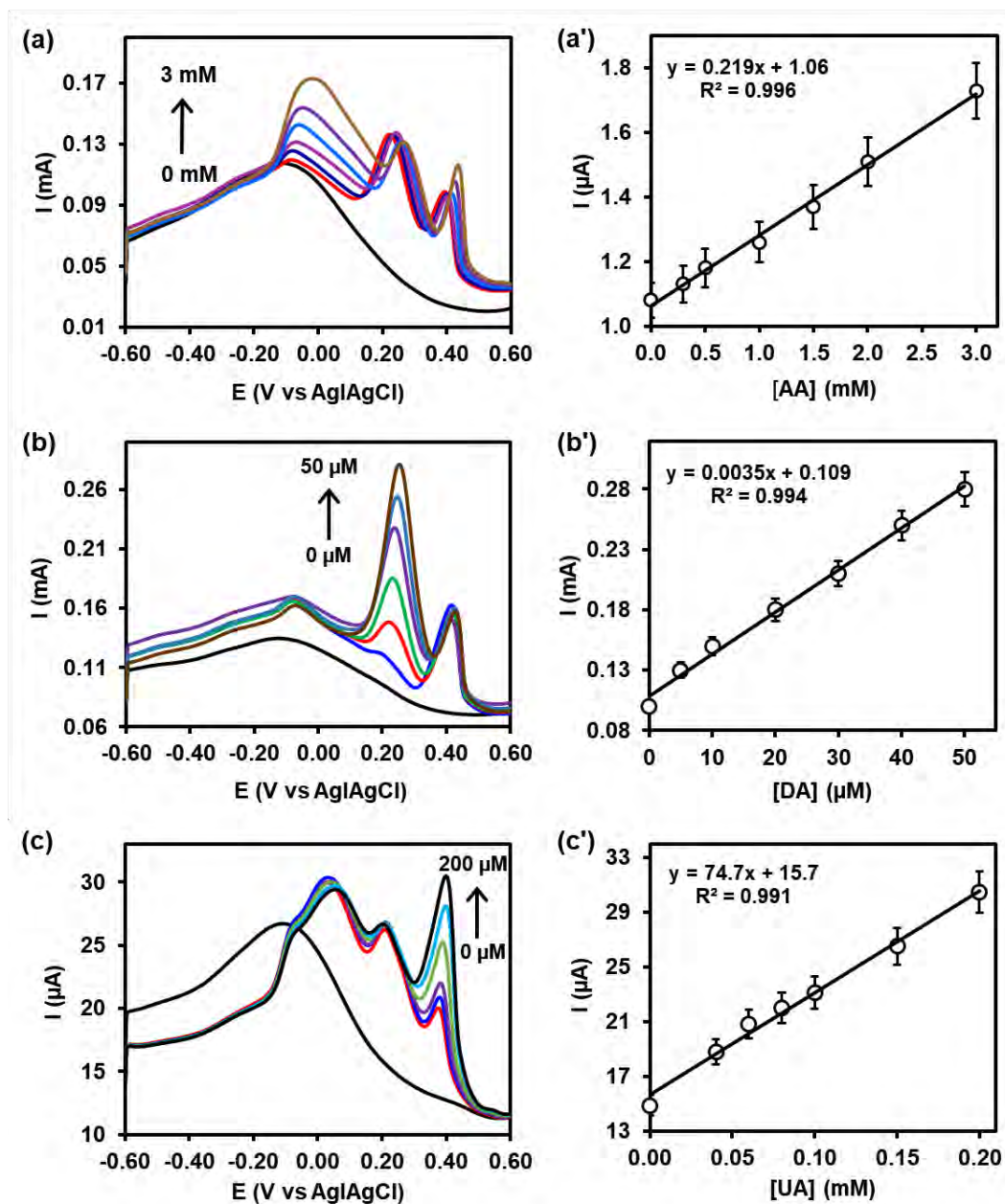


Figure 5.10: DPVs and corresponding calibration curves of GCE-RGO/MoS₂/Ni(OH)₂ in 0.10 M PBS (pH 5.0) for the simultaneous detection of (a) AA (0.10 - 3.0 mM) in the presence of 5.0 μ M DA and 100 μ M UA, (b) DA (1.0 - 50 μ M) in the presence of 1.0 mM AA and 200 μ M UA, and (c) UA (5.0 - 200 μ M) in the presence of 500 μ M AA and 5 μ M DA. Scan rate: 20 mV.s⁻¹, pulse interval 200, pulse amplitude 25 mV, pulse width 50 ms. (n = 3)

5.3.9 Reproducibility and stability of the GCE-RGO/MoS₂/Ni(OH)₂

The reproducibility of the GCE-RGO/MoS₂/Ni(OH)₂ was studied by a series of ten repetitive CV responses of 1.0 mM AA, 0.30 mM DA and 0.50 mM UA in PBS (pH 5.0), **Fig. 5.11(a)**. The modified sensor was scanned multiple times from -0.20 V to 0.60 V for 10 scans in 0.10 M PBS (pH 5.0) to remove absorbed oxidized products prior to each measurement. The relative standard deviations (RSD) of the oxidation peak currents obtained after ten consecutive measurements were 0.60%, 0.50% and 1.1%, for AA, DA and UA, respectively, confirming excellent reproducibility of the electrocatalytic signal for GCE-RGO/MoS₂/Ni(OH)₂. This demonstrated that the modified electrode sensor was able to prevent fouling by the oxidized products of AA, DA and UA.

