



**UNIVERSITI PUTRA MALAYSIA**

**DEVELOPMENT OF BIOSENSOR FOR DETERMINATION OF Cd(II) AND  
Hg(II) IONS BY EXPLOITING MWCNT/PEPTIDE NANOHYBRID  
MODIFIED Au ELECTRODE**

**NADIAH BINTI ABDUL RAHMAN I**

**FS 2011 32**

**DEVELOPMENT OF BIOSENSOR FOR  
DETERMINATION OF Cd(II) AND Hg(II) IONS BY  
EXPLOITING MWCNT/PEPTIDE NANOHYBRID  
MODIFIED Au ELECTRODE**



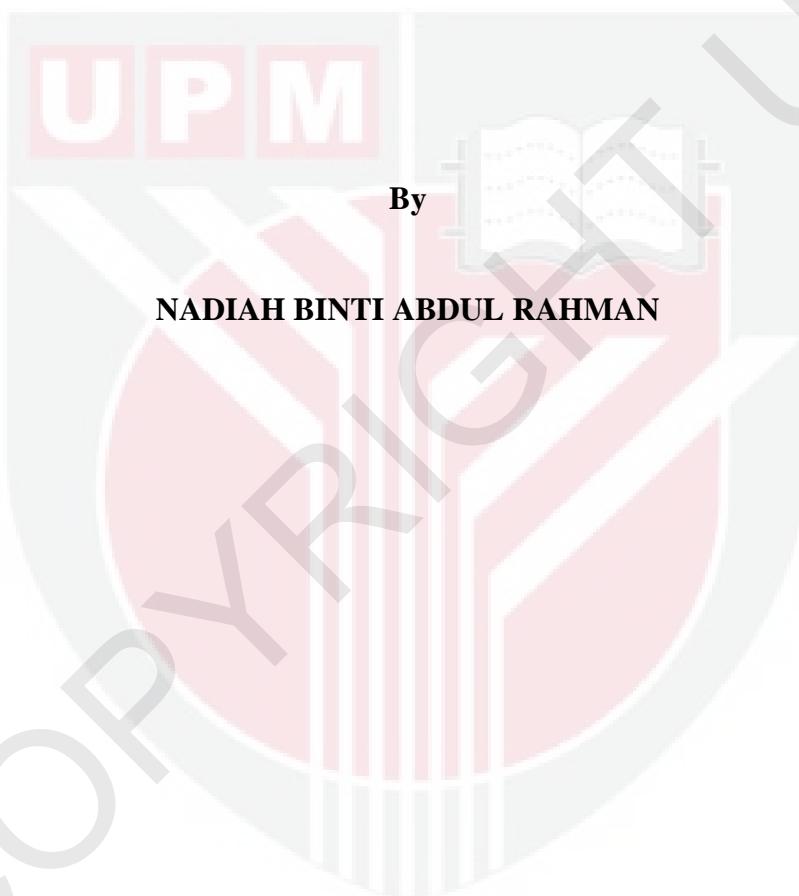
NADIAH BINTI ABDUL RAHMAN

MASTER OF SCIENCE

UNIVERSITI PUTRA MALAYSIA

2011

**DEVELOPMENT OF BIOSENSOR FOR DETERMINATION OF Cd(II) AND Hg(II)  
IONS BY EXPLOITING MWCNT/PEPTIDE NANOHYBRID MODIFIED Au  
ELECTRODE**



**NADIAH BINTI ABDUL RAHMAN**



**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in  
Fulfilment of the Requirements for the Degree of Master of Science**

**May 2011**

Abstract of thesis presented to the Senate of University Putra Malaysia in fulfillment of the requirements for the degree of Master of Science.

**DEVELOPMENT OF BIOSENSOR FOR DETERMINATION OF Cd(II) AND Hg(II)  
IONS BY EXPLOITING MWCNT/PEPTIDE NANOHYBRID MODIFIED Au  
ELECTRODE**

By

**NADIAH BINTI ABDUL RAHMAN**

**May 2011**

**Chairman : Associate Professor Nor Azah Yusof, PhD**

**Faculty : Science**

A biosensor for simultaneous detection of Cd(II) and Hg(II) was developed by using MWCNT/peptides modified Au electrode using cyclic voltammetry (CV). It was observed that the MWCNT/peptide modified gold electrode has significant superior analytical performance in determination of Cd(II) and Hg(II) compared to the unmodified gold electrode.

