DEVELOPMENT OF AS(III) AND HG(II) SENSORS UTILIZING AMINO ACIDS AND PEPTIDES AS RECOGNITION ELEMENTS

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By

NURULHAIDAH BINTI DAUD

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
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DEDICATION

For my beloved parents and family

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

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July 2012

Chairman: Associate Professor Nor Azah Yusof, PhD

Faculty: Faculty of Science

Toxic metal ions in waste water such as arsenic and mercury have lethal effects on the environment and living organisms. This indicates several striking requirements for novel analytical technologies that are fast, portable and cost-effective. In this work, sensitive voltammetric methods for the detection of arsenic and mercury ions are developed based on modified platinum and gold electrode, respectively.

The electrochemical sensors that comprise of metal chelating reagents, together with an ion carrier immobilised within polymeric thin films such as Nafion were developed. The molecule receptors such amino acids (glutamine and leucine) are used for the determination of As(III) ion in water samples using the voltammetric technique. Determination of As(III) by using CNTs/glutamine/Nafion modified platinum electrode was found to be reproducible with R.S.D of 7.3%. The redox peak current of As(III) shows a linear response towards different concentrations of As(III) and linear calibration curves were obtained in the range of 0.1×10^{-8} to

50.5×10^{-8} M. The sensitivity is 1×10^{-4} AμM^{-1} with the limit of detection 3.63×10^{-8} M which is better, compared to CNTs/leucine/Nafion modified Pt electrode in the determination of As(III). The LOD reported here is well within the range defined by the WHO regulation (10 μgL^{-1}). The determination of As(III) by using CNTs/leucine/Nafion modified Pt electrode has optimum response, observed at pH 5 with R.S.D. of 7.6%. The redox peak current of As(III) shows a linear response towards different concentrations of As(III) and linear calibration curves were obtained in the range of 0.02 μM to 2 μM. The limit of detection was determined as 1.67×10^{-8} M and the sensitivity recorded to be 2.1×10^{-5} AμM^{-1}.

A novel sensing strategy, based on the SAM method of MPA-HFHAHFAF peptide and Biotinyl Somatostatin-14 peptide has been used as a receptor for the determination of Hg(II) ion using gold electrode. The reproducibility of MPA-HFHAHFAF peptide modified gold electrode is 4.5%, indicating a reproducible and reliable detection system. A linear working range for the detection of mercury was obtained between 51 to 161 μgL^{-1} with LOD of 1.9 μgL^{-1}. The LOD obtained was below than the WHO guidelines for drinking water of 2 μgL^{-1}. The detection of mercury using Biotinyl Somatostatin-14 peptide /Nafion had been carried out under acidic conditions. Good relative standard deviation of 3.7% was obtained. A linear calibration plot in the range of 40-170 μgL^{-1} was obtained with the sensitivity value of 1×10^{-10} AμM^{-1}. The value of LOD obtained was below WHO guidelines for drinking water, 0.4 μgL^{-1}. In terms of the LOD value, the Biotinyl Somatostatin-14 peptide /Nafion peptide modified gold electrode was better compared to the MPA-HFHAHFAF peptide modified gold electrode for determining Hg(II).

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PEMBANGUNAN PENDERIA As(III) DAN Hg(II) DENGAN MEMANFAATKAN ASID-ASID AMINO DAN PEPTIDA-PEPTIDA SEBAGAI ELEMEN-ELEMEN PENGENALAN

Oleh

NURULHAIDAH BINTI DAUD

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Ion logam toksik di dalam sisa air seperti arsenik dan merkuri mempunyai kesan yang membawa maut terhadap alam sekitar dan organism hidup. Ini menunujukkan keperluan untuk teknologi analisis yang pantas, mudah alih dan kos efektif. Dalam kerja ini, kaedah voltammetri yang sensitif untuk mengesan arsenik dan merkuri dimajukan berdasarkan dengan mengubahsuai elektrod platinum dan emas.

Penderia electrokimia yang terdiri daripada reagen pengikat logam, bersama-sama dengan pembawa ion yang bergerak dalam filem nipis polimer seperti Nafion telah dibangunkan. Penerima molekul seperti asid–asid amino (glutamin dan leusina) digunakan untuk penentuan ion As(III) di dalam sisa air menggunakan teknik voltammetry. Penentuan As(III) dengan menggunakan CNTs/glutamin/Nafion ubahsuai platinum elektrod didapati boleh disalin semula dengan nilai R.S.D. 7.3%. Penentuan As(III) telah diperhatikan bebas gangguan dari ion logam pada keadaan berasid di dalam penimbal asetat. Puncak arus tindak balas pengoksidaan-penurunan