The long-term stability of GCE-RGO/MoS₂/Ni(OH)₂ was investigated by CV responses toward 1.0 mM for AA, 0.30 mM for DA, and 0.50 mM for UA every week for three weeks in **Fig. 5.11(b)**. GCE-RGO/MoS₂/Ni(OH)₂ was stored in 0.10 M PBS (pH 5.0) at 4°C during this period. The electrocatalytic response of the GCE-RGO/MoS₂/Ni(OH)₂ toward AA, DA and UA remained at 85%, 91% and 82% of its initial current response, respectively. These results demonstrated that the GCE-RGO/MoS₂/Ni(OH)₂ showed good stability towards detection of AA, DA and UA in aqueous buffered solutions.

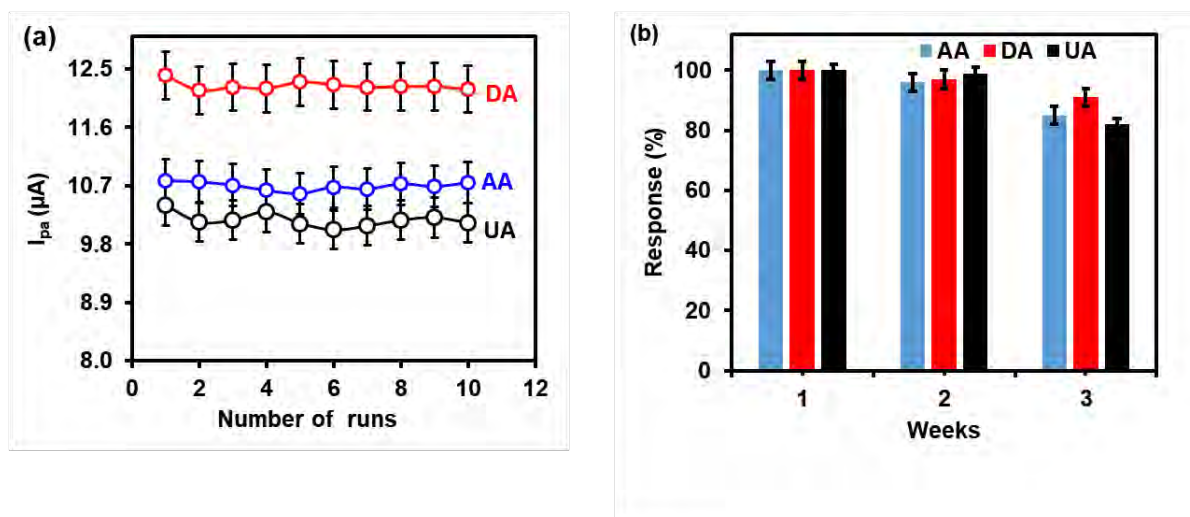


Figure 5.11: Shows (a) ten repetitive CV measurements in 0.10 M PBS (pH 5.0) containing 1.0 mM AA, 0.30 mM DA and 0.50 mM UA at GCE-RGO/MoS₂/Ni(OH)₂, and (b) the stability response of the GCE-RGO/MoS₂/Ni(OH)₂ toward 1.0 mM AA, 0.30 mM DA and 0.50 mM UA measured weekly for three weeks.

5.3.10 Analytical application

The GCE-RGO/MoS₂/Ni(OH)₂ possesses good sensitivity and selectivity for the simultaneous determination of AA, DA and UA. The modified sensor was applied for the analysis DA in the newborn calf serum samples. **Fig. 5.12** depicts the DPVs of 0.10 M PBS (pH 5.0) in the (i) absence and (ii) presence 400 μM AA, 20 μM DA and 75 μM UA in the newborn serum samples. A large increase in the oxidation peak currents were observed at -0.101 V, 0.125 V and 0.236 V for AA, DA and UA, respectively. This demonstrated that AA, DA and UA are present in the serum samples. Furthermore, the standard addition method was used to obtain recovery values for the target analytes in newborn calf serum samples. The serum sample (1 mL) was diluted to 100 mL with 0.10 M PBS (pH 5.0) and then a 10 mL aliquot was spiked with known concentrations of AA, DA and UA.

