



**MARMARA UNIVERSITY
INSTITUTE FOR GRADUATE STUDIES
IN PURE AND APPLIED SCIENCES**



DEVELOPMENT OF ELECTROCHEMICAL SENSORS FOR PESTICIDE DETECTION

YELİZ İPEK

PhD THESIS

Department of Chemical Engineering

Thesis Supervisor

Prof. Dr. Atif KOCA

Thesis Co-Supervisor

Prof. Dr. Ayhan MERGEN

ISTANBUL, 2015



MARMARA UNIVERSITY
INSTITUTE FOR GRADUATE STUDIES
IN PURE AND APPLIED SCIENCES



DEVELOPMENT OF ELECTROCHEMICAL SENSORS FOR PESTICIDE DETECTION

YELİZ İPEK
(724508002)

PhD THESIS

Department of Chemical Engineering

Thesis Supervisor
Prof. Dr. Atif KOCA

Thesis Co-Supervisor
Prof. Dr. Ayhan MERGEN

ISTANBUL, 2015

ACKNOWLEDGEMENT

I would like to acknowledge Council of Higher Education for financial support with Associate Training Program (OYP), Turkish Republic Premiership, The Scientific & Technological Research Council of Turkey (TUBITAK) (Project No. 111T063), TUBA, TUBITAK Münir Bırsel Charitable Institution and research fund of Marmara University (Project Nos. FEN-C-DRP-070211-0011 and FEN-A-150513-0166) for their financial supports.

I am deeply indebted to my research advisor, Prof. Dr. Atıf KOCA, for continuously encouraging and advising me to complete my research studies. I would also like to thank him for his teaching, guidance precious suggestions, comments, and also his meticulous review of my thesis. Also, I wish to thank to my co-supervisor Prof. Dr. Ayhan MERGEN, Prof. Dr. Fatma KARACA ALBAYRAK and Aydın ALBAYRAK for their help.

I would also like to thank Prof. Dr. Halit KANTEKİN, Prof. Dr. M. Kasım ŞENER and Assoc. Prof. Dr. Hatice DİNÇER for synthesizing the valuable compounds I used for designing novel pesticide electrochemical sensor electrodes.

Thanks also go to my loyal friend Duygu ARICAN for being always there and sharing her friendship.

Finally, my special appreciations to:

My honorable and devoted parents İdris KOCA and Hatun KOCA, my dear spouse Vural İPEK, my brothers Erhan, Ayhan, Serkan, Volkan and Vedat for all their love and incessantly support and my seven month old baby Ülgen Deniz İPEK for sleeping all night. They are very special!

December, 2014

Yeliz İPEK

TABLE OF CONTENTS

	PAGE
ACKNOWLEDGEMENT	i
TABLE OF CONTENTS	ii
ÖZET	vi
ABSTRACT	vii
CLAIM FOR ORIGINALITY	viii
SYMBOLS	ix
ABBREVIATIONS	xi
LIST OF FIGURES	xiv
LIST OF TABLES	xxii
1. INTRODUCTION	1
1.1. What is Pesticide	1
1.2. Detection of Pesticides	2
1.3. Introduction to Sensors	3
1.3.1. Biosensors	3
1.3.1.1. 1 st Generation biosensors	6
1.3.1.2. 2 nd Generation biosensors	7
1.3.1.3. 3 rd Generation biosensors	7
1.3.2. Electrochemical sensors	8
1.3.2.1. Amperometric sensors	9
1.3.2.2. Potentiometric sensors	10
1.3.2.3. Impedimetric/Conductometric sensors	10
1.3.3. Electrochemical cell design	11
1.3.3.1. Cell	11

1.3.3.2. Solvent	12
1.3.3.3. Electrolytes	12
1.3.3.4. Electrodes	13
1.3.4. Electrochemical sensor design	16
1.3.5. Immobilization techniques	18
1.3.6. Phthalocyanines	20
1.3.6.1. Sensor application of phthalocyanines	21
1.4. Electroanalytical Methods	23
1.4.1. Cyclic voltammetry (CV)	24
1.4.2. Square wave voltammetry (SWV)	26
1.4.3. Chronoamperometry/Chronocoulometry (CA/CC)	27
1.4.4. Electrochemical impedance spectroscopy (EIS)	29
2. MATERIAL AND METHOD	33
2.1. Carbon Paste Electrode Preparation Technique	33
2.1.1. Materials	34
2.1.2. Electrode preparation	35
2.1.3. Electrochemical measurements	36
2.1.4. Sensor measurements	37
2.2. Thin Film Formation with Langmuir-Blodget Coating Technique	37
2.2.1. Materials	38
2.2.2. Electrode preparation	39
2.2.3. Electrochemical measurements	43
2.2.4. Sensor measurements	43
2.3. Electropolymerization Technique	44
2.3.1. Materials	45

2.3.2.	Electrode preparation	46
2.3.3.	Electrochemical measurements	47
2.3.4.	Sensor measurements	47
2.4.	Click Chemistry and Click Electrochemistry Techniques	48
2.4.1.	Materials	49
2.4.2.	Electrode preparation	50
2.4.2.1.	GCE/PANI-N ₃ /TA-MPc electrode preparation with CC	51
2.4.2.2.	GCE/PANI-N ₃ /TA-MPc electrode preparation with CEC	51
2.4.3.	Electrochemical measurements	52
2.4.4.	Sensor measurements	53
2.5.	LB-Click Electrochemistry Technique	54
2.5.1.	Materials	54
2.5.2.	Electrode preparation	54
2.5.3.	Electrochemical measurements	55
2.5.4.	Sensor measurements	56
3.	RESULTS AND DISCUSSION	57
3.1.	Modified Carbon Paste Electrode Sensor Prepared with Moulding Technique	57
3.2.	ITO/MPc-AQ Sensor Prepared with Langmuir-Blodget Coating Technique	60
3.2.1.	Electrochemical characterization of CoPc-AQ	60
3.2.2.	Sensor measurement of ITO/CoPc-AQ, ITO/CoPc-AQ-nPt and ITO/CoPc-AQ-nAu electrodes	61
3.2.3.	Electrochemical characterization of CuPc-AQ	72
3.2.4.	Sensor measurements of ITO/CuPc-AQ, ITO/CuPc-AQ-nPt and ITO/CuPc-AQ-nAu electrodes	72

3.3.	GCE/poly-MPc Sensor Prepared with Electro-Polymerization Technique	81
3.3.1.	Electrochemical characterization of H ₂ Pc-THQD and NiPc-THQD	81
3.3.2.	Sensor measurements of GCE/polyH ₂ Pc-THQD and GCE/polyNiPc-THQD electrodes	84
3.4.	GCE/PANI-N ₃ /TA-MPc (CEC) Sensor Prepared with Click Electrochemistry Technique	93
3.4.1.	Electrochemical characterization of TA-MPc	93
3.4.2.	Sensor measurements of GCE/PANI-N ₃ /TA-MPc electrodes	101
3.5.	ITO/TA-CoPc/PANI-N ₃ Sensor Measurements Prepared with LB-Click Electrochemistry Technique	117
4.	CONCLUSIONS	121
	REFERENCES	124
	AUTOBIOGRAPHY	

ÖZET

PESTİSİT TAYİNİ İÇİN ELEKTROKİMYASAL SENSÖRLERİN GELİŞTİRİLMESİ

Çiftçiler, ürünlerini ve tohumlarını korumak ve hasat verimini arttırmak için sayısız türde pestisit kullanmaktadır. Ancak, pestisit kalıntıları ekosistem ve gıda zincirine; hava, su ve toprak yoluyla girebilmektedir. İnsanlar ve hayvanlar için kanserojen ve sitotoksik olmaları nedeniyle, tarımda giderek artan bir şekilde kullanılmakta ve en önemli çevre kirliliği etkeni olmaktadır. Bu kimyasalların saptanması için geleneksel metotlar zaman kaybettirici, pahalı olmakta ve donanımlı teknisyenlerin varlığını gerektirmektedirler. Bu zorlukların üstesinden gelmek için, son zamanlarda pestisitlerin analizi için elektrokimyasal biyosensörler gibi yeni alternatif metotlar üzerinde çalışılmaktadır. Bu amaçla, bu çalışmada yeni biyosensör hazırlama teknikleri geliştirilmiştir. Bu teknikler; kalıplama tekniği ile karbon pasta elektrot hazırlama tekniği, Langmuir-Blodgett kaplama ile ince film oluşturma tekniği, elektropolimerizasyon tekniği, klik elektrokimyası tekniği (CEC) ve LB-klik elektrokimyası tekniğidir.

Hazırlanan elektrotlar; voltametrik teknikler, elektrokimyasal empedans spektroskopisi (EIS), ve taramalı elektron mikroskobu (SEM) ile karakterize edilmiştir. GCE/PANI-N₃/TA-CoPc elektrodu eserine karşı 28.67 V/ μ M hassasiyetiyle birlikte en düşük saptama limiti olan 3.84×10^{-10} M değerine sahiptir. Seçicilik parametresine göre, CEC tekniği ile hazırlanmış GCE/PANI-N₃/TA-CoPc elektrodu, bu çalışmada tasarlanan elektrotlar arasında seçiciliği en yüksek elektrottur. Elektrotların stabiliteleri karşılaştırıldığında GCE/PANI-N₃/TA-MPc elektrotlarının kimyasal tekniklerle modifiye edilmiş olmasından dolayı raf ömürlerinin daha yüksek olduğu görülmüştür. CEC tekniği ile modifiye edilmiş elektrot oda koşullarında iki ay saklanmış olduğu halde, pestisitlere karşı kayda değer elektrokimyasal yanıtlar vermiştir. Tasarlanan tüm elektrotların yanıt süreleri analitleri birkaç saniyede algılamış olmalarından dolayı tatmin edicidir.

ABSTRACT

DEVELOPMENT OF ELECTROCHEMICAL SENSORS FOR PESTICIDE DETECTION

Farmers use numerous types of pesticides to protect crops and seeds and to increase the harvesting yield. However, the pesticide residues may enter into the ecosystem and food chain through air, water and soil. Their increasing use in agriculture makes them the most important environmental pollutants since they are carcinogenic and cytotoxic for animals and human. For detection of these chemicals, traditional methods are time consuming, expensive and require highly equipped technicians. To overcome these difficulties, recently new alternative methods such as electrochemical biosensors for the analysis of pesticides are being studied. For this purpose, novel biosensor preparation techniques were developed in this study. These techniques are; carbon paste electrode preparation with moulding technique, thin film formation with Langmuir-Blodgett coating technique, electropolymerization technique, click electrochemistry technique (CEC) and LB-Click Electrochemistry technique.

Prepared electrodes were characterized with voltammetric techniques, electrochemical impedance spectroscopy (EIS), and scanning electron microscopy (SEM). GCE/PANI-N₃/TA-CoPc electrode has the lowest **detection limit** and **sensitivity** of 3.84×10^{-10} M and 28.67 V/ μ M respectively against eserine. According to **selectivity** parameter, GCE/PANI-N₃/TA-CoPc electrode prepared with CEC technique is the most selective electrode among these designed electrodes. When the **stability** of the electrodes were compared, GCE/PANI-N₃/TA-MPc electrodes had longer shelf lives than the other electrodes because of chemically modification of the electrode. Even two months later, the modified electrode with CEC technique stored at room conditions gave valuable electrochemical responses to the pesticides. The **response time** of the designed electrodes were all satisfactory that they recognized analytes in a few seconds.

CLAIM FOR ORIGINALITY

Detection of pesticides at the levels established by the Environmental Protection Agency (EPA) remains a challenge[1, 2]. Traditional detection methods have been used because of their sensitivity and reliability[3, 4]. However, instead of these time-consuming and requiring expensive equipment and highly trained technicians new detection methods are required. Thus, for pesticide detection, AChE enzyme has frequently been used as the recognition element of the biosensor because of the inhibitory effect of pesticides on this enzyme. However, difficulties of immobilization of the receptor material on the substrates, leakage from the substrate surface, high cost, and low chemical and thermal stabilities are some disadvantages of enzyme biosensors[5].

In this study, different pesticide biosensors were designed with some novel phthalocyanines, as biomimetic recognition elements, using five novel immobilization techniques. These immobilization techniques are:

1. Moulding Technique
2. Langmuir-Blodgett Coating Technique
3. Electropolymerization Technique
4. Click Electrochemistry Technique
5. LB-Click Electrochemistry Technique.

Although, the electropolymerization and Langmuir-Blodgett techniques are used as sensor preparation methods in the literature, the phthalocyanines; **H₂Pc-THQD**, **NiPc-THQD**, **CoPc-AQ** and **CuPc-AQ** was not reported as pesticide biosensor recognition materials before. Moulding technique, click electrochemistry and LB-Click Electrochemistry are developed in the this study for the first time. Also the novel phthalocyanines (MPc-THQD, MPc-AQ and TA-MPc) as recognition materials were used with these techniques first-ever. The lowest detection limit was achieved by **GCE/PANI-N₃/TA-CoPc** electrode with a LOD of 3.84×10^{-10} M against eserine.

.

SYMBOLS

A	: Amper
AC	: Sinusoidal current (alternative current)
C	: Counter electrode
C	: Bulk concentration
C_{LOD}	: Smallest detectable concentration
E (t)	: Potential at time t
E	: Potential (V)
E°	: Standard electrode potential
E₀	: Amplitude of the signal
Ep(a)	: Anodic peak potential
Ep(c)	: Cathodic peak potential
f	: Frequency
F	: Faraday's constant
I	: Current (A)
Ip(a)	: Anodic peak current
Ip(c)	: Cathodic peak current
i	: Current
k_m	: Mass transfer coefficient
M	: Molarity
n	: Electron transfer number
O^a	: Anodic oxidation reaction/peak
O^c	: Cathodic oxidation reaction/peak
P^a	: Anodic polymerization peak

P^c	: Cathodic polymerization peak
Q	: Relative activity of the ion
R	: Electrical resistance
R	: Gas constant
R	: Reference electrode
R^a	: Anodic reduction reaction/peak
R^c	: Cathodic reduction reaction/peak
S	: Sensitivity
std_{background}	: Standard deviation
t	: Time
T	: Temperature (°C)
V	: Volt
W	: Working electrode
WS	: Working sense electrode
Z	: Impedance
φ	: Shift in phase
Ω	: Ohm
ω	: Radial frequency

ABBREVIATIONS

ACh	: Acetyl Choline
AChE	: Acetyl Choline Esterase enzyme
Amp.	: Amperometric
ANSI	: American National Standards Institute
AQ	: Anthraquinone substituted
CA	: Chrono-Amperometry
CC	: Chrono-Coulometry
CC	: Click Chemistry
CEC	: Click electrochemistry
CPE	: Carbon Paste Electrode
CV	: Cyclic Voltametry
DCM	: Dichloromethane
DDT	: Dikloro difenil trikloroethan
DMF	: Dimethlyformamide
DMSO	: Dimethyl sulfoxide
DPSCC	: Double potential step chronocoulometry
EIS	: Electrochemical impedance spectroscopy
EPA	: Environmental Protection Agency

EPS	: Electrochemical pesticide sensors
GC	: Gas Chromatography
GCE	: Glassy carbon electrode
GOD	: Glucose oxidase
HPLC	: High Performance Liquid Chromatography
Imp.	: Impedimetric
ITO	: Indium tin oxide coated glass electrode
IUPAC	: The International Union of Pure and Applied Chemistry
LB	: Langmuir-Blodgett coating technique
LOD	: Limit of detection
MPcs	: Metallophthalocyanines
MS	: Mass Chromatography
PBS	: Phosphate buffer solution
Pc	: Phthalocyanine
Pot.	: Potentiometric
SA	: Stearic acid
SCE	: Saturated calomel electrode
SDS	: Sodium dodecyl sulfate
SEM	: Scanning electron microscopy
SWV	: Square Wave Voltametry

TA	: Terminal alkynyl
TBAP	: Tetrabutylammonium perchlorate
THQD	: Peripherally 2-(2,2,4,7-tetramethyl-3,4-dihydroquinolin-1(2H)-yl) ethanol
Tw	: This work
USDA	: U.S. Department Agriculture

LIST OF FIGURES

	PAGE
Figure 1.1. Classification of pesticides.	2
Figure 1.2. Commercial examples of biosensors; (A) Blood glucose sensor, (B) Pregnancy test sensor.	4
Figure 1.3. A typical biosensor structure.	5
Figure 1.4. Hierarchical classification of biosensors according to transducer type.	5
Figure 1.5. Set up of an electrochemical cell.	12
Figure 1.6. 2-electrode cell setup.	14
Figure 1.7. Measured (sample) potential map across a whole cell: The Working lead is at point A and the Counter lead at point E.	14
Figure 1.8. 3-electrode cell setup.	15
Figure 1.9. 4-electrode cell setup.	16
Figure 1.10. Basic chemical structure of phthalocyanines.	20
Figure 1.11. Specific electrochemical techniques.	23
Figure 1.12. A typical scan of potential as a function of time.	24
Figure 1.13. A typical reversible cyclic voltammogram.	25
Figure 1.14. The cyclic voltammogram of paracetamol.	26
Figure 1.15. Diagram of applied potential for SWV technique.	26
Figure 1.16. A typical SWV measurement result.	27

Figure 1.17. Potential wave form for chronoamperometry and chronocoulometry.	28
Figure 1.18. The diffusion effect at an electrode for a potential step redox process.	28
Figure 1.19. The current-time profile associated with two potential steps redox process.	29
Figure 1.20. Sinusoidal current response.	30
Figure 1.21. Origin of Lissajous Figure.	31
Figure 1.22. A Nyquist Plot with impedance vector.	32
Figure 1.23. Bode Plot with one time constant.	32
Figure 2.1. Carbon paste electrode.	34
Figure 2.2. The chemical structure of synthesized CoPc-AQ .	34
Figure 2.3. Mechanical drawing of designed pelletizing mould.	35
Figure 2.4. Carbon paste electrode preparation.	36
Figure 2.5. KSV NIMA Langmuir-Blodgett deposition trough.	39
Figure 2.6. (A) The surface pressure (π)-area isotherm of CoPc-AQ , SA, and CoPc-AQ +SA mixture (1:1), (B) Transfer ratio-layer no changes of CoPc-AQ and CoPc-AQ +SA mixture (1:1) during upstroke and down stroke film transition on to ITO substrates.	39
Figure 2.7. Chemical structure of CuPc-AQ .	41
Figure 2.8. LB studies of CuPc-AQ : (A) the surface pressure-Mean molecular area isotherm of SA, CuPc-AQ and CuPc-AQ +SA mixture and (B) transfer ratios of CuPc-AQ and CuPc-AQ +SA.	41

Figure 2.9. Construction of electrochemical pesticide sensor electrodes, ITO/ CoPc-AQ , ITO/ CoPc-AQ -nPt, and ITO/ CoPc-AQ -nAu.	42
Figure 2.10. Chemical structure of the complexes of H₂Pc-THQD and NiPc-THQD phthalocyanines.	45
Figure 2.11. Electropolymerization of H₂Pc-THQD on the electrode with CV technique.	46
Figure 2.12. Basic reaction mechanism of click chemistry.	48
Figure 2.13. Chemical structure of terminal alkynyl-substituted cobalt phthalocyanine (TA-CoPc:2,9(10),16(17),23(24)-tetrakis(pent-4-ynoxy) phthalocyanine.	50
Figure 2.14. Electrode modification via the “click chemistry” (CC) and the “click elec-trochemistry” (CEC).	51
Figure 2.15. LB-Click Electrochemistry modification technique.	55
Figure 3.1. SWV analysis of eserine with CPE/ CoPc-AQ biosensor.	57
Figure 3.2. Amperometric (A) and potentiometric (B) calibration lines derived from SWV measurements of the CPE/ CoPc-AQ electrode titrated with eserine.	58
Figure 3.3. EIS measurement result of the eserine titration with CPE/ CoPc-AQ and its calibration line in the inset graph.	59
Figure 3.4. DPSCC analysis responses of eserine titration with CPE/ CoPc-AQ biosensor.	60
Figure 3.5. CV and SWV of CoPc-AQ (5.0×10^{-4} M) at 100 mVs ⁻¹ scan rate on Pt in DCM/TBAP electrolyte system.	1

Figure 3.6. SWV responses of ITO/ CoPc-AQ electrodes in PBS (pH= 7.0) during the gradual addition of eserine (A) and carbofuran (B).	62
Figure 3.7. SWV responses of ITO/ CoPc-AQ electrodes in PBS (pH= 7.0) (A) during gradual addition of a mixture of eserine, E and carbofuran, C and (B) during sequential addition of carbofuran and eserine.	63
Figure 3.8. (A) EIS responses of ITO/ CoPc-AQ electrodes in PBS (pH= 7.0) during gradual addition of eserine (B) calibration line ((eserine) vs Z_{real} graph) of eserine sensing measurements derived from EIS responses of ITO/ CoPc-AQ electrodes.	65
Figure 3.9. DPSCC responses of ITO/ CoPc-AQ electrodes in PBS (pH= 7.0) during the gradual addition of eserine.	66
Figure 3.10. SEM images of ITO/ CoPc-AQ -nPt electrode; A) Top view of the film, B) Side view of the film.	67
Figure 3.11. SWV responses of the ITO/ CoPc-AQ -nPt electrode in PBS (pH= 7.0) during the gradual addition of eserine.	68
Figure 3.12. (A) EIS responses of ITO/ CoPc-AQ -nPt electrodes in PBS (pH= 7.0) during the gradual addition of eserine (B) calibration line ([eserine] vs Z_{real} graph) of the eserine sensing measurements derived from EIS responses of ITO/ CoPc-AQ -nPt electrodes.	69
Figure 3.13. DPSCC responses of ITO/ CoPc-AQ -nPt electrodes in PBS (pH= 7.0) during the gradual addition of eserine; (A) first DPSCC cycle, (B) all DPSCC cycles.	70
Figure 3.14. SWV responses of ITO/ CoPc-AQ -nAu electrodes in PBS (pH= 7.0) during the gradual addition of eserine.	71
Figure 3.15. Electrochemical responses of CuPc-AQ in DCM solution.	72

Figure 3.16. The SWV responses of ITO/ CuPc-AQ while gradual addition of (A) carbofuran and (B) eserine.	73
Figure 3.17. (A) The EIS responses of ITO/ CuPc-AQ while gradual addition of carbofuran and (B) its calibration line.	75
Figure 3.18. DPSCC analyses of the sensing measurements for ITO/ CuPc-AQ electrodes; (A) carbofuran and (B) eserine.	77
Figure 3.19. The SWV measurement results of ITO/ CuPc-AQ-nPt during gradual addition of (A) carbofuran and (B) eserine.	78
Figure 3.20. (A) The EIS responses of ITO/ CuPc-AQ-nPt during gradual addition of eserine and (B) its calibration line.	79
Figure 3.21. DPSCC sensor measurement results of ITO/ CuPc-AQ-nPt electrode against (A) carbofuran and (B) eserine.	80
Figure 3.22. SWV diagrams of ITO/ CuPc-AQ-nAu electrode with addition of (A) carbofuran and (B) eserine.	81
Figure 3.23. CV responses of H₂Pc-THQD at 100 Vs ⁻¹ scan rate.	82
Figure 3.24. CV responses of NiPc-THQD at 100 Vs ⁻¹ scan rate.	82
Figure 3.25. Repetitive CVs of NiPc-THQD during the anodic potential scan at 100 mVs ⁻¹ .	83
Figure 3.26. SEM images of the (A) poly-H₂Pc-THQD thin film, (B) poly-NiPc-THQD thin film and (C) Scrapped poly-H₂Pc-THQD thin film formed with CV technique.	84
Figure 3.27. SWV responses of GCE/ poly-H₂Pc-THQD sensor against four different pesticides: (A) carbaryl, (B) carbofuran, (C) eserine and (D) parathion.	85

Figure 3.28. (A) DPSCC result of GCE/ poly-H₂Pc-THQD electrode that titrated with eserine and (B) its calibration line.	86
Figure 3.29. (A) The EIS responses of GCE/ poly-H₂Pc-THQD electrode against eserine and (B) its calibration line.	87
Figure 3.30. SWV measurent results of GCE/ poly-NiPc-THQD electrode with four different pesticides: (A) carbofuran, (B) carbaryl, (C) eserine and (D) parathion.	89
Figure 3.31. (A) DPSCC responses of GCE/ poly-NiPc-THQD electrode against parathion pesticide and (B) ist calibration line.	90
Figure 3.32. (A) EIS results of the parathion titration with GCE/ poly-NiPc-THQD electrode and (B) its calibration line.	91
Figure 3.33. (A) CV and (B) SWV of TA-CoPc (5.0×10^{-4} mol dm ⁻³) at various scan rates on GCE in DMSO/TBAP electrolyte system.	94
Figure 3.34. Repetitive CVs of ANI-N ₃ (5.0×10^{-4} mol dm ⁻³) at 100 mV s ⁻¹ scan rate on GCE in DCM/TBAP electrolyte system.	95
Figure 3.35. Repetitive CVs of GCE/PANI-N ₃ working electrode after 10 min constant applied potential at -0.15 V (CA) in TA-CoPc and CuSO ₄ dissolved DMSO/TBAP electrolyte system at 0.100 V s ⁻¹ scan rate.	96
Figure 3.36. SWV responses of bare GCE, GCE/PANI-N ₃ , GCE/PANI-N ₃ / TA-CoPc (CC), and GCE/PANI-N ₃ / TA-CoPc (CEC) electrodes in (A) DMSO/TBAP and (B) H ₂ O/LiClO ₄ electrolytes	98
Figure 3.37. (A) UV-vis spectra of ITO/PANI-N ₃ and ITO/PANI-N ₃ / TA-CoPc electrodes in DMSO, (B) EIS of bare GCE, GCE/PANI-N ₃ , GCE/PANI-N ₃ / TA-CoPc (CC), and GCE/PANI-N ₃ / TA-CoPc (CEC) electrodes in H ₂ O/LiClO ₄ electrolyte.	99

Figure 3.38. SEM images of (A) ITO/PANI-N ₃ and (B) ITO/PANI-N ₃ /TA-CoPc (CEC) electrodes.	100
Figure 3.39. SWV of (A) TA-H ₂ Pc (5.0×10^{-4} mol dm ⁻³) and (B) TA-ZnPc (5.0×10^{-4} mol dm ⁻³) on GCE in DMSO/TBAP electrolyte system.	101
Figure 3.40. SWV responses of (A) GCE/PANI-N ₃ /TA-H ₂ Pc, (B) GCE/PANI-N ₃ /TA-ZnPc and (C) GCE/PANI-N ₃ /TA-CoPc electrodes in PBS (PH=7.0) during the gradual addition of eserine.	103
Figure 3.41. SWV responses of (A) GCE/PANI-N ₃ /TA-H ₂ Pc, (B) GCE/PANI-N ₃ /TA-ZnPc and (C) GCE/PANI-N ₃ /TA-CoPc electrodes in PBS (PH=7.0) during the gradual addition of parathion	105
Figure 3.42. SWV responses of (A) GCE/PANI-N ₃ /TA-H ₂ Pc, (B) GCE/PANI-N ₃ /TA-ZnPc and (C) GCE/PANI-N ₃ /TA-CoPc electrodes in PBS (PH=7.0) during the gradual addition of carbaryl.	107
Figure 3.43. SWV responses of (A) GCE/PANI-N ₃ /TA-H ₂ Pc, (B) GCE/PANI-N ₃ /TA-ZnPc and (C) GCE/PANI-N ₃ /TA-CoPc electrodes in PBS (PH=7.0) during the gradual addition of carbofuran.	108
Figure 3.44. Calibration curves of potentiometric and amperometric sensing measurements derived from the SWV titration of GCE/PANI-N ₃ /TA-CoPc with eserine.	114
Figure 3.45. (A) EIS responses and (B) Impedimetric calibration line of GCE/PANI-N ₃ /TA-CoPc electrode in PBS (PH=7.0) during the gradual addition of eserine.	115
Figure 3.46. DPSCC responses of GCE/PANI-N ₃ /TA-CoPc electrodes in PBS (PH=7.0) during the gradual addition of with eserine.	116
Figure 3.47. SWV responses of ITO/TA-CoPc/PANI-N ₃ electrodes in	

PBS (PH=7.0) during the gradual addition of eserine. 117

Figure 3.48. EIS responses of ITO/TA-CoPc/PANI-N₃ electrodes in PBS (PH=7.0) during the gradual addition of eserine 118

Figure 3.49. DPSCC responses of ITO/TA-CoPc/PANI-N₃ electrodes in PBS (PH=7.0) during the gradual addition of eserine 119

LIST OF TABLES

	PAGE
Table 3.1. The linear detection range and limit of detection LOD values of CPE/CoPc-AQ electrode for eserine analysis.	58
Table 3.2. Basic sensing paremeters obtained with different techniques for the sensing of the pesticides with various modified electrodes.	64
Table 3.3. Sensor paremeters of modified CPE/CuPc-AQ electrodes for pesticide detection.	76
Table 3.4. Amperometric, potentiometric, impedimetric and chronocoulometric sensor parameters of GCE/poly-MPcs against four different pesticides.	92
Table 3.5. Detection limits and linear range data obtained with different techniques for the sensing of the pesticides with GCE/PANI-N₃/TA-H₂Pc electrodes .	111
Table 3.6. Detection limits and linear range data obtained with different techniques for the sensing of the pesticides with GCE/PANI-N₃/TA-ZnPc electrodes.	112
Table 3.7. Detection limits and linear range data obtained with different techniques for the sensing of the pesticides with GCE/PANI-N₃/TA-CoPc electrodes.	113
Table 3.8. Detection limit and linear range data obtained with different techniques for the sensing of eserine with ITO/TA-CoPc/PANI-N₃ electrode.	120

1. INTRODUCTION

1.1. What is Pesticide

Pest term is used to utter an organism with damaging or unwanted characteristics, as it harms agriculture through feeding on crops or parasitizing livestock. Pesticide is a term used in broad sense for organic toxic compounds used to control insects, bacteria, weeds, nematodes, rodents and other pests. These chemicals were discovered and synthesized by German scientists during World War II about 1940s. There are two types of pesticides; first-generation and second-generation pesticides. The first-generation pesticides are compounds such as arsenic, mercury, and lead which were used before 1940. They were prohibited because of being highly toxic and ineffective. The second-generation pesticides are synthetic organic compounds. After Paul Müller discovered DDT in 1939, usage of these pesticides accelerated in late 1940s[6].

Although they were synthesized to be used as chemical warfare agents, they were not used during the war but the farmers have been using various pesticides to save the agricultural products and increase the harvesting yield, since then. Hunger is still one of the important problems in the world. The increasing human population and decreasing agricultural areas, because of the urbanization activities, cause food deficiency in the world. Farmers use numerous types of pesticides (herbicides, fungicides, insecticides), to protect crops and seeds and to increase the harvesting yield. However, the pesticide residues may enter into the ecosystem and food chain through air, water and soil and affect the ecosystem.

Pesticides are classified into two main groups called as chemical pesticides and biopesticides (Figure 1.1). Pesticides can also be classified as insecticides, herbicides and fungicides or organophosphorous, organochlorines and carbamates[7]. The pesticides do not only affect human health, but also they are hazardous to the environment and ecosystem. Although the biological pesticides are environmentally safer than the chemical pesticides, they have lower popularity because of less social awareness, comparatively lower crop yields, need for frequent applications, and less worked research area[8].

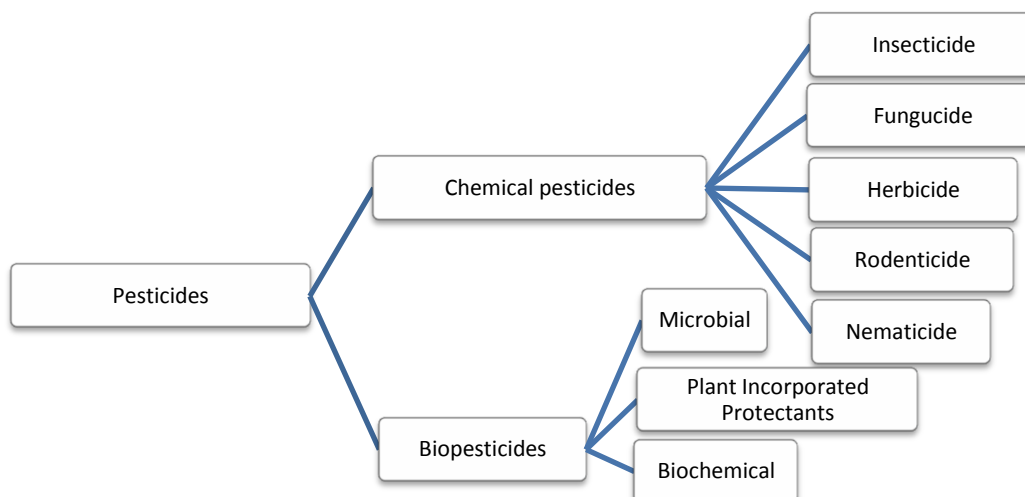


Figure 1.1. Classification of pesticides.

Increasing use of pesticides in agricultural activities makes them the most important environmental pollutants since they are carcinogenic and cytotoxic for animals and human. Their toxicity is based on the inhibition effect on acetylcholinesterase (AChE) enzyme which has an important mission in neurologic system of animals and human. Acetylcholine (ACh) is one of the numerous neurotransmitters in the nervous system and it has important roles in both peripheral nervous system and central nervous system. ACh carries the synaptic pulses between two neurons and when binds to the target synapse AChE which exists in the synaptic space, hydrolyzes ACh into acetate and choline rapidly. Thus, as AChE inhibitor, pesticides lead accumulation of ACh at cholinergic synapses. As a result of this accumulation, bone marrow disorders, nerve disorders such as alzheimer, infertility, and immunological and respiratory diseases may be occurred. Detection of pesticides at the levels established by the Environmental Protection Agency (EPA) remains a challenge.

1.2. Detection of Pesticides

Pesticide residues accumulate up to harmful levels in the body as well as in the environment. Persistent pesticide residuals can exposure through the food chain ranging from meat, poultry, and fish, to vegetable oils, nuts, and various fruits and vegetables.

Detection of pesticides at the levels established by the Environmental Protection Agency (EPA) remains a challenge[1, 2]. For this purpose, there are some traditional detection methods such as gas chromatography (GC), high performance liquid

chromatography (HPLC) combined with mass spectrometry (MS) [3, 4]. Although these detection methods are time-consuming and require expensive equipment and highly trained technicians, they have been used traditionally because of their sensitivity and reliability. The developing science and technology is leading new alternative detection methods for pesticides like electrochemical sensors or biosensors. For designing pesticide biosensors, AChE enzyme has frequently been used because the inhibitory effect of pesticides on this enzyme. However, low stability of the biological species, difficulties of immobilization on the substrates, leakage from the substrate surface, high cost, and low chemical and thermal stabilities are some disadvantages of enzyme biosensors[5].

1.3. Introduction to Sensors

American National Standards Institute (ANSI) describe the sensor term as a device which provides a usable output in response to a specified measurand. The sensor requires a measurable physical quantity to convert it into a processable electrical signal with an active element called transducer. Transducer is a device which converts physical or electrical energies to another. When the transducer convert electrical energy into physical output it is called actuator, otherwise, when it converts physical energy into electrical output it is called sensor. Sensor and transducer terms can be used instead of each other. In 1975 it is stated in the ANSI standards that transducer term should be referred to sensor term but in scientific literature sensor term is preferred[9].

If the material used as an active element in the sensor is developed with developing science and technology, the sensor properties will also be developed as more sensitive, reliable and stable. For instance, Wilhelm von Siemens used a copper resistor to develop a simple temperature sensor in 1860s, but a single-crystal quartz has high resonance stability and piezoelectric properties that made an extraordinarily wide range of high performance temperature sensors possible that have played an important role in everyday life and national defence.

1.3.1. Biosensors

Biosensors can be used in clinical analysis, in online control processes for industry or environment, or even in vivo studies. The difference between biosensor and physical or

chemical sensors is that its recognition element is biological.

The use of biosensors for industrial, medical and environmental analysis is very important. The control of food manufacturing processes, evaluation of food quality, control of fermentation processes and for monitoring of organic pollutants are some of the applications of biosensors. The present popularity of analytical biosensors is due to their specific detection, simple use and low cost. Blood glucose detection devices and pregnancy tests are the most common used biosensors exhibited in Figure 1.2.



Figure 1.2. Commercial examples of biosensors; **(A)** Blood glucose sensor, **(B)** Pregnancy test sensor.

There is a need for simple, rapid and reagentless method for specific determination, both qualitative and quantitative, of various compounds in veterinary medicine, agrifoods, horticulture, pharmaceuticals, petrochemical industries, environment surveillance, defense and security. The requirement for accurate and fast determination is particularly required in human health care. Conventional chemical determinations suffer from lack of specificity and a host of interferences, besides being slow and expensive. Biosensors are important today because they can sense what many other traditional sensing systems cannot. According an IUPAC nomenclature, biosensor is a device that uses specific biochemical reactions mediated by isolated enzymes, immunosystems, tissues, organelles or whole cells to detect chemical compounds usually by electrical, thermal or optical signals.

Beginning of biosensors may be dated to 1962, when Clark, known as the father of the biosensor concept, published an experiment in which glucose oxidase (GOD) was entrapped at a Clark oxygen electrode using dialysis membrane[10]. As bio-components, an enzyme, antibody, nucleic acid, lectine, hormone, cell structure or tissue to interact specifically with the target analyte and the result of biochemical

reaction is consequently transformed through transducer to measurable signal. A typical biosensor structure is exhibited in Figure 1.3.

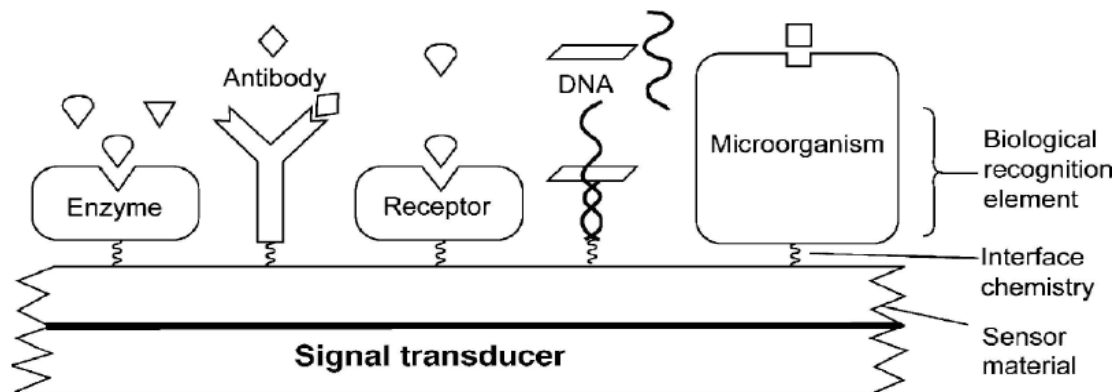


Figure 1.3. A typical biosensor structure[11].

The transducing systems can be electrochemical, optical, piezoelectric, thermometric, ion-sensitive, magnetic or acoustic one. Biosensors can be classified according to the type of the transducer. The basic classification of the biosensors is exhibited in Figure 1.4.

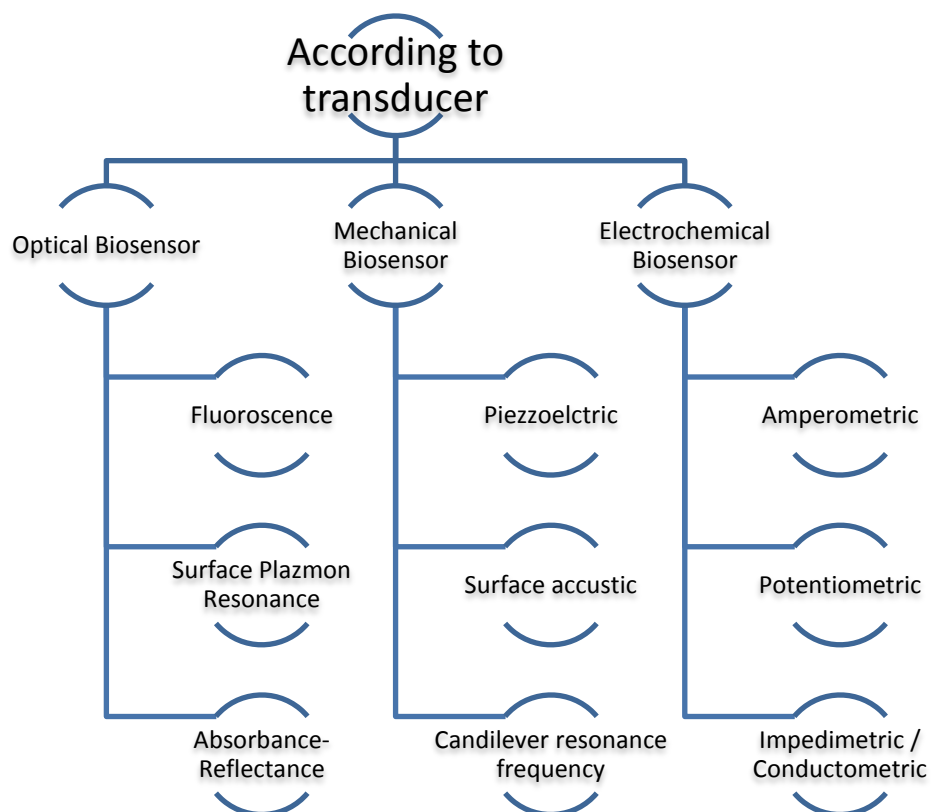


Figure 1.4. Hierarchical classification of biosensors according to transducer type.

Very important part of a biosensor fabrication is the immobilization of biocomponent. Performance of biosensors with immobilized molecules depends also on factors such as the chemical and physical conditions (pH, temperature and contaminants), thickness and stability of the materials[12].

A few advantages of biosensors are listed below:

1. They can measure nonpolar molecules that do not respond to most measurement devices
2. Biosensors are specific due to the immobilized system used in them
3. Rapid and continuous control is possible with biosensors
4. Response time is short (typically less than a minute) and
5. Practical

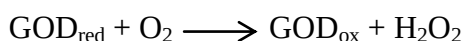
There are also some disadvantages of biosensors:

1. Heat sterilization is not possible because of denaturization of biological material,
2. Stability of biological material (such as enzyme, cell, antibody, tissue, etc.), depends on the natural properties of the molecule that can be denaturalized under environmental conditions (pH, temperature or ions)
3. The cells in the biosensor can become intoxicated by other molecules that are capable of diffusing through the membrane[13].

There are three generation of biosensors:

1.3.1.1. 1st generation biosensors

The production of the reaction diffuses to the transducer and causes electrical response. The glucose oxidase enzyme (GOD) uses glucose to produce H_2O_2 in presence of oxygen. Therefore, the oxygen concentration present on the electrode surface is decreased. The redox group in GOD is FAD (Flavin adenine dinucleotide) that undergoes a $2\text{e}^- 2\text{H}^+$ reduction. The overall reaction is described as follows:



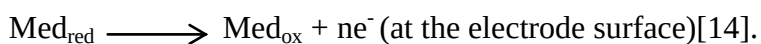
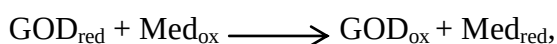
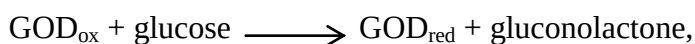
Both FAD and FADH₂ are highly soluble in water but are strongly bound to the enzyme and do not exchange with FAD/FADH₂ in solution. In the above reaction we have therefore shown the redox process of the FAD as that of the enzyme. The decrease in O₂ concentration is proportional to the glucose concentration (under suitable experimental condition). Alternatively, the product formed (H₂O₂) can also be measured at a suitable potential[14].

1.3.1.2. 2nd generation biosensors

To improve the sensor response it includes specific mediators between reaction and transducer.

One potential technical difficulty in using an oxidoreductase is oxygen limitations under conditions of low oxygen tension. To overcome this problem there is a method as replacing the oxygen with an artificial mediator. Electron mediators enhance the rate at which the transfer of electrons takes. Their role is to shuttle electrons between the enzyme and electrode. However, one of the major problems is that it is generally difficult to tailor the redox potential of the molecule and to have high efficiencies (so that the mediator molecule can do the same job repeatedly, as a catalyst). If the potentials do not match well, then there would be energy inefficiency and the mediator may undergo side reactions. Therefore, the selected mediator molecule should have an appropriate redox potential under the specified experimental conditions. The selection of a suitable mediation is often a trial and error process.

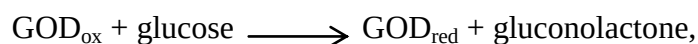
The following reaction scheme can be proposed with a mediator:



1.3.1.3. 3rd generation biosensors

The reaction causes the response. In a mediatorless case, the electrode exchanges electrons directly with the enzyme and hence with the substrate. Thus, the redox potential of the enzyme is realized. However, this is often difficult as the active site of the enzyme is physically distant from the electrode surface. On the other hand, if the

enzyme is very close to the electrode surface, it will have very little conformational freedom and poor access to the substrate, and the environment will be dehydrated. Therefore, it is only by very careful and judicious choice that one can get direct electron transfer to and from the electrode. Another choice, recently advocated, is the use of molecular wires that can be used to connect the enzyme active site to the electrode surface using a chemical link. This reaction scheme can be described as follows:



This process does not involve the mediator or oxygen and depends on the direct oxidation-reduction of the enzyme by the electrode. This is the preferred way, but at the same time the most complex. The elimination of interference, one of the major objectives, is one of the greatest advantages in this design. A steady-state electrochemical signal is reached after a short delay, when the rate of product formation equals the rate at which the product diffuses out of the enzyme membrane. Alternatively, a kinetic measurement can be used in which the rate of change of the electrochemical signal is monitored as a function of time. Therefore the measurement of the signal at the correct time is an important consideration. Third-generation biosensors are promising in order to provide a powerful and inexpensive alternative to conventional analytical strategies for assaying chemical species present in complex matrices. This is because of their ability to discriminate the target analyte from a host of inert and potentially interfering species without the requirement for separating and subsequently identifying all the constituents of the sample. This can be simply achieved by using several different enzymes on the same transducer and scanning the potential (as in polarography) so as to detect different components provided the potentials are sufficiently distinct[15].

1.3.2. Electrochemical sensors

Electroanalysis with electrochemical sensors is a new area in rapid development within electroanalysis. The investigated electrochemical reaction would generate a measurable current (amperometric detection), a measurable potential or charge accumulation (potentiometric detection) or measurable conductivity change of a medium

(conductometric detection) between electrodes. When the current is measured at a constant potential this is referred to as amperometry. If an electrical current is measured while controlled variations of the potential is being applied, this is named as voltammetry. Potentiometric, amperometric and conductometric measurement techniques forms the kinds of electrochemical sensors. Potentiometric sensors have an organic membrane or surface that is sensitive to an analyte. The reaction between them generates a potential proportional to the logarithm of the electrochemically active material concentration. This potential is compared with the reference electrode potential. Amperometric sensors measure the current change resulted by chemical reaction of electroactive materials while a constant potential is being applied. The change of the current is related to the concentration of the species in solution.

Generally biological compounds (glucose, urea, cholesterol, etc.) are not electroactive, so the combination of reactions to produce an electroactive element is needed. This electroactive element leads a change of current intensity. This change is proportional to the concentration of analyte. Conductometric electrochemical sensors can measure the change of the electrical conductivity of cell solution. Most reactions involve a change in the composition of solution. Thus conductometric electrochemical sensors can detect any reactive change occurring in a solution. Electrochemical sensors can sense materials without damaging the system.

1.3.2.1. Amperometric sensors

The working principle of amperometric sensor is based on the measurement of current changes resulting from the electrochemical oxidation or reduction of an electroactive species. It is usually performed by maintaining a constant potential. Amperometric immunosensors detect the concentration dependent current, generated when an electroactive species is either oxidized or reduced at the electrode surface. The current and bulk concentration of the detecting species can be approximated as:

$$I = ZFk_m C$$

where I is the current to be measured, Z and F are constants, k_m is the mass transfer coefficient and C is the bulk concentration of the detecting species[16]. Amperometric sensors are the most common used type of electrochemical sensors and they are more

sensitive than the potentiometric sensors. Working with low potentials is available with amperometric sensors, thus the influences of oxygen and of different interferants on response decrease. An optimal mediator should be stable, able to react rapidly with target molecule, exhibit reversible heterogeneous kinetics, the over potential for the regeneration of the oxidized mediator should be low and pH independent and reduced form should not react with oxygen. Mediators allow to measure at low working potentials and to avoid the interference with unwanted species. Measurements are thus less dependent on oxygen concentration. Mediators are generally inorganic redox ions such a ferricyanide, organometallic compound or organic dyes methylene blue, toluidine blue, or Prussian blue[10].