As(III) menunjukkan tindak balas bergaris ke arah kepekatan As(III) yang berbeza dan garisan keluk penentukuran telah diperolehi dalam julat 0.1×10^{-8} kepada 50.5×10^{-8} M. Kesensitifan adalah 1×10^{-4} AµM^{-1} dengan had pengesanan 3.63×10^{-8} M dimana ianya lebih bagus dibandingkan dengan CNTs/leusina/Nafion ubahsuai platinum elektrod di dalam penentuan As(III). Nilai LOD yang dilaporkan disini adalah dalam lingkungan yang ditakrifkan oleh peraturan-peraturan WHO, iaitu 10 µgL^{-1}. Tindak balas optimum untuk penentuan As(III) dengan menggunakan CNTs/leusina/Nafion ubahsuai platinum elektrod telah diperhatikan pada pH 5 dengan nilai R.S.D. 7.6%. Puncak arus tindak balas pengoksidaan-penurunan As(III) menunjukkan tindak balas bergaris kearah kepekatan As(III) yang berbeza dan garisan keluk penentukuran telah diperolehi dalam julat 0.02 µM kepada 2 µM. Had pengesanan telah ditentukan sebanyak 1.67×10^{-8} M dan kesensitifan ialah 2.1×10^{-5} AµM^{-1}.

Satu strategi baru penderiaan, berdasarkan kaedah SAM peptida MPA-HFHAHFAF dan Biotinyl Somatostatin-14 peptida telah digunakan sebagai reseptor untuk penentuan Hg (II) ion menggunakan elektrod emas. Kebolehulangan isyarat analsis adalah 4.5% menunjukkan pengesanan sistem penghasilan dan boleh dipercayai. Peringkat garis lurus untuk pengesanan merkuri adalah antara 51 kepada 161 µgL^{-1} dengan LOD pada 1.9 µgL^{-1}. LOD yang diperolehi berada bawah daripada garis panduan WHO untuk air minuman 2 µgL^{-1}. Pengesanan merkuri menggunakan peptida Biotinil Somatostatin-14/Nafion telah dijalankan di bawah keadaan berasid. Sisihan piawai relative yang bagus sebanyak 3.7% telah diperolahi. Satu plot penentukuran garisan dalam lingkungan 40-170 µgL^{-1} telah diperolehi dengan nilai sensitivity pada 1×10^{-10} AµM^{-1}. Nilai LOD yang diperolehi adalah 0.4 µgL^{-1} dimana

dibawah garis panduan WHO untuk air minuman. Di dalam istilah nilai LOD, Biotinil Somatostatin-14/Nafion peptide ubahsuai elektrod emas lebih bagus dibandingkan dengan MPA-HFHAHFAF peptide ubahsuai electrod emas untuk penetuan Hg(II).

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I certify that a Thesis Examination Committee has met on 9 July 2012 to conduct the final examination of Nurulhaidah binti Daud on his (or her) thesis entitled "Development of As(III) And Hg(II) Sensors Utilizing Amino Acids and Peptides as Recognition Elements" in accordance with the Universities and University Colleges 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 Doctor of Philosophy.

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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 previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

NURULHAIDAH BINTI DAUD

Date: 9 July 2012

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A	Electrode surface area
C	Concentration of analyte or reactant in the bulk solution
CE	Counter electrode
CNTs	Carbon nanotubes
CV	Cyclic voltammetry
DPV	Differential pulse voltammetry
E	Potential applied to electrode
$E^{\circ\prime}$	Formal reduction potential
E_i	Initial potential
E_{pa}	Anodic peak potential
E_{pc}	Cathodic peak potential
ΔE_p	Peak potential separation
GC	Glassy carbon
I	Current
I_{pa}	Anodic peak current
I_{pc}	Cathodic peak current
n	Number of electrons
Pt	Platinum
Q	Quantity of charge
RE	Reference electrode
SAM	Self-assembled monolayer
T	Temperature (K)
Γ	Surface coverage of the electrode reaction substance
t	Time

Q_{dl}	Capacitive charge (double layer), (in Coulomb)
V	Volt
ν	Scan rate
WE	Working electrode

CHAPTER 1

INTRODUCTION

1.1 Heavy Metals

Metals are defined chemically as "*elements which conduct electricity, have a metallic cluster, are malleable and ductile, form cations, and have basic oxides*". These elements can be found in chemical compounds as positive ions and with a density over 5 kg/dm^3. Heavy metals can harm the natural environment even at low concentration.

Toxic metals in water can be classified into two different health viewpoints. There are metals with an unpleasant effect like iron and other metals giving a very toxic effect such as lead. Heavy metal poisoning comes from industrial pollution, cooking utensils, deodorants, pesticides etc with devastating effects on the human body. Toxic metals also have a predisposition to gather in the human body and can result in chronic damage. One example of a toxic metal in water is cadmium, which can accumulate in the liver and kidney.

Some heavy metals are essential for life, for example they are physiologically essential for plants and animals, although it is needed in trace amount only. They have a direct bearing on human health and agricultural productivity and many are significant pollutants in the ecosystem throughout the world (Adegoke *et al.*, 2009). They are essential for maintaining the metabolism of the human body at trace concentrations, such as Cu, Fe, Mg, Mn, and Zn (Lan *et al.*, 1998). Some are considered to be both very toxic above recommended levels and are relatively accessible, such as As, Cd, Hg, Pb and Sn (Hasan *et al.*, 1998). Heavy metals can enter a water supply from industrial and consumer wastes. They can even enter from acidic rains and breaking down soils which release heavy metals into streams, rivers, lakes and ground water.