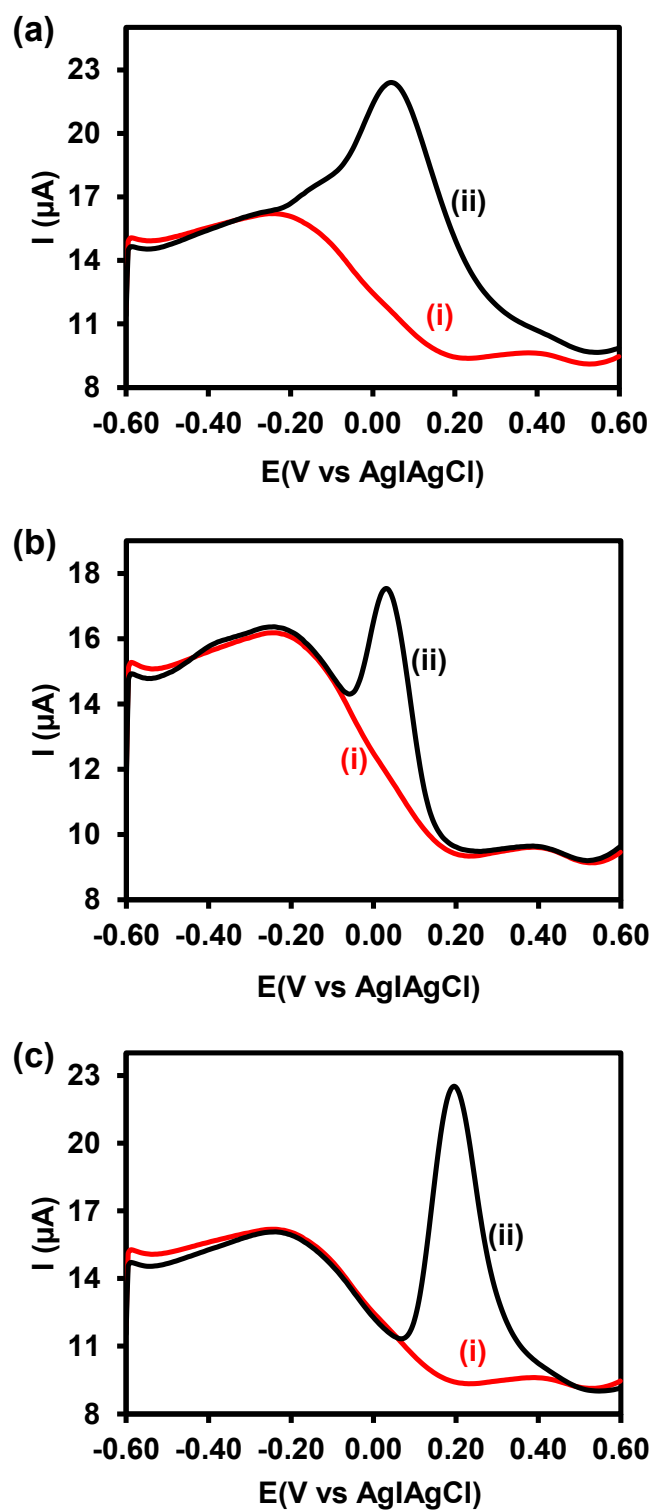


Figure 5.12: DPVs of GCE-RGO/MoS₂/Ni(OH)₂ in 1 % newborn serum in PBS (0.10 M, pH 5.0) (i) alone and (ii) containing (a) 0.40 mM AA, (b) 20 μM DA, and (c) 75 μM UA.

Table 5.3 shows the percentage recovery values ranging from 96.0 - 100.8% for AA, 92.8 - 104.2% for DA, and 99.4 - 102.3% for UA. The results demonstrated that the GCE-RGO/MoS₂/Ni(OH)₂ was successfully applied for the detection of AA, DA and UA in real sample analysis. The measured concentration were 0.40 mM, 0.80 mM and 1.0 mM for AA, 10 μ M, 15 μ M and 20 μ M for DA, and 50 μ M, 75 μ M and 0.10 mM for UA.

Table 5.3: Summary of the detection of AA, DA, and UA in newborn calf serum (n = 3) for the following concentration for AA (0.40 mM, 0.80 mM, and 1.0 mM), DA (10 μ M, 15 μ M and 20 μ M) and for UA (50 μ M, 75 μ M and 0.10 mM).

Analyte	Spiked (μ M)	Found $\pm$ SD (μ M)	Recoveries (%)
AA	400	386.2 $\pm$ 6.0	96.5
	800	805.6 $\pm$ 4.6	100.8
	1000	959.5 $\pm$ 9.7	96.0
DA	10	10.4 $\pm$ 1.6	104.2
	15	14.6 $\pm$ 1.0	97.1
	20	18.6 $\pm$ 1.0	92.8
UA	50	50.4 $\pm$ 2.5	100.8
	75	76.8 $\pm$ 4.3	102.3
	100	99.4 $\pm$ 4.6	99.4

5.4 Conclusions

A composite electrode based on reduced graphene oxide - molybdenum sulfide coated with electrocatalytic nickel hydroxide thin film was successful fabricated. The electrode was characterized using various techniques and successfully evaluated for the simultaneous detection of AA, DA and UA. The oxidation peak potentials of AA, DA and UA shifted

negatively at the GCE-RGO/MoS₂/Ni(OH)₂ with well-defined peak potential differences between AA and DA, AA and UA, and DA and UA of 97 mV, 120 mV and 217 mV, respectively. The well-resolved oxidation peak potentials demonstrate that the modified sensor is very suitable and ideal for simultaneous detection of AA, DA and UA in their ternary mixture. Comparable and low detection limits (especially for AA) were obtained at GCE-RGO/MoS₂/Ni(OH)₂ for AA, DA and UA to the previously reported work. The modified sensor, GCE-RGO/MoS₂/Ni(OH)₂, showed excellent reproducibility, and good stability. The proposed sensor was applied for the simultaneous detection of AA, DA and UA in newborn serum samples (representing real sample). Excellent results were obtained with high and reproducible percentage recoveries.