1.3.2.2. Potentiometric sensors

Potentiometric sensors measure the potential difference between working electrode and reference electrode. When the reference electrode has a defined potential, the working electrode potential changes depending on the analyte concentration in the gas or solution phase. A stable and accurate reference electrode is required for this type of electrochemical sensors.

The measured potential is given by the Nernst equation:

$$E = E^{\circ} - \frac{RT}{nF} \ln Q$$

Where E is the potential to be measured, n is electron transfer number, Q is relative activity of the ion of interest and the others (E° , R, T and F) are constants.

1.3.2.3. Impedimetric/Conductometric sensors

Conductance is the inverse value of resistance for this reason such systems have also been named as as conductometric by some investigators. These sensors has two electrodes consist of reference electrode and working electrodes. Reference electrode is a half-cell with an accurately known potential that is independent of the analyte or any other oin concentration. Also there is a salt bridge preventing components of the analyte solution from mixing with reference electrode solution. Working electrode is immersed in a solution of analyte that produced potential depending on the analyte concentration.

The working electrode should be selective to the analyte. When ions or electrons are produced during the course of chemical reaction, the overall conductivity or resistivity of the solution is changing. The measured parameter when using this transducer is the electrical conductance/resistance of the solution. Conductance measurements have relatively low sensitivity. When using a sinusoidal voltage (AC) the electric field is generated which finally minimize undesirable effects such as Faradaic processes, double layer charging and concentration polarization[10].

1.3.3. Electrochemical cell design

1.3.3.1. Cell

Electrochemical measurements are made in an electrochemical cell (Figure 1.5) consisting of two or more electrodes and the electronic circuit for controlling and measuring the current and the potential. The simplest electrochemical cell uses two electrodes. The potential of one electrode is sensitive to the analyte's concentration, and is called the working electrode or the indicator electrode. The second electrode, which we call the counter electrode, completes the electrical circuit and provides a reference potential against which we measure the working electrode's potential. Ideally the counter electrode's potential remains constant so that we can assign to the working electrode any change in the overall cell potential. If the counter electrode's potential is not constant, we replace it with two electrodes: a reference electrode whose potential remains constant and an auxiliary electrode that completes the electrical circuit.

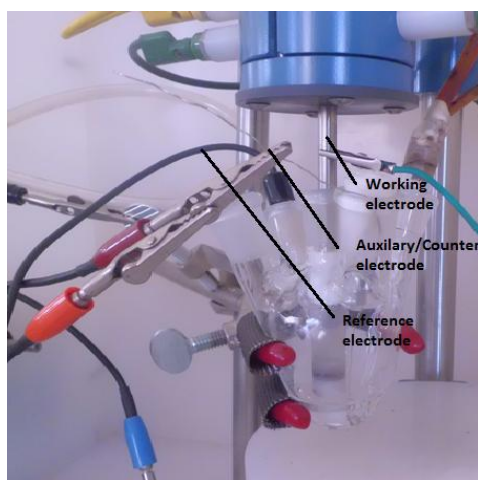


Figure 1.5. Set up of an electrochemical cell.

Except from the electrochemical equipment the cell design contains solvent, electrolyte, one or more electroactive materials and added reagents that will undergo reactions with the electrolytic products. Removing the dissolved oxygen, that has a cathodic signal that can interfere with observed current, should be removed with an inert gas (N_2 or Ar) purging. If the electroactive materials are sensitive to moisture and air, the experiment can be performed in a glove box, glove boxes or in vacuum.

1.3.3.2. Solvent

Before an electrochemical experiment we should choose the solvent determining conductance, solubility of the electrolyte and electroactive substance and reactivity of the solvent with electrolytic products. Also a solvent mixture can be used to have average properties of the mixed solvents. An example is the nonaqueous mixture of methanol and dimethylformamide (DMF) which was used to achieve a balance between solubility and conductivity. When low temperature studies are performed solvent properties should be compatible with the studied temperature. For example DMF dimerizes at the temperatures below $-40^{\circ}C$. Also acetonitrile, ethanol, methanol and methylene chloride are good oxidative (anodic) electrochemical solvents while acetonitrile, DMF and dimethyl sulfoxide (DMSO) are reductive (cathodic). Acetonitrile is known as the best nonaqueous solvent because of its electrochemical properties and relatively nontoxicity. Acetonitrile is one of the volatile solvents and the evaporation of these kind of solvents should be minimized. When the solvent evaporates during the electrochemical measurements, the concentration of the electroactive components in the solution increases.

1.3.3.3. Electrolytes

The electrolytes are added to enhance the conductivity, minimize double layer and migration current effects. An electrolyte should be soluble in the solvent and inert toward the electroactive substance and electrolytic products. For example, lithiumperchlorate is soluble in water and used for aqueous applications while tetrabutyl ammonium perchlorate is used with nonaqueous solvents such as dimethyl sulfoxide and dichloromethane.

1.3.3.4. Electrodes

There are three electrodes in the electrochemical cell. Working electrode (WE), reference electrode and auxiliary/counter electrode. As a working electrode solid disc electrodes made of platinum, glassy carbon, silver and gold are used. Mercury drop electrode is useful for aqueous systems at large negative potentials because of the reduction of hydrogen. Generally, solid electrodes are preferred for nonaqueous solvents and positive potentials. Solid electrodes should be polished often and carefully by polishing, sonicating or cycling the potential of WE. The working electrode can be modified with different immobilization techniques.

There are several reference electrodes used in electroanalytical experiments, silver-silver chloride (Ag-AgCl) and the calomel electrode are the most common. Silver-Silver Chloride (Ag-AgCl) consists of a piece of silver wire anodized with silver chloride in a glass tube. The wire is in contact with concentrated KCl or NaCl solution. A semi-permeable salt bridge protects the electrode from the bulk solution. An important thing to decide the reference electrode is that aqueous based references should not be used for nonaqueous solutions. Otherwise, large junction potentials may effect the experimental results. While many electrodes could be well poised there are several that are very commonly used and commercially available: Silver/Silver Chloride, Saturated Calomel, Mercury/Mercury (mercurous) Oxide, Mercury/Mercury Sulfate, Copper/Copper Sulfate, and more. There are other couples that are often referenced but are not often used today such as the Standard Hydrogen Electrode.

The third electrode of the cell is auxiliary electrode, which provides circuit over which current is either applied or measured. All electrochemistry experiments (with non-zero current) must have a working – auxiliary electrode pair. The potential of the auxiliary electrode is usually not measured and is adjusted so as to balance the reaction occurring at the working electrode. This configuration allows the potential of the working electrode to be measured against a known reference electrode without compromising the stability of that reference electrode by passing current over it. In most experiments, the auxiliary electrode is simply the current source/sink and so relatively inert materials like graphite or platinum are ideal, though not necessary. In some experiments the auxiliary electrode is part of the study and the material composition and setup will vary

accordingly[17].

Two-Electrode Experiments: Two-electrode experiments are the simplest cell setups, but often have far more complex results, and corresponding analysis. In a two electrode setup the current carrying electrodes are also used for sense measurement. The physical setup for two-electrode mode will have the current and sense leads connected together: Working (W) and Working Sense (WS) are connected to a (working) electrode and Reference (R) and Counter (C) are connected to a second (aux, counter, or quasi-/pseudo-reference) electrode. A diagram of 2-electrode cell setup can be seen in Figure 1.6.

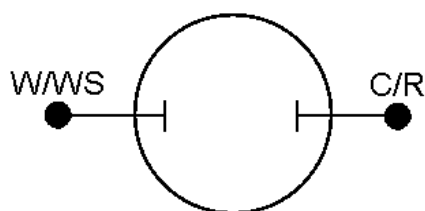


Figure 1.6. 2-electrode cell setup.

Two-electrode experiments measure the whole cell, that is, the sense leads measure the complete voltage dropped by the current across the whole electrochemical cell: working electrode, electrolyte, and counter electrode. If the whole cell potential map looks like Figure 1.7, then 2-electrode setup will have the Working Sense lead at point A and the Reference lead at point E, and so measure the voltage drop across the whole cell.

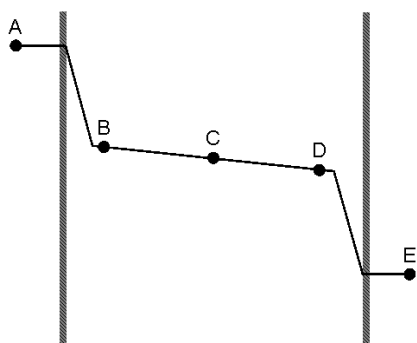


Figure 1.7. Measured (sample) potential map across a whole cell: The Working lead is at point A and the Counter lead at point E.

Two-electrode setups are used in a couple general cases. One is where measurement of

the whole cell voltage is significant, for example electrochemical energy devices (batteries, fuel cells, super caps). The other is where the counter electrode potential can be expected not to drift over the course of the experiment. This is generally in systems which exhibit very low currents and/or relatively short timescales and which also have a well poised counter, e.g. a micro working electrode and a much larger silver counter electrode[17].

Three-Electrode Experiments: In three electrode mode, the Reference lead is separated from the Counter and connected to a third electrode. This electrode is most often positioned so that it is measuring a point very close to the working electrode (which has both Working and Working Sense leads attached). A diagram of a 3-electrode cell setup can be seen in Figure 1.8.

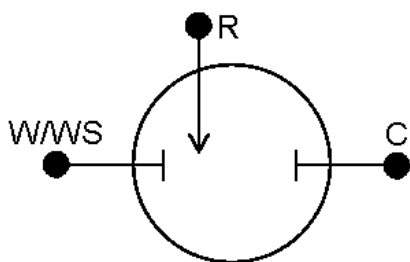


Figure 1.8. 3-electrode cell setup

In Figure 1.8 the sense points are located at A and roughly B. Three-electrode setups have a distinct experimental advantage over two electrode setups: they measure only one half of the cell. That is, the potential changes of the working electrode are measured independent of changes that may occur at the counter electrode.

This isolation allows for a specific reaction to be studied with confidence and accuracy. For this reason, 3-electrode mode is the most common setup used in electrochemical experimentation[17].

Four-Electrode Experiments: In four-electrode mode the Working Sense lead is decoupled from the working electrode, as was (and in addition to) the Reference lead. A diagram of a 4-electrode setup can be seen in Figure 1.9.

Four-electrode setups are measuring potential along the B-D line in Figure 1.9, where there may be some "obstruction" at C. This setup is relatively uncommon in electrochemistry, though it does have its place. In 4-electrode mode the potentials for

any electrochemical reactions that are occurring at the working (and counter) electrode(s) are not being measured. What is being measured is the effect of an applied current on the solution itself or some barrier in that solution.

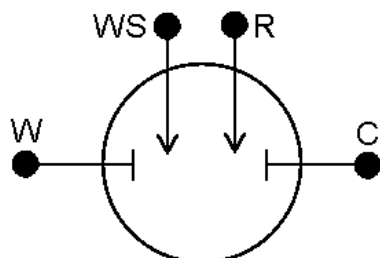


Figure 1.9. 4-electrode cell setup

The most common use of this setup is for measuring impedance across some solution phase interface, such as a membrane or liquid-liquid junction. This setup may also be used to make very accurate measures of solution resistance or the resistance across the surface of some material (solid state cells). [17].

1.3.4. Electrochemical sensor design

When attempting to design a new electrochemical sensor, the firstly the transducer type should be chosen; optical, mechanical or electrochemical transducers can be used for construction. Then, the material which will be used as recognition element should be chosen according to the analyte. After that stage, the immobilization method is decided to immobilize the recognition element on the sensor material.

After construction of an electrochemical sensor, systematically there are many parameters to characterize an electrochemical sensor.

When developing chemical or biological sensors some analytical and physical properties must be evaluated:

(1) **Sensitivity** is the response of the sensor to per unit change in analyte concentration. Sensitivity is also defined as the slope of change in signal with change in concentration:

$$S = \frac{d(\text{signal})}{d(\text{concentration}, M)}$$

This equation is only acceptable for the sensor responses between the linear range. The working capacity of a sensor corresponds to the linear range[18].

(2) **Selectivity** is the ability of the sensor to respond only to the target analyte. That is, lack of response to other interfering chemicals is the desired feature. It is rare to find a sensor which will respond to only one analyte. Also the sensor response may be to a group of analytes with similar chemical structures.

(3) **Range** is the concentration range over which the sensitivity of the sensor is good. Sometimes this is called dynamic range or linearity. It is important to know what concentration range is covered and from the calibration point of view, over what section of this is range response is linear. The importance of the range can be illustrated from the example of the determination of the glucose in blood. This needs to be at least 0.2-20 mM to cover the blood glucose levels found in normal and diabetic persons.

(4) **Response time** is the necessary time for having 95% of the response. Analytical devices require some time to allow the system to come to equilibrium, the response time. If time becomes too long it can materially affect the usefulness of the method for repetitive routine analyses. However, chemical or biochemical sensors this response time is greatly offset by the simplicity of the measurement and the minimal sample preparation time. For electrochemical sensors, the response times can vary from a few seconds to a few minutes. Up to 5 minutes may be acceptable, but if the time exceeds 10 minutes this may be too long.

(5) **Reproducibility** is the accuracy with which the sensor's output can be obtained.

(6) **Limit of detection (LOD)** is the lowest concentration of the analyte to which there is a measurable response. It is one of the most important parameters to be determined for electrochemical sensors. The LOD is typically defined as;

$$C_{LOD} = \frac{3 \text{ } std_{background}}{S}$$

C_{LOD} is the smallest detectable concentration of an analyte, $std_{background}$ is the standard deviation of the background signal and S is the slope of calibration line or the sensitivity of the sensor. If the sensitivity of the electrochemical sensor increases, C_{LOD} will decrease[18].

(7) **Life time** of a sensor can be regarded in several different ways. Even the most robust pH electrodes this the time period over which the sensor can be used without significant deterioration in performance characteristics.

(8) **Stability** characterizes the change in its baseline or sensitivity over a fixed period of time.

(9) **Linearity** is maximum linear value of the sensor calibration curve. Linearity of the sensor must be high for the detection of high substrate concentration. Linear ranges are generally much larger for potentiometric sensors. Thus a pH meter electrode has a practical range from pH 0 to pH 12. Other ion selective electrodes cover quiet considerable ranges that many of them cover six or seven powers of ten[19, 20].

1.3.5. Immobilization techniques

Electrochemical sensors use biomolecules as recognition elements which must be immobilised (or coupled) to the transducer. The transducer is the electrode (or set of electrodes) in the case of the electrochemical sensor. The specificity and selectivity that an electrochemical sensor provides is attributed to this immobilised biological recognition element.

The most commonly used immobilization techniques for construction of electrochemical sensors are

- physical adsorption,
- covalent binding,
- matrix entrapment,
- inter molecular cross-linking and
- membrane entrapment.

1. *Adsorption*: The physical adsorption utilizes a combination of Van der Waals and hydrophobic forces, hydrogen bonds, and ionic forces to attach the biomaterial to the surface of the sensor. This method is very simple, however, employed forces are not very strong and biomolecules attached by this method may be released or not persist.

2. *Covalent binding*: The sensor surface is modified to acquire a reactive group to which the biological materials can be attached. In case of enzymatic biosensors it is through the functional group in the enzyme which is not essential for its catalytic activity.

This method improves uniformity, density and distribution of the bioelements, as well as reproducibility and homogeneity of the surfaces. Covalent immobilization may decrease or eliminate some common problems such as instability, diffusion and aggregation, or inactivation of biomolecules. This occurs when biomolecules are immobilized on sensor surfaces by polymer matrices. For this purposes the reagents such as glutaraldehyde, carbodiimide, succinimide esters, maleinimides and periodate are often used for covalent immobilization.

3. *Matrix entrapment*: In this case biomolecules are trapped within the polymeric gel matrix. For this method the polyacrylamide, starch, alginate, pectate, polyvinyl alcohol, polyvinyl chloride, polycarbonate, polyacrylamide, cellulose acetate and silica gel are often used. Matrix entrapment has disadvantage of possible leakage of the biological species during use, resulting in a loss of activity.

4. *Cross-linking*: For intermolecular cross-linking of biomolecules bi-functional or multi-functional reagents such as glutaraldehyde, hexamethylene di-isocyanate, 1,5-difluoro 2,4-dinitrobenzene and bisdiazobenzidine-2,2-disulphonic acid, etc., are used. The most common cross-linking agent in sensor applications is glutaraldehyde, which couples with the lysine amino groups of enzymes. This method has also some disadvantages; the enzyme layer formed is not rigid, there are higher demands for amount of biological material and cross-linking can cause the formation of multilayers of enzyme, which negatively affects the activity of the immobilized layers. Moreover larger diffusional barriers may delay interactions.

5. *Encapsulation (or membrane entrapment)*: In this method a porous encapsulation matrix (e.g. lipid bilayers) is formed around the biological material and helps in binding it to the sensor. Other approach for encapsulation uses sol-gel method for the immobilization of biological molecules in ceramics, glasses, and other inorganic materials using. In the sol-gel procedure; biological molecules are entrapped in a porous matrix, such as a polymeric oxo-bridged SiO₂ network. These matrices allow optical monitoring of the chemical interactions since they are optically transparent. The

sol-gel process can be performed at room temperature and which protects biomolecules against denaturation. Biomolecules immobilized by this procedure are very stable, but achieving of sol-gel with reproducible pore sizes seems to be still an obstacle. Problems such as diffusional limitations inside the porous network, brittleness of the glassy matrix, reproducibility or discrepancies in the preparation procedures has to be solved before this procedure can be used for routine application[10].

1.3.6. Phthalocyanines

Phthalocyanines are two-dimensional 18π electron aromatic porphyrin synthetic analogues, consisting of four isoindole subunits linked together through nitrogen atoms. Because of these 18π electron conjugated ring system in the structure of the molecule, they have efficient electron transfer abilities and utilized in a wide application areas. Phthalocyanines and their metalloderivatives (MPcs) have recently attracted an increasing interest not only for the preparation of dyes and pigments but also as building blocks for the construction of new molecular materials for chemical sensors, electrochromism, batteries, photodynamic therapy, semiconductive materials, optical discs, catalytic processes, liquid crystals, synthetic metals, solar cells and liquid crystals[21-23]. A Phthalocyanine molecule usually has blue or green colour with aromatic macrocyclic compound structure (Figure 1.10.).

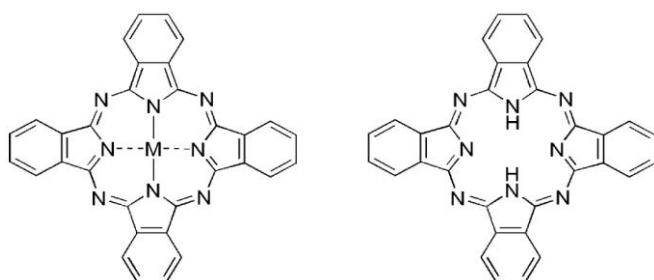


Figure 1.10. Basic chemical structure of phthalocyanines.

Phthalocyanines form coordination complexes with 70 different metal atoms into the Pc ring that offers additional features to optimize the physical responses. On the other hand, their thermal and environmental stability are important characteristics that make them promising candidates to be incorporated into devices. An important point to be addressed in order to reach this goal is the control of the supramolecular arrangement of these macrocycles in the solid state[22].

1.3.6.1. Sensor application of phthalocyanines

Metallophthalocyanines (MPcs) have extensively been studied as electrochemical catalysts and sensor recognition materials because of their properties such as excellent redox behavior, thermal and chemical stability, ability of easy modification with different metal ions and substituents, and ability of easy coating on different substrate with various techniques. MPcs which are artificially derivate of natural protein, porphyrin, have been deeply used as mimetic enzyme receptors for the sensing of many harmful biological target species, such as pesticides. Recently there is a tendency towards finding new materials which can be used as recognition element of sensor instead of enzymes[24-27]. Harbeck et al. chemically modified the core of phthalocyanine to increase sensor sensitivity for organophosphorus and organonitrogen pesticides such as fenthion and methiocarb and they obtained a detection limit down to 0.03 mg dm^{-3} [28]. Pu-Hong Wang et al. designed and synthesized a new phthalocyanine 2,9,16,23-tetrakis [4-(hexafluoroisopropanol)-anilinomethyl]phthalo-cyaninato zinc for electrochemical sarin gas sensor and obtained the detection limit as 205.8 Hz/mg/m^3 [29]. Koca et al., used a terminal-alkyl substituted cobalt phthalocyanine and constructed a biomimetic electrochemical sensor with a new electrode modification technique, “click electrochemistry” (CEC) based on a functional cobalt phthalocyanine and they detected eserine pesticide with a sensitivity of $1.15 \text{ A dm}^3 \text{ mol}^{-1}$ and LOD of $1.75 \cdot 10^{-7} \text{ mol dm}^{-3}$ [30].

For designing new sensing devices, metallophthalocyanines (MPcs) have been extensively studied because of their properties such as excellent redox behavior, thermal and chemical stability, ability of easy modification with different metal ions and substituents, and ability of easy coating on different substrate with various techniques[31-34]. These versatile features of MPcs are account for preferring of MPcs as electrochemical catalysts and sensors. They have been used for the sensing or detection of many target species including hydrazine, glucose, ascorbic acid, and pesticides. For the sensing of these types of biological species, different enzyme based biosensors have been studied. Especially acetylcholinesterase enzyme that catalyzes the oxidation of organic substances is preferred. It is clearly shown in the literature that biological receptors, especially enzymes, used in biosensors have well-known

limitations. These include low stability of the biological species, difficulties of immobilization on the substrates, leakage from the substrate surface, and low chemical and thermal stabilities. Hence, researches start to tend on replacing the enzymes with artificial receptors, which are called as mimetic enzymes. In last decade, MPcs which are artificial derivatives of natural protein, porphyrin, have been deeply used as mimetic enzyme receptors for the sensing of many harmful biological target species, such as insecticide and pesticides. The use of biomimetic sensors is especially advantageous for pesticide analysis, since the enzymes commonly used to detect pesticides, such as acetylcholinesterase and peroxidase are not selective for one target specie. They can only sense a group species with similar structure, such as the class of organophosphate pesticides. Usage of these enzyme biosensors is based on the enzymatic activity inhibition, thus the enzyme gives similar responses for all pesticide inhibitors. Consequently researches are nowadays condensed on the solving of these selectivity problems by using various mimetic receptor elements, such as MPcs. For example Sotomayor M. D. P. T. reported sensor activity of various porphyrin derivatives for different pesticide and insecticides. She and her coworkers studied the sensor activity of CoPc complex coted on GCE and indicated catalytic oxidation of dipyrone at 0.34V on a CoPc based sensor[35]. Sotomayor M. D. P. T. and her coworker also reported the sensing of herbicide (2,4-D) with a $2.1 \times 10^{-6} \text{ mol L}^{-1}$ limit of detection on an iron porphyrin based mimetic sensor. Kobayashi N. and his coworkers illustrated the electrocatalytic activity of cobalt phthalocyanine (CoPc), cobalt tetra-carboxy phthalocyanine (CoTCPC) and cobalt octa-carboxy (CoOCPc), towards the detection of dicrotophos[36]. They stated that catalytic activity of CoPc was found to be dependent on the pH, as well as the substitution on the phthalocyanine ring. They reported $1.25 \times 10^{-7} \text{ M}$ detection limit for the sensing of dicrotophos with CoPc. Sotomayor M. D. P. T. reported the use of iron tetrapyridinoporphydrazine as a catalyst in the construction of an electrochemical sensor for estradiol valerate determination[37]. As it can be seen from all these studies, MPcs were used effectively in recent electrochemical sensor or biomimic sensor researches. However, the electrode selectivity studies are insufficient for many of these kind of studies.

1.4. Electroanalytical Methods

Electrochemical techniques are static techniques and dynamic techniques. In a static technique we do not allow current to pass through the analyte's solution. Dynamic techniques, in which we allow current to flow through the analyte's solution, comprise the largest group of interfacial electrochemical techniques as exhibited in Figure 1.11.

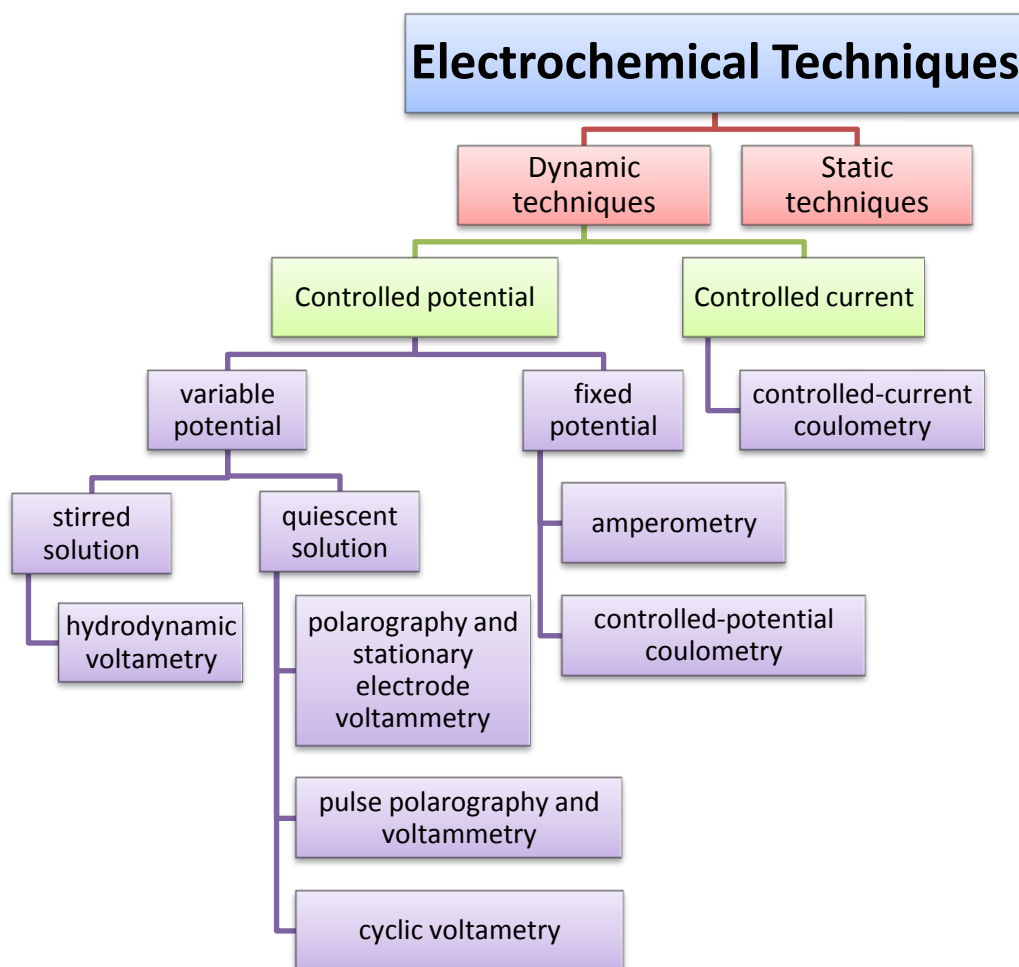


Figure 1.11. Specific electrochemical techniques[38].

For sensor applications Cyclic Voltammetry (CV), Square Wave Voltammetry (SWV), Chrono-Amperometry (CA) and Electrochemical impedance spectroscopy (EIS) are the most used techniques.

1.4.1. Cyclic voltammetry (CV)

A more commonly used variation of the technique is cyclic voltammetry, in which the

direction of the potential is reversed at the end of the first scan. This has the advantage that the product of the electron transfer reaction that occurred in the forward scan can be probed again in the reverse scan.

While the amount of Ox at the electrode surface becomes depleted by the reduction process, it is, of course, replaced by the reduced species, which diffuses away into the solution. Hence, if we reverse the potential sweep from the positive side of the peak, we shall observe the reverse effect. As the potential sweeps back towards the redox potential the reduced species will start to be re-oxidized to Ox. The current will now increase in the negative (oxidizing) direction until an oxidation peak is reached. Figure 1.12 shows the resulting scan of potential against time, while the overall cyclic voltammogram is shown in Figure 1.13.

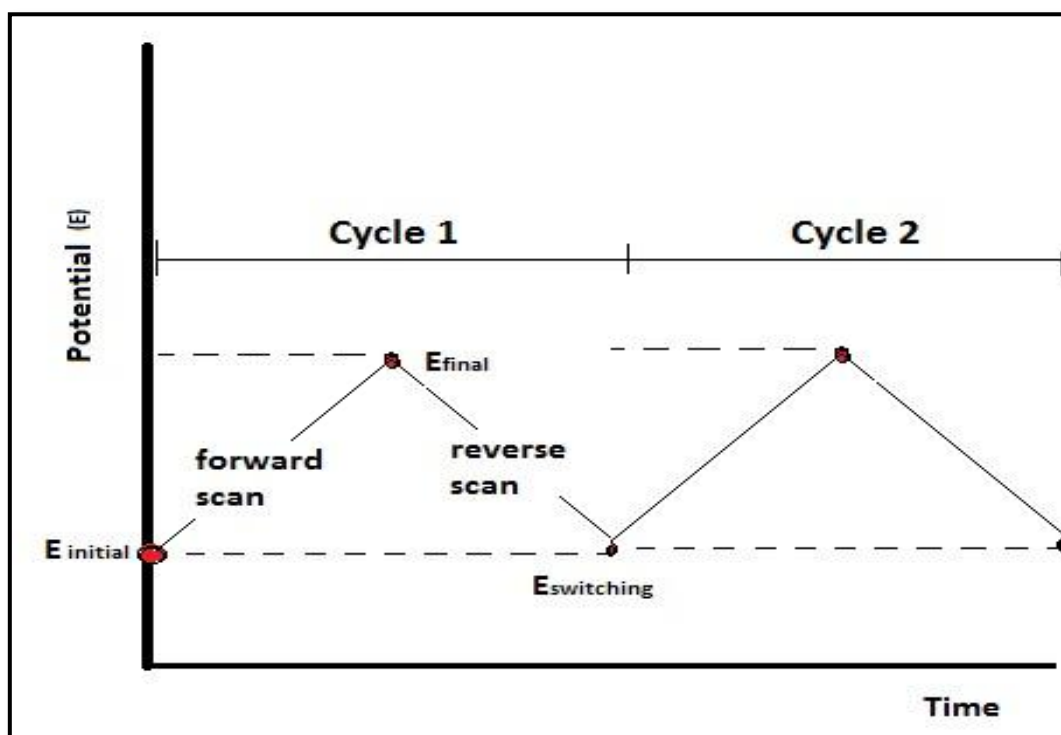


Figure 1.12. A typical scan of potential as a function of time.

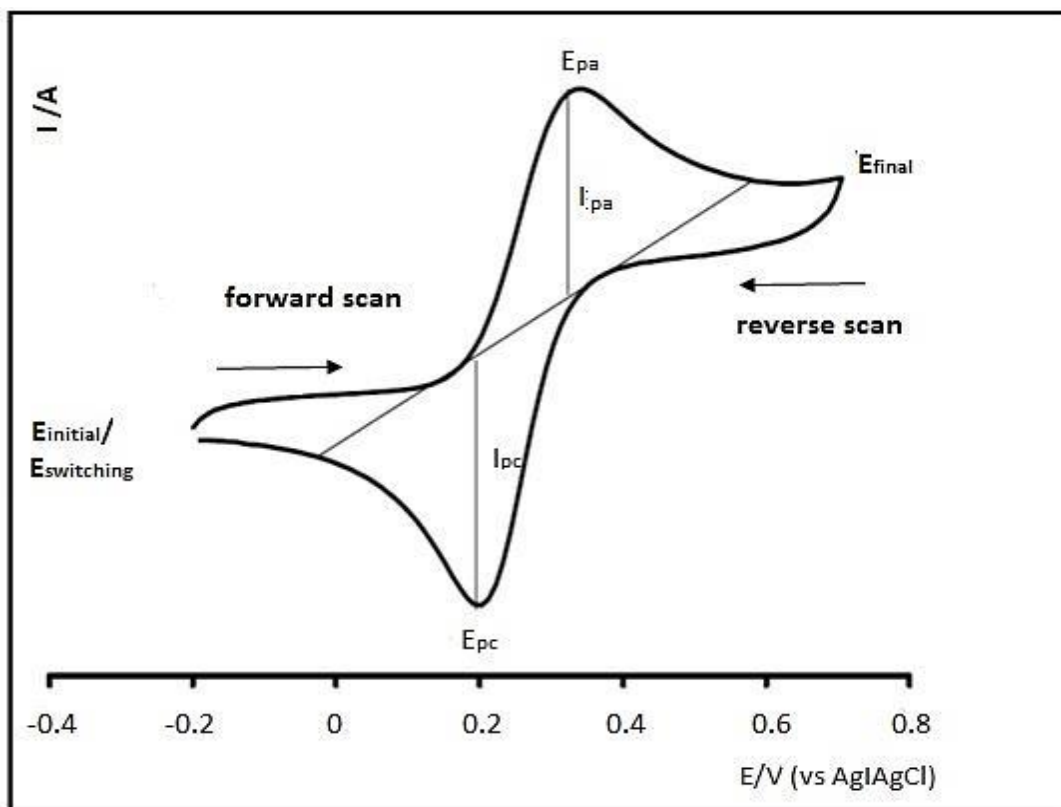


Figure 1.13. A typical reversible cyclic voltammogram.

A cyclic voltammogram should have some properties to be accepted as reversible:

1. The peak potential separation ($E_{p(a)} - E_{p(c)}$) is equal to $59/n$ mV for all scan rates where n is the number of electron equivalents transferred during the redox process;
2. The peak width is equal to $28.5/n$ mV for all scan rates;
3. The peak current ratio ($I_{p(a)}/I_{p(c)}$) is equal to 1 for all scan rates.

If the electrode process is irreversible, the peaks will be shifted away from each other, so that $E_{p(c)} - E_{p(a)} > 0.059/n$. However, the average of the two peak potentials is still a good measure of E^0 . Sometimes, the reverse peak will not be present or will have a different or distorted shape. This indicates complete irreversibility due to further reaction of the initially formed reduction product. This is best shown by the example of 4-acetylaminophenol (paracetamol) which is easily oxidized electrochemically at a carbon paste electrode[20]. The resulting cyclic voltammogram is shown in Figure 1.14.

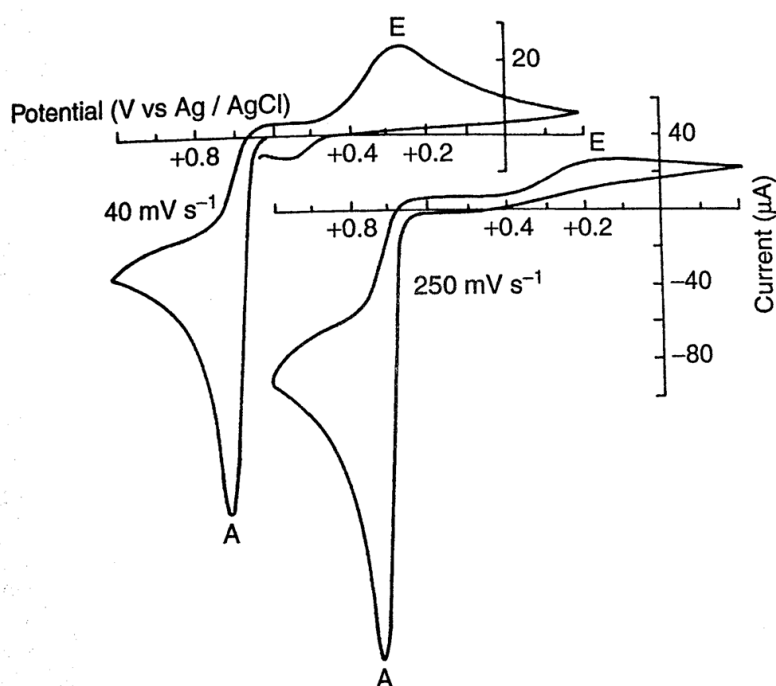


Figure 1.14. The cyclic voltammogram of paracetamol[39].

1.4.2. Square wave voltammetry (SWV)

Square Wave Voltammetry (SWV) is a useful method for studying redox reactions with overlapping waves. It is a derivative of linear sweep voltammetry. In linear sweep voltammetry, the current at the working electrode is measured while the potential between the working electrode and the reference electrode is swept linearly in time. In square wave voltammetry, a staircase potential is applied as exhibited in Figure 1.15.

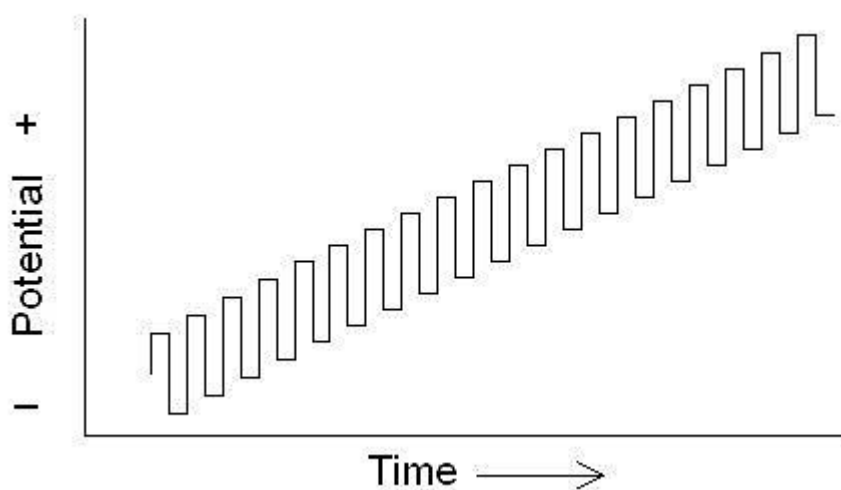


Figure 1.15. Diagram of applied potential for SWV technique.

Oxidation or reduction reactions of materials are obtained as a peak or trough at potentials which the species begin to be oxidized or reduced. A typical SWV measurement result is given in Figure 1.16.

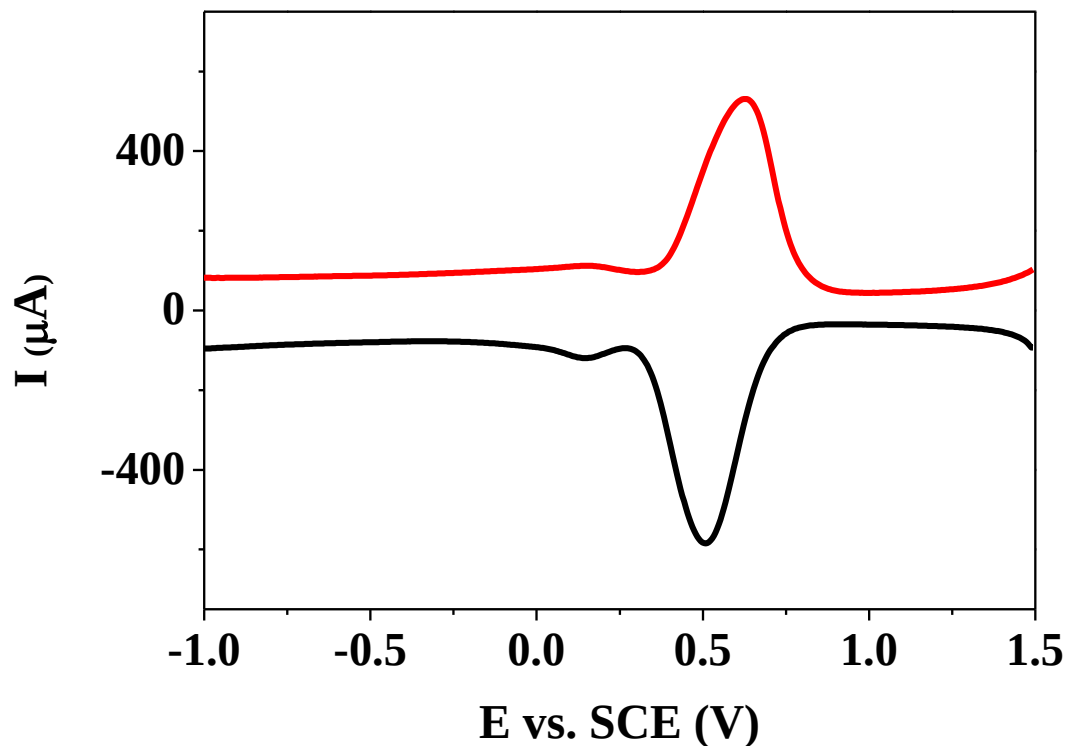


Figure 1.16. A typical SWV measurement result.

The current response is measured right before the next potential change. Therefore the contribution to the current coming from the capacitive charging is minimized and due to the less contribution the detection limits are at nanomolar levels. SWV eliminates the charging current caused by the electrode double layer, thus an increased signal-to-noise ratio results. Therefore shortcomings observed with CV, e.g. overlapping or closely spaced peaks with poor resolution are overcome by SWV. Square wave voltammetry (SWV) is a sensitive electroanalytical technique used to investigate redox reactions that may otherwise be impossible with CV.

1.4.3. Chronoamperometry/Chronocoulometry (CA/CC)

Chronoamperometry (CA) and chronocoulometry (CC) have the same potential wave and one of the simplest potential wave forms. As shown below (Figure 1.17), the potential is changed instantaneously from the initial potential to the first step potential, and it is held at this value for the first step time.

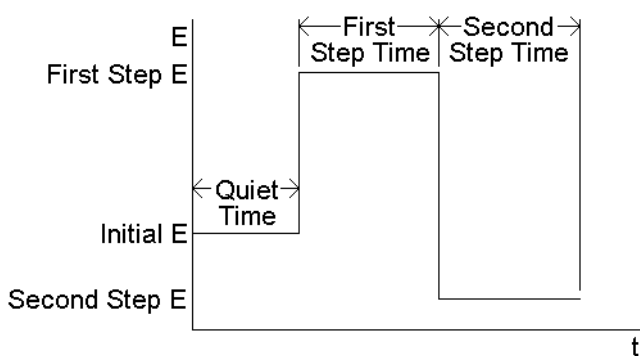


Figure 1.17. Potential wave form for chronoamperometry and chronocoulometry.

In a double potential step experiment, the potential is changed to the second step potential after the first step time, and it is then held at this value for the second step time. In CA, the current is monitored as a function of time, whereas in CC, the charge is monitored as a function of time. The data can also be displayed as charge as a function of time. The charge is calculated by integrating the current. Hence, chronocoulometry data can be obtained[40].

Chronoamperometry is a method particularly useful for electrochemical sensor studies. The current is monitored as a function of time. Decay occurs because of the collapse of the diffusion layer as shown in Figure 1.18.

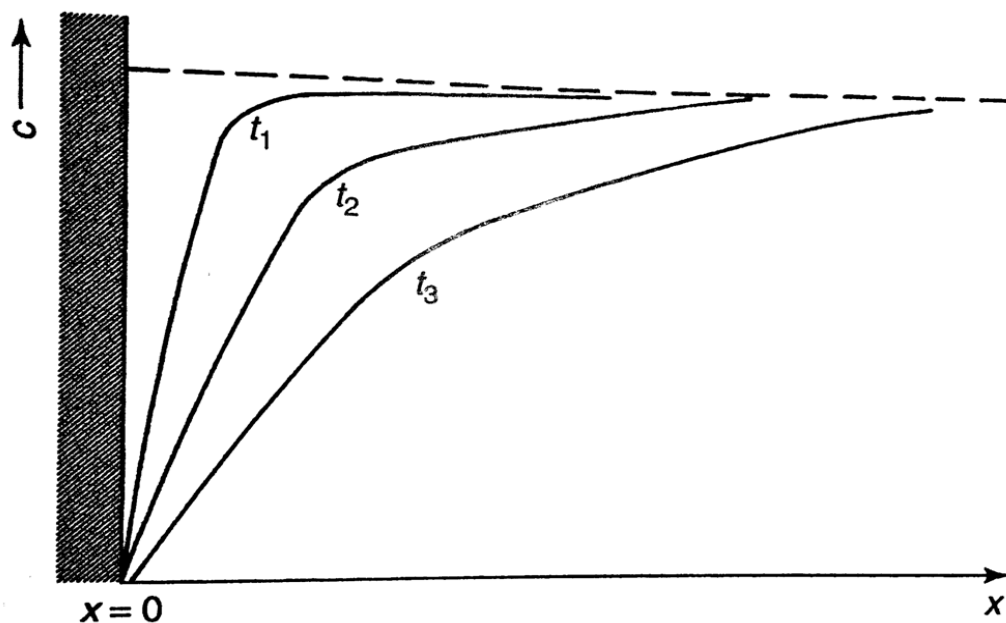


Figure 1.18. The diffusion effect at an electrode for a potential step redox process.

The simplest solution to the diffusion equation, which can be obtained analytically, shows that the decay is proportional to the reciprocal of the square root of time as shown by the Cottrell equation[37]:

$$i = \frac{nFADC_{ox}}{\pi^{1/2}t^{1/2}}$$

Chronoamperometry can be used to determine n , A , D or C providing the other variables known. $i t^{1/2}$ term can be obtained from current-time graph by interpolating the area under the curve as shown in Figure 1.19.

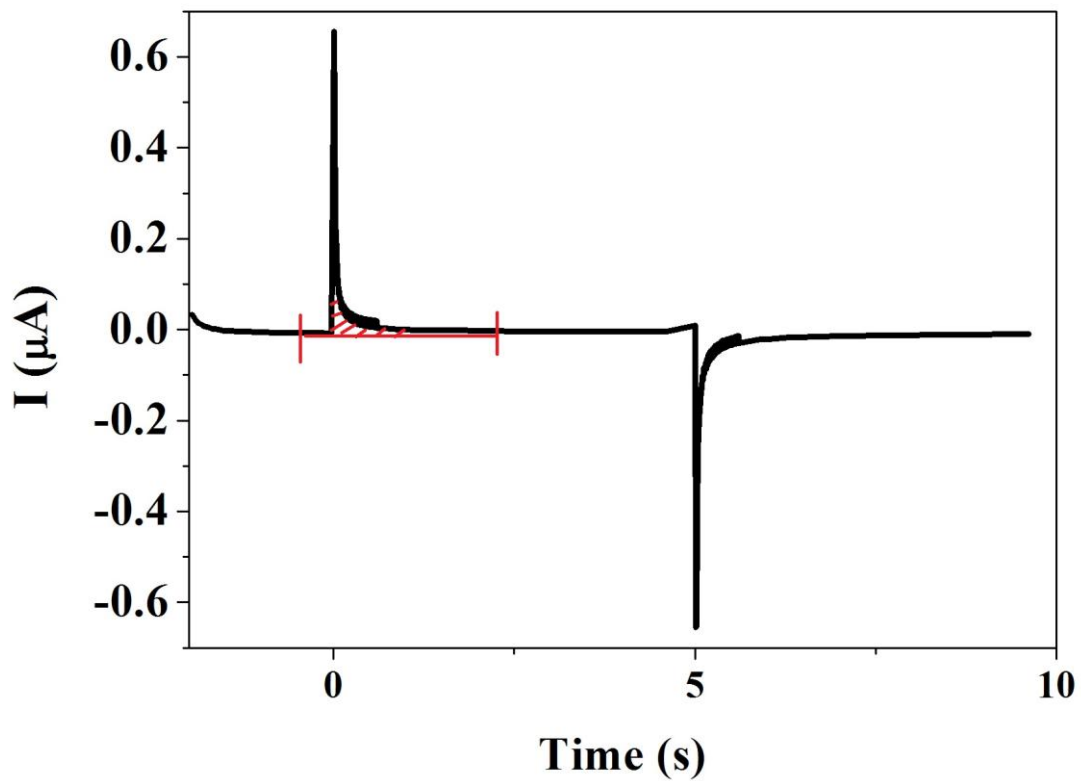


Figure 1.19. The current-time profile associated with two potential steps redox process.

1.4.4. Electrochemical impedance spectroscopy (EIS)

Electrical resistance is the ability to resist the flow of electrical current. Ohm's law defines the resistance as:

$$R = \frac{E}{I}$$

This resistance value is independent from frequency. However, in its place we use impedance for electrochemical studies. Like resistance, impedance is a measure of the ability of a circuit to resist the flow of electrical current. To measure impedance usually an AC potential is applied to an electrochemical cell and the current flowing through the cell is measured. When a sinusoidal potential is applied, the response to this excitation is an AC current signal containing the excitation frequency. This signal is a sum of sinusoidal functions (Fourier series). The current response to a sinusoidal potential will be a sinusoid at the same frequency but shifted in phase (Figure 1.20)

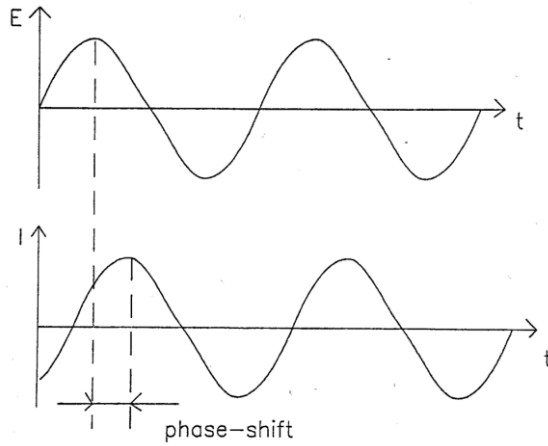


Figure 1.20. Sinusoidal current response.