In developing countries, on average, 90% to 95% of all domestic sewage and 75% of all industrial wastes are discharged into surface waters without undergoing any treatment whatsoever (Hinrichsen *et al.*, 1997). In Thailand and Malaysia, water pollution is so serious as lakes often contain 30 to 100 times more pathogens, heavy metals, and poisons from the industry and agriculture than those permitted by government health standards (Suhaimi-Othman *et al.*, 2001; Ebrahimpour & Mushrifah, 2008). Most industries located in the western coast of the Peninsular Malaysia are dealing with electroplating, electronics, batteries and metal treatment/fabrication and become the major sources of heavy metal contamination (Onundi *et al.*, 2010). Industrial waste water usually contains a specific, readily identified chemical compounds and it carries more than 'twice as much degradable organic matter' as the municipal councils combined. It is found that one-third of the total water pollution comes in the form of effluent discharge, solid wastes and other hazardous wastes (Lokhande et *al.*, 2011).

The presence of heavy metal ions in the environment is a major concern due to their high toxicity. The ability to maintain quality water supplies and the need for water monitoring continues to grow as larger populations require increasing volumes of water (Flavel *et al.*, 2010). On-site monitoring is important because the measurements can be carried out in real time without the need for the sample to be taken back to the laboratory for analysis, so that immediate actions can be taken if guideline levels are exceeded. Therefore, simple and reliable instruments/methods for example, chemical and biosensor are highly potential to be developed as an alternative for these tedious and laborious procedures of metal analysis.

1.2 Biosensor

International Union of Pure and Applied Chemistry (IUPAC, 1999) defines a biosensor as a self-contained integrated device, which is capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element (biochemical receptor) which converts a biological response into an electrical signal. Biosensor device incorporates a biological sensing element connected to a transducer. A transducer converts an observed change (physical or chemical) into a measurable signal, usually an electronic signal which magnitude is proportional to the concentration of a specific chemical or a set of chemical (Eggins, 1996).

The first component in biosensor is biological molecule that can specifically interact and recognize the molecules present in the sample analyte. The second component is an electrochemical or optical device known as a transducer. In Figure 1.1, the molecular sensing element in biosensor recognizes the target analyte and the transducer converts the recognition event into a measurable signal. The output from the transducer is amplified, processed and displayed. The transducer makes use of a physical change that accompanies the reaction, where the changes in the distribution of charges can cause an electrical potential to be produced in potentiometric biosensors. Electrochemical, optical, and thermal biosensors can be identified based on the type of signal used for gathering information about the analyte (Chaki and Vijayamohanan, 2002).

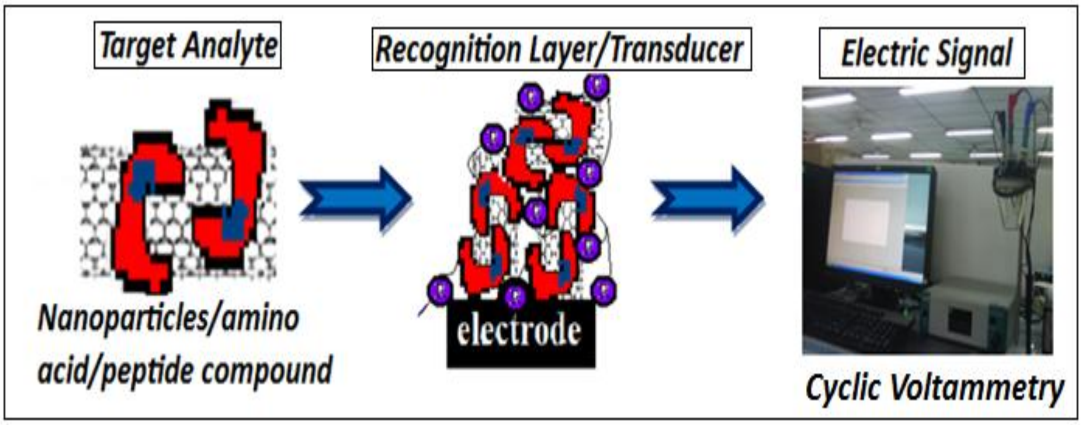

Figure 1.1: Schematic diagram showing the main components of a biosensor.

The specificity of the signal is guaranteed by the bio-receptor and the intensity is most often related to the concentration of the analyte. A good biosensor should possess a reproducible bioreceptor with a very high specificity of bioreceptor towards the analyte, which is stable under different experimental conditions. The transducer should be stable, easily modified and have good properties. The pre-treatment phase should be minimal, to allow fast and easy utilization while the output should be accurate, easily understandable, reproducible, free of background noise, and within the range of interest (Soldà, 2009). The main advantages of a biosensor are the simplicity; it is able to provide rapid, simple and low-cost on-site detection. The use of biological molecule to detect metals enables biosensors to provide an indication of how metal ion interacts with a particular organism (Chow & Gooding, 2006).