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CHAPTER 6

6 Conclusions and future perspectives

6.1 Conclusions

In this thesis, we developed simple, sensitive, selective, stable electrochemical methods based on graphene and its derivative composite electrodes on gold electrode (AuE) and glassy carbon electrode (GCE). The AuE was electrografted with a thin film of 5-diazonium isophthalic acid (IPA), which was then activated with carbodiimide to immobilize aminated graphene quantum dots (AmGQDs). Cobalt tetra carboxyphenoxy phthalocyanines (CoTCPhOPc) was immobilized using amide coupling to yield AuE-IPA-AmGQDs-CoTCPhOPc. In addition to this electrode the GCE was also fabricated using electrodeposition method to form electrochemically reduced graphene oxide (ERGO) and further cobalt tetra aminophthalocyanines (CoTAPc) was polymerized to yield GCE-ERGO/polyCoTAPc. The last surface was modified with reduced graphene oxide-molybdenum disulfide (RGO/MoS₂), and onto this nickel hydroxide thin film was electrodeposited to yield GCE-RGO/MoS₂/Ni(OH)₂. The developed graphene derivatized composite electrodes were fully characterized by spectroscopic and electrochemical techniques. The composite electrodes were evaluated their sensing properties towards the detection of catecholamine neurotransmitters.

The 5-diazonium isophthalic acid electrografted gold electrode immobilized with aminated graphene quantum dots and cobalt tetra carboxyphenoxy phthalocyanine was developed (AuE-IPA-AmGQDs-CoTCPhOPc) for in-sera selective detection of dopamine (DA), epinephrine (EP) and norepinephrine (NOR). The AuE-IPA-AmGQDs-CoTCPhOPc displayed enhanced electroactivity towards DA, EP and NOR catecholamine neurotransmitters. The modified

sensor provided excellent linear response over the range of 1.0 - 50 μM , with lower oxidation potentials. The LoDs were 0.20 μM , 0.45 μM and 0.23 μM for DA, EP and NOR, respectively and were within the ranges in biological fluids. The presence of interfering species AA did not greatly affect the sensitivity of the modified electrode for the detection of the EP and NOR catecholamine neurotransmitters. However, for DA a broad peak appeared in the presence of AA due to the electrostatic interaction between DA and AA. The developed sensor was successfully applied for detection of the three analytes in newborn calf serum samples.

The simultaneous detection of DA and PA was carried out on electroreduced graphene oxide-cobalt phthalocyanine polymer glassy electrode (GCE-ERGO/polyCoTAPc). The electrochemical behaviour of the bare GCE and modified GCE surfaces was investigated by CV and EIS in $[\text{Fe}(\text{CN})_6]^{3-/4-}$ redox probe. The GCE-ERGO/polyCoTAPc displayed excellent electrochemical performance due to excellent conductivity of ERGO and high electrocatalytic activity of polyCoTAPc. The modified sensor showed enhanced oxidation and reduction of DA and PA. The central metal cobalt was involved in the catalytic oxidation of DA and PA. The simultaneous electroanalysis at the GCE-ERGO/polyCoTAPc toward target analytes was conducted by DPV. The LoDs were 95 nM and 0.10 μM for DA and PA, respectively. The obtained LoDs were lower than the physiological levels in the human body. Furthermore, the modified sensor screened off AA and UA interferents in the detection of the target analytes. The oxidation peak currents variations were almost negligible after addition of 50 times AA and four times UA higher than the target analytes except for a broad oxidation peak for PA in the presence of UA. The modified sensor was successfully applied for detection of DA and PA in synthetic urine samples with high percentage recoveries.

Simultaneous detection of AA, DA and UA was demonstrated by using glassy carbon electrode modified with reduced graphene oxide-molybdenum disulfide-nickel hydroxide thin film, GCE-RGO/MoS₂/Ni(OH)₂. The modified electrode successfully separated the oxidation potentials of the three analytes with peak separation of 97 mV, 120 mV and 217 mV for AA-DA, DA-UA, and AA-UA, respectively. The GCE-RGO/MoS₂/Ni(OH)₂ displayed enhanced electrocatalytic activity towards DA and UA. The significantly higher electrocatalytic activities of GCE-RGO/MoS₂/Ni(OH)₂ for DA and UA was due to the honeycomb structure, which provided a larger surface area as well as affinity adsorption of DA and UA onto RGO/MoS₂ via π - π interactions and hydrogen bonding mechanisms. Since DA and UA are aromatic structured molecules, unlike AA. This led to faster electron transfer kinetics for their oxidation processes. The LoDs obtained for AA, DA and UA were 22.4 μ M, 0.030 μ M and 2.1 μ M, respectively. The high LoD for AA, indicates that the method was not very sensitive for the detection of AA.