The gold electrode surfaces are modified with peptides that were synthesized by self assembly of cysteine monomers in the presence of diphenylphosphoryl azide (DPPA). This self assembly approach leads to rapid access of cyclic dimer and linear tripeptide of cysteine. The yielded peptides together with the MWCNT are attached to the Au electrode for further characterization and optimization with CV. The experimental conditions such as pH, supporting electrolyte, reproducibility and scan rates of the modified Au electrode were optimized.

Modification of peptide and MWCNT on the Au electrode surface has increased the sensitivity and selectivity of the electrochemical sensor for simultaneous determination of Cd(II) and Hg(II) ion. The reproducibility of the electrochemical sensor was good with relative standard deviation (R.S.D) value of 2.52%. The linear response for Cd(II)( in presence of borate buffer at pH 3) was obtained in the range of 0.1 ppm to 50.5 ppm whereas for Hg(II)( in presence of acetate buffer at pH 2) the linear response was obtained in the range of 0.1 ppm to 50.0 ppm.

The slope value of  $\log I_p$  versus  $\log v$  is 0.61-0.85 demonstrate that the modified Au electrode undergoes diffusion-adsorption controlled process. The sensitivity expressed as the slope of linear region of calibration curve was  $6 \times 10^{-6}$  A/ppm in borate buffer (pH 3) and  $8 \times 10^{-7}$  A/ppm in acetate buffer (pH 2). The limit of detection (L.O.D) for Cd(II) is  $2.75 \times 10^{-8}$  M whereas the limit of detection for Hg(II) is  $9.07 \times 10^{-10}$  M. The developed electrochemical sensors were applied in analysis of electroplating and wood industrial waste water for simultaneous determination of toxic metal Cd(II) and Hg(II). The results of the waste water sample analysis based on the developed method showed a comparable result with the ICP-OES method.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains.

**PEMBANGUNAN PENDERIABIO UNTUK PENGESANAN Cd(II) DAN Hg(II) ION  
DENGAN MENGEKSPLOITASI MWCNT/PEPTIDA PENGUBAHSUAIAN Au  
ELEKTROD**

Oleh

**NADIAH BINTI ABDUL RAHMAN**

**Mei 2011**

**Pengerusi : Prof Madya Nor Azah Binti Yusof, PhD**

**Fakulti : Sains**

Satu pengesan penderiabio untuk pengesan serentak Cd(II) dan Hg(II) ion telah dibangunkan dengan menggunakan MWCNT/ peptida Au elektrod yang dikaji dengan menggunakan teknik voltamentri siklik. Dapat dilihat bahawa pengesan elektrokimia yang telah diubahsuai dengan MWCNT/peptida Au elektrod mempunyai pencapaian yang luar biasa dalam pengesanan Cd(II) dan Hg(II) berbanding Au elektrod yang tidak diubahsuai.

Permukaan Au elektrod telah diubahsuai dengan peptida yang telah disintesis menggunakan teknik self assembly iaitu menganalisis sisteina dengan kehadiran DPPA. Kaedah sintesis ini menjurus kepada penghasilan yang cepat gelang dimer dan rantai panjang dipeptida sisteina. Hasil peptida yang telah diperolehi bersama dengan MWCNT dilekatkan pada permukaan Au elektrod untuk diklasifikasikan dengan lebih mendalam menggunakan voltammetri siklik(CV).

Keadaan eksperimen seperti perubahan pH, jenis elektrolit penyokong yang sesuai, kebolehan penghasilan semula, kadar imbasan telah di optimakan.

Penambahan peptida dan MWCNT ke atas Au elektrod telah berjaya meningkatkan sensitiviti dan selektiviti alat pengesan elektrokimia dalam pengesanan serentak ion Cd(II) dan Hg(II). Elektrod yang telah diubahsuai mempunyai kebolehan penghasilan semula yang bagus dengan nilai sisihan piawai relatif 2.52%. Garis lurus penentu ukur untuk Cd(II) dengan kehadiran elektrolit penyokong asid borat pada pH 3 adalah dalam lingkungan 0.1 ppm ke 50.5 ppm manakala untuk Hg(II) dengan kehadiran asid asetik pada pH2 nilai yang diperoleh adalah dalam lingkungan of 0.1 ppm ke 50 ppm.