1.3 Electrochemical Sensors for the Determination of Heavy Metals

Electrochemical biosensors can be classified according to the specific electrochemical method employed, whether potentiometric or amperometric. A potentiometric biosensor is based on the measurement of variations of the electrode potential with respect to a reference electrode, or the potential difference between two reference electrodes separated by a permeoselective membrane (Koncki, 2007).

An amperometric biosensor measures the current generated by an applied potential between two electrodes. The oldest bioanalytical application of amperometry is the measurement of dissolved oxygen concentration. It exploits a gas-permeable membrane for selectivity, across which oxygen diffuses and is reduced at the electrode at a constant potential (Hahn, 1998). For the purpose of the determination of heavy metals, electrochemical (bio) sensor and optical chemical (bio) sensors are the two most widely used types (Cammann *et al.*, 1996).

Cyclic voltammetry is the most widely used technique for acquiring qualitative information about chemical reactions (Wang, 2000), where the electrode potential ramps at a constant rate to a peak value and then decreases at the same rate to the initial value. The electroactive material in the solution may reach the electrode in three modes of mass transport which are diffusion; spontaneous movement under the influence of concentration in an unstirred solution, convection; transportation by a force movement in stirred solution, and migration; charged particle movement along an electrical field (Wang, 2000).

The utility of cyclic voltammetry is highly dependent on the analyte being studied. The analyte has to be redox-active within the experimental potential window and displays a reversible wave. If the heterogeneous electron transfer is rapid, and the oxidized and reduced species are stable, the redox process will be known as electrochemically reversible.

There has been a growing need in recent years for constructing electrochemical sensors for fast, on-time and cost-effective monitoring of environmental samples. The research and development (R&D) in the sensor area has expanded exponentially in terms of financial investment, number of papers published, and the number of active researchers worldwide (Wolfbeis, 2004). Electrochemical sensors can be portable, simple to use, *in-situ* and miniature in size. These features are ideal for real-time on field measurements, thus the errors caused by the sample transportation and storage can be largely reduced (Cammann *et al.*, 1996).

1.4 Recognition Elements

A recognition unit (receptor) in biosensor is constructed to provide a selective target binding from a mixture of a compound. An appropriate structure allows multi-point non-covalent interactions with the target and it has high specificity and selective binding called molecular recognition. These receptors or recognition unit can be transformed into sensors by making it coupled with a nanoparticle to respond to the presence of the target without affecting the binding affinity. The sensor detects the bound target analyte, and information on the limit of detection on the total concentration of the target analyte present in the system is needed as an additional requirement on sensor affinity. A good selectivity of binding to discriminate from other species that may be close in structure and properties is required for a good sensor characterization.

The majority of researches in the development of detection system for metal ion involve the usage of macrocyclic ligands as a receptor. In nature, however, metal binding is achieved with a high degree of selectivity using peptide rather than macrocyclic ligands. The selectivity of the binding sites of metals in proteins is defined by the sequence of amino acids within the binding site. As a consequence, sensors that use appropriate amino acid sequence as biomimetic ligands for the detection of metal ions could solve some of the difficulties in fabricating sensors using delicate proteins (Wenrong *et al.*, 2001). Electrodes modified with the recognition elements of biological origin have advantages over other approaches for detecting metal ions, because they have the potential to give an estimate of the bioavailability of heavy metals as distinct from total metal concentration using AAS and ICP-MS. Amino acid and peptides represent the simplest biological recognition element for metal binding. Thus, the usage of these compounds in metal ion detection is important.

There are 20 common amino acids found in proteins that differ in their unique side chain and can be arranged in any particular order or length. Peptides present an almost infinite number of ligands for complexing metal ions. The presence of coordinating side chains in a peptide sequence, the combination of steric effect, the pH environment and hard–soft character of metal ions makes it difficult to predict precisely the relative affinity of a ligand for specific metal ion. A suitable way of determining the relative affinity and selectivity of peptide for a particular metal is through experimental approaches. In this thesis, we discuss different binding recognition molecules such as amino acids and peptides to detect As(III) and Hg(II) ions.

1.4.1 Amino Acids as Recognition Elements

Organic molecules such as amino acids and peptides are able to specifically bind metal ions. Moreover, some of these compounds have been shown to recognize particular motives at the interfaces and even on the surfaces of protein molecules (Fletcher & Hamilton 2007; Peczuh & Hamilton 2000).