6.2 Future Perspectives

The excellent biocompatibility of graphene and its derivative composites makes them attractive candidates for detection of biological molecules, but their application for electrochemical detection of catecholamine neurotransmitters still face challenges such as:

- Fouling and stability of graphene and its derivative based sensors in complex biological matrices affect their sensitivity.
- The presence of multiple compounds in real samples which are closely related affect their selectivity.

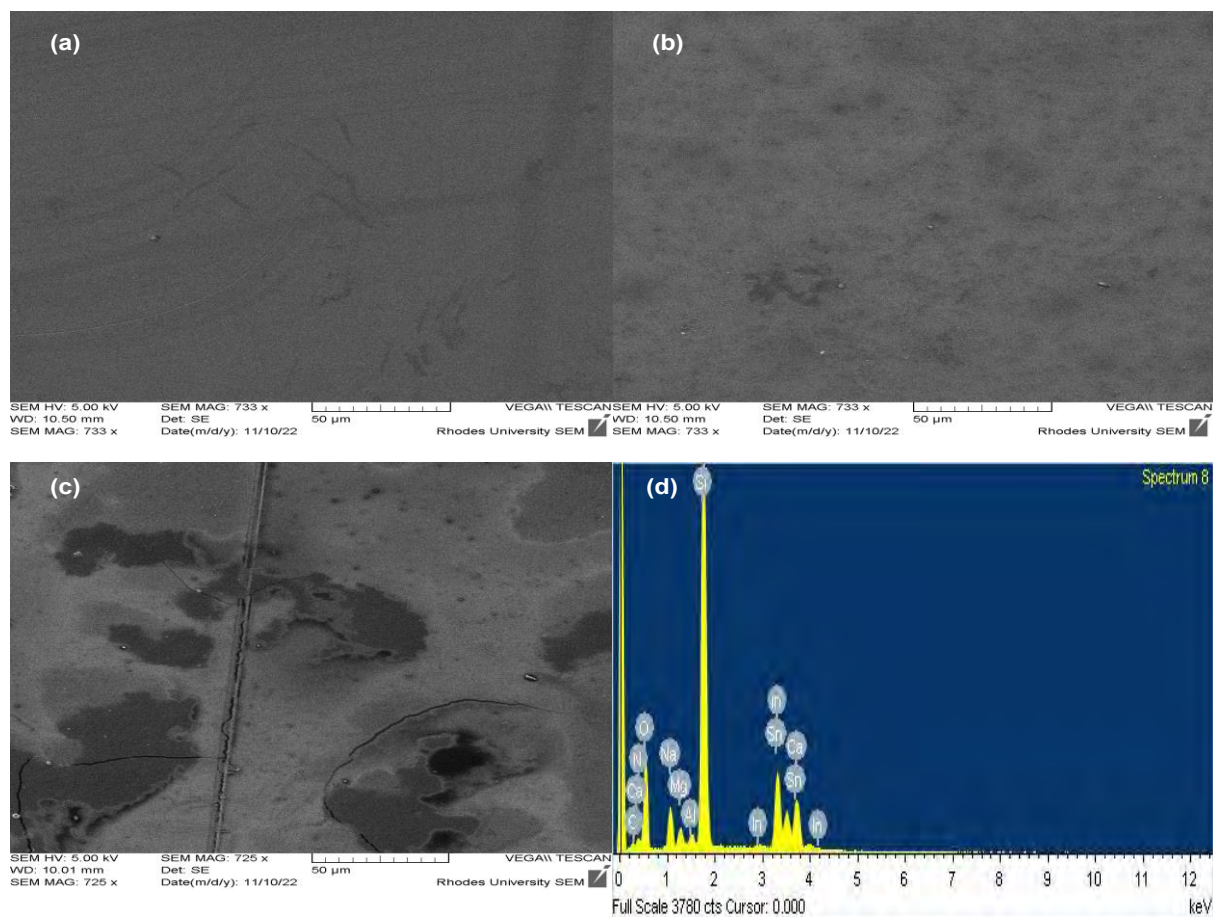
The lack of sensitivity should be improved through functionalization or hybridization with other groups that have specific affinity for given analytes. Further development in the graphene based composite materials will open a path to a development of new methods for assay of simultaneous detection of multiple analytes without any interference from each other. Development of more biocompatible graphene based composite materials that are able to monitor biological systems in real time and in vivo through miniaturization need to be further explored.

Despite the aforementioned challenges, it seems the future application of graphene and its derivative composite electrochemical sensors for detection of catecholamine neurotransmitters will continue to be a mainstay of analytical chemistry as demands for increasingly selective and sensitive detection methods grow.

7 APPENDICES

7.1 Supplementary figures from Chapter 4

The morphology of electrochemically prepared RGO on ITO was firstly characterized by SEM. As shown in **Fig. A1(b)**, a typical wrinkle-like structure appearance of electrochemical reduced RGO can be observed compared to the bare ITO in **Fig. A1(a)**.