Nilai kecerunan  $\log I_p$  melawan  $\log v$  adalah 0.61-0.85 dimana ia adalah proses pembebasan-penyerapan yang terkawal. Sensitiviti yang diperoleh adalah nilai kecerunan garis lurus penentu ukur dimana nilainya adalah  $6 \times 10^{-6}$  A/ppm untuk kehadiran asid borat(pH 3) dan  $8 \times 10^{-7}$  A/ppm untuk kehadiran asid asetik (pH 2). Nilai had pengesanan (LOD) untuk Cd(II) adalah  $2.75 \times 10^{-8}$  M dan nilai had pengesanan untuk Hg(II) adalah  $9.07 \times 10^{-10}$  M. Pengesan elektrokimia yang telah dibangunkan untuk pengesanan serentak Cd(II) dan Hg(II) telah diaplikasikan di dalam sampel sebenar industri iaitu dalam sampel industri kayu dan sampel industri elektro-mendapan. Keputusan yang diperolehi dari teknik yang dibangunkan mempunyai nilai yang hampir sama dengan keputusan yang di peroleh dari teknik ICP-OES.

## **ACKNOWLEDGEMENTS**

In the name of Allah S.W.T, the most Merciful and the most gracious, who sends prophets for the guidance of mankind, million and billion of blessing upon the last prophet and seal of the prophets.

I would like to take this opportunity to express my sincere gratitude and appreciation to my supervisor, Associate Professor Dr Nor Azah Yusof for her guidance, assistance, invaluable advises, ideas and helpful critics throughout the project.

I would also like to express grateful thanks to my co-supervisors, Dr Mariam Mohd Noor and Dr Bimo Ariotejo for their assistance, suggestion, unfailing help and constructive comments throughout the course of this project. My appreciation and grateful thanks also goes to both my parents, Hajar Shaari, Abdul Rahman, my husband Wan Azmir, Abdul Wafiy , Azreen for their inspiration, loves, encouragement and support thoughout the years.

Yet importantly, I wish to convey my deepest gratitude to my ‘lab mates’, Ili Syazana, Hafiz, Mariati Tiansin, and my entire friend who have been helpful in sharing their knowledge, experience, cooperation and concern toward the success of this project.

Last but not least, a special note of appreciation also extended to Pasca Siswazah Scheme Universiti Putra Malaysia for the assistantship.

I certify that a Thesis Examination Committee has met on 19 May 2011 to conduct the final examination of Nadiah Abdul Rahman on her thesis entitled "**Development of Biosensor for Determination of Cd(II) and Hg(II) Ion by Exploiting MWCNT/Peptide Nanohybrid Modified Au Electrode**" in accordance with the Universities and University Collages Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The committee recommends that the student be awarded the (Master of Science).

Members of the Thesis Examination Committee were as follows:

**Name of Chairperson, PhD**

Dr. Nor Azowa Bt Ibrahim  
Faculty of Science  
University Putra Malaysia  
(Chairman)

**Name of Examiner 1, PhD**

Prof Madya Dr. Abdul Halim Bin Abdullah  
Faculty of Science  
University Putra Malaysia  
(Internal Examiner)

**Name of Examiner 2, PhD**

Prof Madya Dr. Tan Wee Tee  
Faculty of Science  
University Putra Malaysia  
(Internal Examiner)

**Name of Examiner 3, PhD**

Prof. Dr. Lee Yook Heng  
School Of Chemical & Food Technology  
Faculty of Science and Technology  
Universiti Kebangsaan Malaysia  
(External Examiner)

---

**NORITAH OMAR, PhD**

Professor and Deputy Dean  
School of Graduate Studies  
Universiti Putra Malaysia  
Tarikh:

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of **Master of Science**. The members of the Supervisory Committee were as follows:

**Nor Azah Yusof , PhD**

Associate Professor

Faculty of Science

University Putra Malaysia

(Chairman)

**Siti Mariam Mohd Noor, PhD**

Senior Lecturer

Faculty of Science

University Putra Malaysia

(Member)

---

**HASANAH MOHD. GHAZALI, PhD**

Professor and Dean

School of Graduate Studies

University Putra Malaysia

Date:



## **DECLARATION**

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at University Putra Malaysia or at any other institutions.