Glutamine is a hydrophilic and a non-essential amino acid present in the human body. The concentration of glutamine in the blood is three to four times greater than all other amino acids, and it is the only amino acid with the ability to easily cross the barrier between both the blood and brain tissue. Glutamine is also one of the most important building blocks in forming the proteins that maintain tissue repair. It can be changed by the body into glutamic acid. It has several important metabolic roles, including as a regulator of nitric oxide synthesis by endothelial cell (Bussolati *et al.*, 1993), as an energy source of intestinal and lymphocytic cells (Leighton *et al.*, 1987) , and a non-toxic transport vehicle to carry ammonia from peripheral tissues to the liver where ammonia is metabolized to urea (Souba, 1991). Glutamine is generally buried in folded proteins and tends to favour the formation of helical structures in proteins.

Leucine is a hydrophobic, non-polar amino acid that is found as a structural element on the interior of proteins and enzymes. It is the second most common amino acid that can be found in proteins and acts in a unique way as it can help burn fat without burning the muscles. It is a neutrally essential ketogenic amino acid and forms an aceoacetate and acetate. Leucine can be synthesized from pyruvate through a multiple-step process in plants. Leucine is one of the three amino acids with a branched hydrocarbon side chain and has one additional methylene group in its side chain compared to valine. It can function as a chain crosslinker via hydrogen bond formation or it can hydrogen-bond to water at the protein surface.

Amino acids and peptide are known for their ability to form complex with metal ions because they possess nitrogen, oxygen and sulfur donor atoms at the main chain, as amino and carboxyl terminals, and at the side chain as in the case of serine and tyrosine (oxygen), lysine and histidine (nitrogen), aspartic acid and glutamic acid (oxygen) or cysteine and methionine (sulfur) (Shimazaki *et al.*, 2009). Thus, the design of peptides that coordinates metals, by the incorporation of modified amino acids, has the potential for applications in the development of selective chemical sensors for metals for use in vivo and in vitro (Mathews *et al.*, 2008; Joshi *et al.*, 2009). In the past, many functional groups such as amine, carboxylate, thiol, hydroxyl, ether and nitrile have been used as a ligand to detect metal ions (Jal et al., 2004).

1.4.2 Peptides as Recognition Elements

Peptides represent an option if one wishes to mimic the molecular recognition mechanism occurring in biomolecules such as enzymes, antibodies, drug receptors, and transmembrane proteins (Tewari & Dubey, 2008; Chiari *et al.*, 2008; Ariga *et al.*, 2007). Peptide could form the basis of biomimetic ligands for the development of sensors for the detection of metal ion.

Peptides have an excellent opportunity for the design of artificial receptors because of (1) the number of different molecules that can be obtained by combining the 21 natural amino acids, (2) the availability of both molecular biology and chemical techniques for the fast screening of peptide libraries, (3) the possibility of automated synthesis, and the low cost for the preparation of relatively large amounts of highly purified peptides, (4) the ease of modification to further enhance binding, and (5) relatively easy modelling (Pavan & Berti, 2012).

NH_2-His-Phe-His-Ala-His-Phe-Ala-Phe-OH (NH_2-HFHAHFAF-OH) peptide is synthesized in order to form a peptide nanotube for further research. NH_2-HFHAHFAF-OH peptide has D,L configuration of eight residues of amino acid. With D,L configuration of amino acid, NH_2-HFHAHFAF-OH peptide has an ability to form a cyclo(HFHAHFAF) and later can be synthesized to form a peptide nanotube (PNTs) sensor.

Peptide nanotubes (PNTs) are fascinating structures because they have the scope for numerous chemical modifications and allow the use of biological system's specificity (Reches M., 2003). Ghadiri and co-workers (Ghadiri *et al.*, 1995; Hartgerink *et al.*, 1996,1998) introduce the PNTs from self-assembly by cyclic peptide consisting D- and L-amino acids (Figure 1.2). To form the PNTs, a highly convergent covalent process was involved. In this stage, the cyclic peptide will rapidly self-assemble, forming well-ordered three-dimensional structures with an appropriate chemical or medium-induced triggering. The choice of the amino acid side chain functionalities and the ring size of the peptide sub-unit employed affect the properties of the outer surface and the internal diameter of peptide nanotubes (Ghadiri et *al.*, 1995).