7.2 Supplementary figures from chapter 5

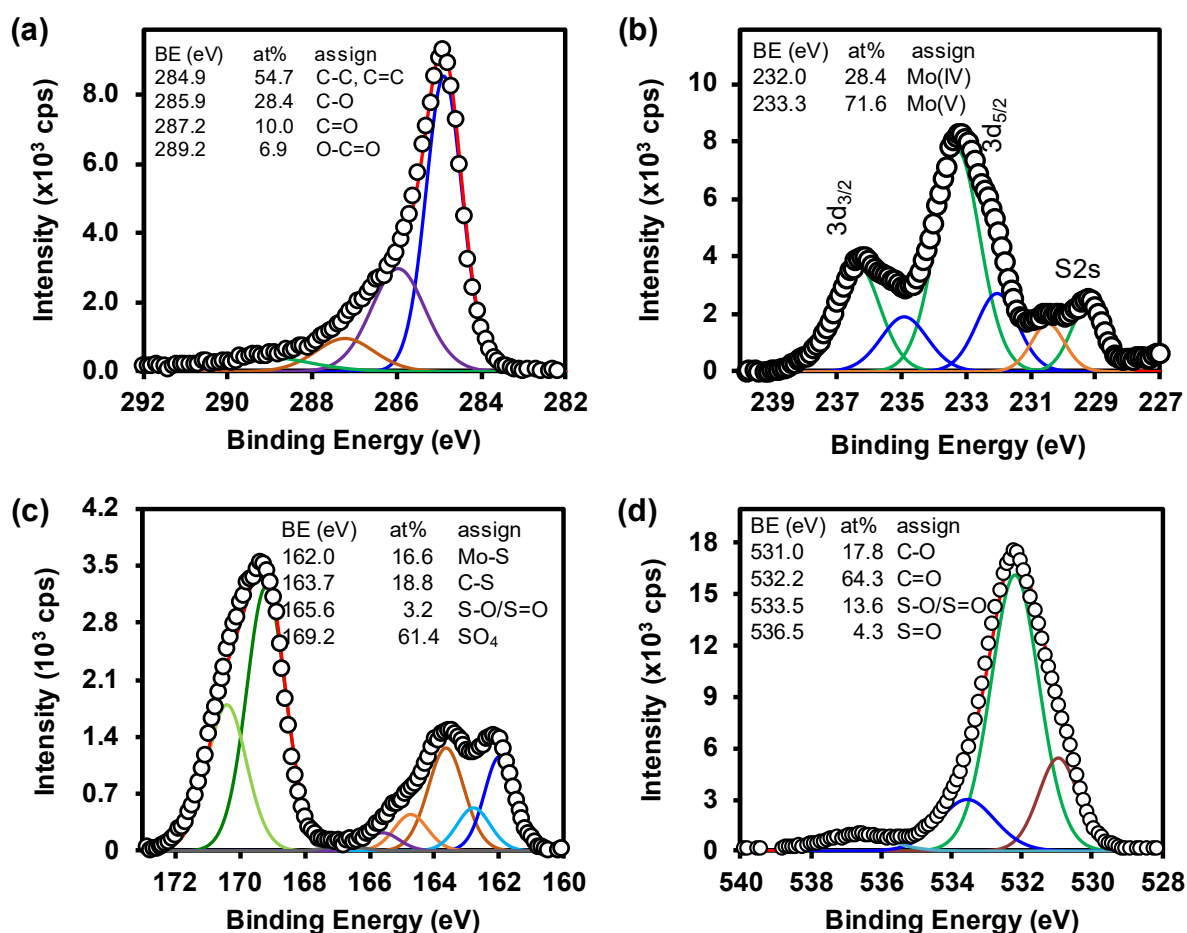


Figure A2: XPS high resolution spectra of (a) C 1s , (b) Mo 3d, (c) S 2p at GCE-RGO/MoS₂ and (d) Ni 2p at GCE-RGO/MoS₂/Ni(OH)₂

Figure A3 (a) shows CV responses of the GCE-RGO/MoS₂/Ni(OH)₂ in the potential range from -0.20 to 0.60 V at different scan rates from 10 to 50 mV.s⁻¹. An increase in oxidation and reduction current was observed and there was no shift in the peak potential confirming a good reversible system. The peak-to- peak potential separation (ΔE_p) was between 78 to 85 mV and a peak current ratio ($I_{a/c}$) was 0.86 – 0.99. The peak-to-peak potential separation is related to the electron transfer (ET) coefficient, and a low ΔE_p value indicates fast ET for a single - electron redox reaction [1]. The obtained ΔE_p values (78 to 85 mV) were with experimental

error close to the ideal value of 59 mV. The plots of oxidation and reduction peak currents against the square root of scan rate ($v^{1/2}$) between 10 – 50 mV.s^{-1} resulted in a relationship in **Fig. A3(b)**. The redox process followed a single - electron Nerstian behaviour which is controlled by semi-infinite linear diffusion from the electrolyte to the electrode surface [1].