**NADIAH BINTI ABDUL RAHMAN**

Date: 19 May 2011



## TABLE OF CONTENT

	Page
<b>ABSTRACT</b>	iii
<b>ABSTRAK</b>	v
<b>ACKNOWLEDGEMENTS</b>	vii
<b>APPROVAL SHEETS</b>	viii
<b>DECLARATION</b>	x
<b>LIST OF TABLES</b>	xv
<b>LIST OF SCHEME</b>	xvii
<b>LIST OF FIGURES</b>	xviii
<b>LIST OF ABBREVIATION</b>	xxi
<b>CHAPTER</b>	
<b>I        INTRODUCTION</b>	1
Objectives of the research	4
<b>II      LITERATURE REVIEW</b>	
Toxicity of Cadmium Ion Cd(II)	5
Toxicity of Mercury Ion Hg(II)	7
Electrochemical Sensors	8
Peptide Based Sensors for Metal Ions	10
Carbon Nanotubes (CNT)	13
Nafion	14
Peptide	15
Cysteine	16
Self Assembly Approach in Peptide Synthesis	17
Cyclic Voltammetry	18

<b>III</b>	<b>MATERIALS AND METHODS</b>	
Chemical Reagents	22	
Instrumentations and Apparatus	25	
Synthesis of Peptide via Self-Assembly Approach		
Peptide Synthesized with Coupling Reagent DPPA	26	
Peptide Synthesized with Coupling Reagent FDPP	27	
Thin Layer Chromatography	27	
Column Chromatography	28	
Spectral Studies		
FTIR Analysis	28	
GCMS Analysis	29	
Characterization and Optimization of the Experimental Parameters Involved for the Modified Gold Electrode in Simultaneous Detection of Cd(II) and Hg(II) Ion by Using Cyclic Voltammetry		
Modification of the Electrode	29	
Gold Electrode Surface Morphology Study	30	
Transmission Electron Microscopy(TEM)	30	
Electrochemical Analysis Procedure	30	
Stock Solution Preparation		
Stock Solution Cd(II)	31	
Stock Solution Hg(II)	31	
Optimization of the Experimental Parameters For Modified Gold Electrode with Cyclic Voltammetry		
Electrochemical Studies of Cd(II) and Hg(II) ion (separate batch)	32	
Simultaneous detection of Cd(II) and Hg(II) ion	32	
Effect of Ratio of Peptide and MWCNT	32	
Effect of Varying Supporting Electrolyte	33	
Effect of Varying pH	34	
Effect of Varying Scan Rates	35	

IV	<b>RESULT AND DISCUSSION</b>	
	Synthesis of Peptide via Self Assembly Approach	40
	Cyclooligomerisation of cysteine using coupling reagent DPPA	41
	FTIR analysis	41
	GCMS analysis	44
	Cyclooligomerisation of cysteine using coupling reagent FDPP	
	FTIR analysis	48
	GCMS analysis	50
	Characterization and Optimization of the Experimental Parameters Involved for the Modified Gold Electrode in Simultaneous Detection Cd(II) and Hg(II) Ion by Using Cyclic Voltammetry	
	Gold Electrode Surface Morphology Study	53
	Transmission Electron Microscopy(TEM)	53
	Optimization of the Experimental Parameters For Modified Gold Electrode with Cyclic Voltammetry	
	Electrochemical Studies of Cd(II) and Hg(II) ion (Separate Batch)	55
	Simultaneous Detection of Cd(II) and Hg(II) ion	57
	Electrochemical Study of Modified and Unmodified Au Electrode	59
	Effect of Varying Ratio Between Peptide and MWCNT	62
	Effect of Varying Analyte Concentration	64
	Effect of Varying Supporting Electrolyte	65
	Effect of Varying pH	71
	Effect of Varying Scan Rates	74
	Interference Study	80
	Response Towards Different Concentration of Cd(II) and Hg(II)	82
	Effect of Different Cd(II) Concentration with Constant Hg(II)	82

Concentration	
Effect of Different Hg(II) Concentration with Constant Cd(II)	84
Concentration	
Reproducibility	85
Validation of the Develop Detection System	87
V      CONCLUSION AND RECOMMENDATION	91
<b>BIBLIOGRAPHY</b>	94
<b>APPENDICES</b>	102
<b>BIODATA OF STUDENT</b>	105