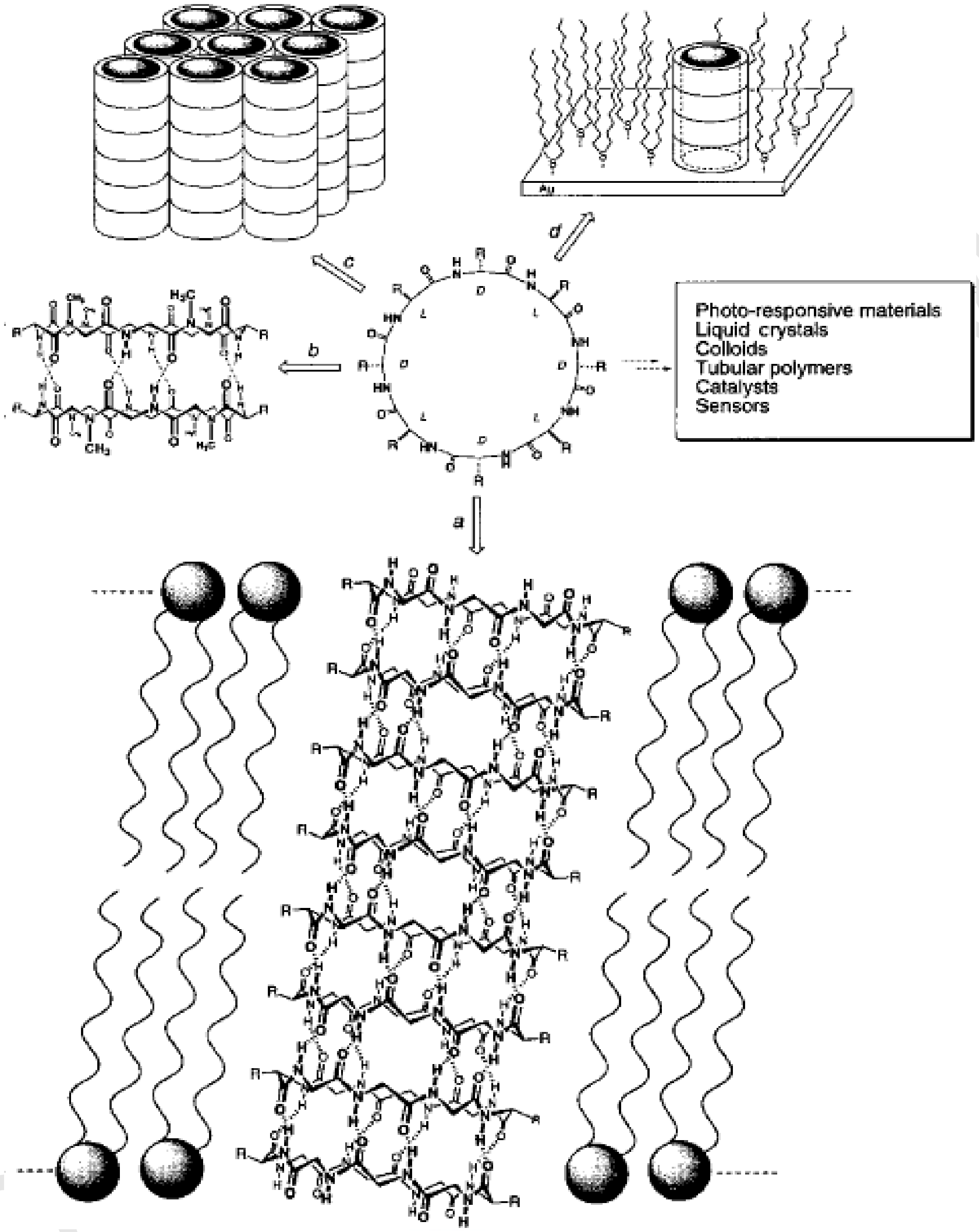

Figure 1.2: (center) Appropriately designed cyclic d,l-a-peptides and bpeptides can adopt a low-energy flat ring-shaped conformation in which the amide backbone moieties lie nearly perpendicular to the plane of the ring structure with side chains radiating around a central pore, the size of which is determined by the number of amino acids employed (for illustrative purposes only an eight-residue cyclic d,l-a-peptide is depicted). Depending on the peptide sequence and conditions employed, peptide subunits can be assembled into: a) transmembrane ion channels and pore structures, b) soluble cylindrical ensembles, c) solid-state tubular arrays, and d) surface-supported composites. Other plausible applications are denoted by the dashed arrow. The representations emphasize the antiparallel hydrogen- bond -directed stacking of the d,l-a-peptide nanotubes (Source: Hartgerink *et al.*, 1998).

In order to minimize non-bonded intramolecular transannular side chain−side chain and side chain−backbone interactions of cyclic peptide, an alternating even number D and L amino acid residues are needed (such as NH_2-[D-His, L-Phe, D-,His, L-Ala, D-His, L-Phe, D-Ala, L-Phe]-OH). With this configuration, the peptide structure adopts or samples a flat-ring shaped conformation, in which all backbone amide functionalities lie approximately perpendicular to the plane of the ring structure. In this flat ring-shaped structure, the subunit components can be stacked on top of each other, resulting in a hollow nanotube. The rings are glued together by complementary attractive hydrogen bonding interactions between the amide groups of the peptide backbone. All amino acid side-chains will point outward, away from the center of the peptide ring structure because of the local conformational and steric constraints imposed by the alternating amino acid backbone configuration, leaving it free to form a tubular core structure.