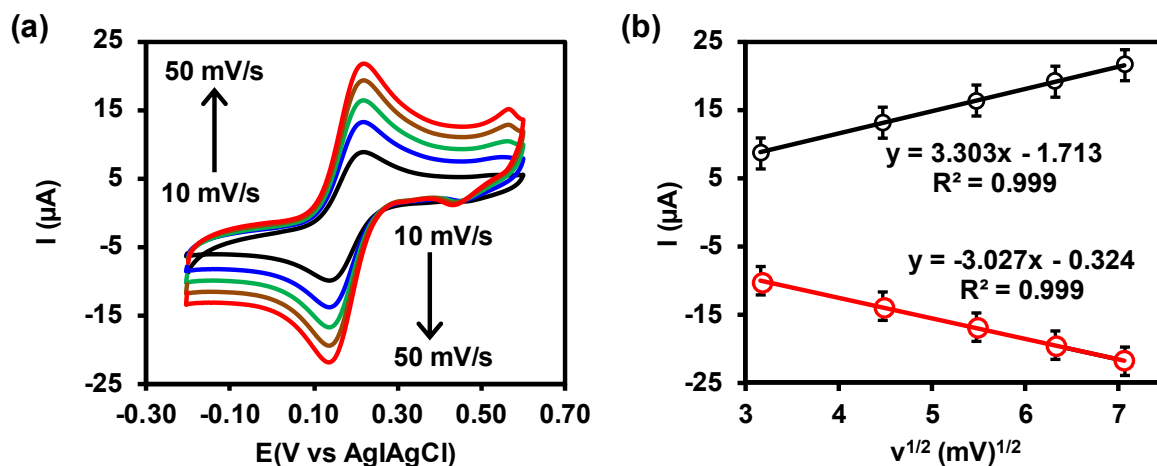


Figure A3: (a) CVs of the GCE-RGO/MoS₂/Ni(OH)₂ in 2.0 mM Fe(CN)₆³⁻/Fe(CN)₆⁴⁻ containing 0.10 M KCl with different scan rates from 10 to 50 mV.s^{-1} . (b) Oxidation and reduction peak currents versus the square root of scan rates.

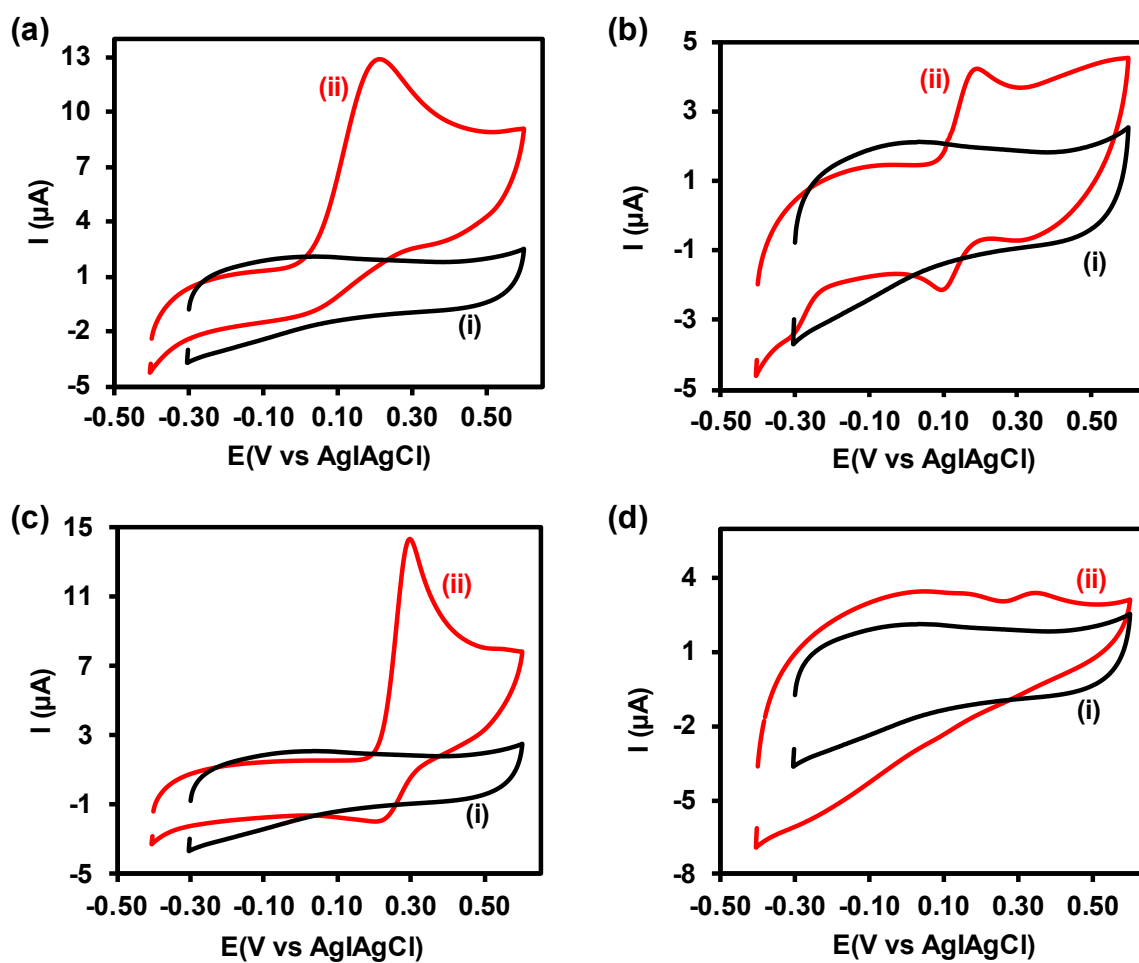


Figure A4: CVs of GCE in (i) 0.10 M PBS (pH 5.0) and (ii) containing (a) 1.0 mM AA, (b) 0.30 mM DA, and (c) 0.5 mM UA. Scan rate: $50 \text{ mV}\cdot\text{s}^{-1}$. (d) Mixture of 1.0 mM AA, 0.30 mM DA, and 0.50 mM UA.