The excitation signal as a function of time has the form:

$$E(t) = E_0 \cos(\omega t)$$

$E(t)$ is the potential at time t , E_0 is the amplitude of the signal, and ω is the radial frequency. The relationship between radial frequency ω (expressed in radians/second) and frequency f (hertz) is:

$$\omega = 2\pi f$$

In a linear system, the response signal I_t , is shifted in phase (ϕ) and has a different amplitude, I_0 :

$$I(t) = I_0 \cos(\omega t - \phi)$$

An expression analogous to Ohm's Law allow us to calculate the impedance of the

system as:

$$Z = \frac{E(t)}{I(t)} = \frac{E_0 \cos(\omega t)}{I_0 \cos(\omega t - \phi)} = Z_0 \frac{\cos(\omega t)}{\cos(\omega t - \phi)}$$

The impedance is therefore expressed in terms of a magnitude Z_0 and a phase shift ϕ . If we plot sinusoidal signal-sinusoidal response signal $I(t)$ an oval forms. In Figure 1.21, the exhibited oval is known as Lissajous figure.

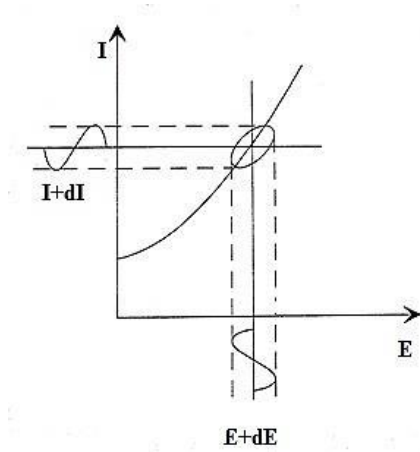


Figure 1.21. Origin of Lissajous Figure.

Using Euler's relationship,

$$\text{Exp}(j\phi) = \cos \phi + j\sin \phi$$

It is possible to express the impedance as a complex function. The potential is described as:

$$E(t) = E_0 \exp(j\omega t)$$

And the current response as:

$$I(t) = I_0 \exp(j\omega t - j\phi)$$

The impedance is then can be written as a complex number:

$$Z = \frac{E}{I} = Z_0 \exp(j\phi) = Z_0(\cos \phi + j\sin \phi)$$

In this equation Z is composed of a real part and an imaginary part. If the real part is

represented in X-axis and imaginary part is represented in Y-axis a Nyquist Plot is obtained as shown in Figure 1.22.

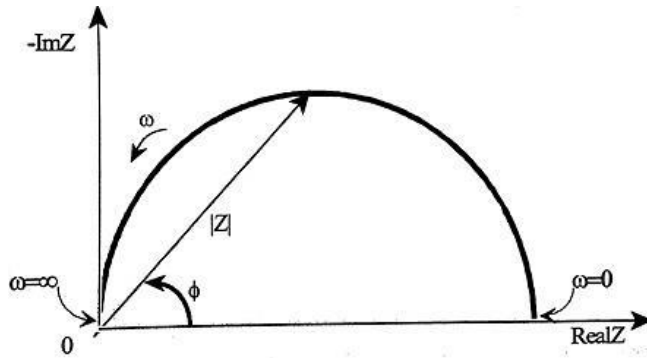


Figure 1.22. A Nyquist Plot with impedance vector.

Low frequency data are on the right side of the plot and higher frequencies are on the left. On the Nyquist Plot the impedance can be represented as a vector of length $|Z|$. The angle between this vector and the X-axis is ϕ which is the phase angle. The semicircle in the plot is characteristic of a single time constant. Electrochemical impedance plots often contain several time constants.

Bode Plot exhibited in Figure 1.23 is another most common presentation method of impedance.

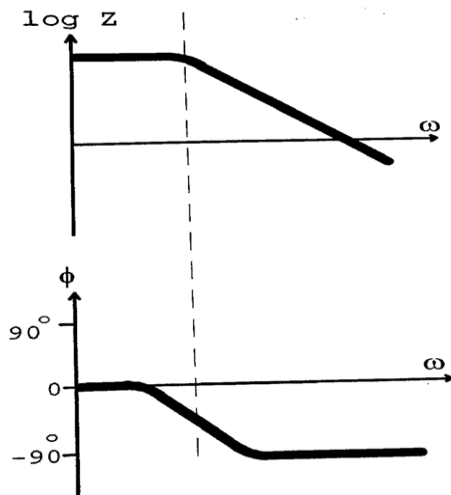


Figure 1.23. Bode Plot with one time constant.

The impedance is plotted with log frequency on the X-axis and both the log of absolute value of the impedance, $\log |Z|$, and phase shift ϕ on the Y-axis. Unlike the Nyquist Plot, the Bode Plot explicitly shows frequency information[41].

2. MATERIAL AND METHOD

2.1. Carbon Paste Electrode Preparation Technique

The ease of surface renewal is the main reason for developing carbon paste electrode (CPE). Also CPE is convenient for chemical modification and composite electrode preparation. Chemically useful functionalities can be introduced during physical mixture of graphite and pasting liquids.

Modifiers can be grouped into at least three different classes, depending on how they affect the interactions of solution species with the electrode.

- Modifiers such as stearic acid introduce an anionic surface charge which affects local electrostatics. The resulting anionic surface promotes redox reactions of cations over anions.
- A modifier such as a zeolite or complexing agent may cause selective adsorption of molecules with particular sizes or charges. Zeolite Y in a CPE preconcentrated methyl viologen and Cu^{2+} in the electrode, but excluded heptyl viologen and $\text{Fe}(\text{CN})_6^{3-/4-}$.
- Modifiers such as metal particles and redox mediators can act as catalysts for normally slow redox reactions at the CPE.

The major advantages of the CPE for such modification schemes include the ease of preparation and surface renewal, and the variety of possible modifying agents[42].

Usually microcrystalline graphite paste has a porous surface and to omit this problem an inert, viscous and nonvolatile liquid is used to fill the pores to achieve a smooth carbon paste electrode surface.

In this study, a drop of liquid hexane is mixed with the graphite powder and packed into an inert shallow well (into an medical injector) with an electrical contact at the back made with a copper wire that was immersed into the carbon paste to make the electrode connection to the potentiostat (Figure 2.1). The paste surface is polished with pressing in a circular motion on a smooth and hard material.

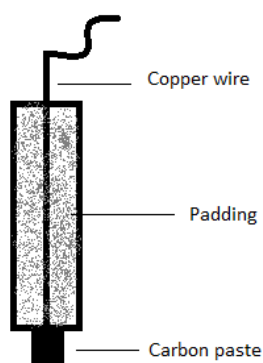


Figure 2.1. Carbon paste electrode.

However, the experimental results were not consistent with each other. Because the rough electrode surface and thus electrodes with different surface areas had different interactions. Flat surface is very important for having a reproducible carbon paste electrode. For this purpose, a new carbon paste electrode preparation technique was developed in this study. Graphite powder was pelletized by compression without addition of any fluid and a reproducible, flat, shiny and tight CPE was produced.

2.1.1. Materials

Graphite powder that was used for the main material of the pelletized carbon paste electrode purchased from Sigma Aldrich (CAS 7782-42-5). Anthraquinone substituted CoPc (**CoPc-AQ**) phthalocyanine was synthesized by Sezer et. al.[43] and used as biosensing material. The chemical structure of the **CoPc-AQ** was exhibited in Figure 2.2 below.

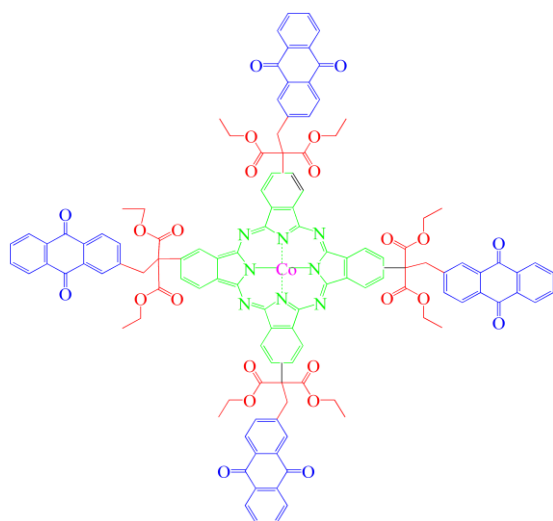


Figure 2.2. The chemical structure of synthesized **CoPc-AQ**.

In this technique a pelletizing mould is designed as exhibited in Figure 2.3. Graphite powder and biosensing material were mixed and pelletized together with the aid of a hydrolic press machine using this mould. The mould material was stainless spring steel and the mould was produced by Parsan Forging and Machining Company.

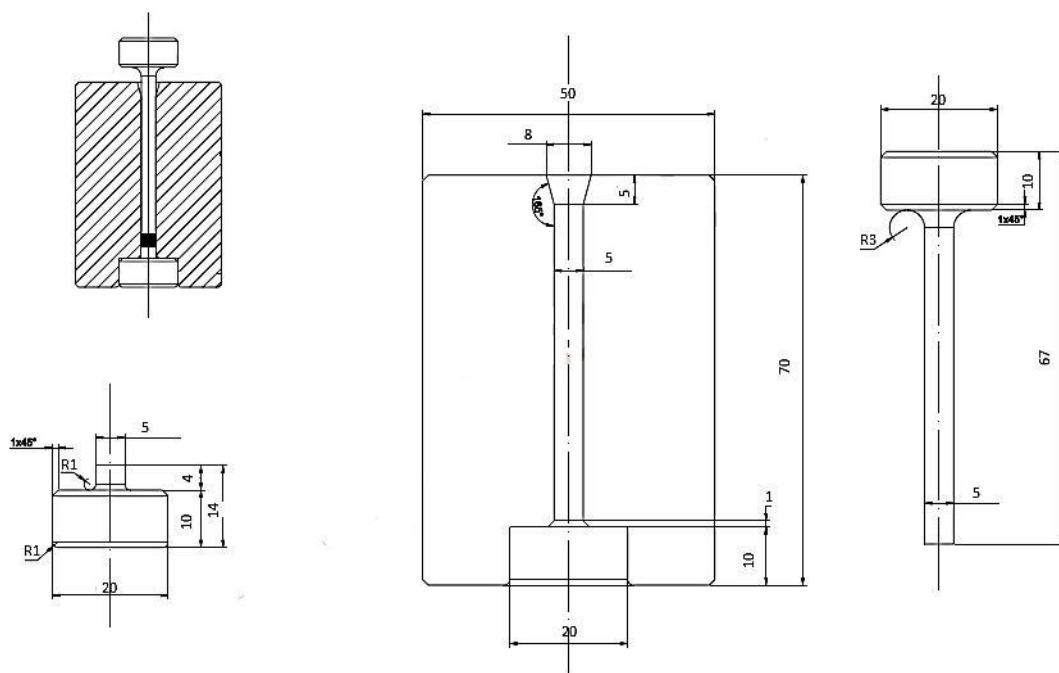


Figure 2.3. Mechanical drawing of designed pelletizing mould.

Also as an analyte eserine (Sigma, E8375) pesticide, was wanted to be detected in phosphate buffer solution (PBS, pH7.0).

2.1.2. Electrode preparation

0.1 g of graphite powder was filled into the mould and 1 ton pressure was applied on it by a hydrolic press to compress and achieve a carbon pellet. This paste electrode was pure carbon paste electrode. To obtain a composite electrode the other component (**CoPc-AQ**) was added into to the carbon powder and mixed well for 5 minutes. After having a homogenous mixture of graphite powder and **CoPc-AQ**, it was pelletized under 1 ton pressure. The produced carbon paste electrode was put into the rotatable electrode tip. The tip was produced and monted to the rotating electrode system as shown in Figure 2.4.

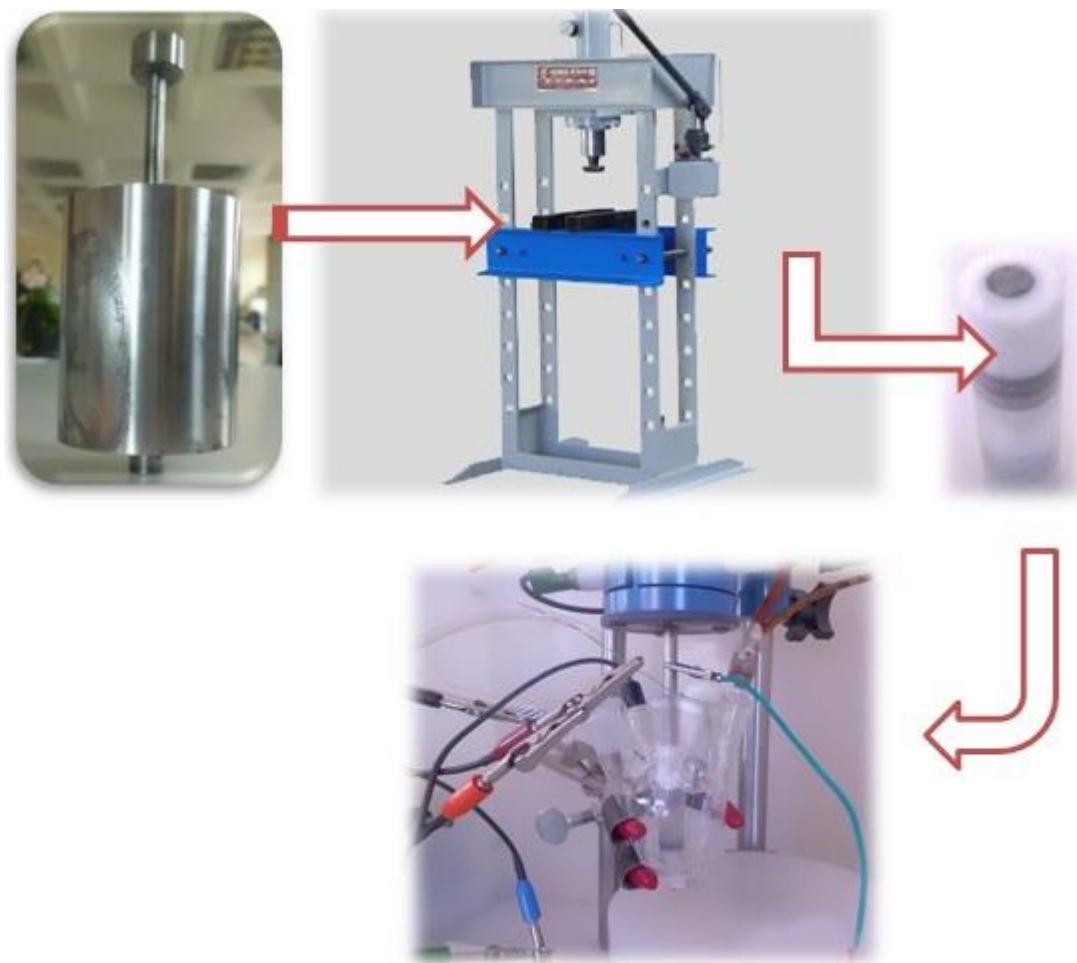


Figure 2.4. Carbon paste electrode preparation.

2.1.3. Electrochemical measurements

Gamry Reference 600 potentiostat/galvanostat utilizing a three-electrode cell configuration at 25 °C was used. The working electrode was a produced carbon paste electrode (CPE) with a surface area of 0.2 cm². Surface of CPE was polished with a smooth paper before each run. A Pt wire served as the counter electrode. Saturated calomel electrode (SCE) was employed as the reference electrode and separated from bulk of the solution by a double bridge. Ferrocene was used as an internal reference. Lithium perchlorate (LiClO₄) in PBS was employed as the supporting electrolyte at a concentration of 0.10 M. High purity N₂ was used to remove dissolved O₂ for at least 15 minute prior to each run and to maintain a nitrogen blanket during the measurements. IR compensation was applied to the CV and SWV scans to minimize the potential control error. Electrochemical impedance spectroscopy (EIS) measurements

were performed in PBS (pH= 7.0) under 10 mV AC voltage versus open circuit potential (open circuit voltage is 0.50 V) in the AC frequency range 300 kHz - 10 Hz with a 10 mV excitation AC voltage.

2.1.4. Sensor measurements

Sensor measurements of CPE/**CoPc-AQ** electrode were applied with square wave voltammetry (SWV), electrochemical impedance spectroscopy (EIS) and double potential step chronocoulometry (DPSCC) analysis to detect eserine pesticide. The three-electrode cell configuration and the solution of 0.1 M lithium perchlorate (LiClO_4), as the electrolyte, dissolved in 0.1 M PBS were used for sensor measurements. Before sensing measurements, the blank measurements were stabilized for SWV, DPSCC, and EIS techniques. Then, eserine solution was added gradually and the working electrode was rotated with 200 rpm to mix and homogenize the solution for 5 seconds. SWV, DPSCC, and EIS techniques were performed sequentially. The detection linear range and detection limit parameters of the electrode was calculated from the results of SWV, DPSCC, and EIS measurements.

2.2. Thin Film Formation with Langmuir-Blodgett Coating Technique

In the 1930s, Irving Langmuir and Katharine Blodgett invented a method for creating highly ordered monolayer films at the air/water interface and then transferring these films to substrate surfaces. The LB method entails the use of a molecule with a polar "head group" (e.g., a carboxylate) and a hydrophobic "tail" (e.g., an alkyl chain). When such amphiphilic molecules (called surfactants) are dispersed onto the surface of water, the head groups point down, because they are strongly solvated by water, and the hydrophobic tails point up. The LB method has recently been used to coat electrode surfaces with monolayer and, after multiple transfers, multilayer films. In particular, surfactants with electroactive ions as the head group have been used, and the electrochemical and photoelectrochemical properties of the resulting films have been investigated. The self-assembly and LB methods can, in principle, produce analogous structures on electrode surfaces. The self-assembly method seems, however, to be more versatile and, because of the formation of the chemisorptive chemical bond, should produce a more stable film [42].

The advantages of Langmuir-Blodgett coating technique are listed below:

- Excellent film thickness control
- Homogenous film formation
- Multilayer structures with varying layer compositions
- High degree of orientational order, which make them potential candidates for application in electronic devices and sensors

Disadvantages of this coating technique are:

- Setup of the coating process is difficult
- There is a need of amphyphilic material for coating

Generally only amphyphilic materials are coated on the substrate with this method. In the present study, phthalocyanines were used as coating material even they are not amphyphilic. Therefore, stearic acid was added to the coating solution, since it is electrochemically inert and amphyphilic material.

2.2.1. Materials

Dichloromethane (DCM), chloroform (CHCl_3), LiClO_4 , $\text{CdCl}_2 \cdot \text{H}_2\text{O}$, stearic acid (SA), KCl, H_2PtCl_6 , sodium dodecyl sulfate (SDS), eserine, carbofuran, and tetrabutylammonium perchlorate (TBAP) were purchased from Sigma Aldrich. Ultra-pure water of 18.2 M Ω purified from a Direct-Q purification system was used for aqueous solutions. The stearic acid solution was used as a supporting agent for LB coating. KCl and H_2PtCl_6 materials were dissolved in ultra-pure water for Pt coating solution and sodium dodecyl sulfate (SDS) was added to this solution for nano Pt coating of ITO electrode. On the other hand, a gold solution (Sigma, 1000941718) was used for nano gold coating process. **CoPc-AQ** (Figure 2.2) and **CuPc-AQ** are the materials which were used as the functional material of the electrochemical sensor in this study were synthesized with the procedure declared by Sezer et. al.[43]. Eserine (Sigma, E8375) and carbofuran (Aldrich, 426008) pesticides were the analytes of modified electrochemical sensor. All sensing measurements were carried out in phosphate buffer solution at pH 7.0.

2.2.2. Electrode preparation

Langmuir-Blodgett technique was applied for coating **CoPc-AQ** and **CuPc-AQ** on ITO electrode using a KSV NIMA Langmuir-Blodgett coating device showed in Figure 2.5.

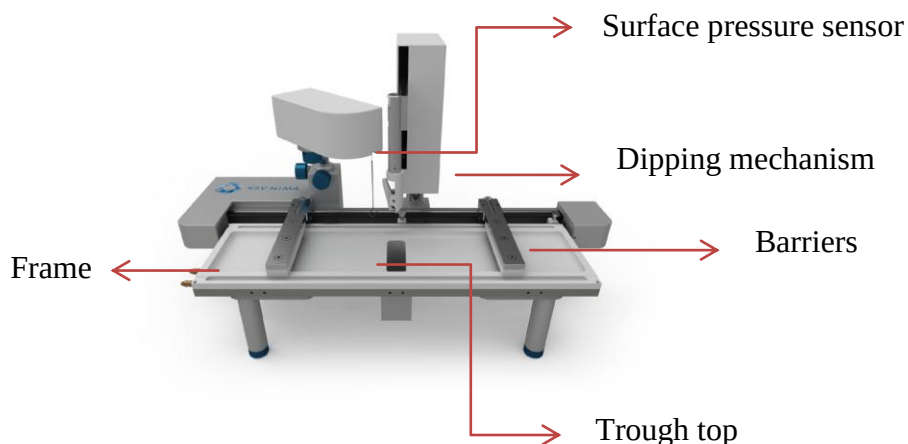


Figure 2.5. KSV NIMA Langmuir-Blodgett deposition trough.

An aqueous subphase is confined in a shallow chamber in the trough top and the monolayer is compressed by the barriers. Surface pressure changes during compression are measured by the surface pressure sensor. The dipping mechanism is used to transfer the monolayer to ITO substrate. The Interface Unit and KSV NIMA LB software enable data transfer and recording.

Figure 2.6 shows the surface pressure (π)-area isotherm of **CoPc-AQ** and **CoPc-AQ-SA** mixture and transfer ratios.

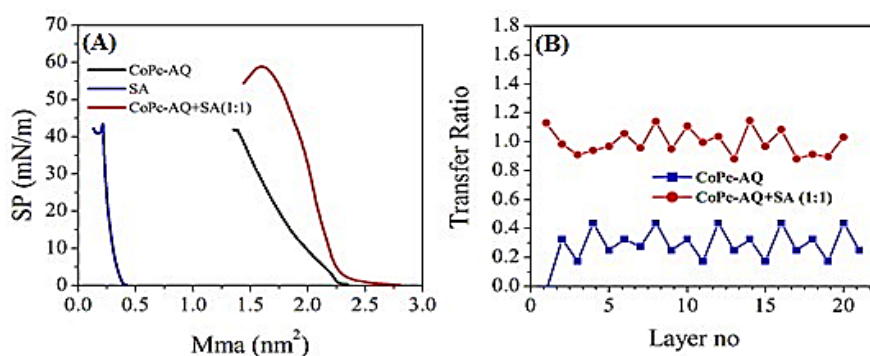


Figure 2.6. (A) The surface pressure (π)-area isotherm of **CoPc-AQ**, SA, and **CoPc-AQ+SA** mixture (1:1), (B) Transfer ratio-layer no changes of **CoPc-AQ** and **CoPc-AQ+SA** mixture (1:1) during upstroke and down stroke film transition on to ITO.

A subphase solution filled in the trough was obtained with aqueous $\text{CdCl}_2 \cdot \text{H}_2\text{O}$ solution at concentration of 1 mg/ml. The coating material **CoPc-AQ** solution was prepared at concentration of 1 mg/ml with chloroform solvent and mixed with SA solution at concentration of 1 mg/ml at 1:1 volumetric ratio. The coating mixture was added on the subphase solution drop by drop until the surface pressure was raised to 0.5mN/m. To detect the ideal surface pressure for monolayer film formation on the subphase, the surface pressure (SP)-mean molecular area (Mma) isotherm was obtained and the ideal surface pressure was detected as 35mN/m. 20 layers of **CoPc-AQ**:SA were coated on ITO electrodes with 5 mm s^{-1} up and down stroke rates and the electrode was waited for 5 minutes at up position. The coated electrodes were abbreviated as ITO/**CoPc-AQ**.

Although **CoPc-AQ** formed well-defined monolayer on the water subphase of the LB trough, it could not be transferred on the ITO electrode properly. As shown in Figure 2.6(B), transfer ratios of **CoPc-AQ** are at around 0.4 for all “up stroke” and “down stroke” which indicates that **CoPc-AQ** did not transferred as well resolved monolayers on the ITO substrate surface, thus SA solution was added to **CoPc-AQ** to facilitate the formation of monolayers on the ITO substrates. When 1:1 by volume mixture of SA and **CoPc-AQ** is used, all transfer ratios during both “up stroke” and “down stroke” are recorded as 1.0 ± 0.1 , which indicate coating of monolayer film with a Y type transfer. 20 LB monolayers could be coated on ITO surface with all **CoPc-AQ** (21 μl) sprayed on the tray of the LB instrument in one trial. The area per **CoPc-AQ** molecule is calculated from the slope of the isotherm curve as $205 \text{ \AA}^2$ which is in agreement with those of similar phthalocyanines in the literature. Limiting areas of the complex indicate face-on orientations of the complex on the water subphase. Presence of SA in **CoPc-AQ** just increases the limiting area to $225 \text{ \AA}^2$.

The same immobilization techniques were applied for **CuPc-AQ** material to compare the effect of copper central metal of phthalocyanine with cobalt. **CuPc-AQ** complex as shown in Figure 2.7 was synthesized and it is used for mimetic biosensor or electrochemical sensor studies.

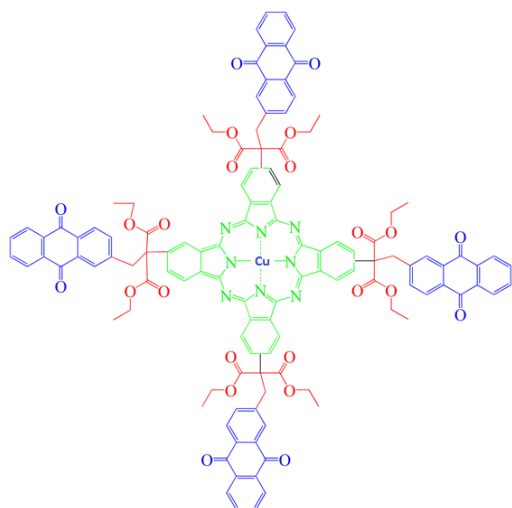


Figure 2.7. Chemical structure of **CuPc-AQ**.

Figure 2.8 shows the surface pressure-mean molecular area isotherm of **CuPc-AQ** and **CuPc-AQ-SA** mixture and transfer ratios.

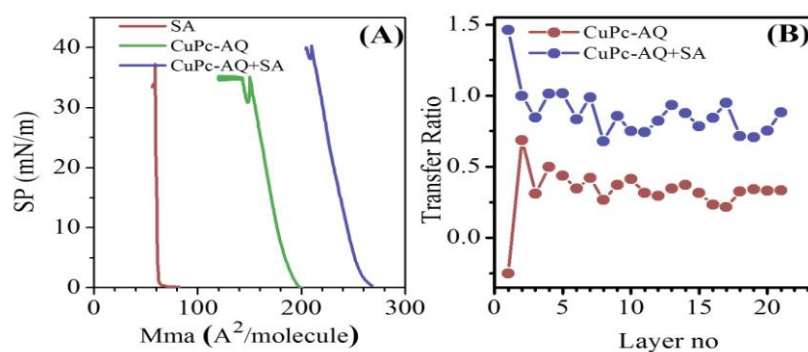


Figure 2.8. LB studies of **CuPc-AQ**: (A) the surface pressure-Mean molecular area isotherm of SA, **CuPc-AQ** and **CuPc-AQ+SA** mixture and (B) transfer ratios of **CuPc-AQ** and **CuPc-AQ+SA**.

In Figure 2.8(A), **CuPc-AQ** seems to be formed as monolayer on the subphase of the LB trough, but it could not be transferred on the ITO electrodes with 1.0 transfer ratio. In Figure 2.8(B), transfer ratios of **CuPc-AQ** were at around 0.4 for all “up stroke” and “down stroke” coating. SA solution was added to **CuPc-AQ** to improve the formation of monolayers with 1.0 transfer ratio on the ITO substrates. When 1:1 mixture of SA and **CuPc-AQ** is used, a Y type transfer occurred and both “up stroke” and “down stroke” transfer ratios are recorded as 1.0 ± 0.1 . Y type transfer means, material transfer onto ITO electrode during upward motion and downward motion of the ITO. 20 LB monolayers were coated on ITO surface with 21 μl **CuPc-AQ** solution sprayed on the

tray of the LB instrument in one trial. When the numbers of coated monolayers were increased more than 20 layers the electrochemical measurements were not very successful because of the thick film which obstructs ion exchange during the measurements. The molecular area of **CuPc-AQ** is calculated from the slope of the isotherm curve as $184 \text{ \AA}^2$ which is in agreement with those of similar phthalocyanines in the literature. The exact molecular area of the complex indicates that it orientated horizontally face-on on the subphase. Presence of SA in **CuPc-AQ** just increases the limiting area to $255 \text{ \AA}^2$ do not affect the electrochemical measurements.

To determine possible practical usage of the **CoPc-AQ** and **CuPc-AQ** as active electrochemical devices, they were coated on ITO electrodes with Langmuir Blodgett (LB) technique (ITO/**CoPc-AQ** and ITO/**CuPc-AQ**), and these electrodes were then modified with nano-platinum and nano gold as shown in Figure 2.9.

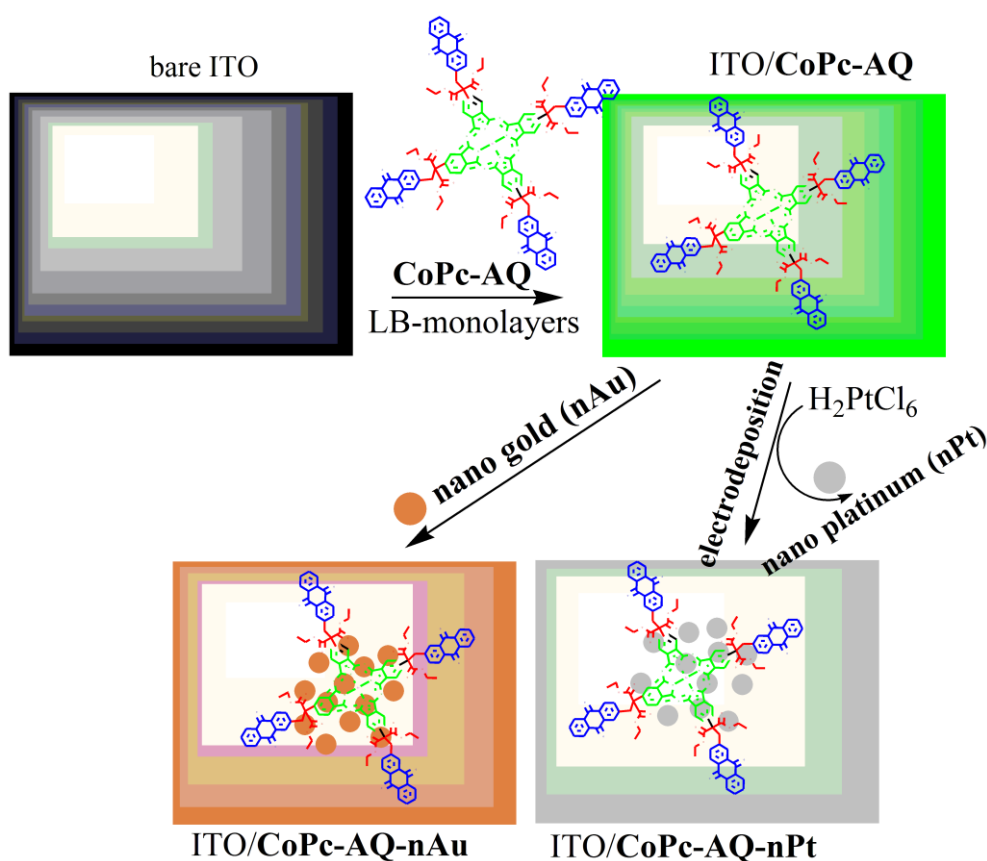


Figure 2.9. Construction of electrochemical pesticide sensor electrodes, ITO/**CoPc-AQ**, ITO/**CoPc-AQ**-nPt, and ITO/**CoPc-AQ**-nAu.

ITO/**CoPc-AQ** and ITO/**CuPc-AQ** electrodes were modified with nano platinum and nano gold. ITO/**CoPc-AQ**-nPt modified electrode was prepared with coating n-Pt on ITO/**CoPc-AQ** with a mixture of 0.1 M KCl and 1 mM H_2PtCl_6 in 10 ml ultra-pure water. 0.8 mg (1% of total weight of solution) sodium dodecyl sulfate (SDS) surfactant that prevented agglomeration of nano-Pt particles was added into this electrolyte before the addition of 1 mM H_2PtCl_6 . 5 repetitive CV cycles were applied between 0.30 V and -0.70 V at 0.100 V s^{-1} scan rate for coating nano-Pt on the ITO/**CoPc-AQ** electrode. ITO/**CoPc-AQ**-nAu electrode was prepared by dropping 10 μL of nano-gold solution on the 0.64 cm^2 area of ITO/**CoPc-AQ** electrode and dried in a vacuum oven at room temperature.

2.2.3. Electrochemical measurements

The electrochemical measurements of the **AQ-CoPc** and **AQ-CuPc** were carried out with a Gamry Reference 600 potentiostat/galvanostat utilizing a three-electrode cell configuration at 25°C . For cyclic voltammetry (CV), and square wave voltammetry (SWV) measurements, the working electrode was a glassy carbon working electrode (GCE) with a surface area of 0.071 cm^2 . Surface of GCE was polished with a diamond suspension before each run. A Pt wire served as the counter electrode. Saturated calomel electrode (SCE) was employed as the reference electrode and separated from bulk of the solution by a double bridge. Ferrocene was used as an internal reference. Tetrabutylammonium perchlorate (TBAP) in dichloromethane (DCM) was employed as the supporting electrolyte at a concentration of 0.10 M. High purity N_2 was used to remove dissolved O_2 for at least 15 minute prior to each run and to maintain a nitrogen blanket during the measurements. IR compensation was applied to the CV and SWV scans to minimize the potential control error. Electrochemical impedance spectroscopy (EIS) measurements were performed in PBS (pH= 7.0) under 10 mV AC voltage versus open circuit potential (open circuit voltage is 0.50 V) in the AC frequency range 300 kHz - 10 Hz with a 10 mV excitation AC voltage.

2.2.4. Sensor measurements

Pesticide sensor measurements of ITO/**CoPc-AQ**, ITO/**CoPc-AQ**-nPt, ITO/**CoPc-AQ**-nAu, ITO/**CuPc-AQ**, ITO/**CuPc-AQ**-nPt, and ITO/**CuPc-AQ**-nAu electrodes as

electrochemical pesticide sensors were performed with square wave voltammetry (SWV), electrochemical impedance spectroscopy (EIS) and double potential step chronocoulometry (DPSCC) analysis with two different pesticides (eserine and carbofuran). The three-electrode cell configuration and the solution of 0.1 M lithium perchlorate (LiClO_4), as the electrolyte, dissolved in 0.1 M PBS were used for sensor measurements. Before sensing measurements, the blank measurements were stabilized for SWV, DPSCC, and EIS techniques. Then, pesticide solutions were added with increasing amounts, gradually and electrochemical measurements of SWV, DPSCC, and EIS techniques were performed with a sequence manner, respectively. The detection linear range and detection limit parameters of the electrodes were calculated from the analysis of all SWV, DPSCC, and EIS results and compared with each other for selectivity and sensitivity.

2.3. Electropolymerization Technique

Film electrode preparation with electropolymerization technique is the most versatile approach for preparing chemically modified electrodes. In many of the recent studies, polymer film method is used for chemical modified electrodes. When the experimental conditions arranged, preparing multi layer films and controlling the thickness of the film is easy. Another advantage of the polymer films is being insoluble in solution phase, thus, there is not any loss of film material and film degradation. Desired electrochemical properties can be obtained by modifying the chemical structure of the polymer.

A number of different methods can be used to prepare polymer film-coated electrodes.

- The simplest is to dip the surface to be coated into a solution of the polymer, remove the electrode from the solution, and allow the solvent to evaporate. While this method is simple, it is difficult to control the amount of material that ends up on the electrode surface.
- Alternatively, a measured volume of solution can be applied to the surface to be coated. This allows for accurate control of the amount of polymer applied.
- The polymer film may also be spin-coated onto the electrode surface. Spin-coating is used extensively in the semi-conductor industry and yields very uniform film thicknesses.

- Polymer films can also be electropolymerized directly onto the electrode surface (Thiophen, pyrrole and aniline are three of these kind of materials).
- The electronically conductive polymers can also be electrosynthesized from the corresponding monomer (polyacetylene, polypyrrole and polyaniline)[42].

2.3.1. Materials

The experimental studies were carried on with high purity chemicals. Dichloromethane (DCM), tetra-butyl ammonium perchlorate (TBAP), phosphate buffer solution (PBS) at pH 7.0 and lithium perchlorate (LiClO₄) were purchased from Sigma Aldrich. Ultra-pure water of 18.2 MΩ purified from a Direct-Q purification system was used for aqueous solutions. **H₂Pc-THQD** and **NiPc-THQD** (Figure 2.10) are the main materials which were used as the functional material of the biomimic electrochemical sensor in this study was synthesized[44]. Eserine (Sigma, E8375), carbofuran (2,3dihydro-2,2-dimethyl-7 benzofuranol N-methyl carbamate) (Aldrich, 426008-25G), carbaryl (Fluka, 36856, 100ng/μl in cyclohexane) and parathion (Fluka, 45607-100MG) pesticides were the analyts of modified biomimic electrochemical sensors. All sensing measurements were carried out in phosphate buffer solution (PBS) at pH 7.0.

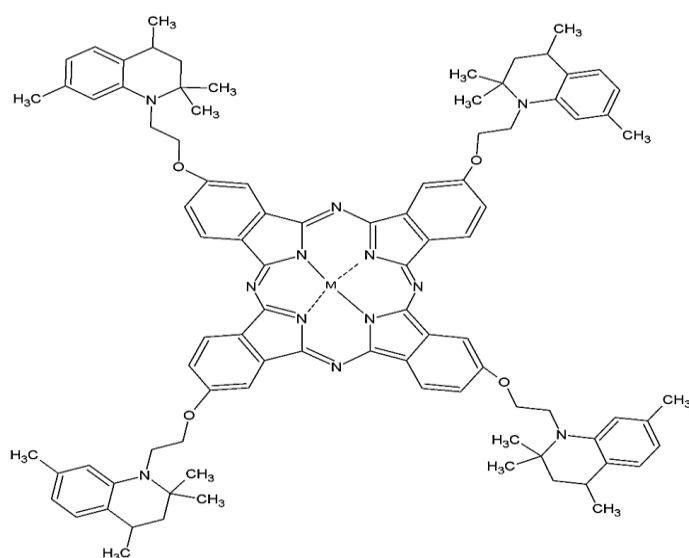


Figure 2.10. Chemical structure of the complexes of **H₂Pc-THQD** and **NiPc-THQD** phthalocyanines.

2.3.2. Electrode preparation

Peripherally 2-(2,2,4,7-tetramethyl-3,4-dihydroquinolin-1(2H)-yl) ethanol substituted phthalocyanine complex was synthesized and its spectroscopic and electrochemical characterizations were performed[44]. When the electrochemical responses of the synthesized complex is studied, the CV and SWV responses of **H₂Pc-THQD** and **NiPc-THQD** exhibited two diffusion controlled and quasi-reversible reductions processes which indicates that the complex can be used as potential electrosensor for the detection of various target species.

However, the polymerization process was observed at anodic potentials as represented in Figure 2.11.

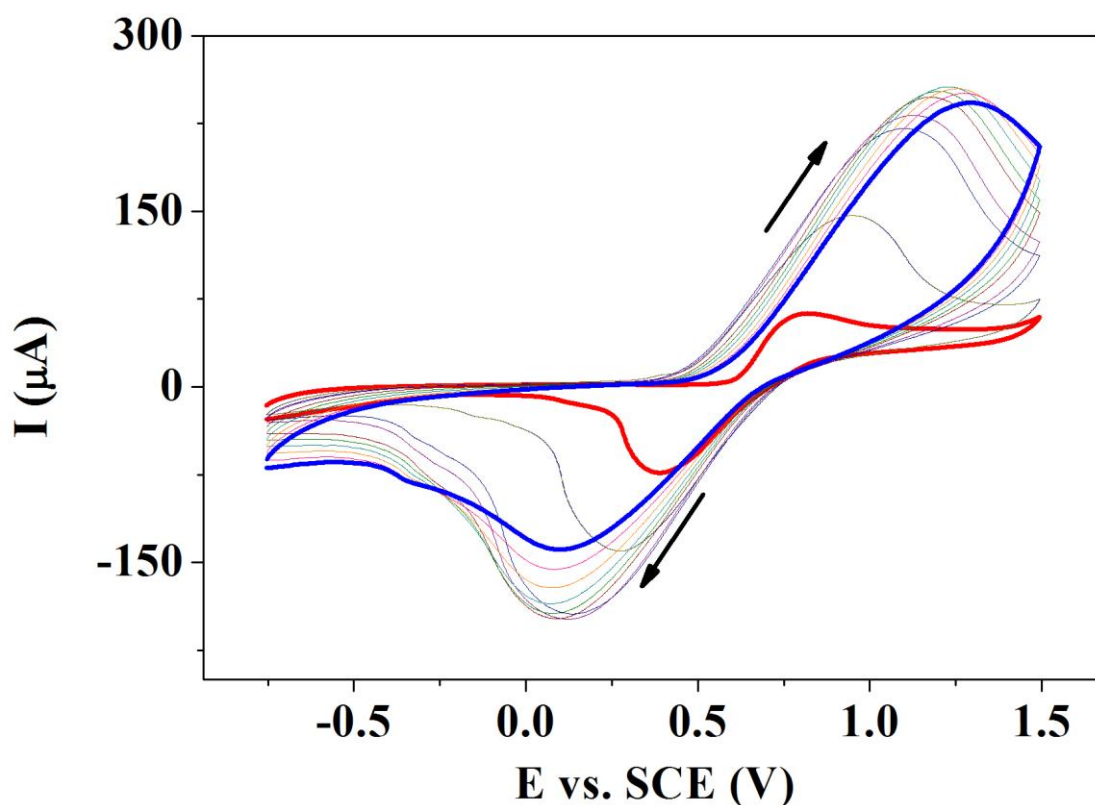


Figure 2.11. Electropolymerization of **H₂Pc-THQD** on the electrode with CV technique.

For the GCE/poly- **H₂Pc-THQD** electrode preparation, the GCE was immersed into the coating solution containing 1mg **H₂Pc-THQD** dissolved in 4 ml of DCM/TBAP and cyclic voltammetry technique was applied between -0.75 V and 1.50 V for 10 cycles. During the CV scans a significant anodic peak was observed at 0.80 V and its cathodic

couple at 0.38 V. The peak potentials were shifted and current intensities were increased continuously. After 7 cycles, the current intensity began decreasing because the thickness of the polymer layer on the electrode was increased enough and after this thickness supporting electrolyte (TBAP) ions were hardly diffused into/out of this polymer film.

2.3.3. Electrochemical measurements

The electrochemical and spectroelectrochemical measurements were carried out with a Gamry Reference 600 potentiostat/galvanostat utilizing a three-electrode cell configuration at 25 °C. For square wave voltammetry (SWV) measurements, the working electrode was a glassy carbon working electrode (GCE) with a surface area of 0.071 cm². Surface of GCE was polished with a diamond suspension before each run. A Pt wire served as the counter electrode. Saturated calomel electrode (SCE) was employed as the reference electrode and separated from bulk of the solution by a double bridge. Ferrocene was used as an internal reference. Tetrabutylammonium perchlorate (TBAP) in dichloromethane (DCM) was employed as the supporting electrolyte at a concentration of 0.10 M. High purity N₂ was used to remove dissolved O₂ for at least 15 minute prior to each run and to maintain a nitrogen blanket during the measurements. IR compensation was applied to the SWV scans to minimize the potential control error. Electrochemical impedance spectroscopy (EIS) measurements were performed in PBS (pH= 7.0) under 10 mV AC voltage versus open circuit potential (open circuit voltage is 0.50 V) in the AC frequency range 300 kHz - 10 Hz with a 10 mV excitation AC voltage.

2.3.4. Sensor measurements

The electrochemical and spectroelectrochemical measurements were carried out with a Gamry Reference 600 potentiostat/galvanostat utilizing a three-electrode cell configuration at 25 °C. GCE/poly-**H₂Pc-THQD** and GCE/poly- **NiPc-THQD** electrodes (GCE/poly-**H₂Pc-THQD** and GCE/poly-**NiPc-THQD**) were tested with four different pesticides (carbamate pesticides: Carbaryl, carbofuran, eserine and organophosphate pesticide: Parathion) whether they can be used as an selective electrochemical pesticide sensor or not. Square wave voltammetry (SWV), double potential step

chronocoulometry (DPSCC) and electro impedance spectroscopy (EIS) responses of GCE/poly- **H₂Pc-THQD** and GCE/poly- **NiPc-THQD** electrodes to varying concentration of different pesticides were recorded. The electrolyte solution used in the electrochemical cell of three-electrode cell configuration was prepared with dissolving 0.10 M lithium perchlorate (LiClO₄) in phosphate buffer solution (PBS, pH=7.0). High purity N₂ gas was used to remove undesired dissolved O₂ from the solution before each run and to form a nitrogen atmosphere in the cell during the measurements. Before sensing measurements, blank tests of modified electrodes were taken with SWV, DPSCC, and EIS techniques, respectively. When the blank measurements were stabilized, pesticide solutions were added with increasing amounts gradually. Then, all electrochemical measurements SWV, DPSCC, and EIS, were carried out again with a sequence manner. Basic sensor parameters were derived from the analysis of all of SWV, DPSCC, and EIS separately and compared with each other with respect to selectivity and sensitivity.

2.4. Click Chemistry and Click Electrochemistry Techniques

Click chemistry is a new method for quickly and reliably synthesis of new substances by joining small modular units. The term was coined by K. Barry Sharpless for the first time in 2001. The 1,3-dipolar cycloaddition of azides with alkynes was first discovered by Huisgen in 1963 (Figure 2.12). However, it did not attract much interest until it was demonstrated that this high temperature reaction could also be carried out under mild conditions using Cu^I as the catalyst, and with tremendous regio-selectivity. Thus, this technique can be used to modify sensor electrodes to immobilize the recognition substance on the electrode surface[45].

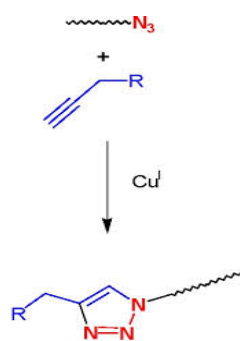


Figure 2.12. Basic reaction mechanism of click chemistry.

Some of the advantages of click chemistry are listed below:

- Click Chemistry reaction is very selective that it takes place only between azide and alkyne components. Interference does not occur with any other organic groups.
- The reaction is biocompatible and it can be used in living cells. Aqueous DMSO, DMF, alcohols, acetonitrile, water and buffers can be used as solvent for the reaction.
- Reaction is quick and quantitative.
- The reaction is pH-insensitive that there is no need to control pH in reaction mixture. There is no need to add any special buffer, acid or base - Click Chemistry works well in pH interval of 4-11.
- Simple process[45].

The only disadvantage of the method is that copper catalyst has adverse effects according to its toxicity.

In this study a new version of the click chemistry is applied with electrochemical reduction of Cu^{II} to Cu^{I} in the reaction media and this method is entitled as “click electrochemistry” (CEC).

2.4.1. Materials

The experimental studies were carried on with high purity chemicals. Dimethylsulfoxide (DMSO) (Merck, K38436331 807, 99.9% purity) and ultra-pure water ($\geq 18 \text{ M}\Omega$, Milli-Q, Millipore) were used as solvents. Tetrabutylammonium perchlorate (TBAP) (Fluka Analytical, 86893, $\geq 99\%$ purity) and lithium perchlorate (Aldrich, 431567-50G) were used as ion carrier reagents in the solutions. Carbaryl (Fluka, 36856, 100 ng/ μl cyclohexane), carbofuran (Aldrich, 426008-25G), eserine (Sigma, E8375-5G) and parathion (Fluka, 45607-100MG), H_2SO_4 (Merck, K33449513 426, 95-98% purity), 4-Azidoaniline.HCl (Aldrich, 359556-250MG), CuBr (Merck Millipore, 8146580050) and CuSO_4 (Merck Millipore, 1027910250) were used as received. Terminal alkynyl-substituted phthalocyanine with cobalt, zinc and H_2 in the central region were used as recognition elements of the biomimic electrochemical

sensor. TA-CoPc: 2,9(10),16(17),23(24)-tetrakis(pent-4-ynoxy)phthalocyanine exhibited in Figure 2.13 was synthesized according to the procedure developed by Dinçer et. al.[46].