Two most important factors in the peptide self-assembly process are the ring strain and conformational stability which are influenced by the size of cyclic peptide employed. A cyclic D,L-peptides with more than six residues may have an internal diameter that is too small, and may have prohibitively large ring strain to allow the peptide backbone to adopt the required geometry for stacking and intermolecular hydrogen-bonding interactions (Ghadiri *et al.*, 1995). On the contrary, due to the greater flexibility of the peptide backbone, a large ring structure may not be sampled in the flat ring shape to take part effectively in the nanotubes' self-assembly process (Hartgerink *et al.*, 1996). Molecular modeling and experimental studies have indicated that eight-residue cyclic peptides possess the optimum balance of a low-strain ring structure and the desired flat ring-shaped conformational stability

(Hartgerink *et al.*, 1996). Ghadiri et al. (1993) publish the first well characterized eight residue peptide cycle [-(D-Ala-Glu-D-Ala-Gln)$_2$-] that is self assembled to form crystalline nanotubes with a uniform 7.5 Ǻ internal diameter.

The backbone−backbone intermolecular hydrogen-bonding interactions can proceed, in principle, via either parallel or antiparallel β-sheet-like ring-stacking arrangements (Figure 1.3) (Hartgerink *et al.*, 1996). However, molecular modeling and a number of experimental observations (Ghadiri, M, 1993, 1994, 1995) suggest a marked preference for the antiparallel arrangement. The formation of the antiparallel ring-stacked arrangement in self-assembled nanotubes has been supported by the symmetries observed in the electron diffraction patterns and the position of the amide I bands in the FT-IR spectra (Ghadiri *et al.*, 1995; Hartgerink *et al.*, 1996).

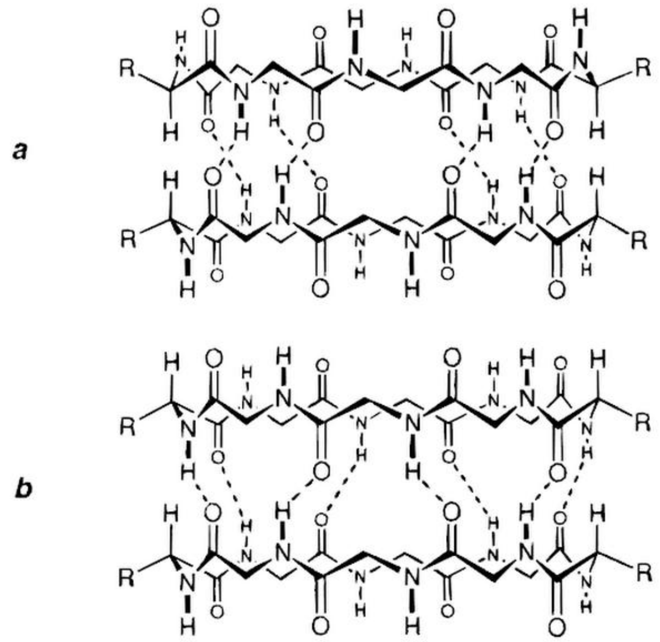

Figure 1.3: Peptide self-assembly producing antiparallel (a) and parallel (b) stacking arrangements (Source: Hartgerink *et al.*, 1996).

In PNTs synthesized, hydrophobic interaction is assumed to play the dominant part in the orientation of nanotubes because it is formed in aqueous solution. With no interaction with water molecules, hydrogen bonding between the peptide subunit is strengthened and results in easier formation of PNTs. With phenylalanine in a peptide sequence (Phe, F), strong covalent bonding is expected and the ring may have a better alignment and have closer stacked aromatic rings in PNTS that will enhance its conductivity.

In single amino acid, there are two donor atoms that can stay complex with metal which are the terminal amine and carboxyl oxygen or amide nitrogen. With a peptide, a stronger binding is achieved when the amide nitrogen is involved. The cyclic voltammetry study from Wenrong *et al.* (2001b) demonstrates the enhanced sensitivity and hence, greater detection limit achievable with an oligopeptide-modified electrode over an electrode in which a single amino acid is used.

In order to study a detection of metal ion with a longer peptide as a modified electrode, another peptide is used in this study which is Biotinyl-Neuromedin S (Biotinyl-Ile-Leu-Gln-Arg-Gly-Ser-Gly-Thr-Ala-Ala-Val-Asp-Phe-Thr-Lys-Lys-Asp-His-Thr-Ala-Thr-Trp-Gly-Arg-Pro-Phe-Phe-Leu-Phe-Arg-Pro-Arg-Asn-NH_2 or Biotin-ILQRGSGTAAVDFTKKDHTATWGRPFFLFRPRN-NH_2).

Biotinyl-Neuromedin S is a 36 amino acid neuropeptide that can be found in human brains and which is related to the neuromedin U. Biotin has low dissociation constant and it can be easily modified using standard peptide coupling methods. Biotin is a B-complex vitamin, a coenzyme in the metabolism of fatty acids and leucine and contains a cyclic urea structure (Figure 1.4) that the sulphur is present in a thio ether linkage. The point of biotinylation can be amino group (NH_2) with a covalent bond that makes the attachment strong and suitable for many different applications.