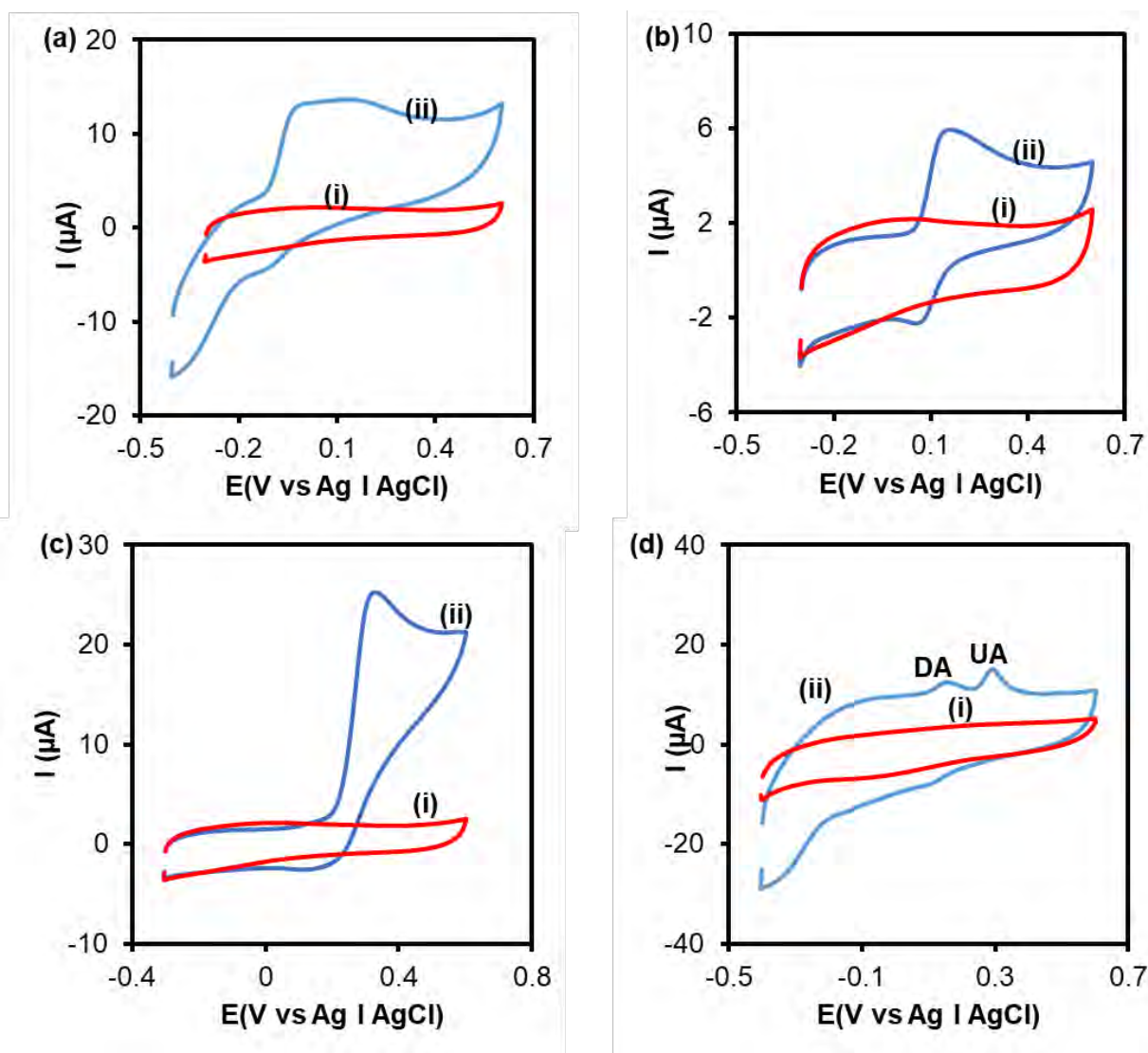


Figure A5: Electrocatalysis of (a) 1 mM AA, (b) 0.3 mM DA , (c) 0.5 mM UA and (d) mixture at GCE-RGO/MoS₂

7.3 References

- [1] N.G. Shang, P. Papakonstantinou, M. McMullan, M. Chu, A. Stamboulis, A. Potenza, S.S. Dhesi, H. Marchetto, Catalyst-free efficient growth, orientation and biosensing properties of multilayer graphene nanoflake films with sharp edge planes, *Adv. Funct. Mater.* 18 (2008) 3506–3514.