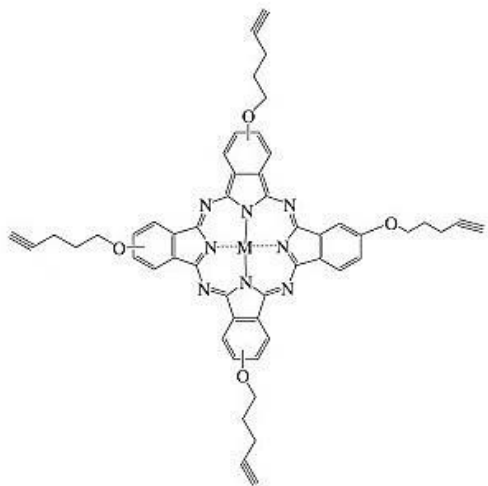


Figure 2.13. Chemical structure of terminal alkynyl-substituted cobalt phthalocyanine (TA-CoPc:2,9(10),16(17),23(24)-tetrakis(pent-4-ynoxy)phthalocyanine).

2.4.2. Electrode preparation

Following solutions were prepared for click chemistry and click electrochemistry reactions.

Solution I: 4.0 mg 4-Azidoaniline.HCl (ANI-N₃) and 0.080 g LiClO₄ was dissolved in 4.0 ml ultra-pure water, then the solution was acidified with 300 µl concentrated H₂SO₄.

Solution II: 0.017 g TBAP, 0.287 mg CuBr, and 0.180 mg Triacetylene-CoPc (TA-CoPc) were dissolved in 2.00 ml DMSO.

Solution III: 0.017 g TBAP, 1.80 mg CuSO₄, and 0.180 mg TA-H₂Pc or TA-ZnPc or TA-CoPc were dissolved in 2.00 ml DMSO.

2.4.3.1. GCE/PANI-N₃ electrode preparation

GCE was coated with PANI-N₃ electrochemically in solution I. For this purpose, repetitive 10 CVs on GCE working electrode were recorded in three-electrode cell containing 4.0 mL “solution 1” as electrolyte. As results of potential cycling between -0.50 V and 1.50 V potentials, ANI-N₃ was electropolymerized on GCE and formed

GCE/PANI-N₃ electrode. GCE/PANI-N₃ electrodes were washed with ultra-pure water to remove unreacted species, then dried at room temperature under vacuum atmosphere, and finally used as azide sources for CC and CEC.

2.4.2.1. GCE/PANI-N₃/TA-MPc electrode preparation with CC

GCE/PANI-N₃ electrode was dipped into the solution II and waited for 3 hours at the room temperature for self-click reaction between azide group of PANI-N₃ and terminally alkyne groups of phthalocyanine (TA-H₂Pc or TA-ZnPc or TA-CoPc) and formation of GCE/PANI-N₃/TA-MPc electrodes. GCE/PANI-N₃/TA-MPc electrodes were washed with DMSO three times to remove unreacted species, then dried at room temperature in a vacuum oven, and finally used as an electrochemical pesticide sensor.

2.4.2.2. GCE/PANI-N₃/TA-MPc electrode preparation with CEC

CV responses of CuSO₄ were firstly tested in DMSO to determine the Cu^I formation potential. It was determined that CuSO₄ gave Cu^{II}/Cu^I couple at -0.15 V in DMSO. CEC was performed in the “solution III” electrolyte on **GCE/PANI-N₃** working electrode. CEC reaction was performed in three steps. First of all, CV of **GCE/PANI-N₃** was recorded between -0.50 and 1.20 V to determine the electrochemical responses of TA-MPc and **PANI-N₃** in the “solution III” before CEC. Then -0.15 V constant potential was applied to the **GCE/PANI-N₃** working electrode for 10 minutes to generate Cu^I ions electrochemically needed for CEC.

Finally CV of **GCE/PANI-N₃** was sequentially recorded between -0.50 and 1.20 V again to determine the electrochemical responses of GCE/PANI-N₃/TA-MPc electrodes after CEC. The modified electrodes, GCE/PANI-N₃/TA-MPc, were washed with DMSO three times to remove unreacted species and then dried in a vacuum oven at room temperature, and finally used as an electrochemical pesticide sensor.

The modification of GCE with PANI-N₃ and then with TA-MPc is illustrated in Figure 2.14.

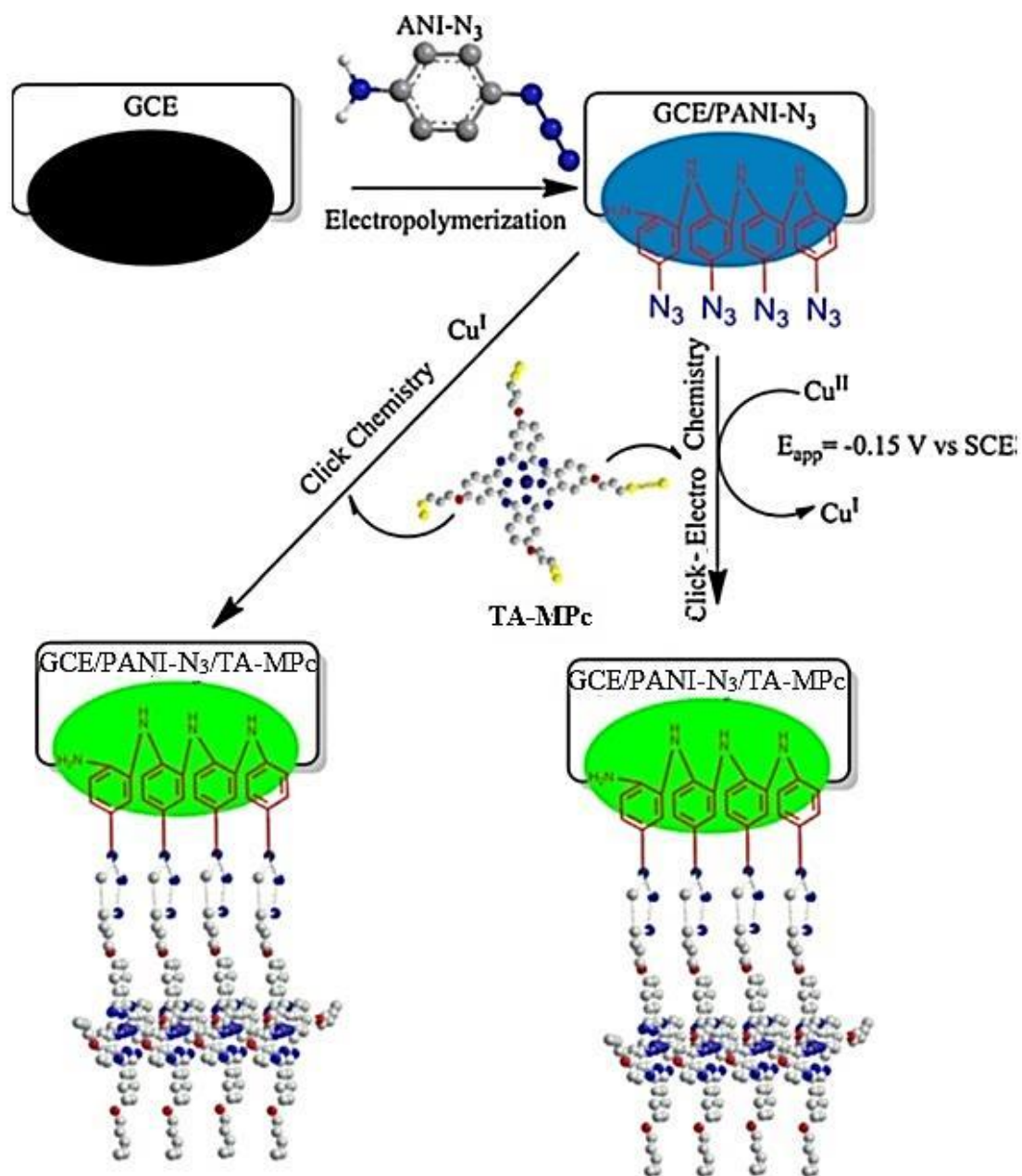


Figure 2.14. Electrode modification via the “click chemistry” (CC) and the “click electrochemistry” (CEC).

2.4.3. Electrochemical measurements

The electrochemical applications and measurements were carried on with a potentiostat (GAMRY Instruments, Reference 600 Potentiostat/Galvanostat/ZRA) utilizing a three-electrode cell configuration at 25 °C. For square wave voltammetry (SWV), electro impedance spectroscopy (EIS) and double potential step chronocoulometry (DPSCC) measurements, the working electrode was a bare or modified glassy carbon electrode

(GCE) with a surface area of 0.196 cm^2 for GCE/**PANI-N₃/TA-H₂Pc**, GCE/**PANI-N₃/TA-ZnPc** and GCE/**PANI-N₃/TA-CoPc** electrodes. A Pt wire was used as the counter electrode. GCE (5 mm disk) was polished before use with polishing paste (Gamma Micropolish II, 0.05micron). Saturated calomel electrode (SCE) was employed as the reference electrode and separated from bulk of the solution by a double bridge. All sensor applications were performed in phosphate-buffer solution (PBS: 0.1 M KH₂PO₄ + 0.1 M K₂HPO₄) containing 0.1 M LiClO₄ as supporting electrolyte. High purity N₂ was used to purge dissolved O₂ for at least 15 minute prior to each run and to maintain a nitrogen atmosphere over the solution during the measurements. DPSCC measurements were taken after SWV sensing measurements by using the peak potentials of newly formed or disappeared peaks. Electrochemical impedance spectroscopy (EIS) measurements of the GCE/**PANI-N₃**, GCE/**PANI-N₃/TA-H₂Pc**, GCE/**PANI-N₃/TA-ZnPc** and GCE/**PANI-N₃/TA-CoPc** electrodes prepared as described below were performed in phosphate buffer solution under 10 mV AC excitation voltage versus open circuit potential (open circuit voltage is 0.50 V) in the AC frequency range 300 kHz - 10 Hz.

2.4.4. Sensor measurements

To test the utilizability of **GCE/PANI-N₃/TA-MPc** electrodes prepared with CC method and CEC method and SWV, DPSCC and EIS analysis of **GCE/PANI-N₃/TA-MPc** electrodes were recorded during titration with four different pesticides (carbaryl, carbofuran, eserine and parathion). 0.1 M lithium perchlorate (LiClO₄) dissolved 0.1 M PBS was used as the electrolyte in the electrochemical cell with three-electrode cell configuration. **GCE/PANI-N₃/TA-MPc** electrodes were used as electrochemical pesticide sensors (EPS). Before sensing measurements, blank tests of the electrodes were taken with SWV, DPSCC, and EIS techniques, respectively. When the blank measurements were stabilized, pesticide solution was added into the electrolyte with increasing amounts gradually using a micro pipette. Then, all electrochemical measurements (SWV, DPSCC, and EIS) were carried out again with a sequence manner. Basic sensor parameters were derived from the analysis of all SWV, DPSCC, and EIS separately and compared with each other with respect to linear range, detection limit, selectivity and sensitivity.

2.5. LB-Click Electrochemistry Technique

2.5.1. Materials

ITO electrode was first coated with the sensor recognition material **TA-CoPc** (Figure 2.16) using Langmuir-Blodgett technique. Terminal alkynyl-substituted cobalt phthalocyanine (TA-CoPc: 2,9(10),16(17),23(24)-tetrakis (pent-4-ynoxy) phthalocyaninatocobalt (II)) exhibited in Figure 2.16 was synthesized according to the procedure developed by Dincer et. al.[46]. Eserine (Sigma, E8375) was the analyte of modified biomimic electrochemical sensor. Chloroform (CHCl_3), $\text{CdCl}_2 \cdot \text{H}_2\text{O}$ and stearic acid (SA) were used for Langmuir-Blodgett technique purchased from Sigma Aldrich. Ultra-pure water of 18.2 M Ω purified from a Direct-Q purification system was used for aqueous solutions. The stearic acid solution was used as a supporting agent for LB coating.

After preparation of ITO/TA-CoPc, the electrode was exposed to click electrochemistry technique to click PANI- N_3 on the electrode. The experimental studies were carried on with high purity chemicals. Dimethylsulfoxide (DMSO) (Merck, K38436331 807, 99.9% purity) and ultra-pure water (≥ 18 M Ω , Milli-Q, Millipore) were used as solvents. Tetrabutylammonium perchlorate (TBAP) (Fluka Analytical, 86893, $\geq 99\%$ purity) and lithium perchlorate (Aldrich, 431567-50G) were used as ion carrier reagents in the solutions. 4-Azidoaniline.HCl (Aldrich, 359556-250MG) and CuSO_4 (Merck Millipore, 1027910250) were used as received. All sensing measurements were carried out in phosphate buffer solution (PBS) at pH 7.0.

2.5.2. Electrode preparation

This electrode modification technique is a combination of Langmuir-Blodgett coating technique and click electrochemistry technique. This method is developed to see the responses of the electrode as a pesticide sensor and whether this combination of the electrochemical sensor is more stable from the *GCE/PANI- N_3 /TA-CoPc* electrode or not when PANI- N_3 coating layer is on the outer side. For this purpose ITO electrode is coated with 20 layers of **TA-CoPc** with Langmuir-Blodgett technique. The subphase $\text{CdCl}_2 \cdot \text{H}_2\text{O}$ was at concentration of 1 mg/ml. The TA-CoPc solution was prepared at concentration of 1 mg/ml with chloroform solvent and mixed with SA solution at

concentration of 1 mg/ml at 1:1 volumetric ratio. The coating mixture was added on the subphase solution until the surface pressure was achieved to 0.5mN/m. For monolayer film formation the surface pressure on the subphase was detected as 35mN/m.

After ITO/**TA-CoPc** electrode was prepared with LB coating method it was immersed into the solution I containing CuSO_4 and -0.15 V constant potential was applied for 10 minutes. Then the ITO/**TA-CoPc/PANI-N₃** electrode was washed with ultra pure water to remove unbonded 4-Azidoaniline.HCl from the electrode surface. ITO/**TA-CoPc/PANI-N₃** electrode preparation procedure is illustrated in Figure 2.15.

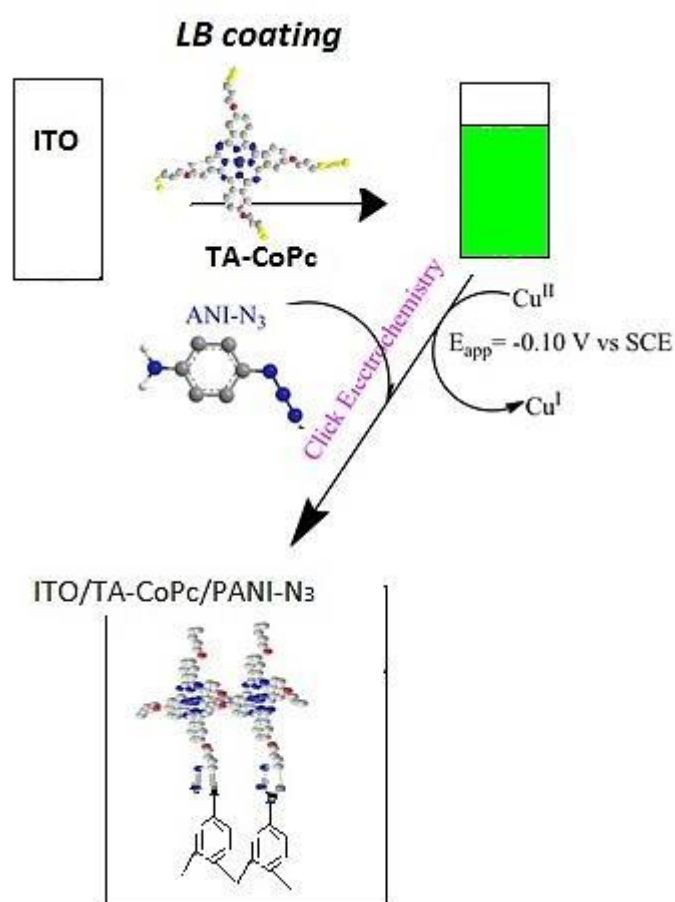


Figure 2.15. LB-Click Electrochemistry modification technique.

2.5.3. Electrochemical measurements

GAMRY Instruments, Reference 600 Potentiostat/Galvanostat/ZRA potentiostat was used for electrochemical applications and measurements at room temperature. For square wave voltammetry (SWV), electroimpedance spectroscopy (EIS) and double

potential step chronocoulometry (DPSCC) measurements, the working electrode was a bare or modified ITO (ITO/**TA-CoPc/PANI-N₃**) electrode with a modified surface area of 0.64 cm². A Pt wire was used as the counter electrode. Bare ITO electrode was cleaned before use with chloroform solvent using isostatic paper to prevent unwanted dust residual on the electrode. Saturated calomel electrode (SCE) was employed as the reference electrode and separated from bulk of the solution by a double bridge. All sensor applications were performed in phosphate-buffer solution (PBS: 0.1 M KH₂PO₄ + 0.1 M K₂HPO₄) containing 0.1 M LiClO₄ as supporting electrolyte. High purity N₂ was used to purge dissolved O₂ for at least 15 minute before each experiment and to maintain a nitrogen atmosphere over the solution during the measurements. DPSCC measurements were performed after SWV sensing measurements by using the peak potentials of newly formed or disappeared peaks. Electrochemical impedance spectroscopy (EIS) measurements of the ITO/**TA-CoPc/PANI-N₃** electrode were performed in phosphate buffer solution under 10 mV AC excitation voltage versus open circuit potential (open circuit voltage is 0.50 V) in the AC frequency range 300 kHz - 10 Hz.

2.5.4. Sensor measurements

ITO/**TA-CoPc/PANI-N₃** electrode prepared with LB-Click Electrochemistry technique was exposure to a series of electrochemical pesticide sensor measurements. SWV, DPSCC and EIS analysis of ITO/**TA-CoPc/PANI-N₃** electrodes were recorded during titration with eserine pesticide. 0.1 M lithium perchlorate (LiClO₄) dissolved 0.1 M PBS was used as the electrolyte in the electrochemical cell with three-electrode cell configuration at 25°C. Before sensing measurements, blank tests of ITO/**TA-CoPc/PANI-N₃** electrodes were taken with SWV, DPSCC, and EIS techniques, respectively. When the blank measurements were stabilized, pesticide solution with a known concentration was added into the electrolyte with gradually increasing amounts using a micro pipette. Then, all electrochemical measurements (SWV, DPSCC, and EIS) were carried out again with a sequence manner. Basic sensor parameters were determined with the SWV, DPSCC, and EIS measurement results.

3. RESULTS AND DISCUSSION

3.1. Modified Carbon Paste Electrode Sensor Prepared with Moulding Technique

Before adding the eserine into the solution, the solved oxygen was purged by N_2 gas flow through the solution and an inert N_2 atmosphere was obtained. Eserine solution was added gradually and the working electrode was rotated with 200 rpm to mix and homogenize the solution for 5 seconds. SWV, DPSCC, and EIS techniques were performed sequentially. The detection linear range and detection limit parameters of the electrode were calculated from the results of SWV, DPSCC, and EIS measurements.

In Figure 3.1 SWV measurement results of eserine titration with carbon paste electrode modified with CoPc-AQ is exhibited.

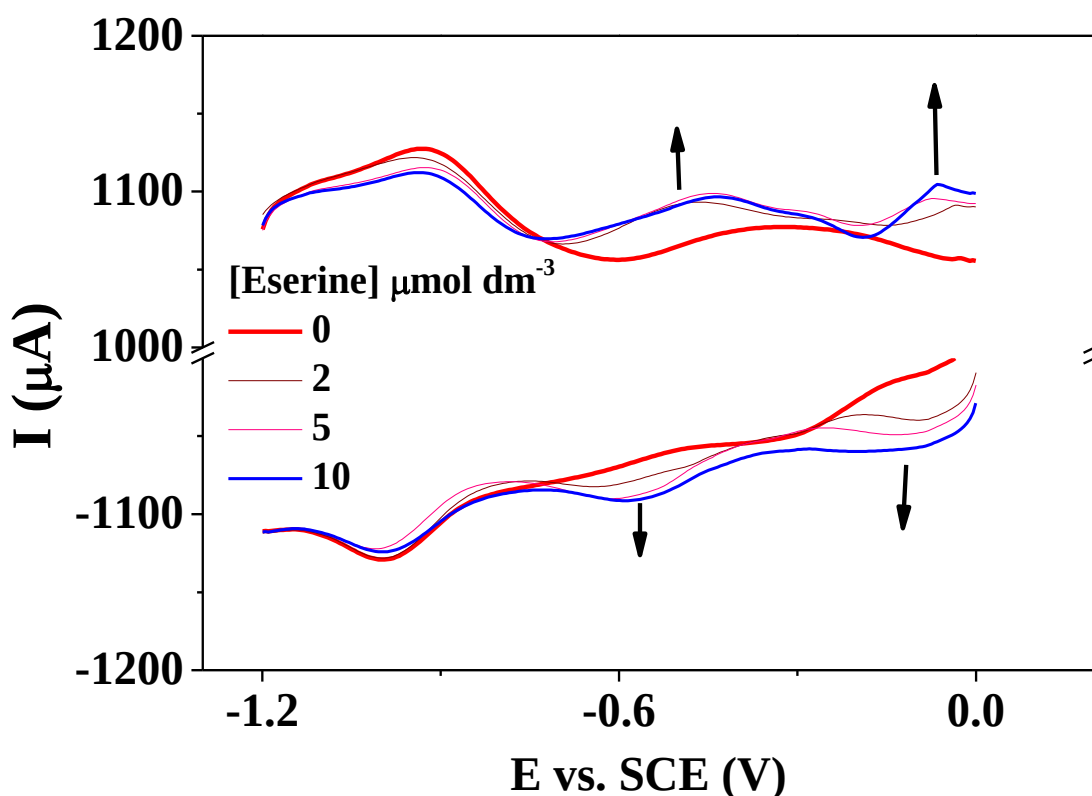


Figure 3.1. SWV analysis of eserine with CPE/CoPc-AQ sensor.

The current intensities are considerably high but there is a significant change in the redox activities of the modified sensor. SWV measurements without eserine addition show two cathodic peaks. These peaks are assigned to Co^{II}/Co^I reduction at around -0.50 V

and AQ reduction at about -1.00 V. After gradually increasing addition of eserine pesticide into the electrolytic solution new peaks are observed at -0.09 V and -0.57 V while the peaks at -0.50 V and -0.32 V were decreased in current intensity. The peak changes can be thought as peak shifts and the changing potentials of the peaks provide potentiometric sensor while the current intensity changes lead to an amperometric sensor. Thus, this modified electrode can be used as a potentiometric or as an amperometric sensor. The calibration lines derived from SWV responses is illustrated in Figure 3.2.

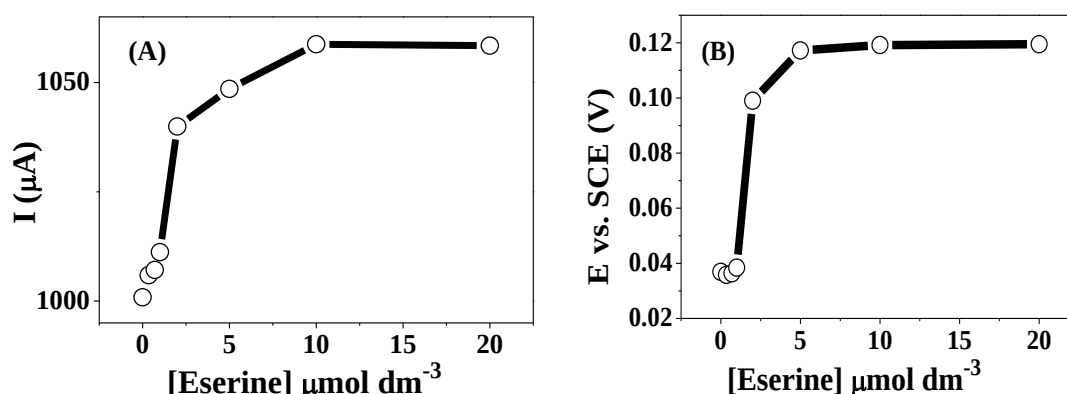


Figure 3.2. Amperometric (A) and potentiometric (B) calibration lines derived from SWV measurements of the **CPE/CoPc-AQ** electrode titrated with eserine.

The linear detection ranges and limit of detection LOD values are represented in Table 3.1 below.

Table 3.1. The linear detection range and limit of detection LOD values of **CPE/CoPc-AQ** electrode for eserine analysis.

Electrode	Sensing technique		Linear range (μM)	LOD (M)	Sensitivity
CPE/CoPc-AQ	SWV	Amperometric	0.204-2.111	3.12×10^{-9}	21.52 A/M
		Potentiometric	0.889-2.035	1.61×10^{-8}	0.01 V/μM
	EIS	Impedimetric	0.041-2.638	1.35×10^{-9}	4.83 ohm/μM
	DPSCC	Amperometric	0.074-1.035	2.26×10^{-8}	3638.95 C/M

According to the lowest LOD result, SWV-amperometric measurement technique is the most sensitive technique for this modified electrode.

EIS measurement results of the eserine titration with **CPE/CoPc-AQ** and its calibration line in the inset graph is given in Figure 3.3. The Radius of the cemicircles were increased with increasing pesticide concentration. From the calibration line this

relationship between concentration and impedance can be followed obviously. The increment of the cemicircle radiuses provides an impedimetric sensor.

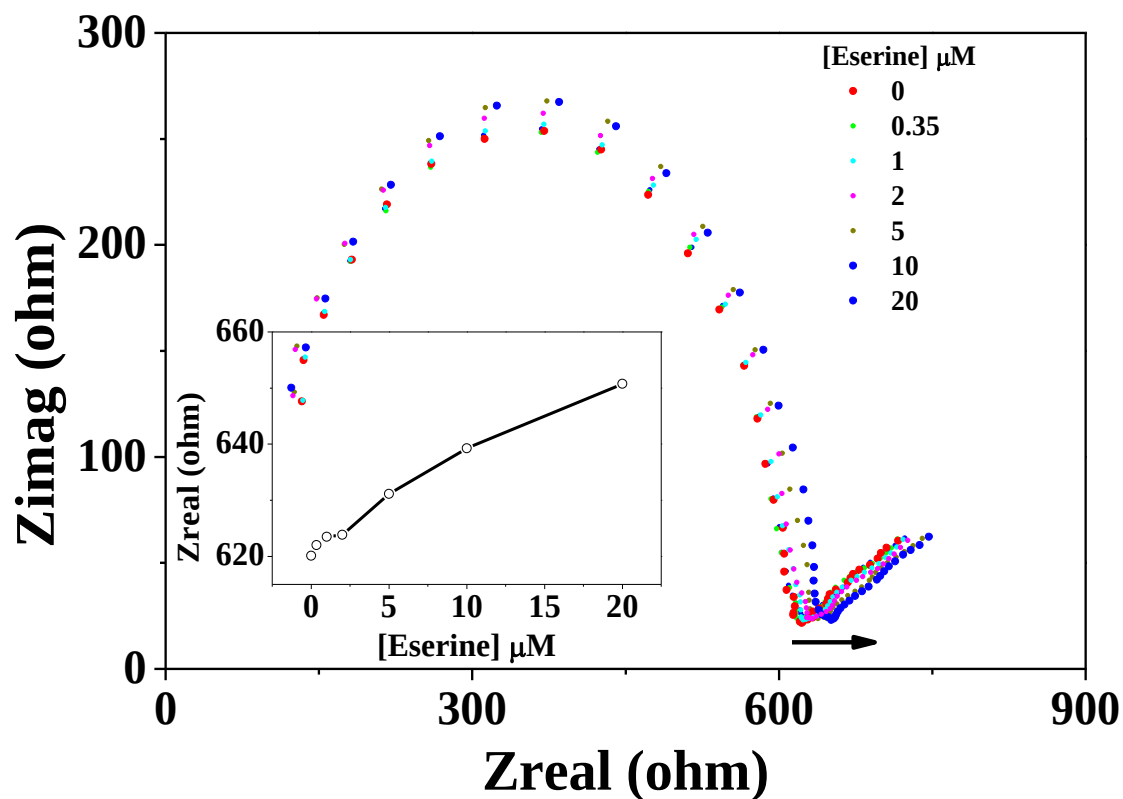


Figure 3.3. EIS measurement result of the eserine titration with **CPE/CoPc-AQ** and its calibration line in the inset graph.

DPSCC analysis responses of eserine titration with **CPE/CoPc-AQ** sensor is presented in Figure 3.4. During gradual addition of eserine, the charge density recorded with DPSCC measurements decreases and gives a linear range between 0.074×10^{-6} - 1.035×10^{-6} M.

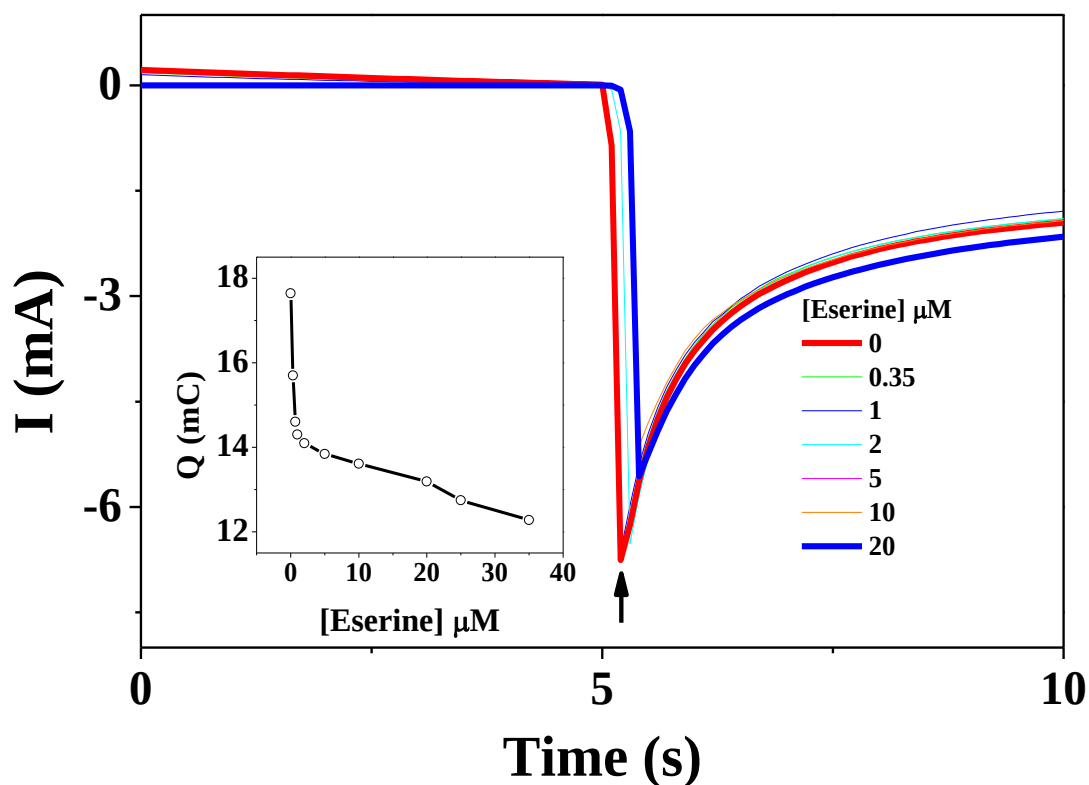


Figure 3.4. DPSCC analysis responses of eserine titration with CPE/CoPc-AQ sensor.

3.2. ITO/MPc-AQ Sensor Prepared with Langmuir-Blodgett Coating Technique

3.2.1. Electrochemical characterization of CoPc-AQ

CoPc-AQ complex was synthesized and its spectroscopic and electrochemical characterizations were performed. **CoPc-AQ** shows a Pc ring based and a $\text{Co}^{\text{II}}/\text{Co}^{\text{III}}$ based oxidation reaction during the anodic potential scans as shown in Figure 3.5.

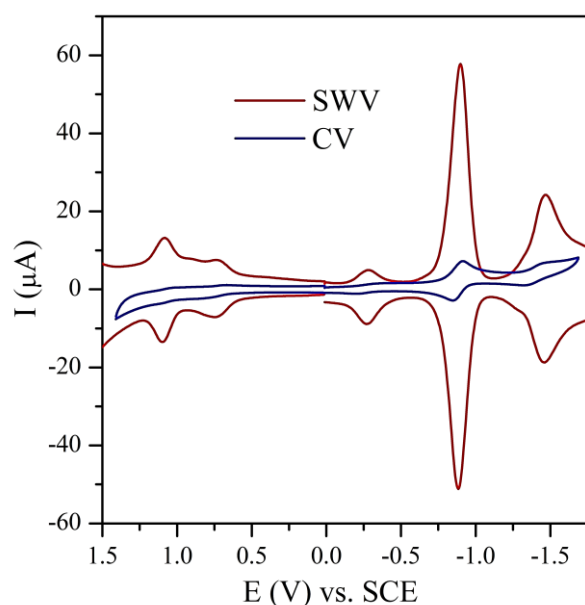


Figure 3.5. CV and SWV of **CoPc-AQ** (5.0×10^{-4} M) at 100 mVs^{-1} scan rate on Pt in DCM/TBAP electrolyte system.

Moreover a $\text{Co}^{\text{II}}/\text{Co}^{\text{I}}$ based reduction at -0.28 V , an **AQ** based reduction at -0.90 V and a Pc based reduction reaction at -1.46 V are recorded during the cathodic potential scans. Totally **CoPc-AQ** gives five reversible and diffusion controlled redox reactions, which indicated worthy of the complex as potential electrocatalyst and/or electrosensor for the detection of various target species.

3.2.2. Sensor measurement of ITO/CoPc-AQ, ITO/CoPc-AQ-nPt and ITO/CoPc-AQ-nAu electrodes

Sensing activity of ITO/**CoPc-AQ** electrode was tested for two different pesticides, carbofuran and eserine, with SWV, EIS, and DPSCC techniques. ITO/**CoPc-AQ** electrode gives different electrochemical responses for carbofuran and eserine, which indicate selectivity of the sensor for these pesticides. While ITO/**CoPc-AQ** electrode senses carbofuran with the voltammetric responses recorded at anodic potentials (1.20 V), interaction of the electrode with eserine decreases the anthraquinone based reduction peaks of the electrode recorded at cathodic potentials (-0.80 V). Figure 3.6 illustrates SWV responses of ITO/**CoPc-AQ** electrode during gradual addition of eserine and carbofuran into the phosphate buffer electrolyte solution.

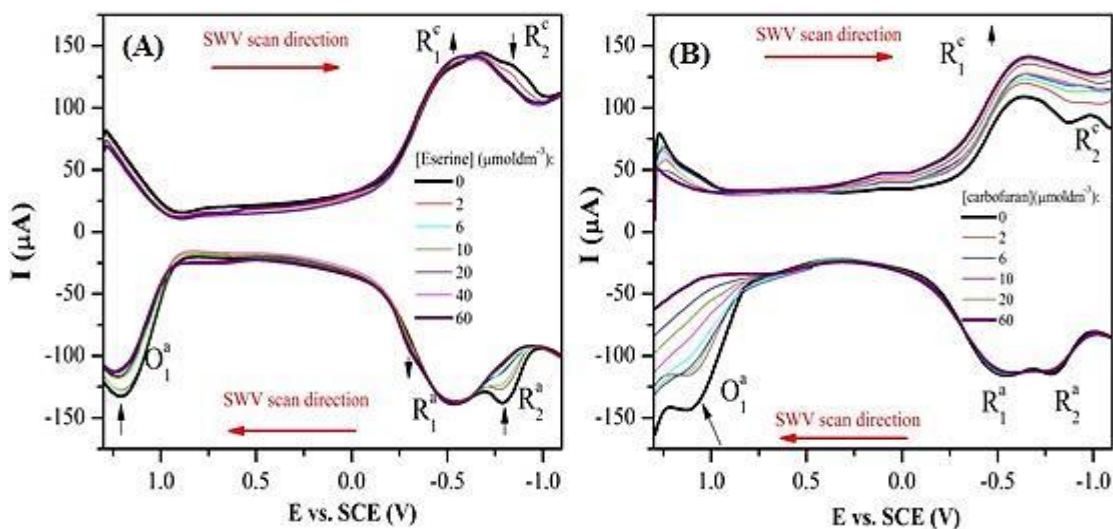


Figure 3.6. SWV responses of ITO/CoPc-AQ electrodes in PBS (pH= 7.0) during the gradual addition of eserine (A) and carbofuran (B).

As shown in Figure 3.6B, voltammetric responses of CoPc-AQ recorded during gradual addition of carbofuran are considerably different than those of the eserine sensing results. While the voltammetric changes of the reduction processes are trivial, the oxidation peak O_1^a continuously decreases with the positive potential shifts during gradual addition of carbofuran. While eserine interaction affect the AQ based reduction peak at the cathodic side of the voltammograms, differently carbofuran interactions affect $\text{Co}^{\text{II}}/\text{Co}^{\text{III}}$ oxidation wave of CoPc-AQ complex, since peak current of this wave only decreases with respect to increasing carbofuran concentration. It can be easily proposed with respect to these SWV results that carbofuran interacts with the Co^{II} center of the complex and this interaction prevents the oxidation of the cobalt ion. These voltammetric analyses illustrate that ITO/CoPc-AQ can also be used as carbofuran sensor electrode at anodic potentials. Different voltammetric responses of ITO/CoPc-AQ for different pesticides indicate selectivity of ITO/CoPc-AQ sensors for carbofuran and eserine. While eserine sensing responses are observed at the cathodic potentials (at -0.80 V), carbofuran sensing responses are observed at the anodic potentials (at 1.20 V).

Selectivity studies were also carried out with the sequential additions and addition of mixture of these two pesticides. As shown in Figure 3.7A, when a mixture of eserine and carbofuran was added to PBS electrolyte, both of the AQ based peak at -0.85 V and $\text{Co}^{\text{II}}/\text{Co}^{\text{III}}$ oxidation peak at 1.20 V decreases in current intensity.

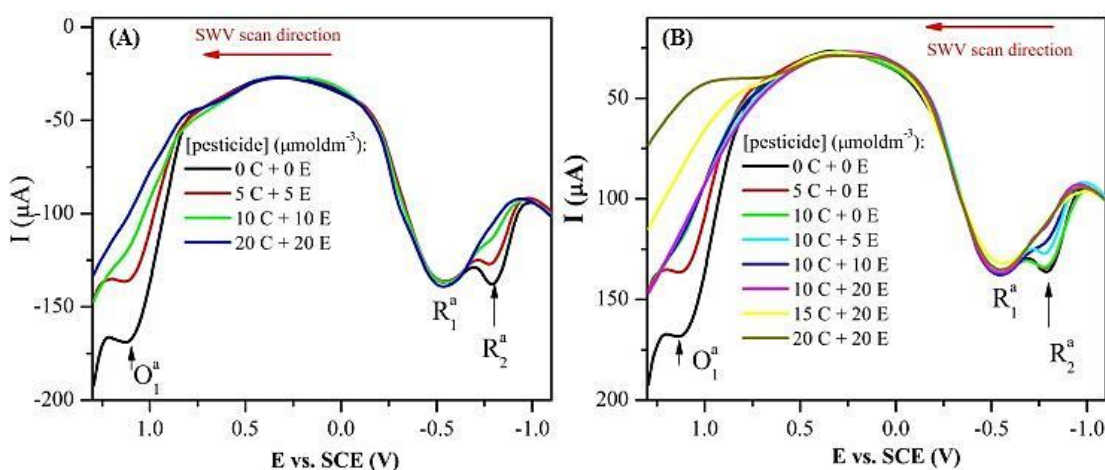


Figure 3.7. SWV responses of ITO/CoPc-AQ electrodes in PBS (pH= 7.0) (A) during gradual addition of a mixture of eserine, E and carbofuran, C and (B) during sequential addition of carbofuran and eserine.

This voltammetric changes indicate that while eserine interacts with AQ groups, carbofuran interacts with cobalt center of CoPc-AQ complex. In order to support this proposition, pesticides were sequentially added and SWV responses were followed with respect to effect of different pesticides. As shown in Figure 3.7A, only O_1^a peak at 1.20 V when carbofuran concentration was gradually increased while the other peaks remain stable (red and green SWVs). Then eserine was added to the same electrolyte and only R_2 peak at -0.80 V decreases (cyan, blue and magenta SWVs). Sequential addition of carbofuran alters again only the O_1^a peak. These voltammetric results support the selectivity of the electrode for these pesticides.

In addition to the selectivity, sensitivity of the electrode should provide exceptions of the application fields for application of ITO/CoPc-AQ as pesticide sensor. As shown in Table 3.2, detection limits and linear ranges of the ITO/CoPc-AQ derived from SWV measurements for sensing of carbofuran and eserine are within acceptable sensing ranges.

Table 3.2. Basic sensing parameters obtained with different techniques for the sensing of the pesticides with various modified electrodes.

Modified electrode	Sensing techniques	Pesticides						Ref.
		Linear range (M) $\times 10^6$	Eserine Detection Limit (M)	Sensitivity	Linear range (M) $\times 10^6$	Carbofuran Detection Limit (M)	Sensitivity	
ITO/CoPc-AQ	SWV Amperometric	0.032 - 2.766	1.65×10^{-7}	8.66 A/M	0.021 - 1.411	1.21×10^{-7}	12.32 A/M	Tw*
	SWV Potentiometric	0.046 - 1.712	1.61×10^{-6}	0.03 V/ μ M	0.054 - 2.120	2.69×10^{-8}	5.57 V/ μ M	Tw
	EIS	0.054 - 2.213	1.16×10^{-7}	3.65 ohm/ μ M	0.034 - 2.261	1.28×10^{-8}	154.94 ohm/ μ M	Tw
	DPSCC	0.054 - 4.601	5.97×10^{-7}	75.43 C/M	0.054 - 2.090	1.41×10^{-8}	2005.34 C/M	Tw
ITO/CoPc-AQ-nPt	SWV Amperometric	0.012 - 2.820	4.69×10^{-7}	1.97 A/M	0.040 - 2.824	5.81×10^{-7}	0.08 A/M	Tw
	SWV Potentiometric	0.012 - 15.012	1.76×10^{-6}	0.01 V/ μ M	0.040 - 5.444	6.77×10^{-8}	0.01 V/ μ M	Tw
	EIS	0.012 - 2.834	2.30×10^{-9}	15.14 ohm/ μ M	0.012 - 1.347	6.00×10^{-8}	3.03 ohm/ μ M	Tw
	DPSCC	0.012 - 2.801	2.79×10^{-7}	939.24 C/M	0.012 - 1.420	4.69×10^{-8}	24.44 C/M	Tw
ITO/CoPc-AQ-nAu	SWV Amperometric	0.001 - 1.421	6.40×10^{-9}	6.53 A/M	0.078 - 2.743	1.74×10^{-7}	1.11 A/M	Tw
	SWV Potentiometric	0.043 - 1.301	1.00×10^{-7}	0.03 V/ μ M	0.032 - 2.885	1.91×10^{-7}	0.01 V/ μ M	Tw
	EIS	0.002 - 0.706	6.90×10^{-9}	22.40 ohm/ μ M	0.014 - 1.391	5.74×10^{-8}	10.73 ohm/ μ M	Tw
	DPSCC	0.032 - 2.511	5.20×10^{-7}	94.78 C/M	0.020 - 2.915	1.95×10^{-7}	108.83 C/M	Tw
ZnPc	CA Amperometric					1.37×10^{-7}		[47]
MWCNT/PANI/AChE	CA Amperometric					1.40×10^{-6}		[48]
AChE/CoPc	CA Amperometric					1.0×10^{-8}		[49]
AChE / TTF-TCNQ- EMIMTCB/CP	CA Amperometric			7 ppt				[50]
TTF-TCNQ-ionic/ionic liquid gel	CA Amperometric			26×10^{-12}				[51]
AChE/PAMAM				0.03 ppb				[51]

*Tw: This work

To support the SWV responses of the electrode and to determine effects of the electrochemical techniques to the basic sensor parameters, sensing measurements were also carried out with EIS and DPSCC analysis methods. Figure 3.8 illustrates EIS responses of ITO/CoPc-AQ electrode during titration with eserine.

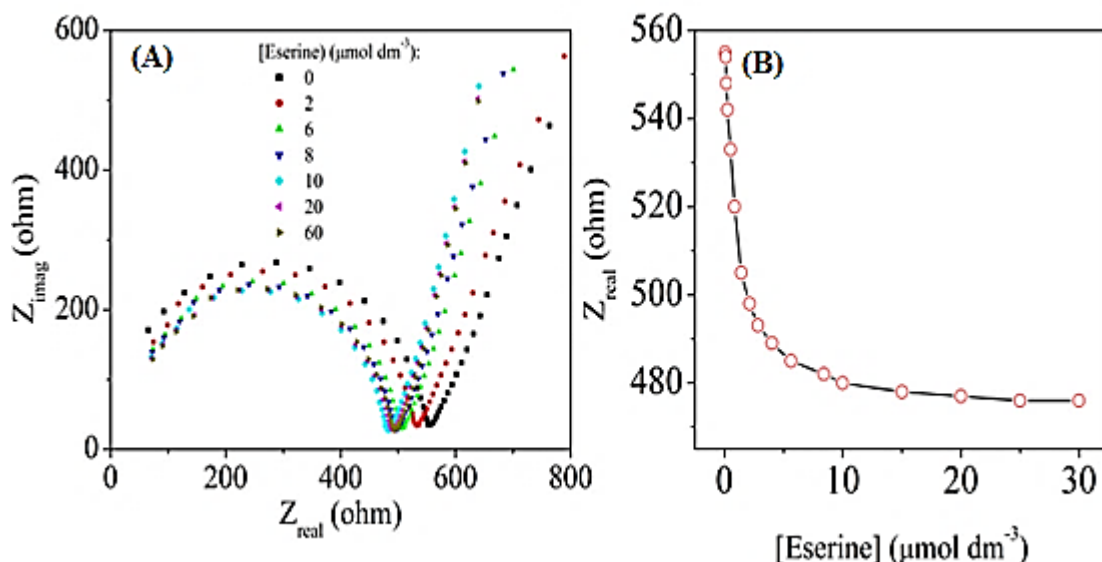


Figure 3.8. **A)** EIS responses of ITO/CoPc-AQ electrodes in PBS (pH= 7.0) during gradual addition of eserine **B)** calibration line ([eserine] vs Z_{real} graph) of eserine sensing measurements derived from EIS responses of ITO/CoPc-AQ electrodes.

As shown in Figure 3.8, radius of the semicircles decreases gradually as a function of the increasing eserine concentration. These EIS responses indicate interaction of ITO/CoPc-AQ with eserine and this interaction affects the conductivity of the modified electrode. Change of the conductivity of the electrode directly alters EIS responses of the electrode. The basic sensor parameters were also derived from the EIS responses of ITO/CoPc-AQ electrode and the results are tabulated on Table 3.2. Calibration lines of the electrode for eserine sensing were given in Figure 3.8B. When compared with SWV analysis, EIS technique behaves more sensitive, since detection limit of the electrode decreases up to 1.16×10^{-7} M. DPSCC responses of the sensing measurements were also performed between 0.0 V and -1.0 V constant potentials repetitively (Figure 3.9).

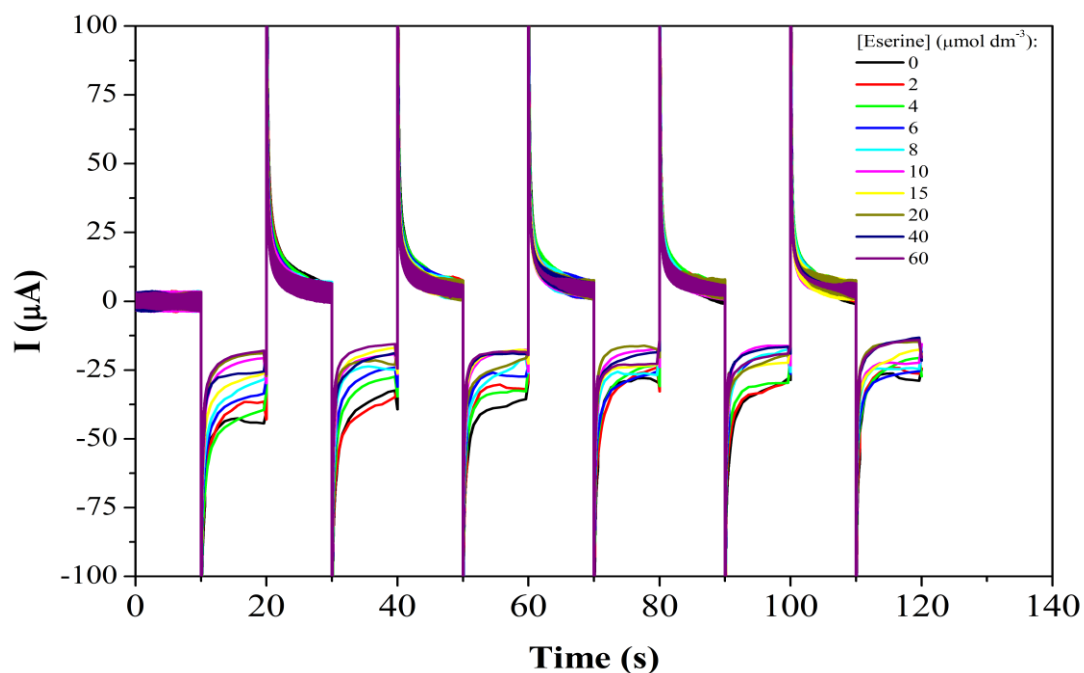


Figure 3.9. DPSCC responses of ITO/CoPc-AQ electrodes in PBS (pH= 7.0) during the gradual addition of eserine.

During gradual addition of eserine, charge density of the electrode recorded with DPSCC measurements decreases and gives a linear range between 0.054×10^{-6} and 4.60×10^{-6} M. When these three techniques were compared, the smallest LOD is recorded with EIS technique.

To increase the sensing activity of **CoPc-AQ** electrode, ITO/CoPc-AQ electrode was electrochemically modified with nano-Pt (ITO/CoPc-AQ-nPt), and sensing measurements were repeated on this electrode. Morphology of the ITO/CoPc-AQ-nPt electrode was determined with SEM. As shown in SEM image of the electrode given in Figure 3.10, nPt clusters were uniformly distributed in the **CoPc-AQ** film and diameter of the nPt clusters are within the nanometer range.

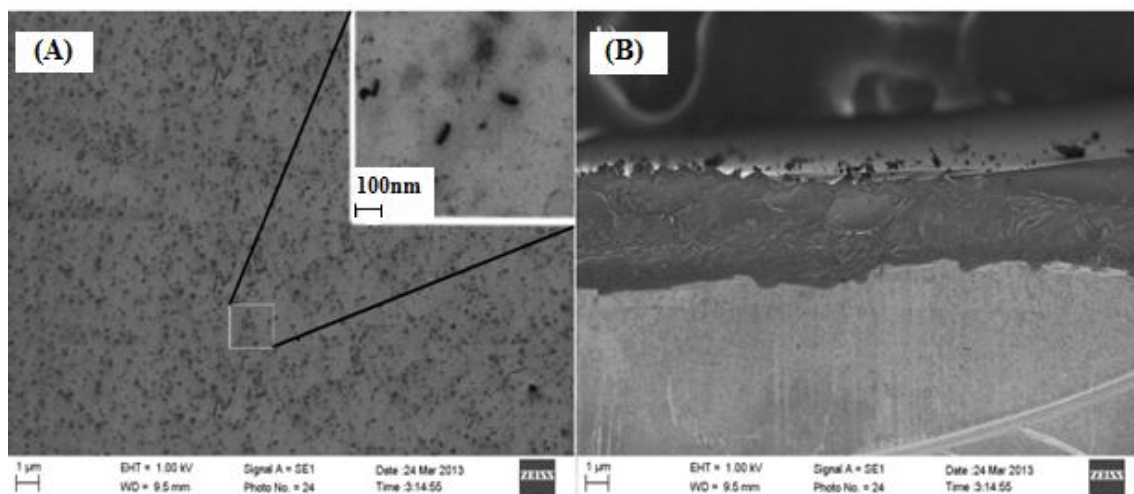


Figure 3.10. SEM images of ITO/**CoPc-AQ**-nPt electrode; **A)** Top view of the film, **B)** Side view of the film.

Figure 3.10 shows that nPt particles has rod shaped structures and the particle size for nPt particles are about 100 nm in length and 10 nm in width. Position of the nanoparticles in the CoPc film and thickness of the film can be observed especially from Figure 3.10B. Film thickness on the ITO electrode is about 3 μm as shown in the side SEM view of the film (Figure 3.10B). In Figure 3.10, nPt particles were immersed into the film and were at nano sizes, thus they did not alter the film thickness considerably.

Figure 3.11 illustrates the SWV responses of ITO/**CoPc-AQ**-nPt during gradual addition of eserine into the phosphate buffer electrolyte solution.

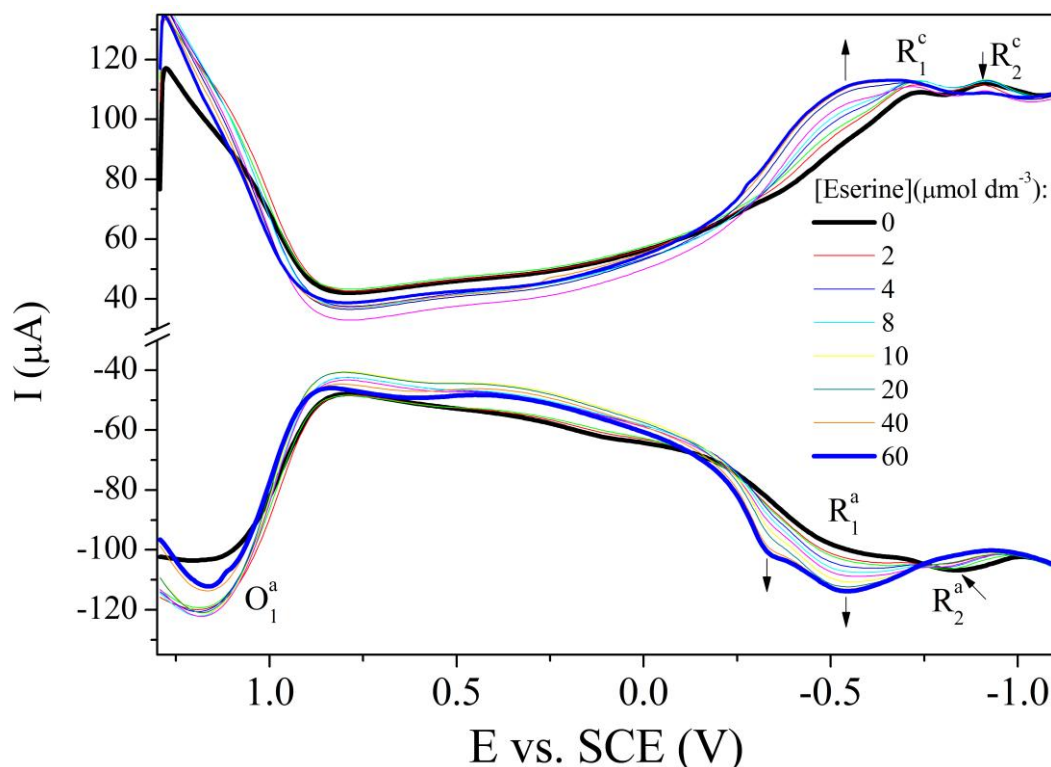


Figure 3.11. SWV responses of the ITO/CoPc-AQ-nPt electrode in PBS (pH= 7.0) during the gradual addition of eserine.

Without eserine, ITO/CoPc-AQ-nPt electrode gives similar voltammetric responses with ITO/CoPc-AQ. Differently the reduction peaks of Co^{II}/Co^I and AQ groups are not separated from each other clearly. Without eserine, ITO/CoPc-AQ-nPt electrode gives Co^{II}/Co^I reduction peak at -0.73 V and AQ reduction peak at -0.92 V during the forward SWV scan. During the reverse SWV scan, the couples of AQ reduction peak and the couple of Co^{II}/Co^I reduction peak are recorded at -0.84 and -0.56 V respectively. Moreover an oxidation peak is also recorded at 1.12 V. Interaction of eserine with ITO/CoPc-AQ-nPt alters the SWV responses of ITO/CoPc-AQ. During titration with eserine, while AQ based redox peaks decrease slightly in current intensity, new redox peaks before Co^{II}/Co^I reduction peaks increases at -0.34 and -0.56 V as a function of increasing eserine concentration. These voltammetric responses illustrate activity of ITO/CoPc-AQ-nPt to sense eserine. The sensor parameters derived from SWV analyses are tabulated on Table 3.2. Limit of detection (LOD) of ITO/CoPc-AQ-nPt is smaller than that of ITO/CoPc-AQ, which indicates that nano Pt coated on CoPc-AQ improves the sensor activity of the ITO/CoPc-AQ electrode. Figure 3.10 illustrates the EIS

responses of ITO/**CoPc-AQ**-nPt during gradual addition of eserine into the phosphate buffer electrolyte.

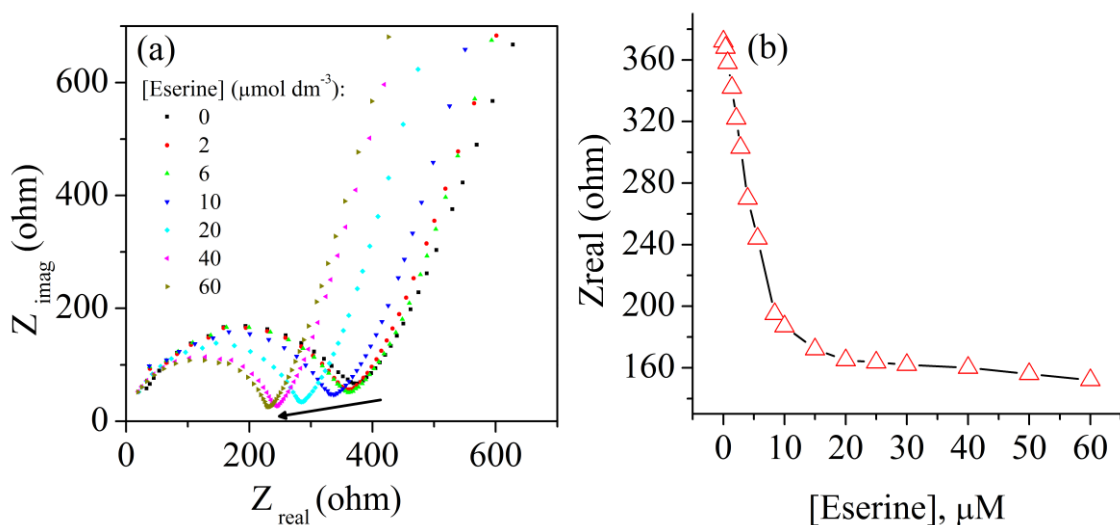


Figure 3.12. (A) EIS responses of ITO/**CoPc-AQ**-nPt electrodes in PBS (pH= 7.0) during the gradual addition of eserine **(B)** calibration line ([eserine] vs Z_{real} graph) of the eserine sensing measurements derived from EIS responses of ITO/**CoPc-AQ**-nPt electrodes.

ITO/**CoPc-AQ**-nPt electrode has a semicircle with a smaller radius than those of ITO/**CoPc-AQ** electrode, which indicates that nPt increases the conductivity of the electrode. During gradual addition of eserine, radius of the semicircles decreases with respect to increasing eserine concentration, which also supports the interaction of eserine with the sensor electrode. LOD of the electrode derived from EIS analysis is also smaller than that of ITO/**CoPc-AQ** electrode. As shown in Figure 3.10b, ITO/**CoPc-AQ**-nPt electrode gives a linear range between 0.0118×10^{-6} and 2.83×10^{-6} M and LOD of 2.30×10^{-9} M.

DPSCC results of ITO/**CoPc-AQ**-nPt electrode are shown in Figure 3.13.

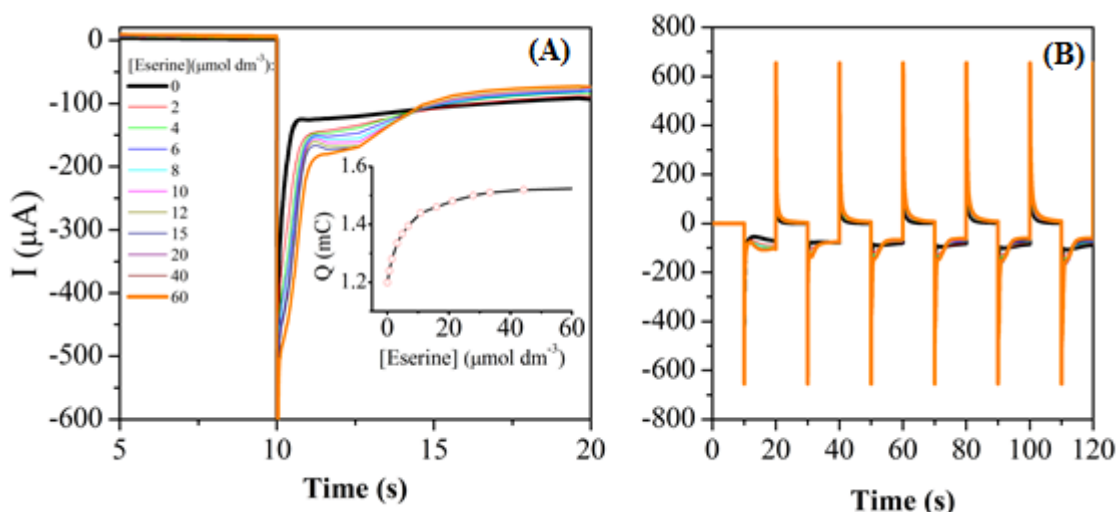


Figure 3.13. DPSCC responses of ITO/CoPc-AQ-nPt electrodes in PBS (pH= 7.0) during the gradual addition of eserine; **(A)** first DPSCC cycle, **(B)** all DPSCC cycles.

A considerable DPSCC response changes is not recorded during the forward step of the technique ($E_o = 0.0$ V and $E_f = -1.00$ V) as a function of eserine concentration. However during the reverse step of the technique ($E_o = -1.0$ V and $E_f = 0.0$ V), completely different DPSCC responses are recorded. When 0.0 V potential was applied, the current passed from the sensor electrode increases immediately and then decreases sharply and reaches to a steady state just after 1 second. When eserine concentration increases, the current does not reach to a steady state and starts to increase again after 1 second and reach to steady state after 5 seconds. These uncommon DSPCC responses are well shown on the extended graph of one CC response Figure 3.13A. These DPSCC responses are in agreement with SWV responses of the electrode (increasing new peaks between 0 and -1.0 V). To determine interaction duration of the electrode with eserine, continuous 5 DPSCC cycles was applied (Figure 3.13B). During the repetitive CC response, very similar current-time responses were observed for all DPSCC cycles as shown in Figure 3.13B.

Similar sensing measurements were carried out with ITO/CoPc-AQ-nAu electrode (Figure 3.14).

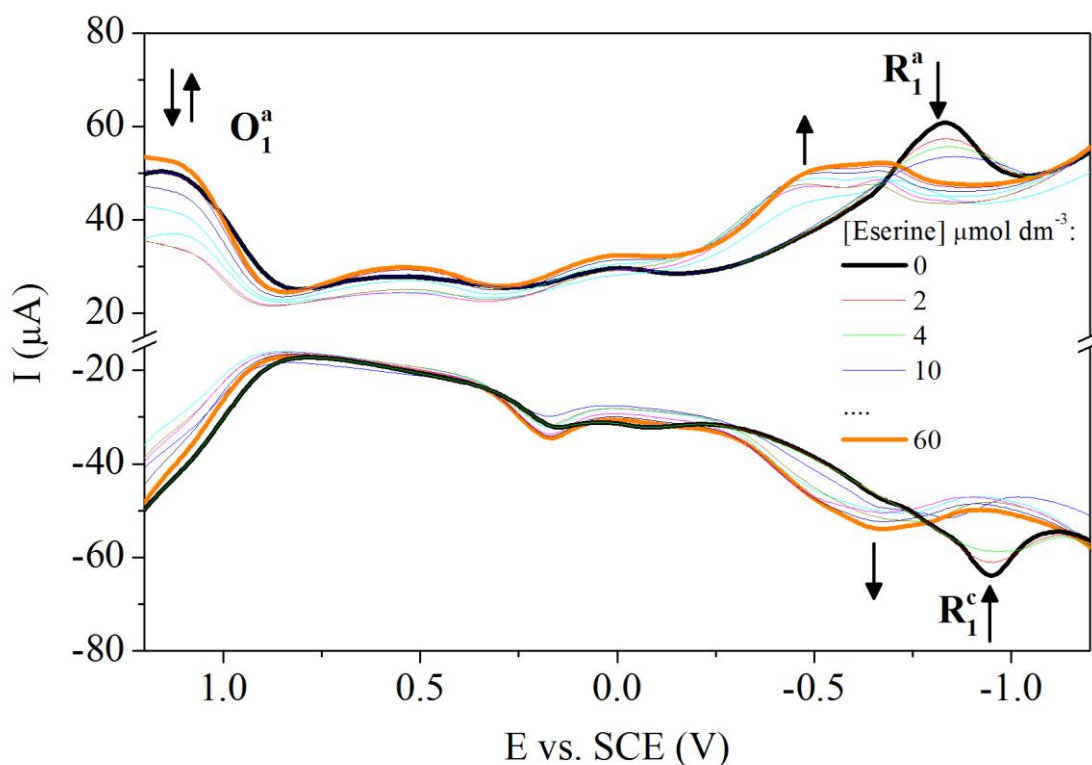


Figure 3.14. SWV responses of ITO/CoPc-AQ-nAu electrodes in PBS (pH= 7.0) during the gradual addition of eserine.

Without eserine, ITO/CoPc-AQ-nAu electrode gives similar SWV responses with those of ITO/CoPc-AQ-nPt electrode. A reduction couple R_1^a/R_1^c at -0.94 V and an oxidation wave O_1^a at 1.20 V are observed. During titration with eserine, while the peaks of R_1^a/R_1^c couple decreases in current intensity, a cathodic peak at -0.64 V and two anodic peaks at 0.47 V and -0.70 V are observed and peak current of these new waves increase gradually as a function of increasing eserine concentration. EIS and DPSCC analysis of the electrode for eserine sensing were also carried out and basic sensor parameters were derived from the responses of these different techniques separately and tabulated on Table 3.2. The LOD value was found as 6.90×10^{-9} M with EIS technique on the ITO/CoPc-AQ-nAu electrode.

3.2.3. Electrochemical characterization of CuPc-AQ

In Figure 2.10 electrochemical responses of **CuPc-AQ** in DCM solution is shown. Two Pc ring based and one **AQ** sustituent based reduction reactions during the cathodic potential scans were observed. An **AQ** based reduction at -0.90 V and two Pc based reduction reaction at -1.2V and -1.46V are recorded during the cathodic potential scans. During the anodic potential scans Pc ring peaks are formed. To sum up, **CuPc-AQ** gives five reversible and diffusion controlled redox reactions, which indicates that the complex can be used as potential electrocatalyst and/or electrosensor for the detection of various target species.

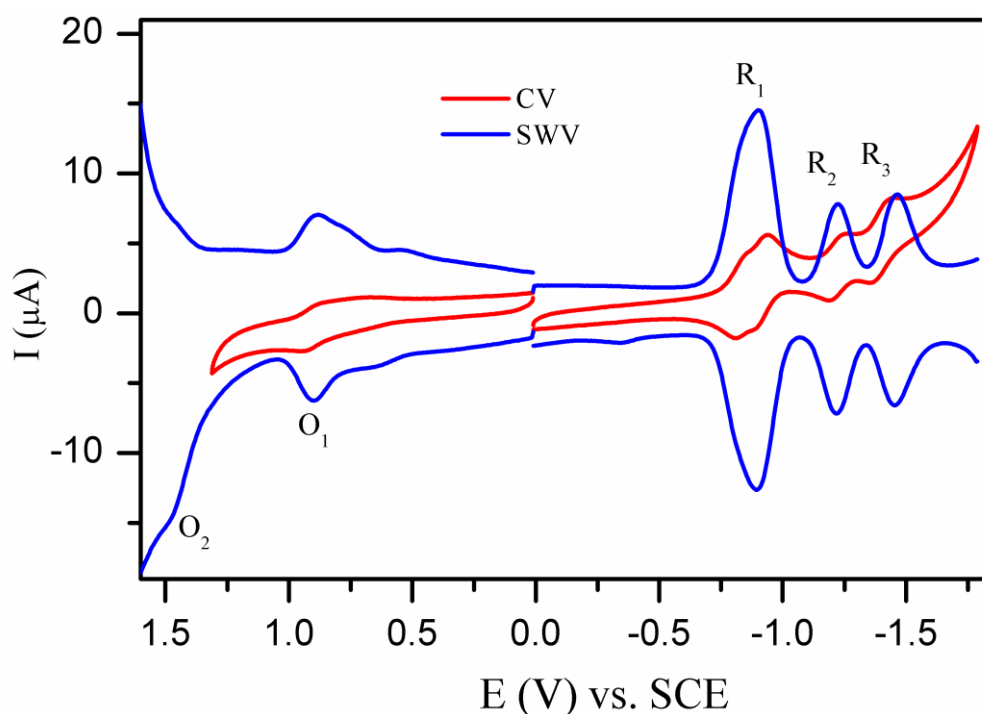


Figure 3.15. Electrochemical responses of **CuPc-AQ** in DCM solution

Synthesized and electrochemically characterized **CuPc-AQ** complex.

3.2.4. Sensor measurements of ITO/CuPc-AQ, ITO/CuPc-AQ-nPt and ITO/CuPc-AQ-nAu electrodes

ITO/**CuPc-AQ** electrode was tested with two different pesticides, carbofuran and eserine, whether it can be used as a pesticide sensor or not. For this purpose sensing activities of the electrode was determined with SWV, EIS, and DPSCC electrochemical

techniques. ITO/**CuPc-AQ** electrode that gives different electrochemical response for carbofuran and eserine behaved as a selective sensor for these pesticides. The SWV responses of ITO/**CuPc-AQ** were shown in Figure 3.16 while gradual addition of eserine and carbofuran into the phosphate buffer electrolyte solution containing electrochemical cell.

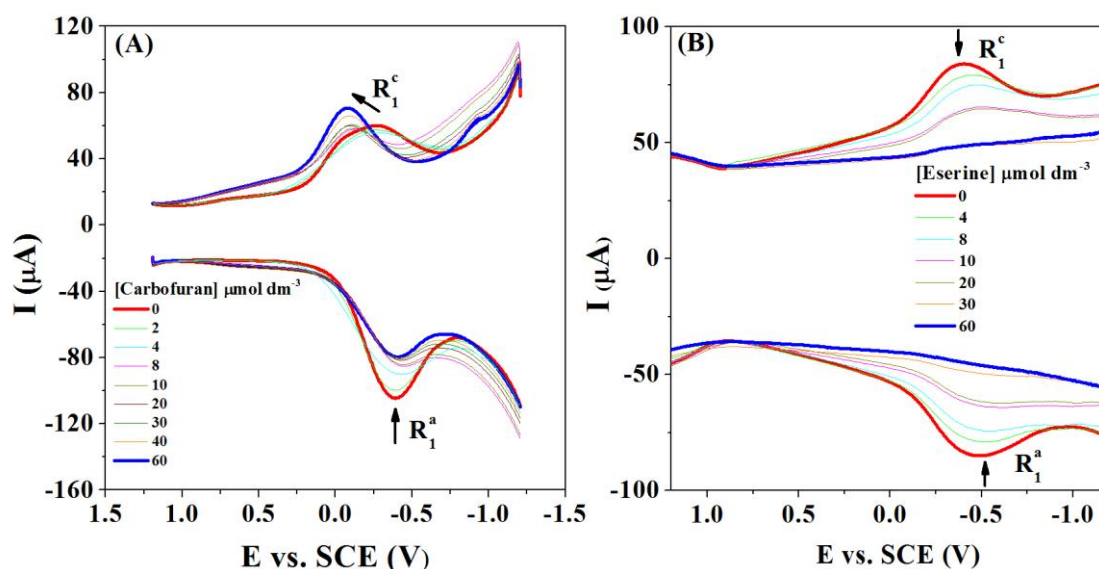


Figure 3.16. The SWV responses of ITO/**CuPc-AQ** while gradual addition of (A) carbofuran and (B) eserine.

The electrochemical responses of the **CuPc-AQ** LB thin film were different than the responses of the material in the dichloromethane solution. The potentials over 1.2V burns the ITO electrode, thus for sensor measurements the applied potentials were between -1.0V and 1.0V. In this potential range a broad cathodic peak was observed about -0.40V which was attributed to an overlapped peak of Pc ring reduction and AQ substituent reduction peaks.

The red colored curves that show the SWV responses of ITO/**CuPc-AQ** electrode without pesticide addition give a reduction couple, R_1^c at about -0.40 V. Figure 3.16A illustrates the SWV responses of ITO/**CuPc-AQ** during gradual addition of carbofuran. As a result of the titration with carbofuran pesticide, the current intensity of R_1^c process at -0.40 V decreased and a new peak was formed at -0.08V while the R_1^a at -0.39 V potential gradually decreased in current intensity. According these changes in SWV responses during the titration process of carbofuran, this electrode has a sensor activity

for carbofuran. The current intensity decrease of the reduction peaks during the sensing measurements indicates that **CuPc-AQ** behaves as artificial sensing material instead of enzymes which were used for pesticide biosensors as organic sensing material. However, **CuPc-AQ** is more preferable than enzymes because of its superior properties as being more stable and having low cost.

In Figure 3.16B, voltammetric responses of **CuPc-AQ** recorded during gradual addition of eserine are considerably different than carbofuran titration results. During the addition of eserine, voltammetric changes of R_1^c and R_1^a reduction current intensities continuously decreased with the increasing concentration of eserine. These voltammetric analysis illustrate that ITO/**CuPc-AQ** electrode can also be used as a selective sensor electrode for carbofuran and eserine while it behaves in a different manner for each pesticides.

The detection limits and linear ranges of the ITO/**CuPc-AQ** electrode were calculated from SWV measurements of carbofuran and eserine titration processes and the results were exhibited in Table 3.3. To support the SWV responses, and to determine the sensor results of the other electrochemical techniques, the sensing responses of ITO/**CuPc-AQ** were also measured with EIS and DPSCC analysis methods.

The EIS sensing measurement of ITO/**CuPc-AQ** with carbofuran and the calibration line that was derived from EIS data were illustrated in Figure 3.17.

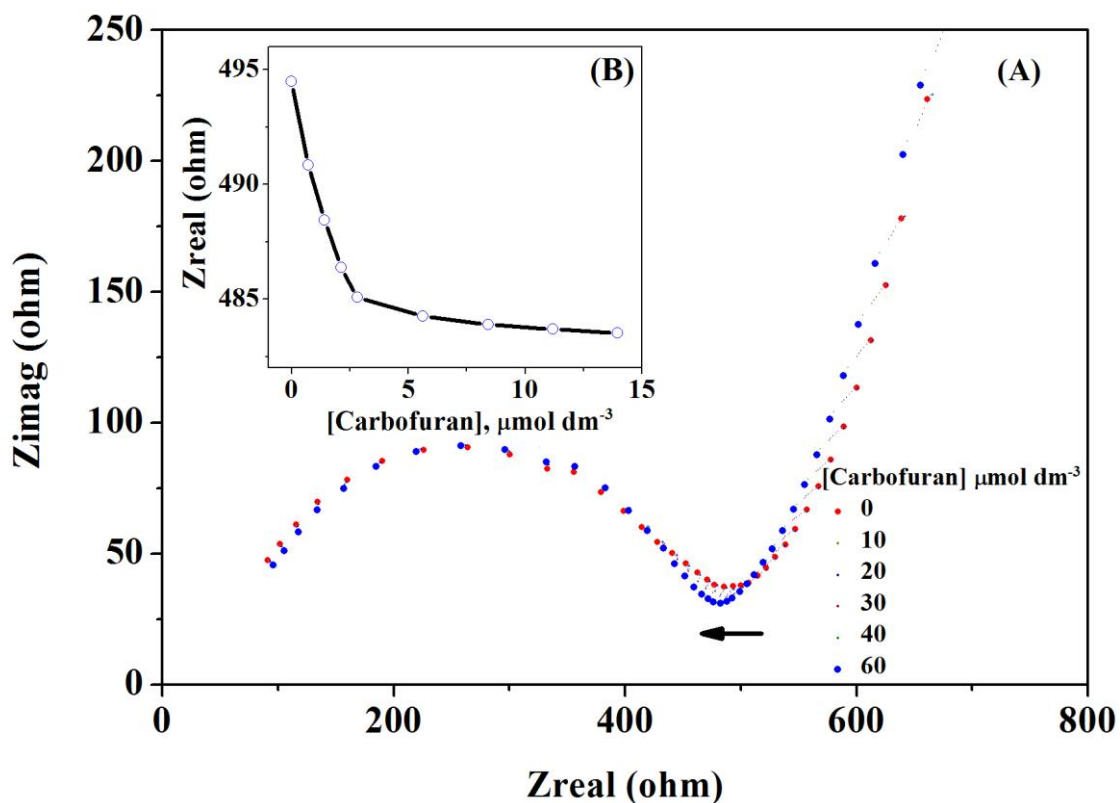


Figure 3.17. (A) The EIS responses of ITO/CuPc-AQ while gradual addition of carbofuran and (B) its calibration line.

The radius of the Nyquist diagram semicircles decreases gradually as a function of the increasing carbofuran concentration in the electrochemical cell solution (Figure 3.17A). The interaction between ITO/CuPc-AQ with carbofuran increases the conductivity of the modified working electrode. The calibration line was given in Figure 3.17B and the detection linear range and detection limit values of this modified electrode obtained from this calibration line were tabulated on Table 3.3.

Table 3.3. Sensor paremeters of modified **CuPc-AQ** electrodes for pesticide detection.

Modified electrode	Sensing techniques	Pesticides						Ref.
		Linear range (M) $\times 10^6$	Eserine Detection Limit (M)	Sensitivity	Linear range (M) $\times 10^6$	Carbofuran Detection Limit (M)	Sensitivity	
ITO/CuPc-AQ	SWV Amperometric	0.014 - 2.811	6.97×10^{-8}	8.87 A/M	2.100 - 16.681	2.08×10^{-7}	8.26A/M	Tw*
	SWV Potentiometric	0.014 - 1.332	3.03×10^{-7}	0.05 V/ μ M	0.003 - 1.276	9.04×10^{-8}	0.12V/ μ M	Tw
	EIS	0.736 – 5.395	5.93×10^{-8}	316.48 ohm/ μ M	0.010 - 1.962	8.44×10^{-9}	2.31 ohm/ μ M	Tw
	DPSCC	0.002 - 2.822	6.58×10^{-8}	155.22 C/M	0.021 - 1.266	2.95×10^{-7}	57.68 C/M	Tw
ITO/CuPc-AQ-nPt	SWV Amperometric	0.030- 1.346	4.38×10^{-8}	5.53 A/M	0.160 - 1.963	4.66×10^{-8}	18.97 A/M	Tw
	SWV Potentiometric	0.044 - 2.613	6.97×10^{-8}	0.05 V/ μ M	0.257 - 2.108	7.53×10^{-8}	0.05V/ μ M	Tw
	EIS	0.165 - 2.758	4.11×10^{-8}	3.86 ohm/ μ M	0.633 - 10.863	5.83×10^{-9}	8.51 ohm/ μ M	Tw
	DPSCC	0.022 - 2.671	4.63×10^{-8}	153.85 C/M	0.039 - 1.351	1.63×10^{-7}	300.4 C/M	Tw
ITO/CuPc-AQ-nAu	SWV Amperometric	0.061 - 1.241	3.87×10^{-8}	30.30 A/M	0.022 - 1.880	3.71×10^{-8}	7.53 A/M	Tw
	SWV Potentiometric	0.149 - 2.969	2.89×10^{-8}	0.01 V/ μ M	0.034 - 2.842	1.62×10^{-8}	0.07 V/ μ M	Tw
	EIS	0.039 - 2.371	1.23×10^{-9}	9.90 ohm/ μ M	0.012 - 2.855	3.99×10^{-9}	6.36 ohm/ μ M	Tw
	DPSCC	0.065 - 14.531	2.41×10^{-8}	5.51 C/M	0.005 - 10.854	1.82×10^{-8}	4.45 C/M	Tw
CoO/rGO/GCE	CA Amperometric					1.9×10^{-8}		[52]
AChE–AuNPs–SF/Pt	CA Amperometric					1.0×10^{-9}		[53]
BSA/Ab/[DpAu/DMD PSE]2/Au	CA Amperometric					2.7×10^{-7}		[54]
AChE / TTF–TCNQ– EMIMTCB/CP	CA Amperometric		2.6×10^{-8}					[55]
AChE–ChO	CA Amperometric		0.1×10^{-9}					[56]
AChE/PAMAM			0.03 ppb					[57]

When compared with SWV analysis, the detection limit of the electrode decreases up to 5.44×10^{-9} M. DPSCC analyses of the sensing measurements for eserine and carbofuran were also performed between 0.0 V and -1.0 V constant potentials consecutively (Figure 3.18).

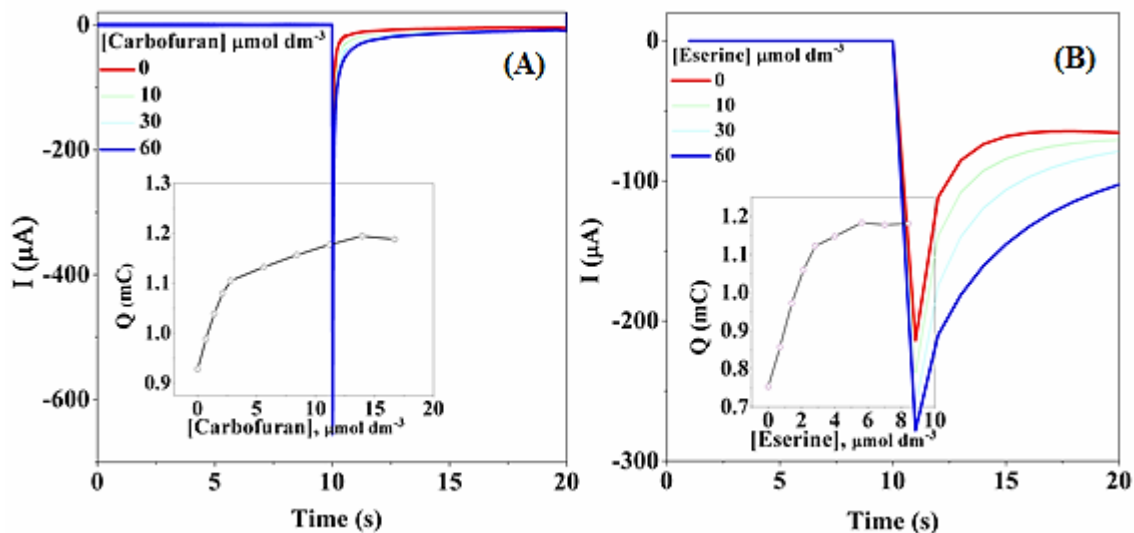


Figure 3.18. DPSCC analyses of the sensing measurements for ITO/CuPc-AQ electrodes; **(A)** carbofuran and **(B)** eserine.

There is an increase of the charge density during gradual addition of eserine and carbofuran with DPSCC measurements. When these three techniques were compared, the lowest LOD is recorded with EIS technique.

To improve the sensing activity of **CuPc-AQ**, the ITO/CuPc-AQ electrodes were electrochemically coated with nano-Pt (ITO/CuPc-AQ-nPt), and sensing measurements were repeated on this electrode with the same conditions and methods. In Figure 3.19, the SWV measurement results of ITO/CuPc-AQ-nPt during gradual addition of carbofuran (Figure 3.19A) and eserine (Figure 3.19B) into the phosphate buffer electrolyte were illustrated.

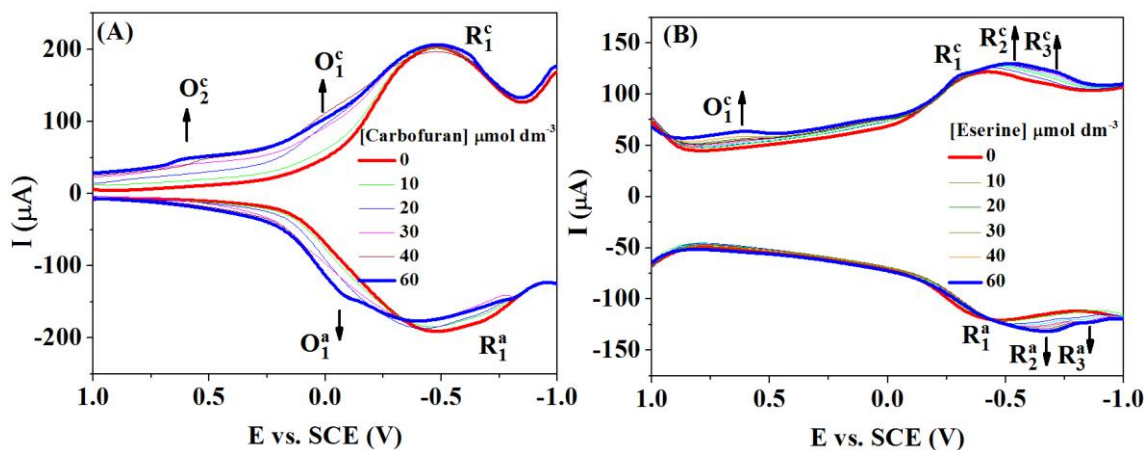


Figure 3.19. The SWV measurement results of ITO/CuPc-AQ-nPt during gradual addition of (A) carbofuran and (B) eserine.

Without pesticide addition, ITO/CuPc-AQ-nPt electrode gives similar voltammetric responses with ITO/CuPc-AQ. ITO/CuPc-AQ-nPt electrode gives AQ reduction peak at -0.42 V during the forward SWV scan and at the reverse SWV scan, the couples of AQ reduction peak is seen at -0.40V. In Figure 3.19A, the SWV response of ITO/CuPc-AQ-nPt seems to be differing with the gradual addition of carbofuran pesticide. After gradually addition of carbofuran, a new reversible peak forms at oxidation region for ITO/CuPc-AQ-nPt, although ITO/CuPc-AQ electrode gives different response. In Figure 3.19B, during titration with eserine, the interaction of pesticide with ITO/CuPc-AQ-nPt affects the SWV responses and two new reduction peaks were observed, at -0.67V and -0.85V while the reduction peaks of ITO/CuPc-AQ electrode decreases and no new peaks form. These voltammetric responses illustrate that **CuPc-AQ** is a selective sensor material against carbofuran and eserine. The sensor parameters derived from SWV analyses are tabulated on Table 3.3. The detection limit (LOD) of ITO/CuPc-AQ-nPt is smaller than that of ITO/CuPc-AQ, which indicates that nano Pt coated on **CuPc-AQ** improves the sensor activity of the ITO/CuPc-AQ electrode. Figure 3.20 exhibits the EIS responses of ITO/CuPc-AQ-nPt during gradual addition of eserine into the phosphate buffer electrolyte.

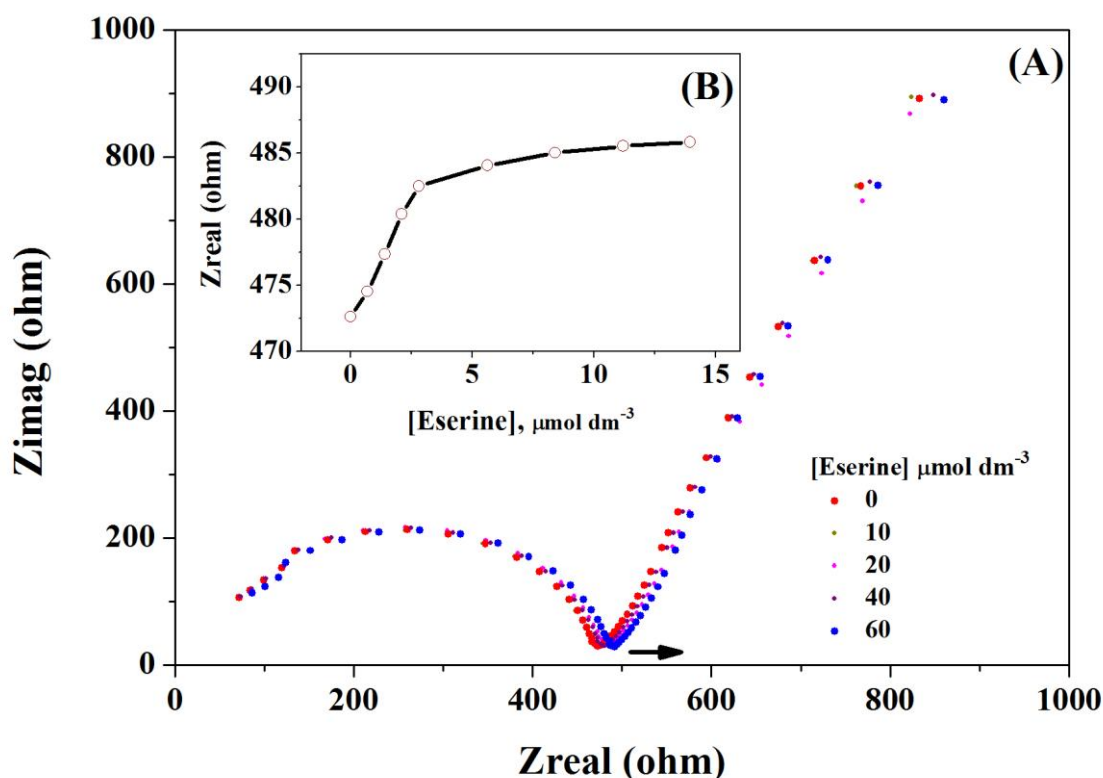


Figure 3.20. (A) The EIS responses of ITO/CuPc-AQ-nPt during gradual addition of eserine and (B) its calibration line.

During gradual addition of eserine, radius of the Nyquist diagram semicircles increases with increasing eserine concentration, which also supports the interaction of eserine with the sensor electrode. Also, Figure 3.20 illustrates that the electrical conductivity of the ITO/CuPc-AQ-nPt electrode has improved since the semicircle radius of the electrode is smaller than those of ITO/CuPc-AQ electrode. On the other side, LOD of the electrode derived from EIS analysis is also smaller than that of ITO/CuPc-AQ electrode.

DPSCC sensor measurement results of ITO/CuPc-AQ-nPt electrode are shown in Figure 3.21.

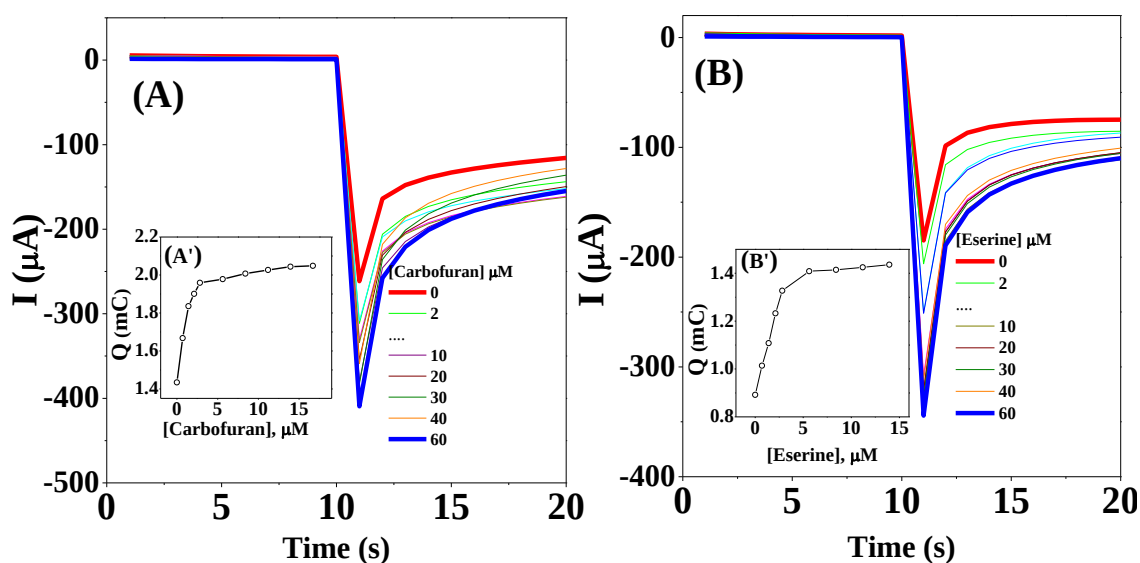


Figure 3.21. DPSCC sensor measurement results of ITO/CuPc-AQ-nPt electrode against **(A)** carbofuran and **(B)** eserine.

The potentials $E_o = 0.0$ V and $E_f = -1.00$ V were applied consecutively after each pesticide addition and considerable DPSCC response changes are recorded during the forward step of the technique as a function of pesticide concentration. As shown in Figure 3.21A, ITO/CuPc-AQ-nPt electrode gives a linear range between 0.0387×10^{-6} and -1.460×10^{-6} M and LOD of 1.63×10^{-7} M for carbofuran. However, in Figure 3.21B, after 8 μ M eserine concentration in the phosphate buffer electrolyte solution, the electrical charge of the working electrode remained almost constant that determined the linear range of the ITO/CuPc-AQ-nPt electrode as 0.022×10^{-6} and -2.87×10^{-6} M and LOD of 4.63×10^{-8} M for DPSCC technique. These DPSCC responses are in agreement with SWV responses of the electrode that two new reduction peaks were observed, at -0.67 V and -0.85 V, lead the charge increase of the working electrodes between 0 and -1.0 V.

Similarly the ITO/CuPc-AQ electrode was modified with Au to improve the sensor parameters and sensing measurements were carried out with SWV, EIS and DPSCC techniques. As shown in Figure 3.22, SWV diagrams of ITO/CuPc-AQ-nAu electrode without any pesticide addition illustrates that ITO/CuPc-AQ-nAu electrode gives similar SWV responses with those of ITO/CuPc-AQ-nPt and ITO/CuPc-AQ electrodes.

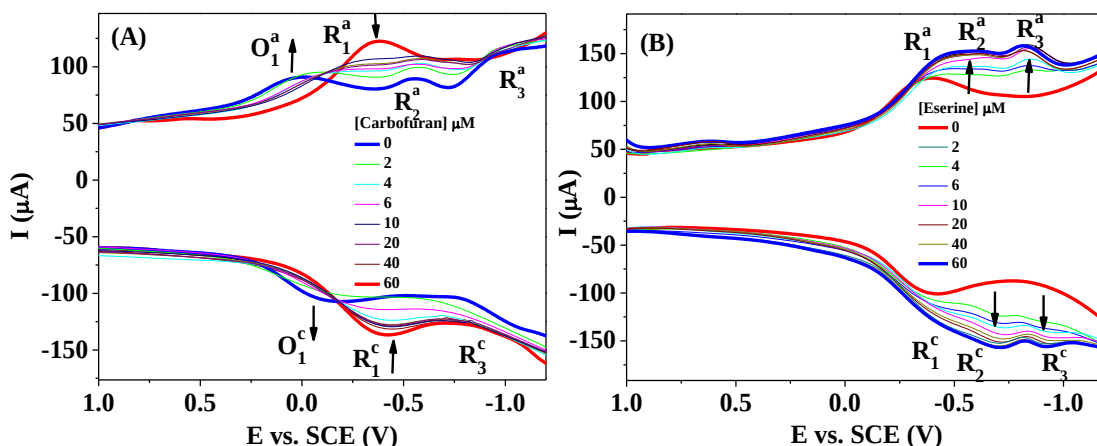


Figure 3.22. SWV diagrams of ITO/CuPc-AQ-nAu electrode with addition of **(A)** carbofuran and **(B)** eserine.

In Figure 3.22A, a reduction couple R_1^a/R_1^c at -0.39 V and -0.43 V are observed. However, during gradual addition of carbofuran, the peaks of R_1^a/R_1^c couple decreases in current intensity, a cathodic peak at -0.56 V, a cathodic couple at -1.0 V, and an anodic couple at 0.04 V and -0.10 V were observed and peak current of these new waves increased gradually as a function of increasing carbofuran concentration. In Figure 3.22B, two new cathodic couples were formed with the increasing concentration of eserine at about -0.60 V and -0.83 V. EIS and DPSCC analysis of the electrode for carbofuran and eserine sensing were also carried out under the same conditions and basic sensor parameters were derived from the responses of these different techniques separately and tabulated on Table 3.3.

3.3. GCE/poly-MPc Sensor Prepared with Electro-Polymerization Technique

3.3.1. Electrochemical characterization of H_2Pc -THQD and $NiPc$ -THQD

Voltammetric analyses of **H_2Pc -THQD** and **$NiPc$ -THQD** were performed to derive possible application of the complexes as electrochemical sensor materials. The voltammetric analyses results were very similar **H_2Pc -THQD** and **$NiPc$ -THQD**; because they have redox inactive H^+ and Ni^{2+} centers, they only give Pc ring based redox processes. Both complexes give two quasi-reversible reduction processes during the cathodic potential scans and they are electropolymerized on the working electrode

during the anodic potential scans in DCM/TBAP electrolyte system. They have different nuclear charges of the Pc centers and thus, the potential of reduction peaks are different.

In Figure 3.23. CV responses of **H₂Pc-THQD** at 100 Vs⁻¹ scan rate is given. The THQD substituents lead polymerization and thin film formation on the working electrode. The coating technique is convenient immobilization technique for biomimic electrochemical sensor electrode preparation.

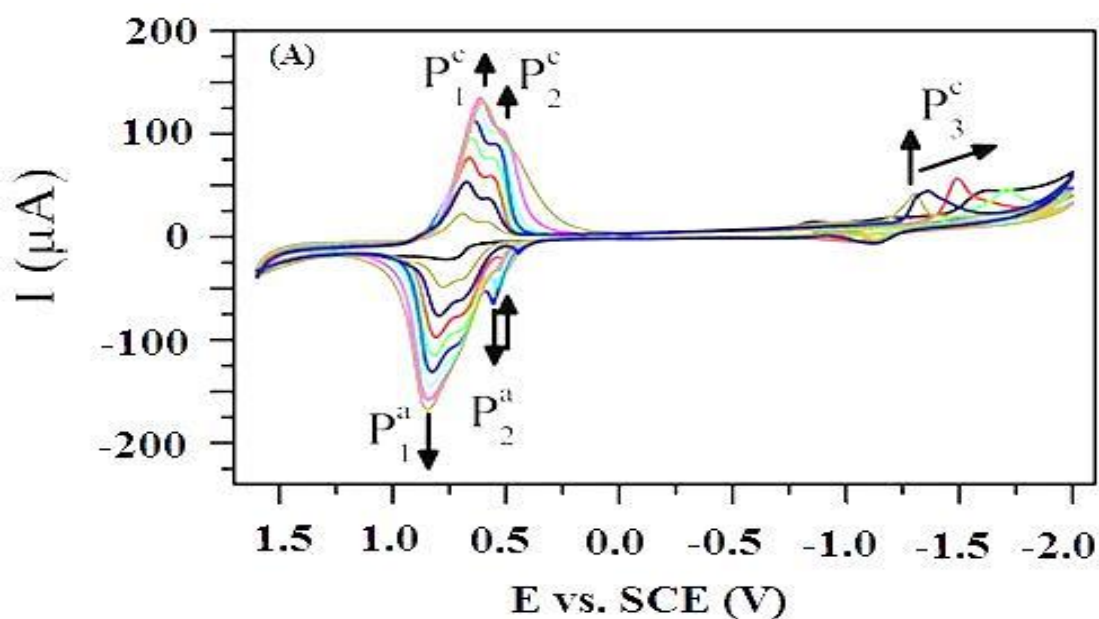


Figure 3.23. CV responses of **H₂Pc-THQD** at 100 Vs⁻¹ scan rate.

Cyclic voltammetry responses of **NiPc-THQD** is given in Figure 3.24.

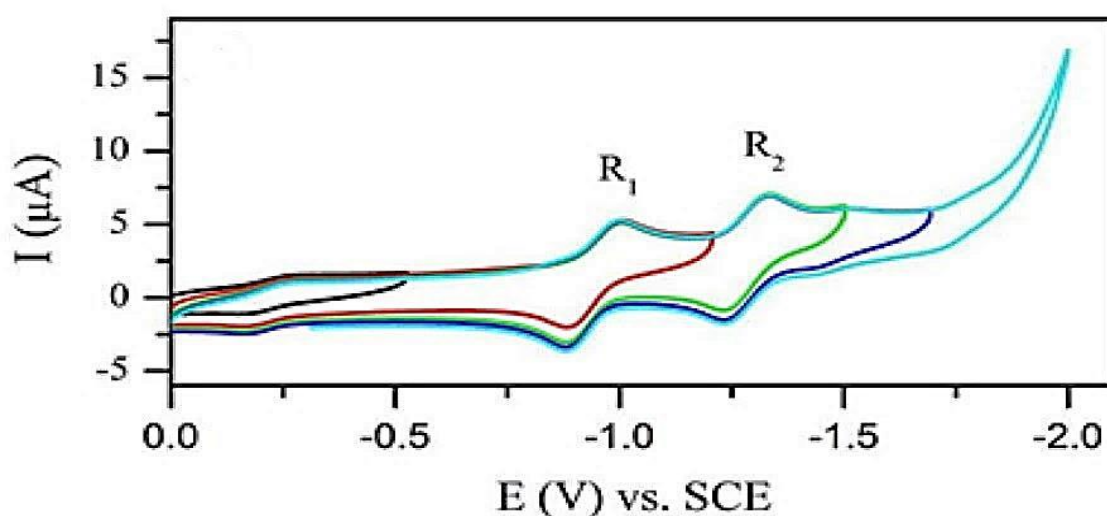


Figure 3.24. CV responses of **NiPc-THQD** at 100 Vs⁻¹ scan rate.

NiPc-THQD has two diffusion controlled and reversible reduction responses in DCM/TBAP electrolyte system: R_1 at -0.94 V and R_2 at -1.29 V. During the anodic potential scan as given in Figure 3.23 the phthalocyanine polymerizes on the working electrode with oxidation reactions.

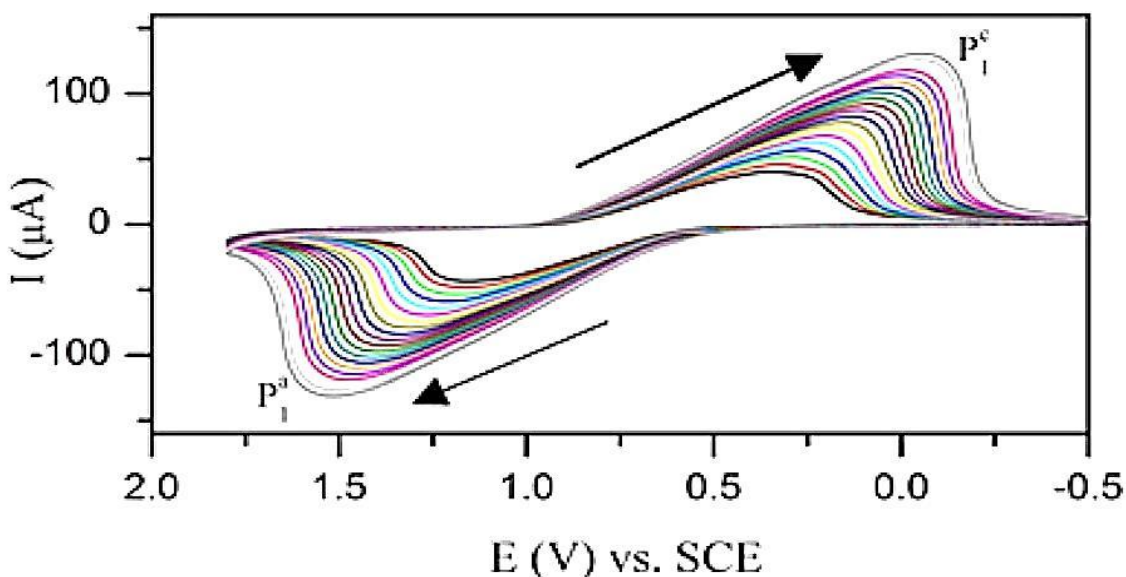


Figure 3.25. Repetitive CVs of **NiPc-THQD** during the anodic potential scan at 100 mVs^{-1} .

CV cycles with increasing intensity indicates electropolymerization and a polymeric thin film formation on the working electrode. Most probably, the **THQD** substituents are induced and polymerized.

The film structure is important for ion diffusion thus SEM micrographes of the film was investigated. In Figure 2.14 the SEM images of the **poly-H₂Pc-THQD** thin film and **poly-NiPc-THQD** thin film were exhibited.

GCE/**poly-H₂Pc-THQD** and GCE/**poly-NiPc-THQD** electrodes were coated with a thin gold layer for SEM investigations. A bulk like polymer structure was observed with this method. When the film was scraped, it was possible to measure the thickness by SEM device. The film thickness was about 350 nm for polymer film formed with 10 cycle CV.

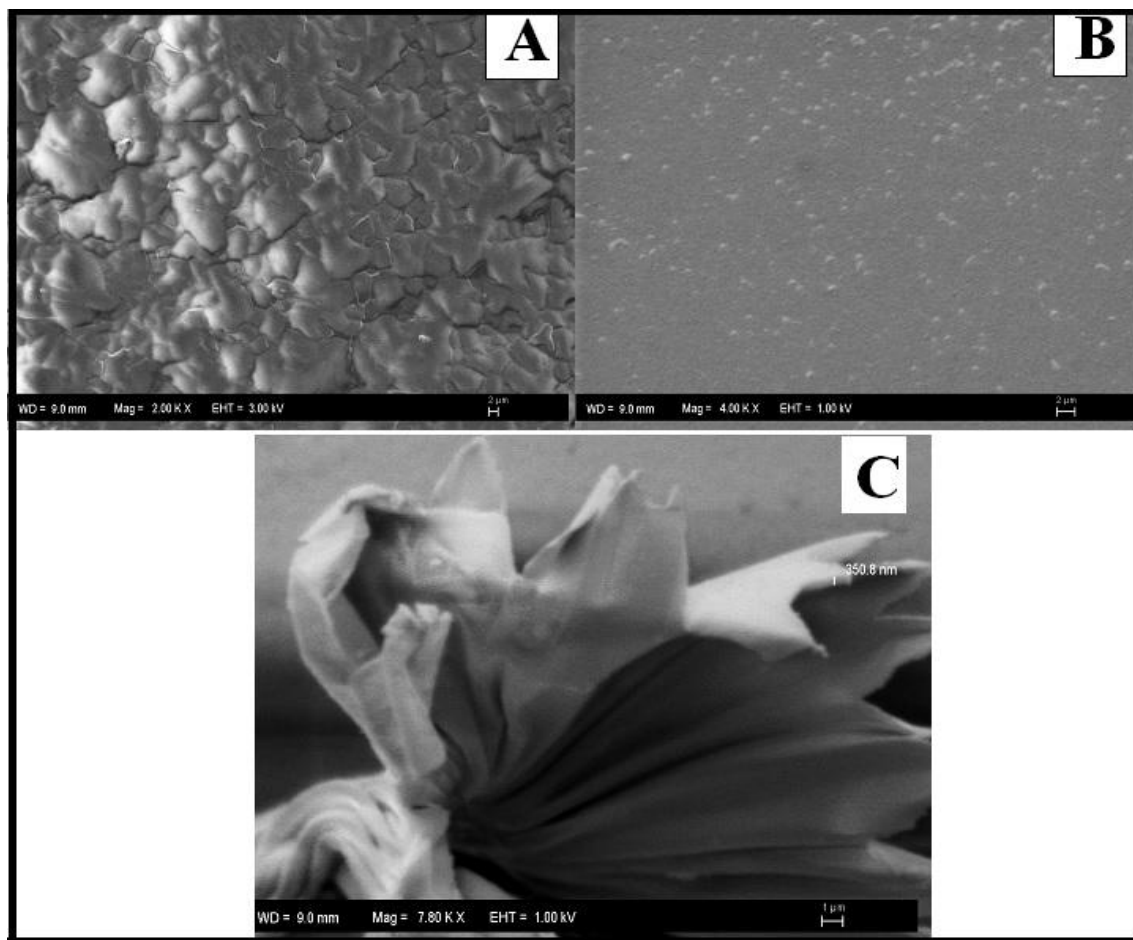


Figure 3.26. SEM images of the (A) **poly-H₂Pc-THQD** thin film, (B) **poly-NiPc-THQD** thin film and (C) Scrapped **poly-H₂Pc-THQD** thin film formed with CV technique.

3.3.2. Sensor measurements of GCE/**polyH₂Pc-THQD** and GCE/**polyNiPc-THQD** electrodes

GCE/**poly-MPc-THQD** electrodes (GCE/**poly-H₂Pc-THQD** and GCE/**poly-NiPc-THQD**) were tested for four different pesticides, carbofuran, carbaryl, eserine and parathion for sensor application. Sensing activities of the electrodes were determined with SWV (amperometric and potentiometric sensor), EIS (impedimetric sensor) and DPSCC (chrono amperometric sensor) techniques.

In Figure 3.27 SWV responses of GCE/**poly-H₂Pc-THQD** sensor against four different pesticides were represented.

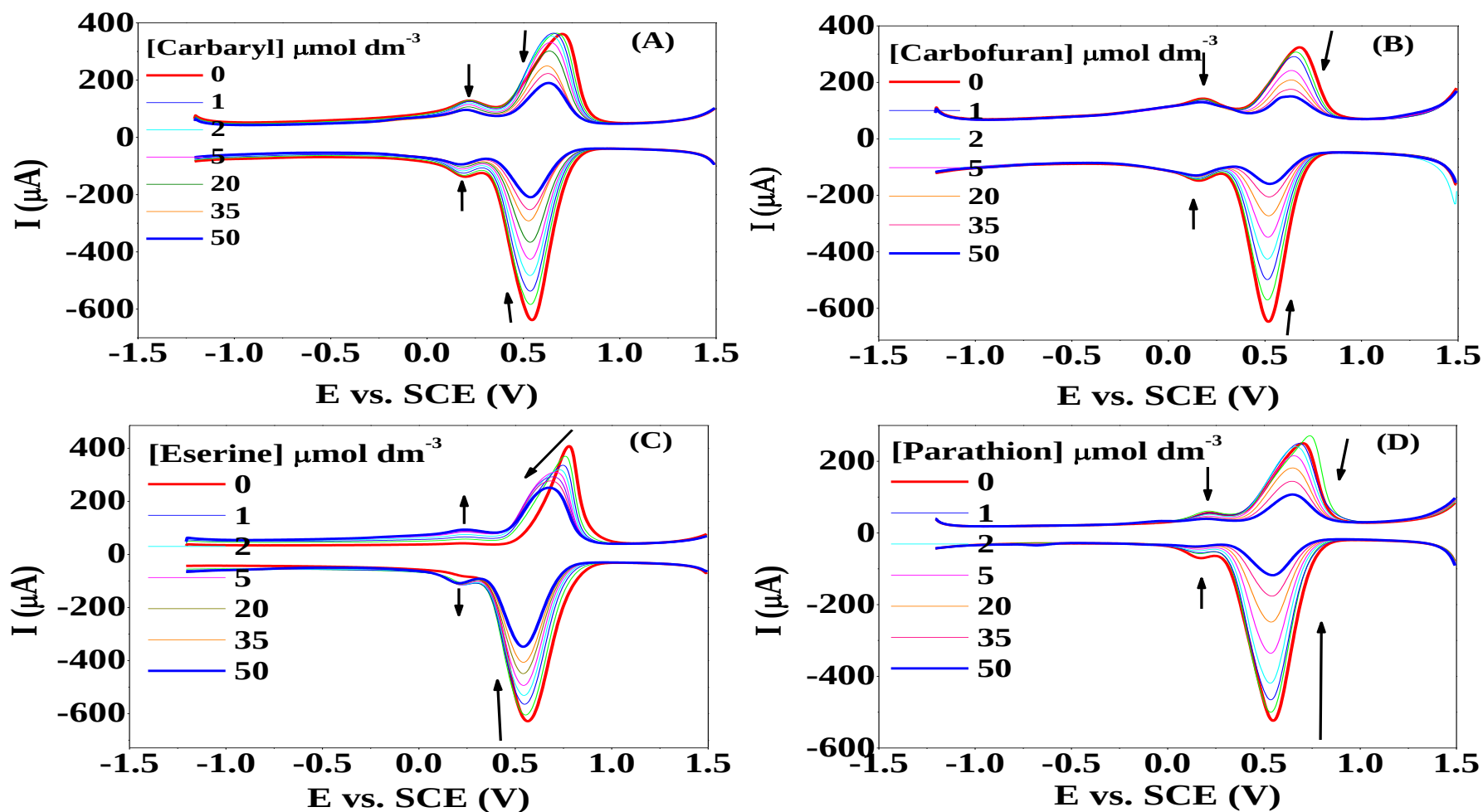


Figure 3.27. SWV responses of GCE/poly- H_2Pc -THQD sensor against four different pesticides: (A) carbaryl, (B) carbofuran, (C) eserine and (D) parathion.

From these results it can be seen that GCE/**poly-H₂Pc-THQD** electrode is behaving as a selective sensor for eserine pesticide. On the other hand, linear detection ranges and detection limit values calculated from these measurements. The detection limit (LOD) was calculated by using the equation: $LOD = 3S/b$ where S is the standard deviation of the blank solution, b is the slope of the analytical curve. The linear detection ranges for eserine were 0.0038×10^{-6} - 0.91×10^{-6} M for amperometric sensor and 0.0375×10^{-6} - 0.98×10^{-6} M for potentiometric sensor. The detection limits were 5.72×10^{-9} M and 1.11×10^{-8} M for amperometric sensor and potentiometric sensor.

In Figure 3.28 the exhibited DPSCC result belong to GCE/**poly-H₂Pc-THQD** electrode that titrated with eserine.

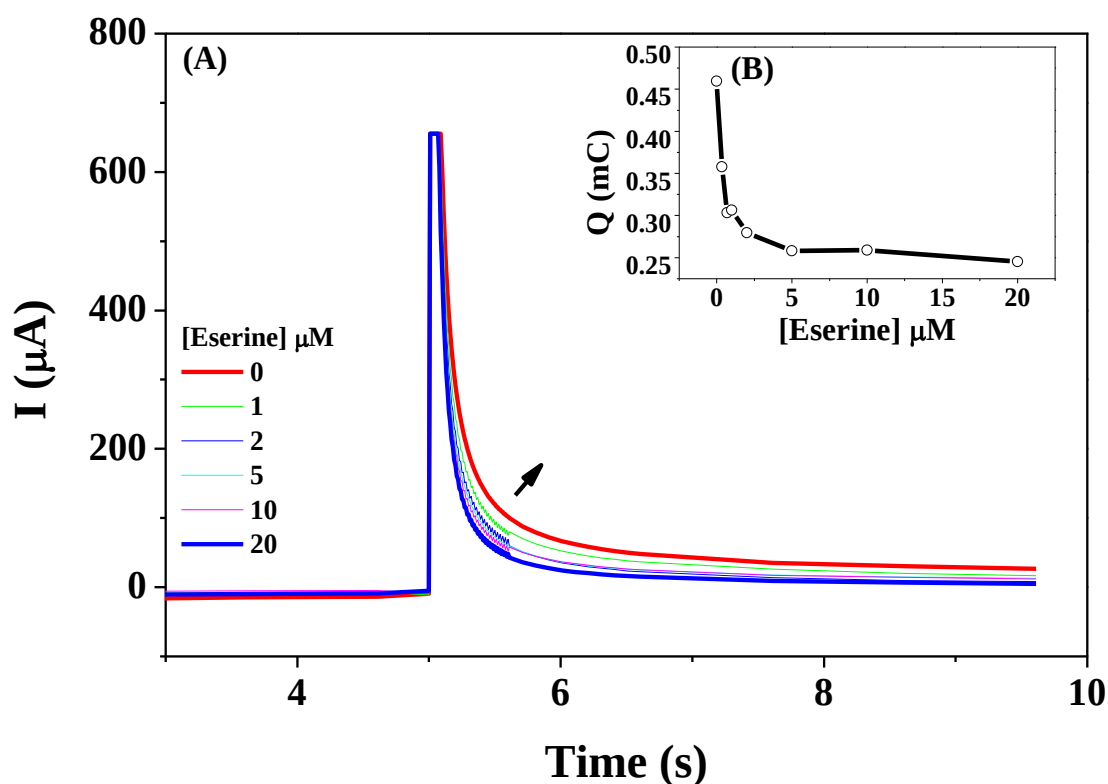


Figure 3.28. (A) DPSCC result of GCE/**poly-H₂Pc-THQD** electrode that titrated with eserine and (B) its calibration line.

0.00 V and 1.50 V potentials were applied respectively for each pesticide addition. According to the calibration line derivated from the DPSCC data the linear detection range was 0.1163×10^{-6} - 1.96×10^{-6} M and detection limit was 6.19×10^{-9} M.

The EIS responses of GCE/**poly-H₂Pc-THQD** electrode against eserine pesticide is

given in Figure 3.29. The charge transfer resistance (R_{ct}) of GCE/poly-H₂Pc-THQD electrode is equal to the diameter of cemicircle in the Nyquist diagram.

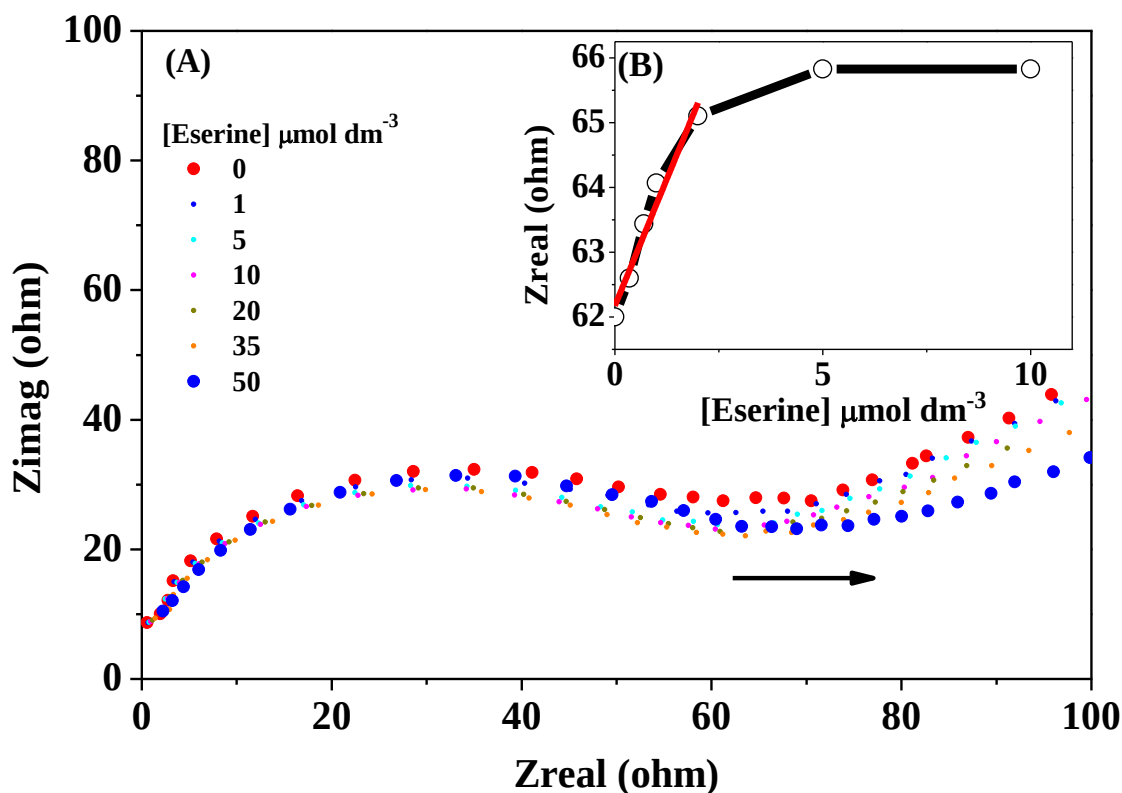


Figure 3.29. (A) The EIS responses of GCE/poly-H₂Pc electrode against eserine and (B) its calibration line.

R_{ct} is generally chosen for impedimetric sensing parameter as it changes by changing pesticide concentration. R_{ct} of the modified electrode increased as the concentration of the analyte increased since the analyte interacted with the poly-H₂Pc film on the electrode. According to the changing R_{ct} values by changing pesticide concentrations, a calibration line (Eserine concentration vs. Z_{real} or R_{ct}) was obtained and linear detection range of the electrode was between $0.075 \times 10^{-6} - 1.930 \times 10^{-6}$ M while the detection limit was calculated as 3.38×10^{-9} M.

In Figure 3.30 SWV measurement results of GCE/poly-NiPc electrode with four different pesticides are illustrated. The modified sensor electrode was obviously a selective and highly sensitive for parathion pesticide when the central metal of the polymerized phthalocyanine was changed as Ni. The SWV responses of the electrode for the other three pesticides (the carbamates; eserine, carbaryl and carbofuran) were similar with each other that the anodic peaks observed at oxidation region shifted and current

intensities were decreased by increasing pesticide concentrations. On the other side, the increasing parathion concentration in the sample lead to a decrease in current intensity not shifting. The linear detection ranges and detection limits were calculated from the calibration lines obtained from the SWV data. The linear detection range for parathion was between 0.208×10^{-6} - 2.100×10^{-6} M for amperometric sensor and 0.063×10^{-6} - 1.170×10^{-6} M with potentiometric sensor. The detection limits were 1.39×10^{-9} M and 1.15×10^{-8} M for amperometric sensor and potentiometric sensor measurements, respectively.

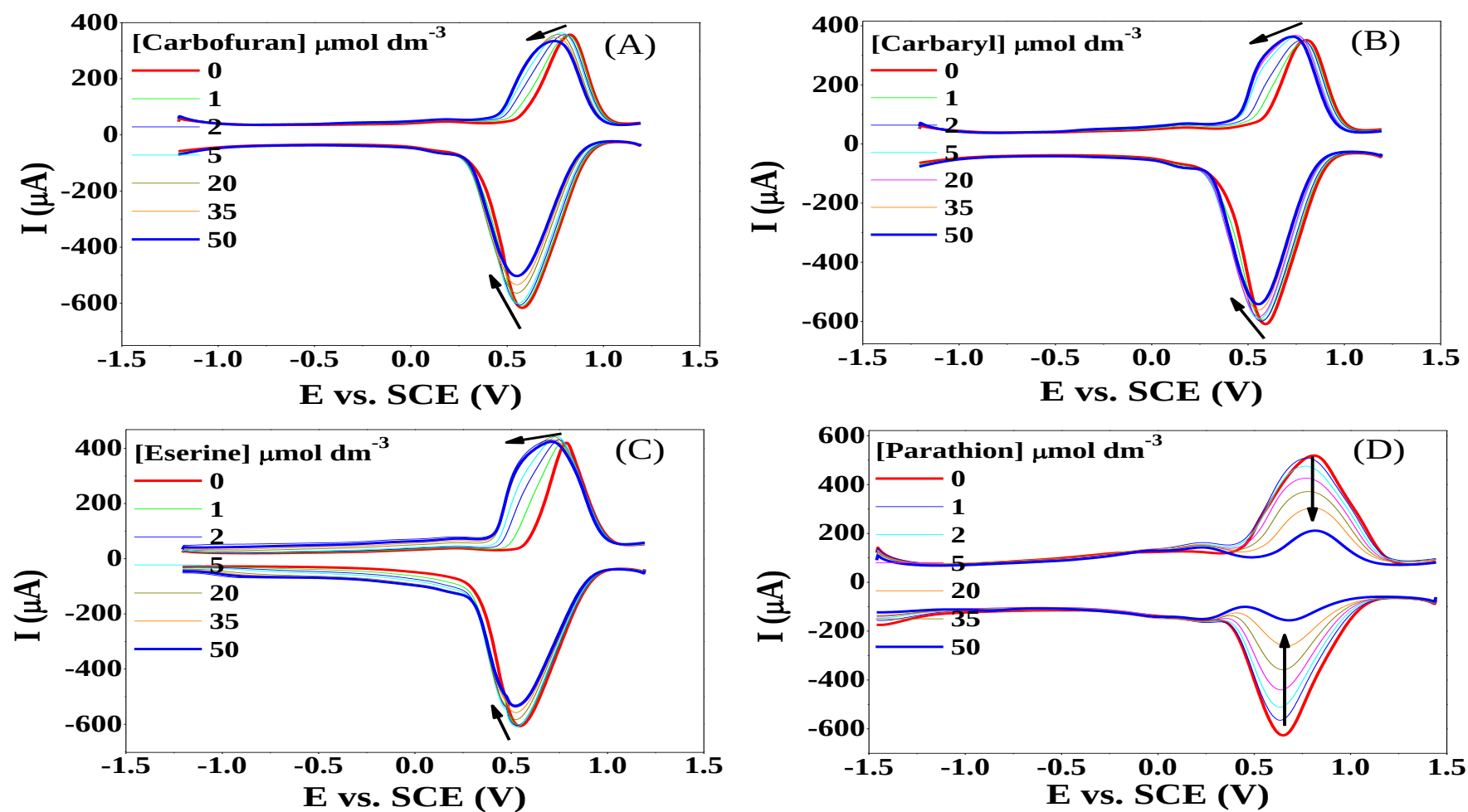


Figure 3.30. SWV measurement results of GCE/poly-NiPc electrode with four different pesticides: (A) carbofuran, (B) carbaryl, (C) eserine and (D) parathion.

DPSCC responses of GCE/poly-NiPc electrode against parathion pesticide is showed in Figure 3.31.

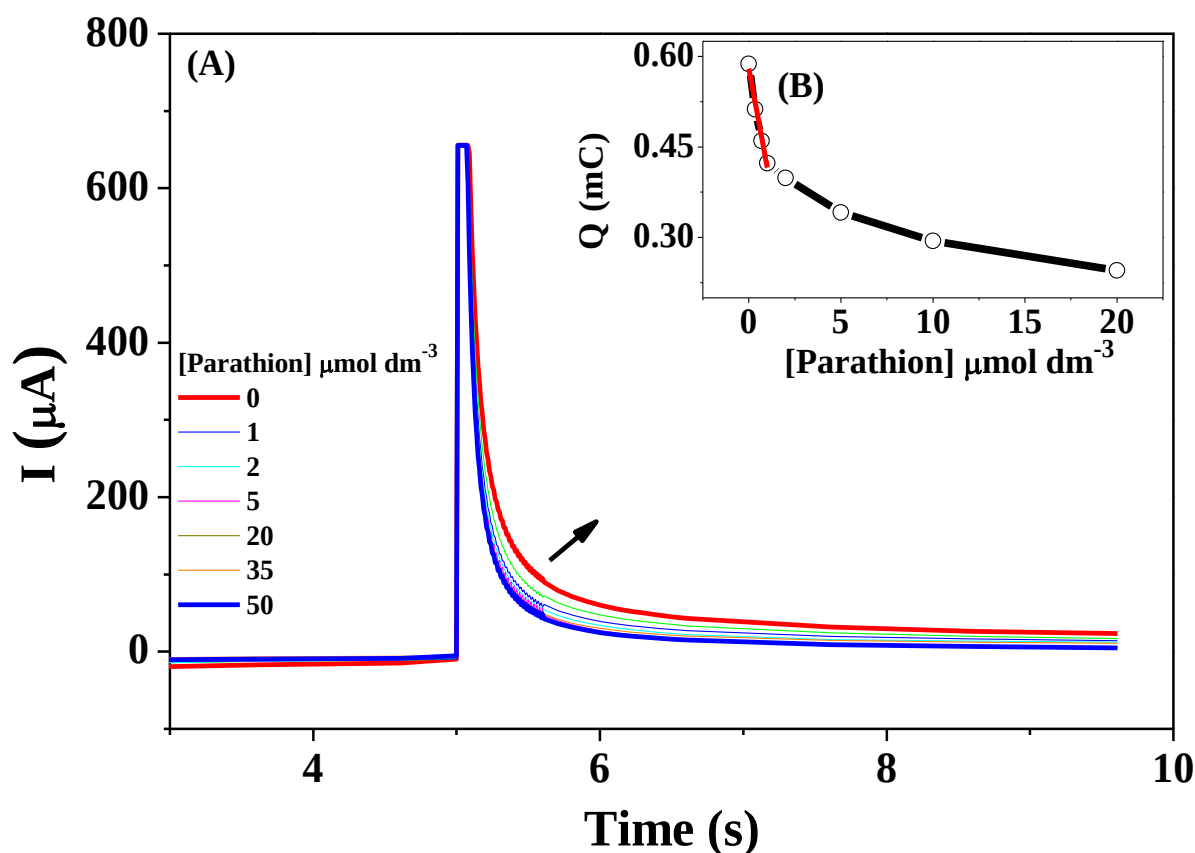


Figure 3.31. (A) DPSCC responses of GCE/poly-NiPc electrode against parathion pesticide and (B) its calibration line.

0.00 V and 1.50 V potentials were applied respectively for each pesticide concentration. As shown in the inner calibration line graph, the electrical charge passing through the working electrode decreased with the increasing parathion concentration that it is in agreement with SWV results. The calibration line derived from the DPSCC data the linear detection range was 0.0539×10^{-6} - 1.04×10^{-6} M and detection limit was 7.28×10^{-9} M for parathion.

In Figure 3.32 EIS result of the parathion titration with GCE/poly-NiPc electrode is illustrated.

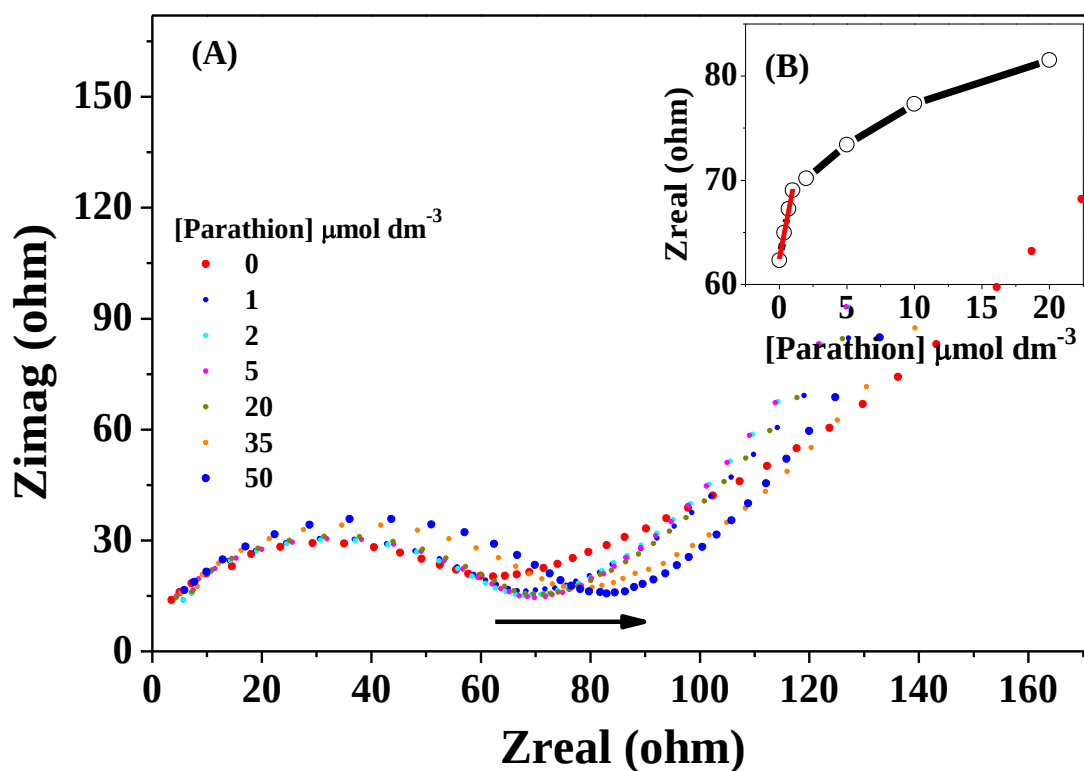


Figure 3.32. (A) EIS results of the parathion titration with GCE/poly-NiPc electrode and (B) its calibration line.

R_{ct} , the impedimetric sensing parameter, is increased with increasing parathion quantity in the sample solution. Linear detection range and detection limit were calculated from the calibration line as 0.071×10^{-6} - 1.22×10^{-6} M and 1.42×10^{-9} M, respectively.

The sensing results illustrated in the figures and tabulated in Table 3.4.

Table 3.4. Amperometric, potentiometric, impedimetric and chronocoulometric sensor parameters of **GCE/poly-MPcs** against four different pesticides.

		Eserine			Parathion		
		Linear Range (μM)	Detection Limit (M)	Sensitivity	Linear Range (μM)	Detection Limit (M)	Sensitivity
GCE/poly- H_2Pc	SWV Amperometric	0.004 - 0.912	5.72×10^{-9}	125.08 A/M	0.331 - 1.904	1.86×10^{-9}	46.12 A/M
	SWV Potentiometric	0.038 - 0.980	1.11×10^{-8}	0.06 V/ μM	0.015 - 1.016	1.98×10^{-8}	0.05 V/ μM
	EIS	0.075 – 1.935	3.38×10^{-9}	1.71 ohm/ μM	0.171 - 0.912	1.27×10^{-9}	7.46 ohm/ μM
	DPSCC	0.079 – 0.956	6.19×10^{-9}	71.70 C/M	0.268 - 1.986	3.93×10^{-9}	13.59 C/M
GCE/poly-NiPc	SWV Amperometric	0.088 - 2.243	4.59×10^{-9}	20.34 A/M	0.208 - 2.102	1.39×10^{-9}	55.65 A/M
	SWV Potentiometric	0.025 - 1.086	7.22×10^{-9}	0.05 V/ μM	0.063 - 1.178	1.15×10^{-8}	0.07 V/ μM
	EIS	0.014 - 1.102	1.65×10^{-9}	3.27 ohm/ μM	0.071 - 1.223	1.42×10^{-9}	6.42 ohm/ μM
	DPSCC	0.037 - 1.253	5.42×10^{-9}	4.31 C/M	0.054 - 1.046	7.28×10^{-9}	180.45 C/M
		Carbaryl			Carbofuran		
		Linear Range (μM)	Detection Limit (M)	Sensitivity	Linear Range (μM)	Detection Limit (M)	Sensitivity
GCE/poly- H_2Pc	SWV Amperometric	0.330 - 3.674	1.85×10^{-9}	17.67 A/M	0.074 – 1.844	1.04×10^{-9}	45.84 A/M
	SWV Potentiometric	0.064 - 1.024	8.59×10^{-9}	0.06 V/ μM	0.049- 1.205	1.19×10^{-8}	0.04 V/ μM
	EIS	0.149 - 1.890	8.31×10^{-9}	5.34 ohm/ μM	0.114 – 2.054	1.22×10^{-9}	3.90 ohm/ μM
	DPSCC	0.116 - 1.113	1.34×10^{-9}	27.4 C/M	0.049 - 1.117	6.70×10^{-9}	6.62 C/M
GCE/poly-NiPc	SWV Amperometric	0.054 – 2.054	5.83×10^{-9}	14.10 A/M	0.013 - 2.331	8.44×10^{-9}	14.95 A/M
	SWV Potentiometric	0.138 - 1.115	1.10×10^{-9}	0.05 V/ μM	0.079 - 1.564	2.70×10^{-8}	0.04 V/ μM
	EIS	0.028 – 0.996	7.29×10^{-9}	16.30 ohm/ μM	0.030 - 1.829	1.15×10^{-9}	5.75 ohm/ μM
	DPSCC	0.648 - 1.774	3.49×10^{-9}	24.57 C/M	0.025 - 1.047	9.04×10^{-9}	13.78 C/M

It can be easily concluded that GCE/poly-H₂Pc and GCE/poly-NiPc electrodes are available to be used as sensitive and selective pesticide sensors for eserine and parathion. Sensing results can be monitored with different methods, SWV, DPSCC and EIS methods that they can be used as potentiometric, amperometric and impedimetric sensors. According to given data on Table 3.4, amperometric detection technique was more sensitive than the other techniques for these two pesticides.

For reproducibility and stability investigation of the sensor electrodes, 10 GCE/poly-H₂Pc and 10 GCE/poly-NiPc electrodes were prepared with electropolymerization technique. The SWV responses of modified electrodes were compared for potential and current differences of redox processes were compared with each other and the highest relative standard deviations of the responses were found as 3.7 and 4.3% for current reproducibility's of the redox processes of GCE/poly-H₂Pc and GCE/poly-NiPc electrodes respectively. The stabilities of the electrodes were tested with comparing the potential and current of the redox processes recorded with SWVs at different times. Storage stabilities of all electrodes were found higher than 90% after 60 days, which indicates long term stability of the sensor electrodes.

3.4. GCE/PANI-N₃/TA-MPc (CEC) Sensor Prepared with Click Electrochemistry Technique

3.4.1. Electrochemical characterization of TA-MPc

As an electrochemical device, redox activity of a functional material should be well known. Therefore the electrochemical activity of TA-MPc in DMSO/TBAP electrolyte system on a GCE in solution has been investigated. CV and SWV of TA-CoPc is given in Figure 3.33.

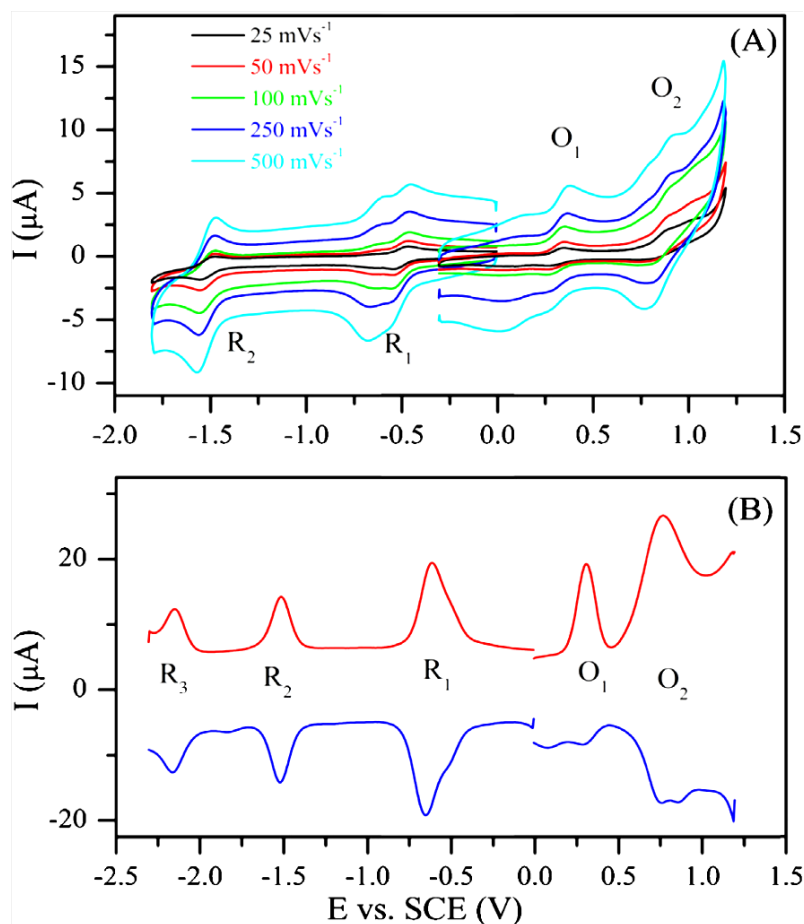


Figure 3.33. (A) CV and (B) SWV of TA-CoPc (5.0×10^{-4} mol dm $^{-3}$) at various scan rates on GCE in DMSO/TBAP electrolyte system.

TA-CoPc gives two oxidation reactions, O₁ ($E_{1/2} = 0.30$ V vs. SCE; $\Delta E_p = 76$ mV; $I_{pa}/I_{pc} = 0.38$) and O₂ ($E_{1/2} = 0.76$ V vs. SCE; $\Delta E_p = 70$ mV; $I_{pa}/I_{pc} = 1.0$), and three reduction reactions, R₁ ($E_{1/2} = -0.62$ V vs. SCE; $\Delta E_p = 76$ mV; $I_{pa}/I_{pc} = 0.92$), R₂ ($E_{1/2} = -1.53$ V vs. SCE; $\Delta E_p = 70$ mV; $I_{pa}/I_{pc} = 0.90$) and R₃ ($E_{1/2} = -2.16$ V vs. SCE) within the potential window of the electrolyte system. SWVs of the complex illustrate these redox processes more clearly as shown in Figure 3.33B. The first reduction process splits into two waves due to the existence of equilibrium between coordinated and non-coordinated TA-CoPc. It is well known that Co^{II} and Co^{III} oxidation states of metal center of TA-CoPc could form tetra and penta coordinated complex and form an equilibrium between these two different forms.

Electrochemistry of TA-CoPc (five reversible and diffusion controlled redox reactions and metal based reduction and oxidation processes) shows value of TA-CoPc as a potential electrochemical device, such as electrocatalyst and/or electrosensor for the

detection of various target species. For the usage of an electrochemical device, functional specie should be coated on a substrate with desired properties. Therefore we have aimed to coat TA-CoPc on an electrode and characterized basic parameters of TA-CoPc based modified electrode. TA-CoPc was coated on GCE with both of CC and CEC techniques. During these processes, first of all, 4-azidoaniline (ANI-N₃) was coated on the GCE with electropolymerization, as the azido source, which is needed for the bounding of alkyne terminal of TA-CoPc. Then GCE/PANI-N₃ electrode was dipped into a DMSO solution of TA-CoPc and CuBr and waited for 3 h at the room temperature for self click reaction between azide group of PANI-N₃ and terminally alkyne groups of TA-CoPc. GCE/PANI-N₃ electrode was also coated with TA-CoPc by using CEC technique in a DMSO solution of TA-CoPc and CuSO₄. Figure 3.34 illustrates the repetitive CV recorded during the electro polymerization of ANI-N₃ on the GCE working electrode in AN/TBAP electrolyte.

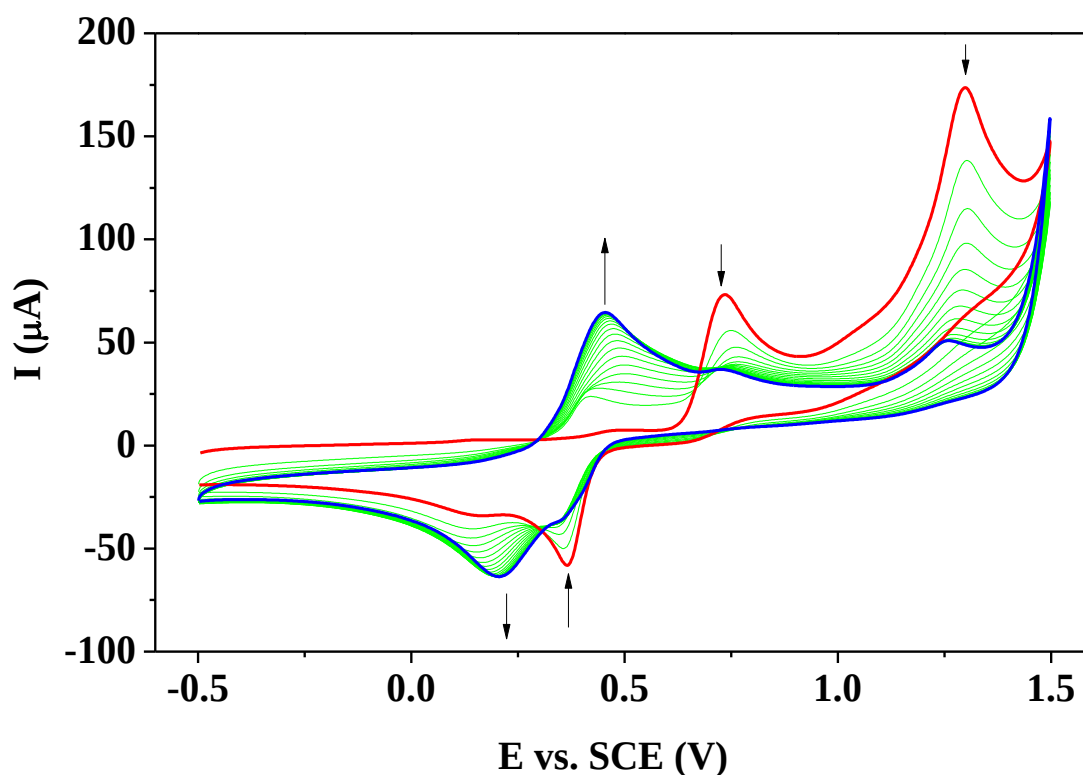


Figure 3.34. Repetitive CVs of ANI-N₃ (5.0×10^{-4} mol dm⁻³) at 100 mV s⁻¹ scan rate on GCE in DCM/TBAP electrolyte system.

During the first anodic CV cycle, ANI-N₃ gives two oxidation waves at 0.74 V and 1.30 V. Oxidative electropolymerization of ANI-N₃ immediately occurs and forms ANI-N₃

polymer (PANI-N₃) on the GCE working electrode (GCE/ANI-N₃). PANI-N₃ on GCE gives a reversible redox couple at 0.33 V ($E_{1/2}$) whose peak current increases as a function of increasing CV cycles. These electrochemical responses indicate formation of a GCE/ANI-N₃ modified electrode. PANI formation is well known and extensively studied in the literature. However, there is only one paper on the electropolymerization of 4-azidoaniline in the literature. Bedioui and his co-workers modified an electrode with electropolymerized azidoaniline and used this electrode for the bounding of ferrocene moieties. However, their CV data about the electropolymerization processes are ill defined and the redox waves of PANI were not clear. Voltammetric responses of ANI-N₃ recorded here give well defined PANI based redox couple which is in harmony with the reported studies on the electropolymerization of PANI.

The redox responses of CuSO₄ were investigated in DMSO/TBAP electrolyte on GCE, which gave Cu^{II}/Cu^I redox couple at -0.15 V and Cu^I/Cu⁰ redox couple at -0.65 V. Cu^{II}/Cu^I redox couple is also recorded in Figure 3.35 at the same potential.

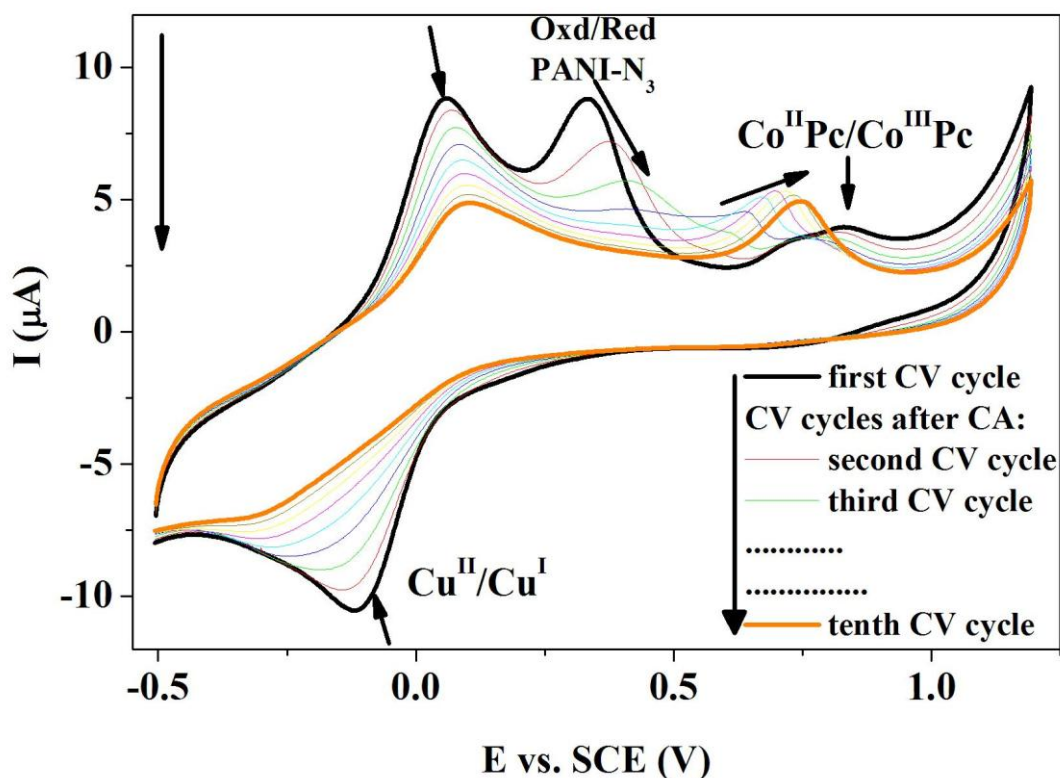


Figure 3.35. Repetitive CVs of GCE/PANI-N₃ working electrode after 10 min constant applied potential at -0.15 V (CA) in TA-CoPc and CuSO₄ dissolved DMSO/TBAP electrolyte system at 0.100 V s⁻¹ scan rate.

These data indicate that applied potential at around -0.15 V produce Cu^{I} ions for the CEC reaction. To perform the CEC reaction, three voltammetric methods were carried out sequentially (Figure 3.35). First of all, CV response of GCE/PANI- N_3 electrode was recorded in TA-CoPc and CuSO_4 dissolved DMSO/TBAP electrolyte between -0.50 V and 1.20 V (step 1: 1 CV cycle in Figure 3.35). Then CA responses of GCE/PANI- N_3 electrode were recorded at -0.15 V during 10 min (step 2) and finally CV response of GCE/PANI- N_3 electrode was recorded sequentially in TA-CoPc dissolved DMSO/TBAP electrolyte between -0.50 V and 1.20 V (step 3: From 2 to 10 CV cycles in Figure 3.35). The voltammetric difference between step 1 and step 3 indicates proceeding of the CEC reaction during step 2 and formation of GCE/PANI- N_3 /TA-CoPc (CEC) electrode. While GCE/PANI- N_3 electrode only gives red/oxd wave of PANI- N_3 at 0.33 V and $\text{Co}^{\text{II}}\text{Pc} / \text{Co}^{\text{III}}\text{Pc}$ wave of TA-CoPc at 0.84 V during the 1 CV cycle, a new wave is recorded at 0.61 V and this wave shifts up to 0.74 V with increasing in current intensity as a function of increasing CV cycles. This new wave was assigned to the $\text{Co}^{\text{II}}\text{Pc}/\text{Co}^{\text{III}}\text{Pc}$ process of binding bound TA-CoPc to the azide groups on the electrode. Moreover the red/oxd wave of PANI- N_3 at 0.33 V decreases in current intensity with positive potential shifts as a function of repetitive CV cycles due to the blockage of PANI- N_3 film on the electrode with TA-CoPc. All of these voltammetric responses support proceeding of the CEC reaction and modification of GCE/PANI- N_3 electrode with TA-CoPc and formation of GCE/PANI- N_3 /TA-CoPc (CEC) electrode modification of GCE with PANI- N_3 and then with TA-CoPc was illustrated in Figure 3.36. GCE/PANI- N_3 and GCE/PANI- N_3 /TA-CoPc electrodes were electrochemically and spectroscopically characterized with SWV, EIS, and UV-vis spectroscopy. Figure 3.36 illustrates SWV responses of bare GCE, GCE/PANI- N_3 , and GCE/PANI- N_3 /TA-CoPc electrodes constructed with CC and CEC methods in DMSO/TBAP and $\text{H}_2\text{O}/\text{LiClO}_4$ electrolytes.

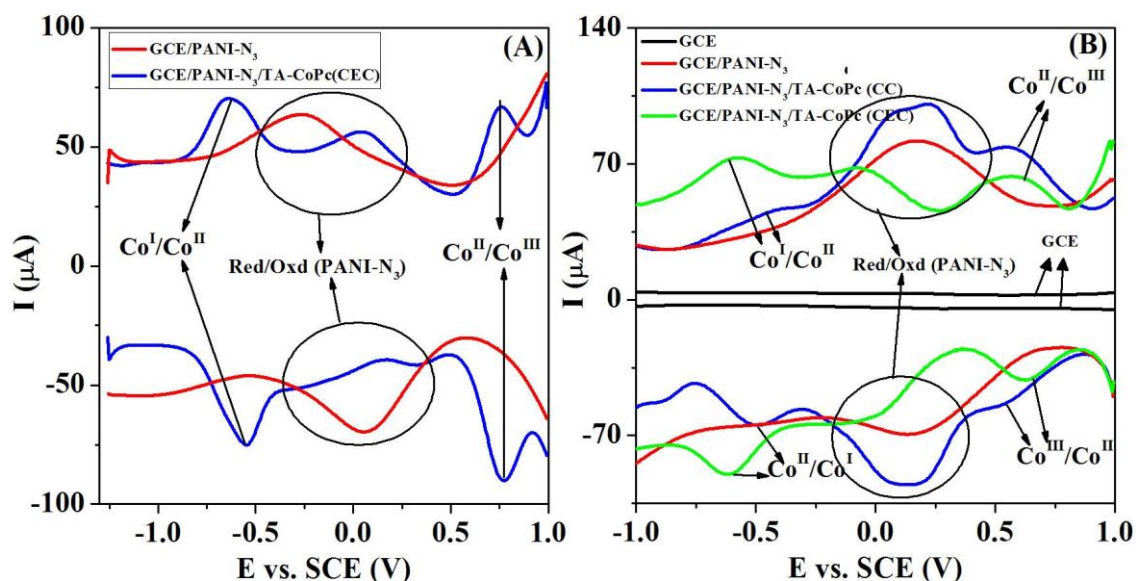


Figure 3.36. SWV responses of bare GCE, GCE/PANI-N₃, GCE/PANI-N₃/TA-CoPc (CC), and GCE/PANI-N₃/TA-CoPc (CEC) electrodes in **(A)** DMSO/TBAP and **(B)** H₂O/LiClO₄ electrolytes.

GCE/PANI-N₃/TA-CoPc electrodes were modified with the CEC and common CC techniques were prepared and they tested for comparisons. While GCE/PANI-N₃ electrode gives redox waves of PANI-N₃ film at around 0 V, GCE/PANI-N₃/TA-CoPc electrodes give TA-CoPc based redox couples at around -0.50 V and 0.70 V in addition to the PANI-N₃ based redox processes. Similar redox processes were recorded with both of the GCE/PANI-N₃/TA-CoPc electrodes modified with the CEC and common CC techniques. These redox responses given in Figure 3.36 illustrate bounding of TA-CoPc to PANI-N₃ with both of the CEC and common CC techniques. The redox responses of modified electrodes were tested in two different electrolyte systems, a nonaqueous organic and an aqueous media, and same redox processes were recorded in both of DMSO (Figure 3.36A) and H₂O (Figure 2.23B) with slight potential shifts. These data indicate that GCE/PANI-N₃/TA-CoPc electrodes give Co^{II}/Co^I and Co^{II}/Co^{III} redox processes of TA-CoPc and red/oxd process of PANI-N₃ indifferent media and this worthy redox activity of the modified electrode indicate usage of the electrodes as electrochemical devices in these media. Figure 3.37 illustrates UV-vis, and EIS spectroscopy responses of the modified electrodes.

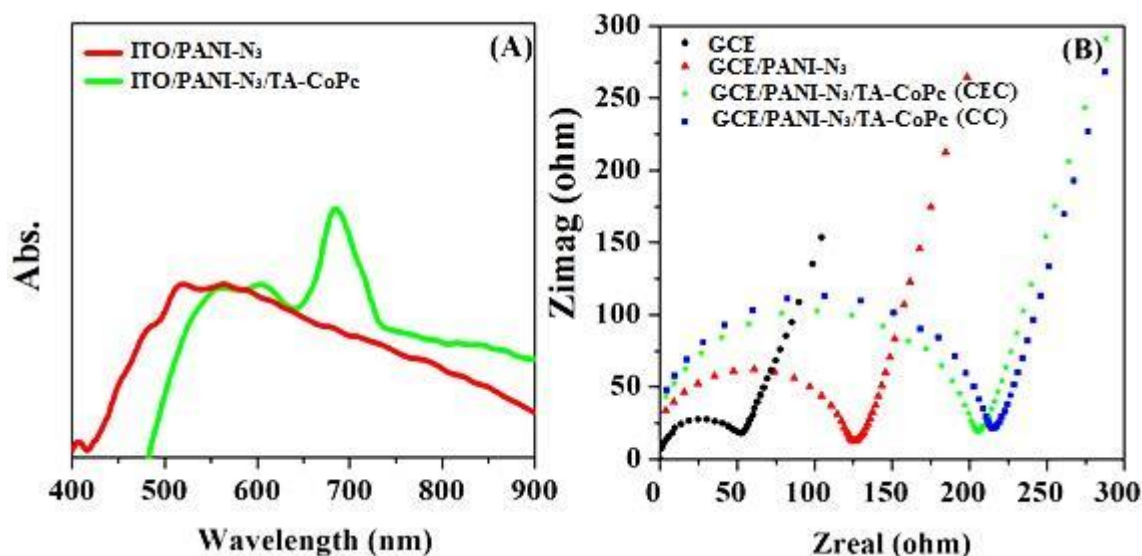


Figure 3.37. (A) UV-vis spectra of ITO/PANI-N₃ and ITO/PANI-N₃/TA-CoPc electrodes in DMSO, (B) EIS of bare GCE, GCE/PANI-N₃, GCE/PANI-N₃/TA-CoPc (CC), and GCE/PANI-N₃/TA-CoPc (CEC) electrodes in H₂O/LiClO₄ electrolyte.

While ITO/PANI-N₃ electrode gives a broad band at around 500 nm assigned to the PANI-N₃, a new band at 684 nm is recorded on GCE/PANI-N₃/TA-CoPc (CC) and GCE/PANI-N₃/TA-CoPc (CEC) electrodes. ITO/PANI-N₃/TA-CoPc electrodes constructed with CC and CEC reactions give same spectroscopic and EIS response. This new band is the Q band of the CoPc complex (Figure 3.37A). When TA-CoPc was bonded to PANI-N₃, the absorption band of PANI-N₃ shifts to longer wave lengths. These spectral changes clearly illustrate presence of TA-CoPc on the modified electrode. Figure 3.37B illustrates the EIS of the bare and modified GCE electrodes. As can be seen, the depressed capacitive semi circle recorded on GCE enlarged when GCE was modified with PANI-N₃ and the biggest semicircle was recorded on GCE/PANI-N₃/TA-CoPc (CC) and GCE/PANI-N₃/TA-CoPc (CEC) electrodes modified with CC and CEC. It implies that PANI-N₃/TA-CoPc film has higher charge transfer resistance (R_{ct}) than PANI-N₃ film. The same shape and different diameters of the semicircle in the EIS of the electrodes (Figure 3.37B) demonstrate that GCE was coated with PANI-N₃ and TA-CoPc was bonded to PANI-N₃ which alters the Faradaic resistance of the modified electrodes. While GCE gives a semicircle with a radius of 20 Ω, coating of the electrode with PANI-N₃ increases the radius of the semicircle to 125 Ω and binding of TA-CoPc to PANI-N₃ increases to about 200 Ω (Figure 3.37B). These EIS data indicate

that coating of GCE electrode with conductive organic film decreases the conductivity of the electrode. The EIS responses of modified electrodes are in harmony with the similar EIS studies in the literature. GCE/PANI-N₃/TA-CoPc (CC) and GCE/PANI-N₃/TA-CoPc (CEC) electrodes give generally similar voltammetric, EIS, and spectroscopic responses. The main difference is the different time interval of these techniques. While at least 3 h is needed for click chemistry reaction, 10 min was enough for the CEC reaction. Another advantage of the CEC is the controllability of the reaction with the cycle number of CV and time interval of CA techniques. Figure 3.38 represents SEM image of ITO/PANI-N₃ and ITO/PANI-N₃/TA-CoPc (CEC) electrodes.

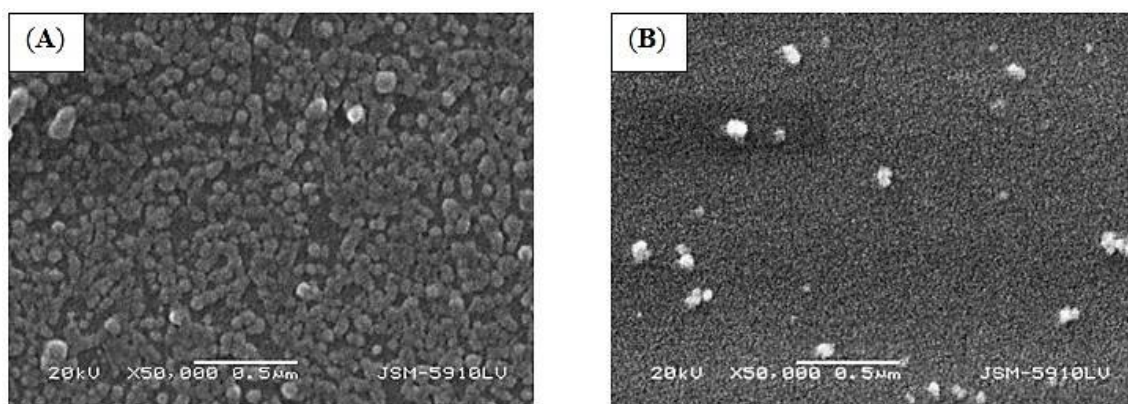


Figure 3.38. SEM images of **(A)** ITO/PANI-N₃ and **(B)** ITO/PANI-N₃/TA-CoPc (CEC) electrodes.

PANI-N₃ and PANI-N₃/TA-CoPc (CEC) films have different structures on ITO electrode as shown in the SEM image, which support modification of PANI-N₃ with TA-CoPc with CEC. Both of the PANI-N₃ and TA-CoPc have uniform film structures on the ITO electrodes. While PANI-N₃ forms approximately 100 nm diameter nano-sized clusters, the size of the clusters decreases when the surface was modified with TA-CoPc.

Also electrochemical characterization of TA-H₂Pc and TA-ZnPc are also investigated. The SWV measurement of these phthalocyanines coated on GCE were obtained in DMSO/TBAP solution. The related results are given in Figure 3.39.

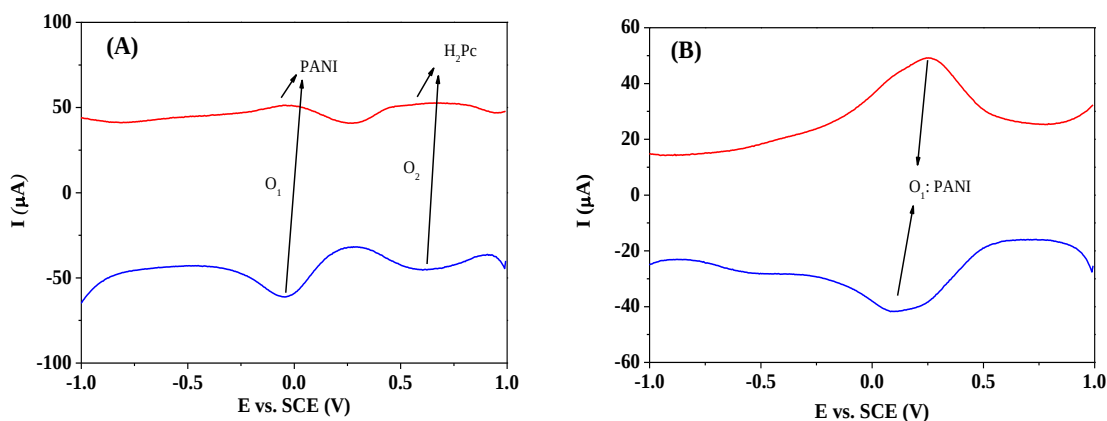


Figure 3.39. SWV of **(A)** TA-H₂Pc (5.0×10^{-4} mol dm⁻³) and **(B)** TA-ZnPc (5.0×10^{-4} mol dm⁻³) on GCE in DMSO/TBAP electrolyte system.

Pesticide sensing responses of GCE/PANI-N₃/TA-MPc (CEC) electrodes were tested with SWV, DPSCC and EIS methods for usability as electrochemical pesticide sensor for eserine, carbaryl, carbofuran, and parathion. It is well known that many pesticides, such as eserine, are known to be cholinesterase inhibitors and it has been used as a pesticide and pharmaceutical. Since sensing of these pesticides with high sensitivity and selectivity has great importance.

3.4.2. Sensor measurements of GCE/PANI-N₃/TA-MPc electrodes

SWV, CA and EIS responses of **GCE/PANI-N₃/TA-MPc** electrodes to varying concentration of carbaryl, carbofurane, eserine and parathion pesticides were recorded to test the utilizability of **GCE/PANI-N₃/TA-MPc** electrodes as electrochemical pesticide sensors. 0.10 M lithium perchlorate (LiClO₄) dissolved in phosphate buffer solution (PBS) (PH=7.0) and it was used as the electrolyte in the electrochemical cell of three-electrode cell configuration. **GCE/PANI-N₃/TA-MPc** electrodes were used as EPS. Before sensing measurements, blank tests of **GCE/PANI-N₃** and **GCE/PANI-N₃/TA-MPc** electrodes were taken with SWV, DPSCC, and EIS techniques, respectively. When the blank measurements were stabilized, pesticide solutions were added with increasing amounts gradually. Then, all electrochemical measurements SWV, DPSCC, and EIS, were carried out again with a sequence manner. Basic sensor parameters were derived from the analysis of all of SWV, DPSCC and EIS separately and compared with each other with respect to selectivity and sensitivity.

GCE/**PANI-N₃/TA-MPc** electrodes was tested with SWV, EIS, and DPSCC techniques for usability as EPS for different pesticides; carbaryl, carbofuran, parathion, and eserine. SWVs of GCE/**PANI-N₃/TA-MPc** electrodes changed considerably during the titration with different pesticides. GCE/**PANI-N₃/TA-CoPc** electrode gives different electrochemical responses for each of the pesticides, carbaryl, carbofuran, parathion, and eserine, which indicates selectivity of the EPS for these pesticides. Figure 3.40 illustrates the SWV responses of GCE/**PANI-N₃/TA-MPc** electrodes during gradual addition of eserine into the phosphate buffer electrolyte.

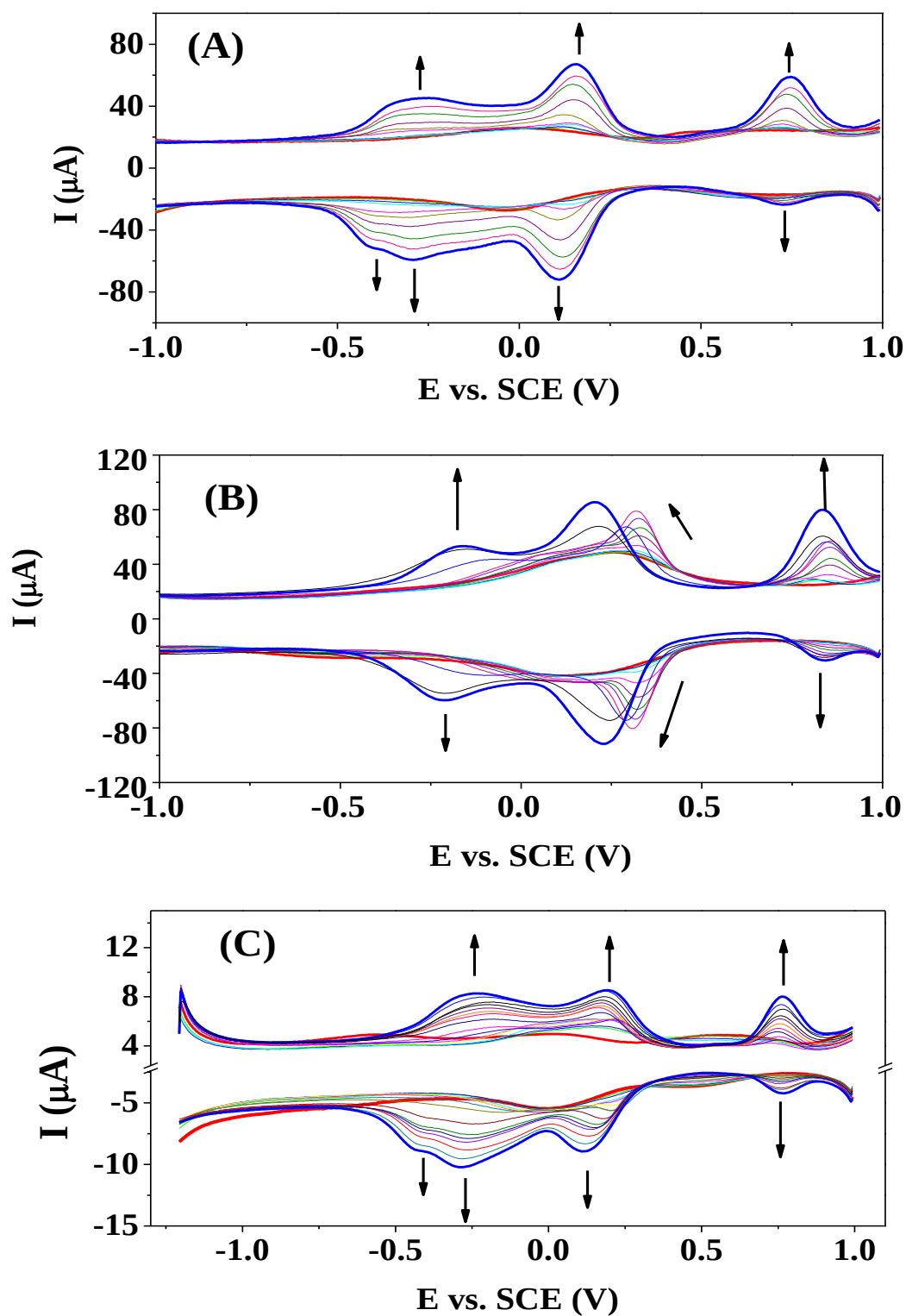


Figure 3.40. SWV responses of A) GCE/PANI-N₃/TA-H₂Pc, B) GCE/PANI-N₃/TA-ZnPc and C) GCE/PANI-N₃/TA-CoPc electrodes in PBS (pH=7.0) during the gradual addition of eserine.

In Figure 3.40A SWV responses of GCE/**PANI-N₃/TA-H₂Pc** electrode with the titration of eserine is shown. Three new redox couples formed with increasing peak intensity and a slightly potential shifting during the addition of eserine indicate that this electrode can be used as a potentiometric or amperometric eserine sensor. In Figure 3.40B the same experiments were repeated for eserine pesticide with GCE/**PANI-N₃/TA-ZnPc** electrode and beside new three redox couple formation the potential shifting of the peaks were more considerable. In Figure 3.40C GCE/**PANI-N₃/TA-CoPc** electrode gives Co^{II}Pc/Co^IPc couple at -0.58 V, Red/Oxd couple of **PANI-N₃** at 0.02 V, and Co^{II}Pc/Co^{III}Pc at 0.66 V without eserine. During titration with eserine, the Co^{II}Pc/Co^IPc and Co^{II}Pc/Co^{III}Pc redox processes decrease in current intensity gradually and completely disappear at the end of the titration. New redox waves increase gradually as a result of the interaction of eserine and the GCE/**PANI-N₃/TA-CoPc** electrode. Especially three redox couples are well resolved with increasing current intensity and potential shifts at -0.23, 0.19, and 0.76 V as a result of the interaction with eserine. Current changes versus increasing pesticide concentration represent usability of the GCE/**PANI-N₃/TA-CoPc** electrode as amperometric sensor and potential shifting of the new waves with respect to increasing eserine concentration indicates usability of the electrode as potentiometric pesticide sensor.

Voltammetric eserine sensing responses of GCE/**PANI-N₃/TA-MPc** electrodes are different from each other because of the central metal of the phthalocyanines. When different pesticides were used, different redox peaks at different potentials are observed. For instances while three redox couples are observed at -0.23, 0.19, and 0.76 V during eserine sensing, only a sharp redox couple is recorded at -0.16 V with parathion and different peaks are observed for the other pesticides. These voltammetric responses of GCE/**PANI-N₃/TA-MPc** electrodes indicate selectivity of the electrodes for different pesticides.

Figure 3.41 shows the SWV responses of GCE/**PANI-N₃/TA-MPc** electrodes during gradual addition of parathion.

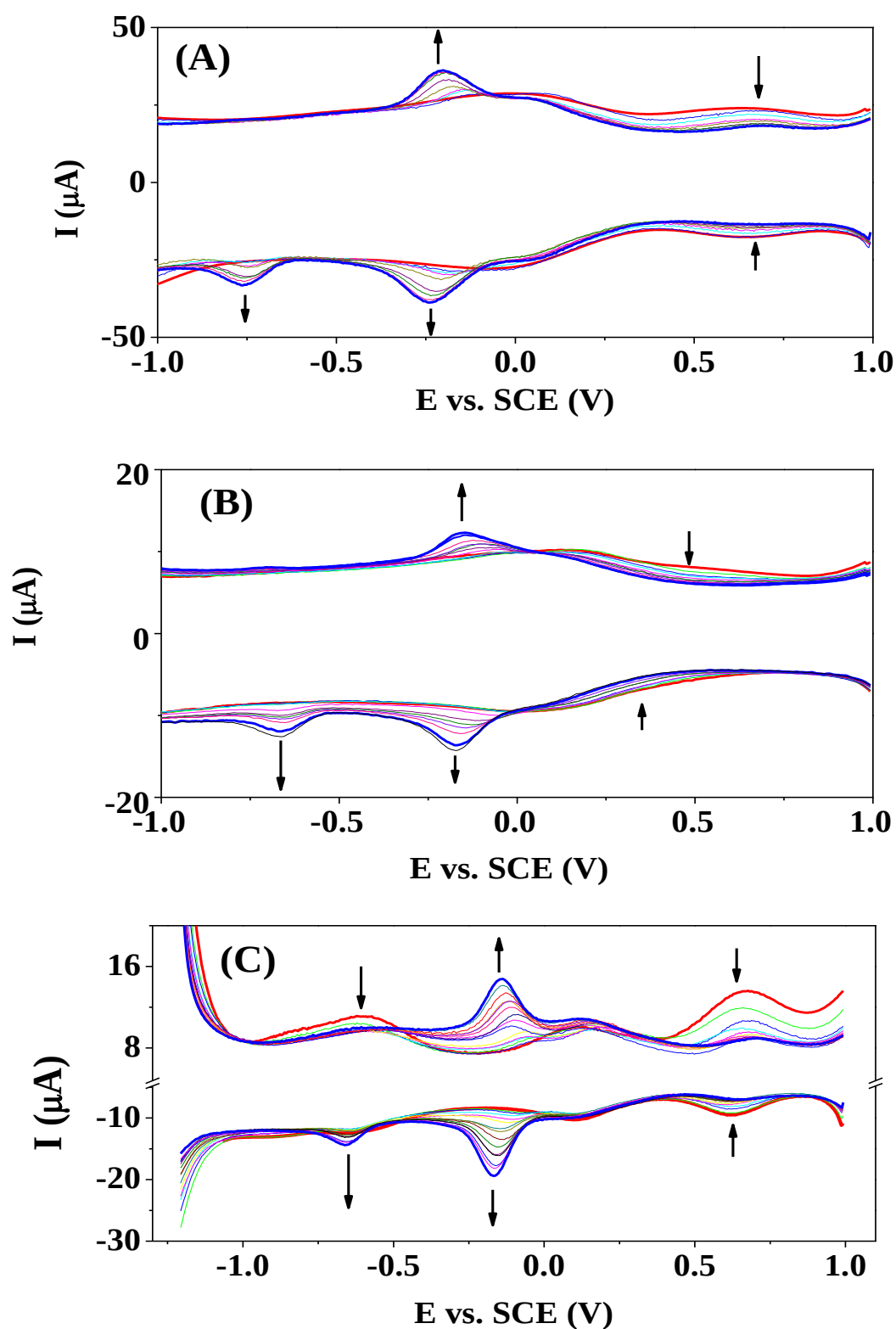


Figure 3.41. SWV responses of (A) GCE/PANI- N_3 /TA- H_2Pc , (B) GCE/PANI- N_3 /TA- ZnPc and (C) GCE/PANI- N_3 /TA- CoPc electrodes in PBS (PH=7.0) during the gradual addition of parathion.

Parathion sensing responses of the electrodes are completely different than those of eserine as shown in Figure 3.40 and Figure 3.41. SWV responses of GCE/**PANI-N₃/TA-H₂Pc** and GCE/**PANI-N₃/TA-ZnPc** are similar and different from those of GCE/**PANI-N₃/TA-CoPc**. While three redox couples at -0.23, 0.19, and 0.76 V are recorded with eserine titration, only a sharp redox couple is recorded at -0.16 V with parathion. Moreover Co^{II}Pc/Co^IPc couple at -0.58 V does not disappear with parathion titration. The differences between the sensing responses of eserine and parathion can be easily used to differentiate these two pesticides from each other in a target sample. Carbaryl and carbafulan sensing experiment results are given in Figure 3.42 and Figure 3.43 for GCE/**PANI-N₃/TA-H₂Pc**, GCE/**PANI-N₃/TA-ZnPc** and GCE/**PANI-N₃/TA-CoPc** electrodes, respectively.

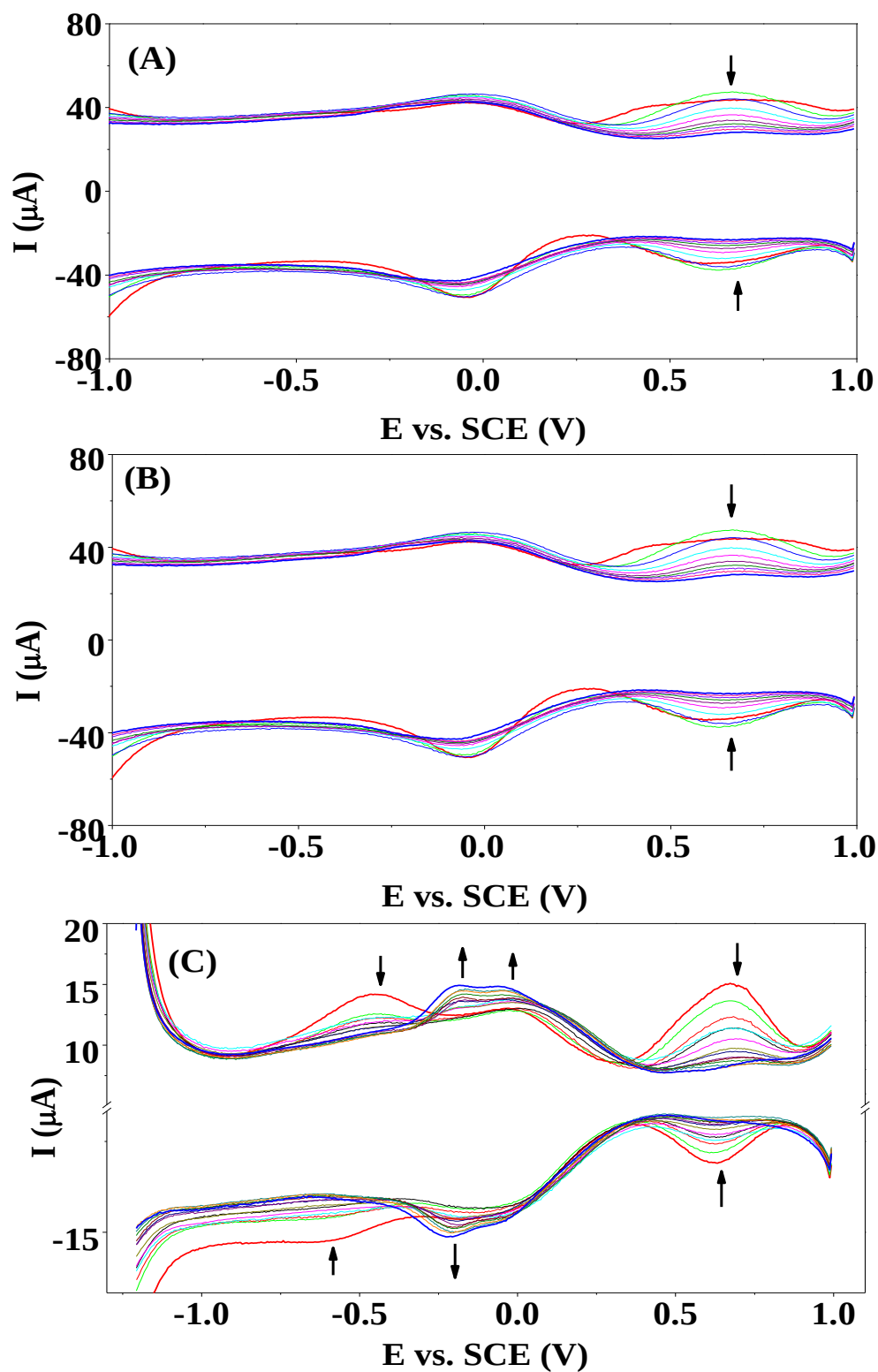


Figure 3.42. SWV responses of (A) GCE/PANI-N₃/TA-H₂Pc, (B) GCE/PANI-N₃/TA-ZnPc and (C) GCE/PANI-N₃/TA-CoPc electrodes in PBS (PH=7.0) during the gradual addition of carbaryl.

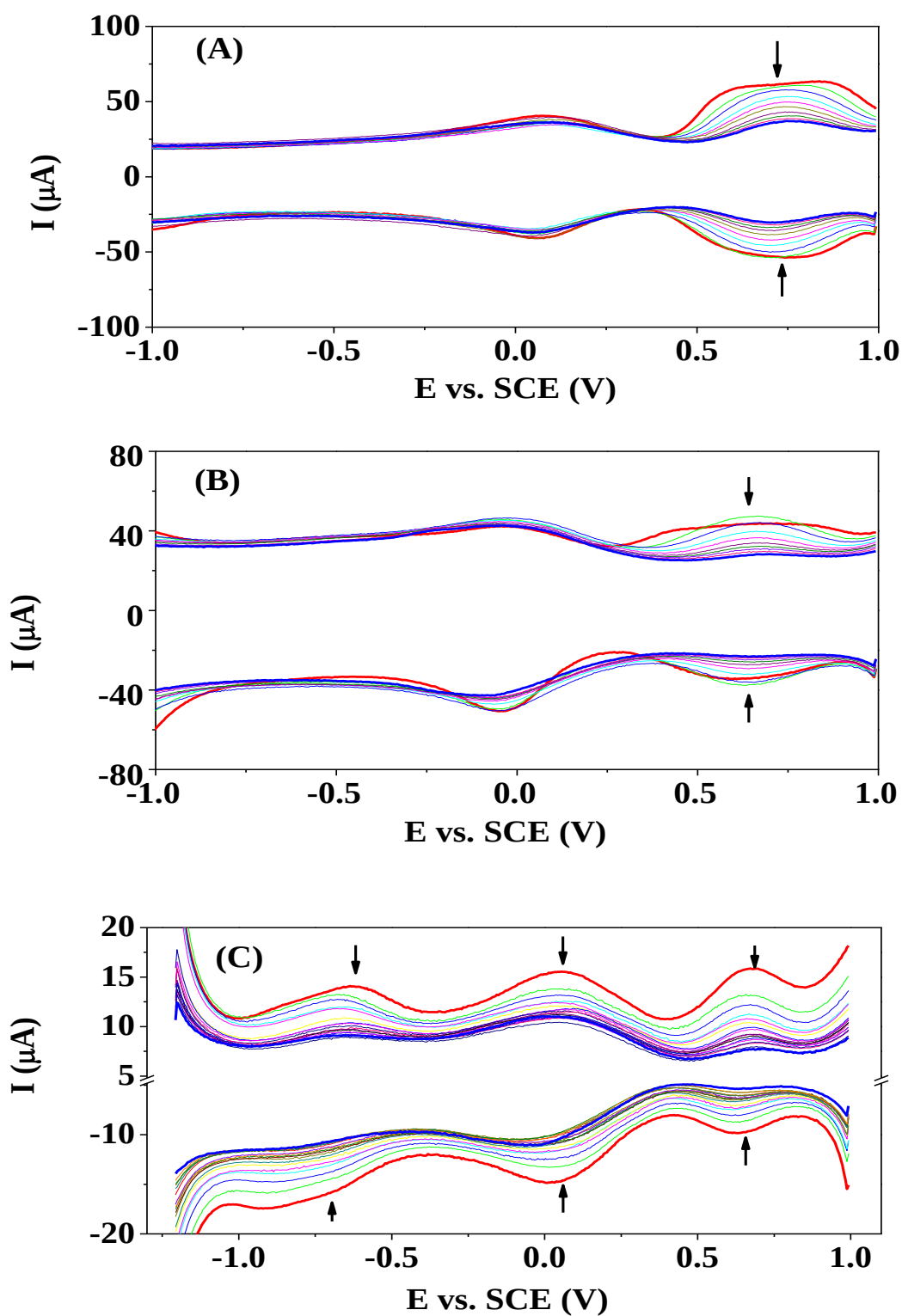


Figure 3.43. SWV responses of (A) GCE/PANI-N₃/TA-H₂Pc, (B) GCE/PANI-N₃/TA-ZnPc and (C) GCE/PANI-N₃/TA-CoPc electrodes in PBS (PH=7.0) during the gradual addition of carbofuran.

In Figure 3.42A and Figure 3.43A the SWV responses of **GCE/PANI-N₃/TA-H₂Pc** against carbaryl and carbofuran are given respectively. However, the peak intensities about 0.65 V decreased for both of the pesticides. Thus, this electrode is not selective for carbaryl and carbofuran. In Figure 3.42B and Figure 3.43B, SWV results of **GCE/PANI-N₃/TA-ZnPc** electrode for carbaryl and carbofuran sensing studies were exhibited. **GCE/PANI-N₃/TA-ZnPc** electrode did not give any response to carbaryl while there are some new peak formations at anodic region for carbofuran. In Figure 3.30C, while Co^{II}Pc/Co^IPc and Co^{II}Pc/Co^{III}Pc couples decrease in current intensity, two small couples are recorded at -0.20 and -0.04 V during gradual addition of carbaryl. These voltammetric responses are completely different than those of eserine and parathion. In Figure 3.43C any new redox process is recorded during carbafuran sensing experiment. All redox processes of **GCE/PANI-N₃/TA-CoPc** electrode decrease in intensity during the gradual addition of carbofuran. Consequently, when we compare these SWV sensing experiments, it can be easily concluded that **GCE/PANI-N₃/TA-H₂Pc** electrode is selective for eserine and parathion, **GCE/PANI-N₃/TA-ZnPc** electrode is selective for eserine, parathion and carbofuran while **GCE/PANI-N₃/TA-CoPc** electrode could be used as a selective EPS for all of these four pesticides.

In literature there is not any selective electrochemical pesticide sensor, which gives different voltammetric, peaks for the sensing of different pesticides. Many selective pesticide sensors were reported in the literature, but those electrodes just gave different intensities for the measured signals. This type of sensors could just sense a known amount sensor selectively. For instance, Pan J. and his coworkers reported a molecular imprint-based fluorescence sensor based on Eu complex modified mesoporous silica particles as selective and sensitive optosensing of λ -cyhalothrin. They only reached this result by comparing fluorescence intensity changes, but this sensing signal also changed with concentration. Similar molecular imprint electrodes were reported as selective sensors, however these sensors sensed only on specific pesticide, which fitted to the imprinted shape of the sensors. Sawant S.N. research group reported a polyaniline-based sensitive electrochemical sensor for selective detection of lindane, a pesticide. But similarly they used only peak current intensity difference for the signal of selectivity. It is well known that a selective electrochemical sensor has to give different redox processes for different targets. Changing of signal intensity can only be used for a

known concentration, since amount of the target species have similar intensity changes. For instance the EPS, introduced here, gave different SWV waves which are specific changes for a specific pesticide, thus can sense these pesticides selectively.

Electrochemical pesticide sensors based on MPc have seldom been reported to have high sensitivity in the 10^{-8} M concentration range. For instance, Harbeck, M. and coworkers reported detection limit of as 1.37×10^{-7} M for sensing carbamate classes of pesticides on the chemically modified ZnPc electrode. Harbeck, M. and co-workers reported similar sensitivity for various pesticides with QCM based sensor based on phthalocyanines. Although detection limits in the range of 1.0×10^{-6} and 1.0×10^{-8} M are generally reported, very sensitive examples are also reported in the literature. For instance, 5.0×10^{-12} M detection limit was reported for the sensing of chlorpyrifos-oxon pesticides on the electrochemical sensor modified with a cobalt (II) phthalocyanine complex. For practical usage GCE/**PANI-N₃/TA-CoPc** electrode should have adequate sensitivity, which can be competed with the proved sensors, in addition to the sufficient stability and reproducibility. Thus to derive these sensor parameters, EIS, and DPSCC measurements were also performed sequentially in addition to the SWV. Basic sensor parameters, calibration lines, and detection limits (LOD) of **GCE/PANI-N₃/TA-H₂Pc**, **GCE/PANI-N₃/TA-ZnPc** and **GCE/PANI-N₃/TA-CoPc** electrodes were derived from SWV, EIS, and DPSCC analyses and the results are tabulated in Table 3.5, Table 3.6 and Table 3.7, respectively.

Table 3.5. Detection limits and linear range data obtained with different techniques for the sensing of the pesticides with **GCE/PANI-N₃/TA-H₂Pc** electrodes.

Sensor	Sensing techniques*		Carbaryl (M)			Carbofuran(M)			Ref.
			Linear Range x10 ⁶	LOD	Sensitivity	Linear Range x10 ⁶	LOD	Sensitivity	
GCE/PANI-N ₃ /TA-H ₂ Pc	SWV	Amp.	0.072-1.744	2.17 10 ⁻⁰⁸	7.69 A/M	0.080-0.934	3.64 10 ⁻⁰⁸	12.08 A/M	tw
		Pot.	0.064-1.521	6.33 10 ⁻⁰⁹	0.02 V/ μM	0.019-0.825	5.32 10 ⁻⁰⁹	0.12 V/ μM	tw
	EIS	Imp.	0.065-1.667	8.20 10 ⁻⁹	1.76 ohm/μM	0.064-1.990	6.16 10 ⁻⁹	5.44 ohm/μM	tw
	DPSCC	Amp.	0.019-0.769	2.47 10 ⁻⁹	19.20 C/M	0.027-0.992	2.50 10 ⁻⁹	8.47 C/M	tw
			Eserine (M)			Parathion (M)			
	SWV	Amp.	0.348-4.378	1.09 10 ⁻⁰⁷	3.63 A/M	0.440-4.543	6.17 10 ⁻⁰⁸	1.09 A/M	tw
		Pot.	0.039-1.901	3.45 10 ⁻⁰⁸	0.02 V/ μM	0.039-0.596	1.011 10 ⁻⁹	0.022 V/ μM	tw
	EIS	Imp.	0.303-1.866	1.16 10 ⁻⁹	2.90 ohm/μM	0.007-1.027	8.92 10 ⁻⁹	1.31 ohm/μM	tw
	DPSCC	Amp.	0.016-1.542	4.41 10 ⁻⁹	4.81 C/M	0.021-0.871	2.56 10 ⁻⁹	8.27 C/M	tw
	AChE/ FePc AChE/ RB-AuNP	CA						1.0 10 ⁻⁰⁸	[58]
		Col.			4.98 10 ⁻⁰⁷				[59]

*Amp.: Amperometric, Pot.: Potentiometric, Imp.: Impedimetric

Table 3.6. Detection limits and linear range data obtained with different techniques for the sensing of the pesticides with GCE/PANI-N₃/TA-ZnPc electrodes.

Sensor	Sensing techniques*		Carbaryl (M)			Carbofuran(M)			Ref.
			Linear Range x10 ⁶	LOD	Sensitivity	Linear Range x10 ⁶	LOD	Sensitivity	
GCE/PANI-N ₃ /TA-ZnPc	SWV	Amp.	0.039-0.942	9.58 10 ⁻⁹	3.50 A/M	0.249-2.301	1.36 10 ⁻⁰⁹	0.42 A/M	tw
		Pot.	0.055-0.871	3.10 10 ⁻⁰⁹	0.01 V/ μM	0.056-2.119	1.20 10 ⁻⁷	0.0030 V/ μM	tw
	EIS	Imp.	0.704-2.124	6.53 10 ⁻⁹	8.60 ohm/μM	0.016-1.946	1.81 10 ⁻⁹	2.63 ohm/μM	tw
	DPSCC	Amp.	0.016-1.002	6.64 10 ⁻⁹	3.74 C/M	0.102-1.465	1.30 10 ⁻⁹	4.16 C/M	tw
			Eserine (M)			Parathion (M)			
	SWV	Amp.	0.027-4.493	1.00 10 ⁻⁸	3.35 A/M	1.051-4.513	8.55 10 ⁻⁹	0.39 A/M	tw
		Pot.	0.374-2.041	1.60 10 ⁻⁰⁸	0.03 V/ μM	0.951-5.194	2.20 10 ⁻⁰⁹	0.020 V/ μM	tw
	EIS	Imp.	0.164-1.632	2.92 10 ⁻⁹	7.27 ohm/μM	0.358-1.667	1.91 10 ⁻⁹	3.41 ohm/μM	tw
	DPSCC	Amp.	0.357-2.135	1.29 10 ⁻⁸	4.77 C/M	0.348-1.665	1.00 10 ⁻⁹	3.25 C/M	tw
ZnPc	CA	Amp.		1.37 10 ⁻⁰⁷					[60]
MWCNT/ PANI/ AChE	CA	Amp.		1.40 10 ⁻⁰⁶					[61]

*Amp.: Amperometric, Pot.: Potentiometric, Imp.: Impedimetric

Table 3.7. Detection limits and linear range data obtained with different techniques for the sensing of the pesticides with **GCE/PANI-N₃/TA-CoPc** electrodes.

Sensor	Sensing technique*		Carbaryl (M)			Carbofuran(M)			Ref.
			Linear Range x10 ⁶	LOD	Sensitivity	Linear Range x10 ⁶	LOD	Sensitivity	
GCE/PANI-N ₃ /TA-CoPc	SWV	Amp.	0.013-0.060	9.73 10 ⁻⁰⁹	32.10 A/M	0.270-5.420	5.90 10 ⁻⁰⁸	0.94 A/M	tw
		Pot.	0.013-0.060	3.84 10 ⁻¹⁰	28.67 V/ μM	0.270-8.920	4.62 10 ⁻⁰⁷	0.010 V/ μM	tw
	EIS	Imp.	0.370-0.860	1.79 10 ⁻⁰⁸	33.8 ohm/μM	1.270-1.480	8.50 10 ⁻⁰⁸	15.3 ohm/μM	tw
	DPSCC	Amp.	0.0013-0.063	4.60 10 ⁻⁰⁹	11.09 C/M	0.068-1.390	2.09 10 ⁻⁰⁷	1.24 C/M	tw
			Eserine (M)			Parathion (M)			
	SWV	Amp.	1.560-7.450	1.75 10 ⁻⁰⁷	1.15 A/M	0.035-1.890	1.00 10 ⁻⁰⁷	5.08 A/M	tw
		Pot.	0.330-9.340	3.33 10 ⁻⁰⁸	0.0087 V/ μM	0.076-1.950	1.33 10 ⁻⁰⁷	0.032 V/ μM	tw
	EIS	Imp.	0.091-3.561	2.26 10 ⁻⁰⁸	14.9 ohm/μM	5.300-12.710	3.21 10 ⁻⁰⁷	0.34 ohm/μM	tw
	DPSCC	Amp.	2.008-12.060	1.75 10 ⁻⁰⁷	16.8 C/M	0.010-0.400	2.62 10 ⁻⁰⁸	21.8 C/M	tw
AChE/CoPc	CA	Amp.	4.5 10 ⁻⁰⁹ M eserine						[62]
Tyr/CoPc	CA	Amp.	10–50 ppb carbaryl	5–90 ppb carbofuran	6–100 ppb parathion				[63]

*Amp.: Amperometric, Pot.: Potentiometric, Imp.: Impedimetric

As shown in Table 3.5, 3.6 and 3.7, LOD values of GCE/**PANI-N₃**/**TA-MPc** electrodes vary with the applied techniques, but all data supply the expected LOD for the practical usage of the electrodes as pesticide sensor. All LOD values are much lower than the maximum residue limits (MRL) as reported in the European Union pesticides database as well as those from the U.S. Department Agriculture (USDA).

Figure 3.44 represents the potentiometric (E vs. [eserine]) and amperometric (I vs. [eserine]) calibration lines derived from the SWV measurements of GCE/**PANI-N₃**/**TA-CoPc** electrode.

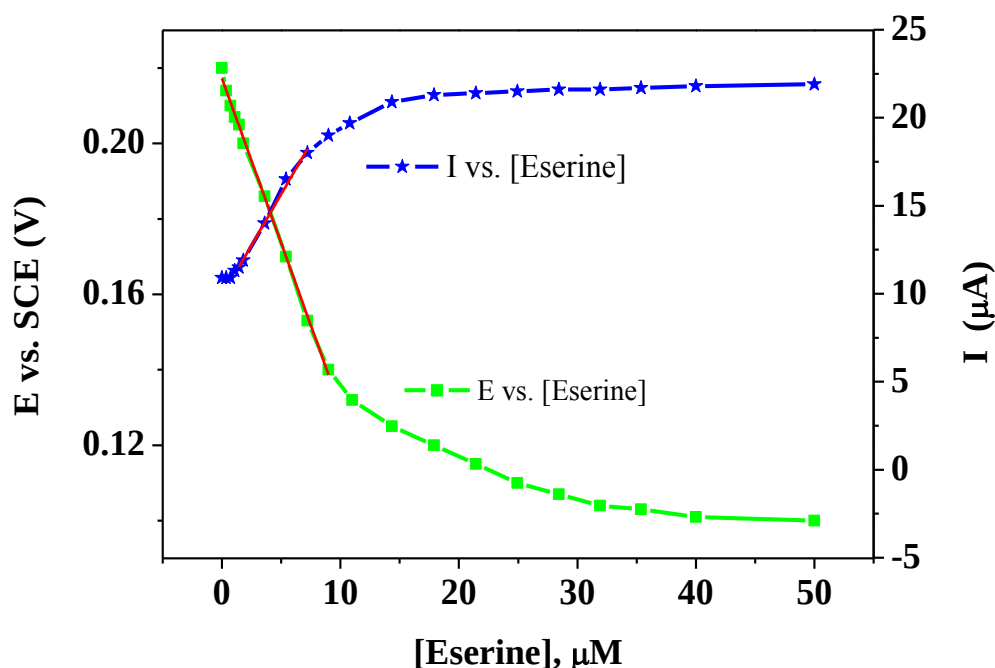


Figure 3.44. Calibration curves of potentiometric and amperometric sensing measurements derived from the SWV titration of **GCE/PANI-N₃/TA-CoPc** with eserine.

Potentiometric sensing analysis gives a linear range between 0.33 and 9.34 $\mu\text{mol dm}^{-3}$ eserine with a sensitivity of 0.0087 V $\text{dm}^3 \mu\text{mol}^{-1}$ and LOD of $3.33 \cdot 10^{-8} \text{ mol dm}^{-3}$. Similarly amperometric sensing analyses gives a linear range between 1.56 and 7.45 $\mu\text{mol dm}^{-3}$ eserine with a sensitivity of 1.15 A/(M) and LOD of $1.75 \cdot 10^{-7} \text{ mol dm}^{-3}$. Potentiometric sensitivity of the electrode is approximately 30 times higher than the amperometric one.

These sensor parameters were also derived from EIS and CC analyses to determine the most sensitive sensing electrochemical technique. GCE/**PANI-N₃**/**TA-CoPc** electrode

gives similar EIS and CC responses for all pesticides (except carbofuran). EIS responses of the electrode recorded during eserine sensing experiments were given as a representative of the pesticides in Figure 3.45. EIS results indicated that radiuses of the semicircles in EIS graphs increase gradually with respect to increasing pesticide concentrations. These EIS responses indicate interaction of the electrode with eserine and this interaction affects the conductivity of the modified electrode. As a result of the analysis of this graph calibration line of the eserine sensing derived from EIS analysis is given as inset graph in Figure 3.33 and LOD was found as 2.26×10^{-8} M.

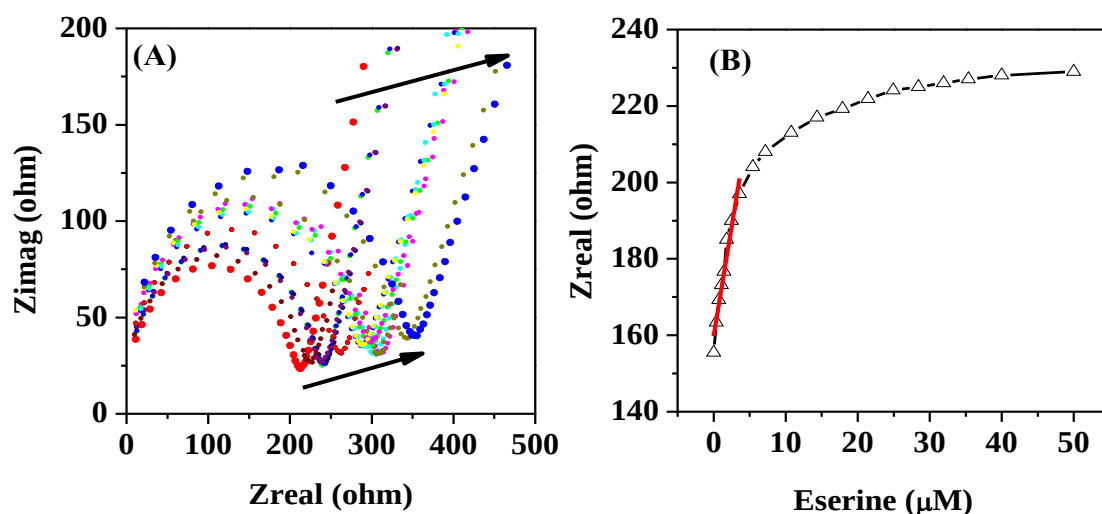


Figure 3.45. (A) EIS responses and (B) Impedimetric calibration line of GCE/PANI-N₃/TA-CoPc electrode in PBS (PH=7.0) during the gradual addition of eserine.

DPSCC analysis of the electrode was also performed and the results of DPSCC were compares with EIS and SWV analyses with respect to linear range and LOD values. As shown in Figure 3.46, current of the electrode recorded with DPSCC also increases as a function of eserine concentration. DPSCC analysis of the electrode gives a linear range between 2 and 12 μM eserine and 1.75×10^{-7} M LOD. All sensor parameters of the electrode were tabulated in Table 3.7.

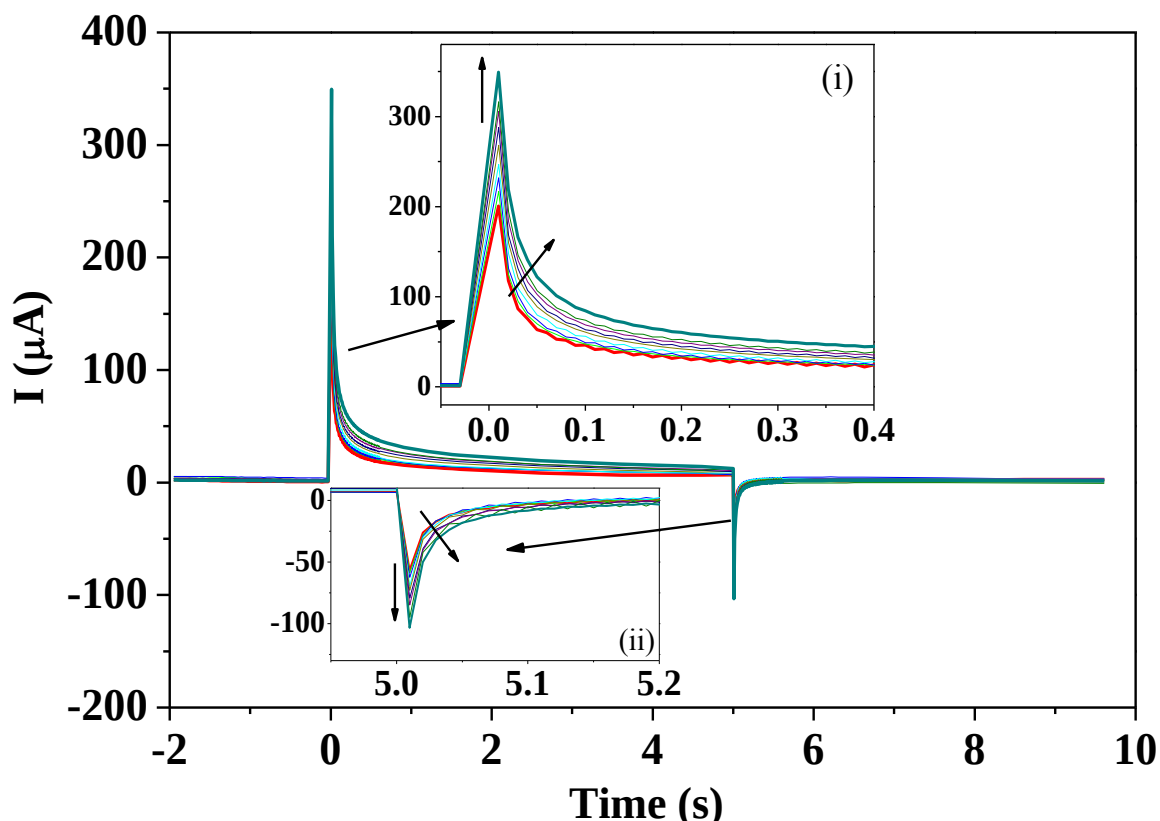


Figure 3.46. DPSCC responses of **GCE/PANI-N₃/TA-CoPc** electrodes in PBS (PH=7.0) during the gradual addition of with eserine.

The reproducibility and stability of the **GCE/PANI-N₃/TA-CoPc** electrode was tested with comparing the SWV responses of constructed 10 electrodes. Potential and current differences of redox processes were compared. The highest relative standard deviations were obtained as 2.2 and 1.6% for potential and current reproducibility's respectively. The stability of the **GCE/PANI-N₃/TA-CoPc** electrode was tested with comparing the SWV responses of 10 electrodes at different times. Stability of **GCE/PANI-N₃/TA-CoPc** electrode decreases just 5% after 60 days. To check reusability of the electrode, a used electrode was washed with pure water efficiently and used again and current responses before and after the washing were compared. Only 2.5 % current decrease was recorded after the first usage and 17% current decrease was achieved after five usages.

3.5. ITO/TA-CoPc/PANI-N₃ Sensor Measurements Prepared with LB-Click Electrochemistry Technique

Figure 3.47 exhibits the SWV responses of ITO/TA-CoPc/PANI-N₃ electrode during eserine into the phosphate buffer electrolyte. In the basic situation, ITO/TA-CoPc/PANI-N₃ electrode gives Co^{II}Pc/Co^IPc couple at -0.63 V, Red/Oxd couple of PANI-N₃ at 0.17 V, and Co^{II}Pc/Co^{III}Pc at 0.78 V. During titration with eserine, while the Co^{II}Pc/Co^IPc and Co^{II}Pc/Co^{III}Pc redox processes decrease in current intensity gradually and completely disappear at the end of the titration, redox waves of PANI-N₃ shifts through the negative potentials with increasing current intensities gradually because of the interaction of eserine with the working electrode. Increases or decreases of the current intensity lead the electrode to be used as an amperometric sensor while peak potential shifts lead the electrode to be used as a potentiometric sensor. Thus, ITO/TA-CoPc/PANI-N₃ electrode can be used for pesticide detection as an amperometric or potentiometric sensor according to the SWV measurements.

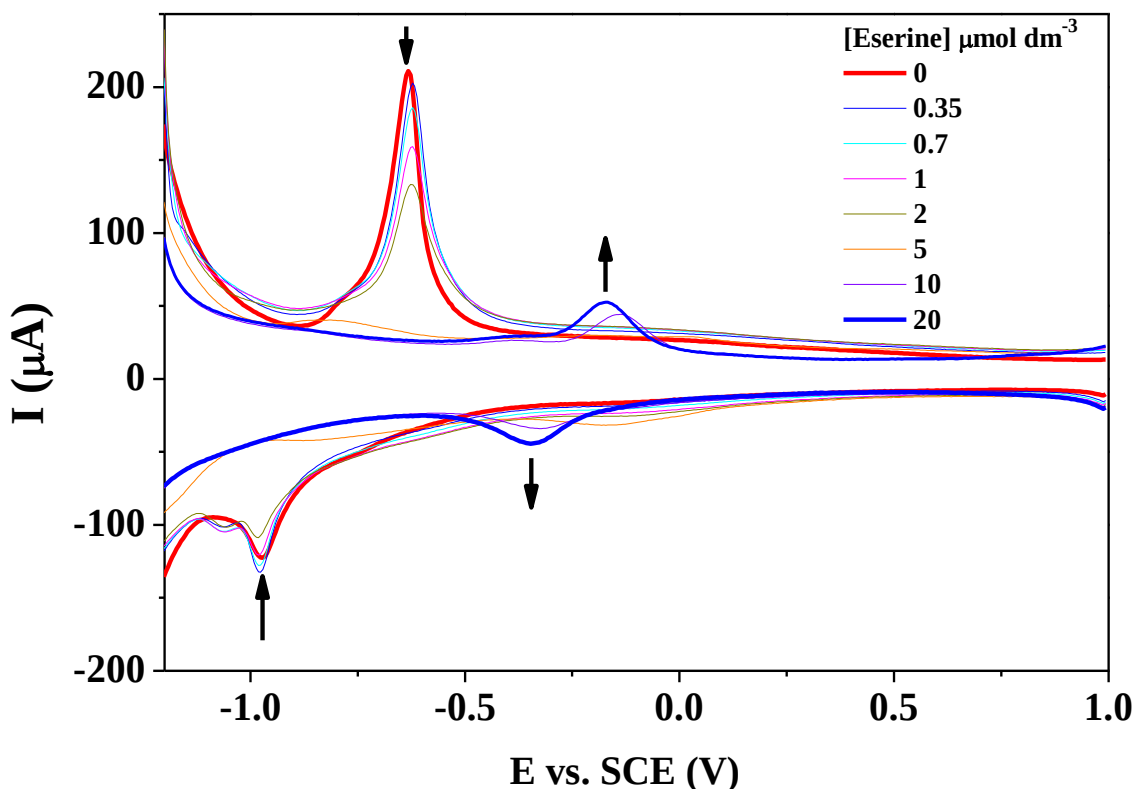


Figure 3.47. SWV responses of ITO/TA-CoPc/PANI-N₃ electrodes in PBS (PH=7.0) during the gradual addition of eserine.

Also EIS and DPSCC analyses were applied to determine the most sensitive sensing electrochemical technique and lowest LOD. EIS responses of the ITO/TA-CoPc/PANI-N₃ electrode recorded during eserine sensing experiments were given in Figure 3.48. EIS results indicated that the semicircles in EIS graphs increase gradually with respect to increasing eserine concentration. The interaction of the electrode with eserine results in the decreasing conductivity of the modified electrode. The calibration line of the eserine sensing derived from EIS analysis is given as inset graph in Figure 3.48.

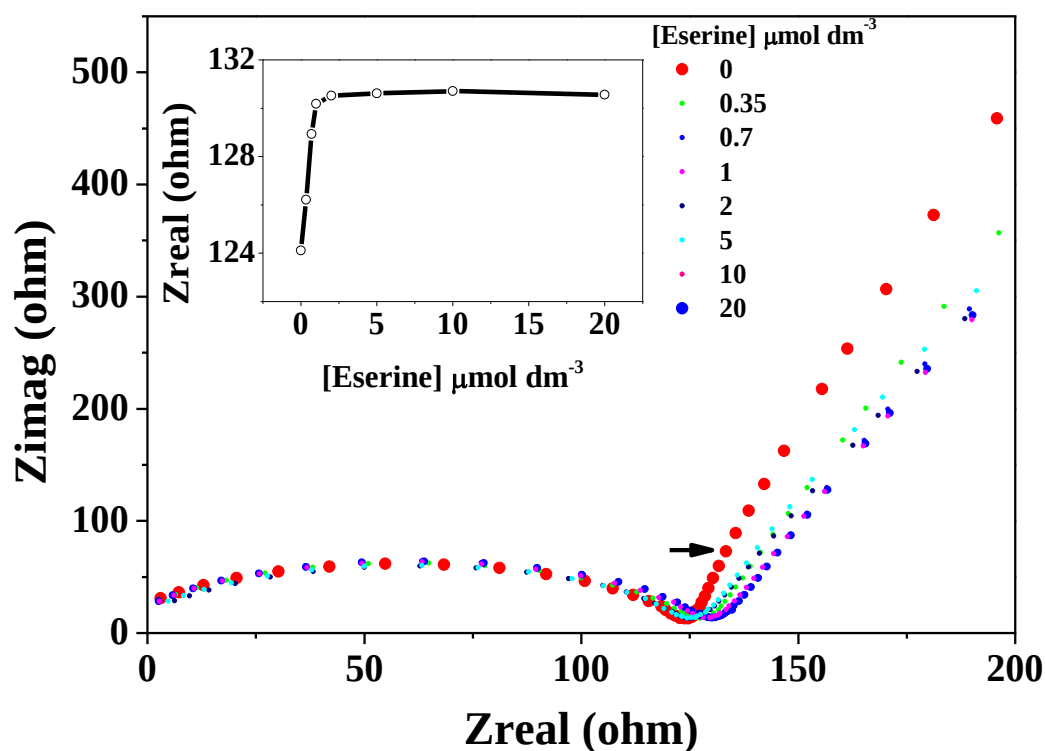


Figure 3.48. EIS responses of ITO/TA-CoPc/PANI-N₃ electrodes in PBS (PH=7.0) during the gradual addition of eserine.

In Figure 3.49 DPSCC responses of ITO/TA-CoPc/PANI-N₃ electrodes in PBS (PH=7.0) during the gradual addition of with eserine.

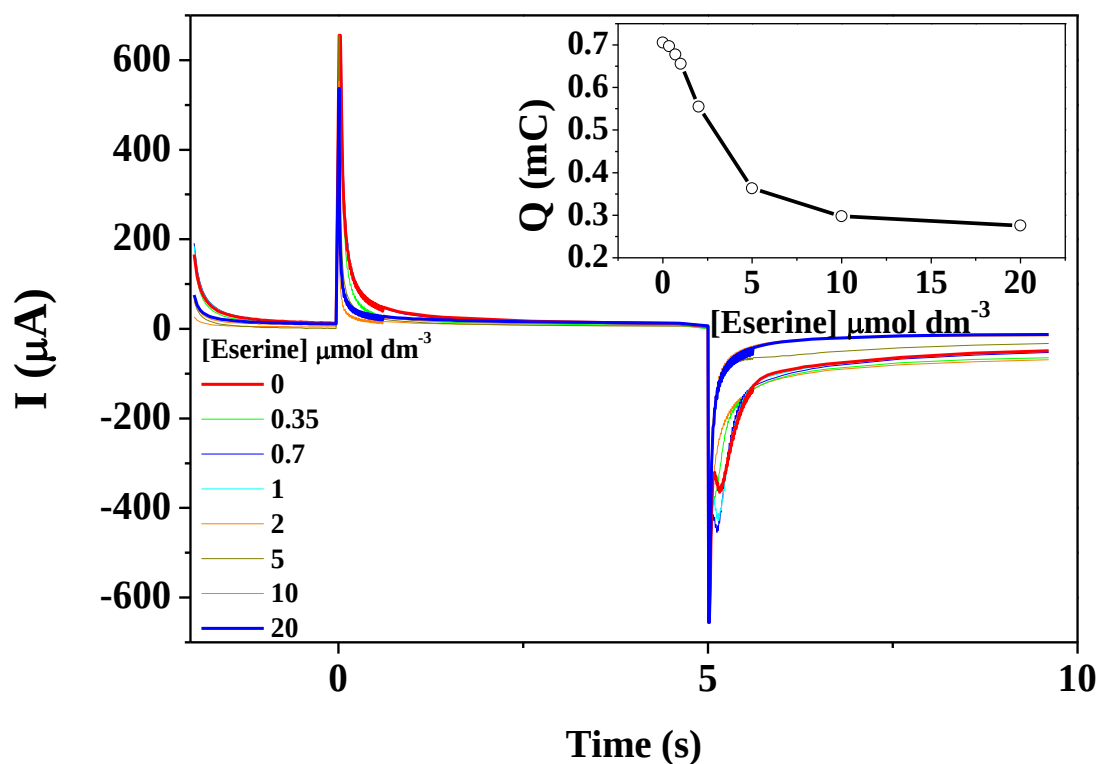


Figure 3.49. DPSCC responses of ITO/TA-CoPc/PANI-N₃ electrodes in PBS (PH=7.0) during the gradual addition of eserine

The current and thereby the charge of the electrode recorded with DPSCC also decreased with addition of eserine. DPSCC analysis of the electrode gives a linear range between 0.213 and 4.691 μM eserine with a sensitivity of 78.23 C/M and 1.300×10^{-9} M LOD. Sensor parameters of the electrode are shown in Table 3.8.

Table 3.8. Detection limit and linear range data obtained with different techniques for the sensing of eserine with **ITO/TA-CoPc/PANI-N₃** electrode.

Electrode	Sensing technique*		Linear range (μM)	LOD (M)	Sensitivity
ITO/TA-CoPc/PANI-N ₃	SWV	Amp.	0.133-2.788	2.14×10^{-9}	48.89 A/M
		Pot.	0.027-0.360	1.12×10^{-8}	0.032 V/ μM
	EIS	Imp.	0.058-1.042	8.91×10^{-9}	6.47 ohm/ μM
	DPSCC	Amp.	0.213-4.691	1.30×10^{-9}	78.23 C/M

*Amp.: Amperometric, Pot.: Potentiometric, Imp.: Impedimetric

4. CONCLUSIONS

Organophosphorous and carbamate pesticides are used in modern agricultural activities very often because of their high insecticidal activity and relatively low persistence. The increasing use of the pesticide in agriculture makes them the most important environmental pollutants. To prevent environmental pollution a continuous observation is needed. There are some conventional approaches to detect pesticides such as gas chromatography (GC), high-performance liquid chromatography (HPLC) and mass spectrometry (MS) that are traditional methods for detection of pesticides. These time-consuming techniques are also require expensive equipment and highly trained technicians. Developing new alternative detection methods is a crucial point to protect the ecosystem and human health. Electrochemical sensors or biosensors are promising applications. However, low stability of the biological species, difficulties of immobilization on the substrates, leakage from the substrate surface, high cost, and low chemical and thermal stabilities form the main disadvantages of pesticide biosensors. To overcome these disadvantages of biosensors, phthalocyanines can be used as biomimetic recognition elements. Because of the 18π electron conjugated ring system in the structure of the phthalocyanine molecule, they have efficient electron transfer abilities and utilized in a wide application areas including biomimic electrochemical sensors. Besides excellent redox behavior, thermal and chemical stability, ability of easy modification with different metal ions and substituents, and ability of easy coating on substrate with various techniques makes them significant for electrochemical sensor applications. The modification technique and designin of the phthalocyanine structure determine the sensor properties such as sensitivity, selectivity, linear range, response time, limit of detection and stability.

In this study, pesticide electrochemical sensors were designed with some novel phthalocyanines synthesized for the first time and five different immobilization techniques according to biomaterial that will be coated on the electrode surface. These immobilization techniques are listed below:

1. Carbon Paste Electrode Preparation with Moulding Technique
2. Thin Film Formation with Langmuir-Blodget Coating Technique
3. Electropolymerization Technique

4. Click Electrochemistry Technique

5. LB-Click Electrochemistry Technique

Electropolymerization technique is used very often in the literature because of being a simple and low cost method. However, carbon paste electrode preparation with a designed mould, click electrochemistry and LB-Click Electrochemistry immobilization techniques were applied in this study for the first time.

The electrochemical measurements of the new designed electrochemical sensor were carried out with a Gamry Reference 600 potentiostat/galvanostat, at 25 °C. A Pt wire served as the counter electrode and saturated calomel electrode (SCE) was employed as the reference electrode and separated from bulk of the solution by a double bridge. Sensor parameters of the sensor electrodes were determined with SWV, EIS and DPSCC methods. The detection linear range and detection limit parameters of the electrodes were calculated from the analysis of all SWV, DPSCC, and EIS results and compared with each other.

In summary, **LOD** values of the almost all sensor electrodes, most of them are smaller than the nanomolar range convey requirements of sensing applications. The lowest LOD value was obtained with **GCE/PANI-N₃/TA-CoPc** electrode as 3.84×10^{-10} M. **Sensitivity** is also an important criteria for an electrochemical sensor and it is proportional to the detection limit. Thus, **GCE/PANI-N₃/TA-CoPc** electrode is the most sensitive electrode against eserine with a sensitivity value of 28.67 V/ μ M. Lower limit of detection (LOD) means higher sensitivity. According to **selectivity** parameter of the electrochemical sensors, GCE/PANI-N₃/TA-CoPc electrode gave different responses to four different pesticides that it is the most selective electrode in these designed electrodes. When the **stability** of the electrodes were compared, GCE/PANI-N₃/TA-MPc electrodes prepared with CEC technique are more stable or in other words they have longer shelf life than the other electrodes because of chemically modification of the electrode. Polymerization technique, LB coating technique and moulding techniques are physically modification techniques and can be easily removed from the electrode surface or broke down (with moulding technique). Even 6 months later, the modified electrode with CEC technique stored at room conditions gave valuable responses to the pesticides. The **response times** of the designed electrodes were all

satisfactoring that they recognized the analytes in a few seconds. When these sensor parameters are taken into account, the electrochemical sensor electrodes prepared with CEC technique are more succesfull because of being selective, stable and having a long shelf life, having a low response time and low LOD values. However, LOD parameter of the GCE/PANI-N₃/TA-MPc electrodes can be improved with using phthalocyanines with different chemical structures and different central metal atoms. Also for immobilization of phthalocyanine different binders instead of PANI-N₃ can be tried.

REFERENCES

- [1] Lee, K., Ga, J., E.K., (2012), Multiresidue pesticide analysis in Korean ginseng by gas chromatography-triple quadrupole tandem mass spectrometry. *Food Chem.*134:2497-503.
- [2] Gaffney, S.H., Curriero, F.C., Strickland, P.T., Glass, G.E., Helzlsouer, K.J. and Breysse, P.N., (2005), Influence of geographic location in modeling blood pesticide levels in a community surrounding a US Environmental Protection Agency Superfund site. *Environ Health Persp.*113 (12):1712-6.
- [3] Fatoki, O.S.A., (2003), Methods for selective determination of persistent organochlorine pesticide residues in water and sediments by capillary gas chromatography and electron-capture detection. *J Chromatogr A* 983(1-2):225-36.
- [4] Xu, M.L., Liu, J.B. and Lu, J., (2014), Determination and Control of Pesticide Residues in Beverages: A Review of Extraction Techniques, Chromatography, and Rapid Detection Methods *Applied Spectroscopy Reviews*.49(2):97-120.
- [5] Eggins, B.R., (2002), *Chemical Sensors and Biosensors* UK
- [6] Gupta, R.C., (2009), *Handbook of Toxicology of Chemical Warfare Agent*. USA: Academic Press.
- [7] Sassolas, A. P-SB, Marty J.-L.,. (2012), Biosensors for Pesticide Detection: New Trends. *American Journal of Analytical Chemistry*.3:210-32.
- [8] Bhadekar, R.P.S., Nirichan, B., (2011), Developments in Analytical Methods for Detection of Pesticides in Environmental Samples. *American Journal of Analytical Chemistry*.2:1-15.
- [9] Council, N.R., (1995), *Expanding the Vision of Sensor Materials*. Washington DC: The National Academies Press.
- [10] Monošíka, R.S.M., and Šturdíka, E., (2012), Biosensors — classification, characterization and new trends. *Acta Chimica Slovaca*.5:109-20.
- [11] Mostafa, G.A.E., (2010), Electrochemical Biosensors for the Detection of Pesticides. *The Open Electrochemistry Journal*.2.
- [12] Monošík, R.S.M., Šturdík, E., (2012), Biosensors — classification, characterization and new trends. *Acta Chimica Slovaca*.5 (1):109-20.
- [13] Koyun, A., Ahlatcıoğlu, E., and Koca İpek, Y., (2012), *A Roadmap of Biomedical Engineers and Milestones*. Croatia.
- [14] Malhotra, B.D., Turner, A.P.F., (2003), *Advances in Biosensors*. USA: JAI Press.
- [15] Malhotra, B.D., Turner, A.P.F., (2003), *Advances in Biosensors-Perspectives in Biosensors*: Elsevier Science B. V.

- [16] E. MGA., (2010), Electrochemical Biosensors for the Detection of Pesticides. *The Open Electrochemistry Journal*.2:22-42.
- [17] <http://www.gamry.com/application-notes/two-three-and-four-electrode-experiments/>.
- [18] Zhang, S.G.W., Yang, Y., (2000), Materials and techniques for electrochemical biosensor design and Construction. *Biosens Bioelectron*.15:273–82.
- [19] Khalid, M.A.A., (2013), Electrochemistry. Croatia: In Tech.
- [20] Eggins, B.R., (2002), Chemical sensors and biosensors. England: John Wiley & Sons Ltd.
- [21] Sakamoto, K., and Ohno-Okumura, E., (2009), Syntheses and Functional Properties of Phthalocyanines. *Key Eng Mater*.2:1127-79.
- [22] Claessens, C. G.H.U., Torres, T., (2008), Phthalocyanines: From Outstanding Electronic Properties to Emerging Applications. *The Chemical Record*. Japan: The Japan Chemical Journal Forum and Wiley Periodicals, Inc.; p. 75-97.
- [23] Agar, E., Sasmaz, S., Agar, A., (1999), Synthesis and Properties of the Phthalocyanines Containing Eugenol (4-Allyl-2-Methoxyphenol). *Turk J Chem*.23:131- 7.
- [24] Bhattachayay, D.P.P., Banerjee, S., Sanyal, S. K., Turner, A. P. F., Sarkar, P., (2008), Electrochemical acetylcholine chloride biosensor using an acetylcholine esterase biomimic. *Anal Lett* 41 (8):1387-97.
- [25] Lin, T.T., Huang, X., Liu, J. Q., (2011), Artificial Enzyme Construction with Temperature Sensitivity. *Comm Com Inf Sc*.214:257-62.
- [26] Vial, L.D.P., (2009), Artificial enzyme-based biosensors. *New J Chem* 33:939-46.
- [27] Wujcik, E.K, Londono, N.J., Duirk, S.E., Monty, C.N. and Masel, R.I., (2013), An acetylcholinesterase-inspired biomimetic toxicity sensor. *Chemosphere*.91(8):1176-82.
- [28] Erbahar, D.D., Gurol, I., Gumus, G., Musluoglu, E., Ozturk, Z.Z., Ahsen, V. and Harbeck, M., (2012), Pesticide sensing in water with phthalocyanine based QCM sensors. *Sensor Actuat B-Chem*.173:562-8.
- [29] Wang, P.H., Yu, J.H., Li, Z.J., Ding, Z.J., Guo, L. and Du, B., (2013), Synthesis and evaluation of a new phthalocyanine as sensor material for sarin detection. *Sensor Actuat B-Chem*.188:1306-11.
- [30] Ipek, Y., Dincer, H. and Koca, A., (2014), Electrode modification based on "click electrochemistry" between terminal-alkynyl substituted cobalt phthalocyanine and 4-azidoaniline. *Sensor Actuat B-Chem*.193:830-7.
- [31] Boni, A.C.W.A., Dutra, R. A. F., Sotomayor, M. D. T., (2011), Cobalt phthalocyanine as a biomimetic catalyst in the amperometric quantification of dipyrone using FIA. *Talanta*.85(4):2067-73.

- [32] Rahim, A.B., Kubota, L. T.; Gushikem, Y., (2011), SiO₂/C/Cu(II)phthalocyanine as a biomimetic catalyst for dopamine monooxygenase in the development of an amperometric sensor. *Electrochimica Acta*.56 (27):10116-21.
- [33] Shahrokhian, S.A., Mohammadpoor-Baltork, I., Tangestaninejad, S., (2000), Potentiometric detection of 2-mercaptobenzimidazole and 2-mercaptobenzothiazole at cobalt phthalocyanine modified carbon-paste electrode. *Electroanal.*12 (11):Potentiometric detection of 2-mercaptobenzimidazole and -mercaptobenzothiazole at cobalt phthalocyanine modified carbon-paste electrode.
- [34] Wong, A.S., (2013), Biomimetic sensor based on 5,10,15,20-tetrakis(pentafluorophenyl)-21H,23H-porphyrin iron (III) chloride and MWCNT for selective detection of 2,4-D. *Sensor Actuat B-Chem.*332-339:332-9.
- [35] Boni, A.C., Wong, A., Dutra, R.A.F. and Sotomayor, M.D.T., (2011), Cobalt phthalocyanine as a biomimetic catalyst in the amperometric quantification of dipyrone using FIA. *Talanta*.85(4):2067-73.
- [36] Vilakazi, S., Nyokong, T., Fukuda, T. and Kobayashi, N., (2012), Electrocatalytic behavior of cobalt phthalocyanine complexes immobilized on glassy carbon electrode towards the reduction of dicotophos pesticide. *J Porphyr Phthalocya.*16(7-8):939-45.
- [37] Batista, I.V., Lanz, M.R.V., Dias, I.L.T., Tanaka, S.M.C.N., Tanaka, A.A. and Sotomayor, M.D.P.T., (2008), Electrochemical sensor highly selective for estradiol valerate determination based on a modified carbon paste with iron tetrapyrrolineporphyrine. *Analyst*.133(12):1692-9.
- [38] Harvey, D., (2002), *Analytical Chemistry 2.0 - Electrochemical Methods*
- [39] Benschoten, J.J., Lewis, J., Heineman, W. R., Roston, D. A., and Kissinger, P. T., (1983), *Chem. Educ. American Chemical Society*.60:772-6
- [40] http://www.basinc.com/mans/EC_epsilon/Techniques/ChronoI/ca.html. 2014.
- [41] Instruments G. *Electrochemistry Software Getting started Guide & Primers and Tutorials*. 2014.
- [42] Kissinger, P.T., Heineman, W.R., (1996), *Laboratory Techniques in Electroanalytical Chemistry*. New York: Marcel Deccer Inc.
- [43] KOCA, A., B. S., Şener, M. K., .A. E. U., (2010), Synthesis, electrochemistry, spectroelectrochemistry and electrocolorimetry of phthalocyanine–anthraquinone hybrids. *Synthetic Met.*160:21-55.
- [44] Arican, D., Aktaş, A., Kantekin, H., and Koca, A., (2014), Electrochromism of Electropolymerized Phthalocyanine-Tetrahydroquinoline Dyads. *J Electrochem Soc.*161 (10):670-6.
- [45] Avti, P.K., Maysinger, D., and Kakkar, A., (2013), Alkyne-Azide “Click” Chemistry in Designing Nanocarriers for Applications in Biology. *Molecules*.18:9531-49.

- [46] Dincer, H., Mert, H., Sen, B.N., Dag, A. and Bayraktar, S., (2013), Synthesis and characterization of novel tetra terminal alkynyl-substituted phthalocyanines and their starpolymers via click reaction. *Dyes Pigments*.98:246-54.
- [47] Erbahar, D.D., Harbeck, M., Gurol, I., Gumus, G., Musluoglu, E., Ozturk, Z. Z. and Ahsen, V., (2013), *J Porphyr Phthalocya*.17:989-95.
- [48] Ahn, H.J., Kim, K.R., (2014), 2D Hall Sensor Array for Measuring the Position of a Magnet Matrix. *Int J Pr Eng Man-Gt*.1(2):125-9.
- [49] Valdes-Ramirez, G., Cortina, M., Ramirez-Silva, M. T. and Marty, J.L., (2008), *Anal Bioanal Chem*.392:699-707.
- [50] Zamfir, L.G., Rotariu, L. and Bala, C., (2013), *Biosens Bioelectronics* 46:61-7.
- [51] Snejdarkova, M., Svobodova, L., Nikolelis, D. P., Wang, J. and Hianik, T., (2003), *Electroanal*.15:1185-91.
- [52] Wang, M.Y., Wang, M., Zhang, D.E., Chen, J., (2014), Electrochemical nonenzymatic sensor based on CoO decorated reduced graphene oxide for the simultaneous determination of carbofuran and carbaryl in fruits and vegetables.151:191-7.
- [53] Yin, H., Xu, J., Shi, W., Zhu, L., (2009), Amperometric biosensor based on immobilized acetylcholinesterase on gold nanoparticles and silk fibroin modified platinum electrode for detection of methyl paraoxon, carbofuran and phoxim. *Journal of Electroanalytical Chemistry*.637:21-7.
- [54] Sun, X., Wang, X., (2012), Amperometric immunosensor based on deposited gold nanocrystals/4, 40-thiobisbenzenethiol for determination of carbofuran. *Food Control*.28:184-91.
- [55] Zamfir, L.-G., Bala, C., (2013), Acetylcholinesterase biosensor for carbamate drugs based on tetrathiafulvalene–tetracyanoquinodimethane/ionic liquid conductive gels. *Biosensors and Bioelectronics*.46:61-7.
- [56] Snejdarkova, M., Evtugyn, G., Budnikov, H., Karyakin, A., Hianik, T., (2004), Acetylcholinesterase sensors based on gold electrodes modified with dendrimer and polyaniline A comparative research. *Analytica Chimica Acta*.514:79-88.
- [57] Snejdarkova, M.L.S., Nikolelis, D.P. Wang, J., Hiani, T., (2003), Acetylcholine biosensor based on dendrimer layers for pesticides detection. *Electroanalytical Chemistry*.15:1185-91.
- [58] Ciucu, A.A, Negulescu, C., and Baldwin, R. P., (2003), *Biosens Bioelectronics*.18:303.
- [59] Liu, D.B., Chen, W. W., Wei, J. H., Li, X. B., Wang, Z. and Jiang, X. Y., (2012), *Anal Chem*.84:4185.

- [60] Erbahar, D.D., Harbeck, M., Gurol, I., Gumus, G., Musluoglu, E., Ozturk, Z.Z, et al., (2013), Zinc phthalocyanines with fluorinated substituents for direct sensing of carbamate and organophosphate pesticides in water. *J Porphyr Phthalocya.*17(10):989-95.
- [61] Cesarino, I., Moraes, F.C., Lanza, M.R.V., Machado, S.A.S., (2012), Electrochemical detection of carbamate pesticides in fruit and vegetables with a biosensor based on acetylcholinesterase immobilised on a composite of polyaniline-carbon nanotubes. *Food Chem.*135(3):873-9.
- [62] Valdes-Ramirez, G., Cortina, M., Ramirez-Silva, M.T., Marty, J.L., (2008), Acetylcholinesterase-based biosensors for quantification of carbofuran, carbaryl, methylparaoxon, and dichlorvos in 5% acetonitrile. *Anal Bioanal Chem.*392(4):699-707.
- [63] Tanimoto de Albuquerque, Y.D., Ferreira, L.F., (2007), Amperometric biosensing of carbamate and organophosphate pesticides utilizing screen-printed tyrosinase-modified electrodes. *Anal Chim Acta.*596(2):210-21.

AUTOBIOGRAPHY

Name Surname : Yeliz İPEK

Birth Place and Date : Alucra - 13.02.1984

Foreign Language : English

E-mail : kocayeliz@gmail.com, yeliz.ipek@marmara.edu.tr

Educational Background :

Degree	Department/Program	University/High School	Graduation year
High School	Science-Maths	Maltepe Kadir Has Anatolian High School	2002
BSc	Chemical Engineering	Yıldız Technical University	2006
MSc	Chemical Engineering	Gebze High Technology Institute	2008

Articles:

1. Koca A., İpek Y., Şener M. K., (2014). “Electrochemical Pesticide Sensor Based on Langmuir-Blodgett Film of Cobalt Phthalocyanine-Anthraquinone Hybrid”. *Journal of Porphyrines and Phthalocyanines*, Article in Press.
2. İpek, Y., Dincer, H. & Koca, A., (2014). Selective Electrochemical Pesticide Sensor Modified with "Click Electrochemistry" between Cobaltphthalocyanine and 4-Azidoaniline. *Journal of Electrochemical Society*, 161, 183-190.
3. İpek, Y., Dinçer, H. & Koca, A., (2014). Electrode modification based on “click electrochemistry” between terminal-alkynyl substituted cobalt phthalocyanine and 4-azidoaniline. *Sensor and Actuators B*, 193, 830–837.
4. Özçeşmeci, İ., Burat, A. K., İpek, Y., Koca, A. & Bayır, Z. A., (2013). Synthesis, electrochemical and spectroelectrochemical properties of

- phthalocyanines having extended π -electrons conjugation. *Electrochimica Acta.*, 89 (1), 270-277.
5. Yanık B., Agustos H., Ipek Y., Koyun A., Uzunsoy D., (2013). Synthesis and Characterization of Aluminium Nanoparticles by Electric Arc Technique. *Arab J Sci Eng*, 38:3587–3592.
 6. İpek, Y., Mergen, A. & Öksüz, H. B. M., (2012). Production of Nano Zinc Borate ($4\text{ZNO} \cdot \text{B}_2\text{O}_3 \cdot \text{H}_2\text{O}$) and Its Effect on PVC. *Journal of the European Ceramic Society.*, 32, 2001-2005.
 7. Koyun, A., Ahlatcioğlu, E., Erdoğan, D. C., İpek, Y., (2012). The DMA, TG/DTA and FT-IR Characteristics of Wood Polymer Composites. *Synthesis and Characterisation of Aluminium Nanoparticles by Electric Arc Technique*, 445, 219-224.
 8. İpek, Y. & Yoruç, A. B. H., (2012). Sonochemical Synthesis of Hydroxyapatite Nanoparticles with Different Precursor Reagents. *Acta Physica Polonica A.*, 121., 230-232.
 9. İpek, Y., Yoruç, A. B. H. & Elkoca, A. K. O., (2012). Zirconia-Hydroxyapatite Composite Coating on CP-Ti Implants by Biomimetic Method. *Advanced Materials Research*, 445, 685-690.
 10. İpek, Y., Yüksek, K. & Sadikoğlu, H., (2009). Solution of counter diffusion problem with position dependent diffusion coefficient by using variational methods. *Journal of Computational and Applied Mathematics*, 232, 285-294.
 11. İpek, Y. & Yoruç, A. B. H., (2009). Double Step Stirring: A Novel Method for Precipitation of Nano-Sized Hydroxyapatite Powder. *Digest Journal of Nanomaterials and Biostructures*, 4, 73-81.

Book Section

- Koyun, A., Ahlatcioğlu & İpek, Y. (June, 2012). Biosensors and Their Principles. *A Roadmap of Biomedical Engineers and Milestones*, InTech (115-142). Croatia.

Congress/Symposium/Conference

1. İpek Y., Koca A, Şener K., (2013). Anthraquinon Substituted Cobalt Phthalocyanine Pesticide Biosensor, 44th World Chemistry Congress, İstanbul, Türkiye.
2. İpek Y., Koca A, Şener K., (2013). Enzymless Pesticide Biosensor, 44th World Chemistry Congress, İstanbul, Türkiye
3. İpek, Y. & Koca, A., (2012). Karbaril Biyosensöründe Gluteraldehitin Elektrokimyasal Davranışlarının İncelenmesi 26. Ulsal Kimya Kongresi. Muğla, Türkiye
4. Yanık, B., Agustos, H., Koyun, A. İpek, Y., & Uzunsoy, D., (2011). Synthesis and Characterisation of Aluminium Nanoparticles by Electric Arc Technique, 14th International Conference on Advances in Materials & Processing Technology. Istanbul, Turkey
5. Koyun, A., Ahlatcioğlu, E. & Erdoğan, D. C. İpek, Y., (2011). The DMA, TG/DTA and FT-IR Characteristics of Wood Polymer Composites, 14th International Conference on Advances in Materials&Processing Technologies-AMPT'11. Istanbul, Turkey
6. Yoruç, A. B. H., Karakaş, A. İpek, Y., & Elkoca, O., (2011). Zirconia-Hydroxyapatite Composite Coating on CP-Ti Implants by Biomimetic Method, 14th International Conference on Advances in Materials&Processing Technologies-AMPT'11. Istanbul, Turkey
7. İpek, Y. & Mergen, A., (2011). Effect of Surfactant Materials on Flame Reterdant Zinc Borate Powder Particle Size, International Symposium on Boron Borides and Related Materials. Istanbul, Turkey
8. İpek, Y. & Mergen, A., (2011). Flame Reterdancy Properties of Composites With Different Combinations of Nano Zinc Borate-PVC, International Symposium on Boron Borides and Related Materials. Istanbul, Turkey
9. İpek, Y., Mergen, A. & Bölek, H., (2011). Production of Nano ZInc Borate ($4\text{ZnO} \cdot \text{B}_2\text{O}_3 \cdot \text{H}_2\text{O}$) and its Effect On Pvc, Engineering Ceramics 2011. Bratislava, Slovakia

10. Yoruç, A. B. H. & İpek, Y., (2011). Sonochemical Synthesis of Hydroxyapatite Nanoparticles with Different Precursor Reagents, 1st. International Advances in Applied Physics and Materials Science (APMAS 2011). Antalya, Turkey
11. Çeken, B., Koca, A. & İpek, Y., (2011). Biyosensör Elektrotlarının Modifikasyonu ve Karakterizasyonu, 25. Ulsal Kimya Kongresi. Erzurum, Türkiye
12. İpek, Y. & Koca, A., (2011). Pestisit Tayininde Biyosensör Elektrotlarının Modifikasyonu, 25. Ulsal Kimya Kongresi. Erzurum, Türkiye
13. İpek, Y., Yoruç, A. B. H., Uzunsoy, D. & Benlioğlu, S., (2010). Farklı Karıştırma Teknikleri ve Başlangıç Maddelerinden Sentezlenmiş Hidroksiapatit Tozunun Özelliklerinin İncelenmesi, 9. Ulusal Kimya Mühendisliği Kongresi. Ankara, Türkiye
14. Yoruç, A. B. H., İpek, Y. & Uzunsoy, D., (2010). Synthesis and characterization of nano hydroxyapatite powders using by different stirring methods and precursor reagents, TechConnect World Conference and Expo 2010. California, USA
15. İpek, Y., Mergen, A. & Eroğlu, M., (2009). Surfactan Enhanced Wet Chemical Precipitation of Fire Retardant Nano Zinc Borate, International Conference on Nanomaterials and Nanosystems (NanoMats2009). Istanbul, Turkey
16. İpek, Y., Mergen A.& Eroğlu, M., (2009). Synthesis of Nano Zinc Borate with Emulsion Technique, 4th International Boron Symposium. Eskişehir, Turkey
17. İpek, Y. & Yoruç, A. B. H., (2008). The Effect of Stirring Technique on Properties of Hydroxyapatite Powder Precipitated with Wet Chemical Synthesis, 14th International Metallurgy and Materials Congress. Istanbul, TURKEY

Scholarships

- OYP Scholarship 2011-2015
- Premiership Scholarship 2008-2012
- YTU Central Lab. Scholarship 2009-2011
- PhD Scholarship of TUBITAK Munir Birsal Charitable Institution 2010-2011