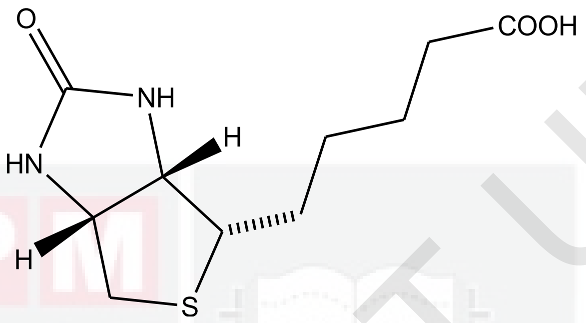

Figure 1.4: Biotin structure.

1.5 Problem Statement

The toxicity of metal ions makes the monitoring of metals in the environment vital. Thus, an accurate development of very sensitive, precise instruments for the detection of heavy metal is becoming a big challenge and important to the regulatory agencies, community and general public.

Current methods of metal ion monitoring involve using a classical elemental analysis technique such cold vapour atomic fluorescence spectrometry (Guimaraes *et al.*, 2000), cold vapour atomic absorption spectrometry (Hight & Cheng, 2005), ICP-AES (Boaventura *et al.*, 1997) and ICP-MS (Ugo *et al.*, 2001). These methods are sensitive, which can provide a wide linear range and low detection limits of heavy metal ions especially arsenic and mercury ions. However, these instruments are expensive, large in size and can only be used in the laboratory only. They require the sample to be transported from the site to a laboratory and therefore, the pre-treatment sample is inevitable. The high cost and slow measurement times are typically associated with the conventional measurements of regulated heavy metals. Hence, the high toxicity of heavy metal has prompted the development of various analytical methods for its determination towards the creation of a sufficiently stable electrode which is simple to operate, inexpensive and which does not require mechanical or chemical treatment before analysis or for regeneration.

Electrochemical sensors with recognition elements of biological origin have received particular attention, which include a very broad range of electrode materials and measurement methodologies with high sensitivity and selectivity, impressive cost effectiveness and possible miniaturization (Fu *et al.*, 2010). A variety of attempts, with more complex fabrication technology are required to develop a more sensitive and inexpensive sensor. The development of such sensors should be given to the efforts to improve the recognition element, where the researchers need to synthesize good molecular receptors that can give a good response to the selective metal ions such as arsenic and mercury without much interference from foreign metal ions. Another aspect is to investigate the immobilization of the new material use as solid matrix with interesting properties such as its easy preparation, inexpensive, non-toxic and multifunctional nature. It is also important to improve the signal processing technologies and instrumentation to qualify of sensor information thus makes it more selective and sensitive for the detection of arsenic and mercury ions.

1.6 Objectives

Objectives of the research are:

1) To develop electrochemicals for As(III) ion sensors using glutamine and leucine as recognition elements.

2) To develop electrochemicals for Hg(II) ion sensors using NH_2-HFHAHFAF-OH peptide and Biotinyl Somatostatin-14 as recognition elements.

3) To characterize the sensing ability of the developed electrochemical sensor for the optimization of experimental conditions.

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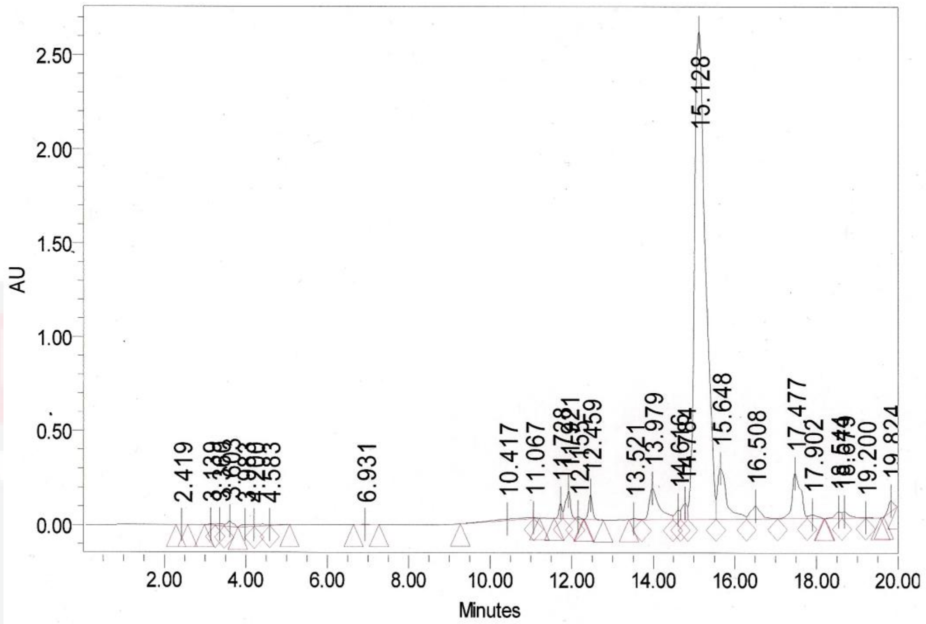
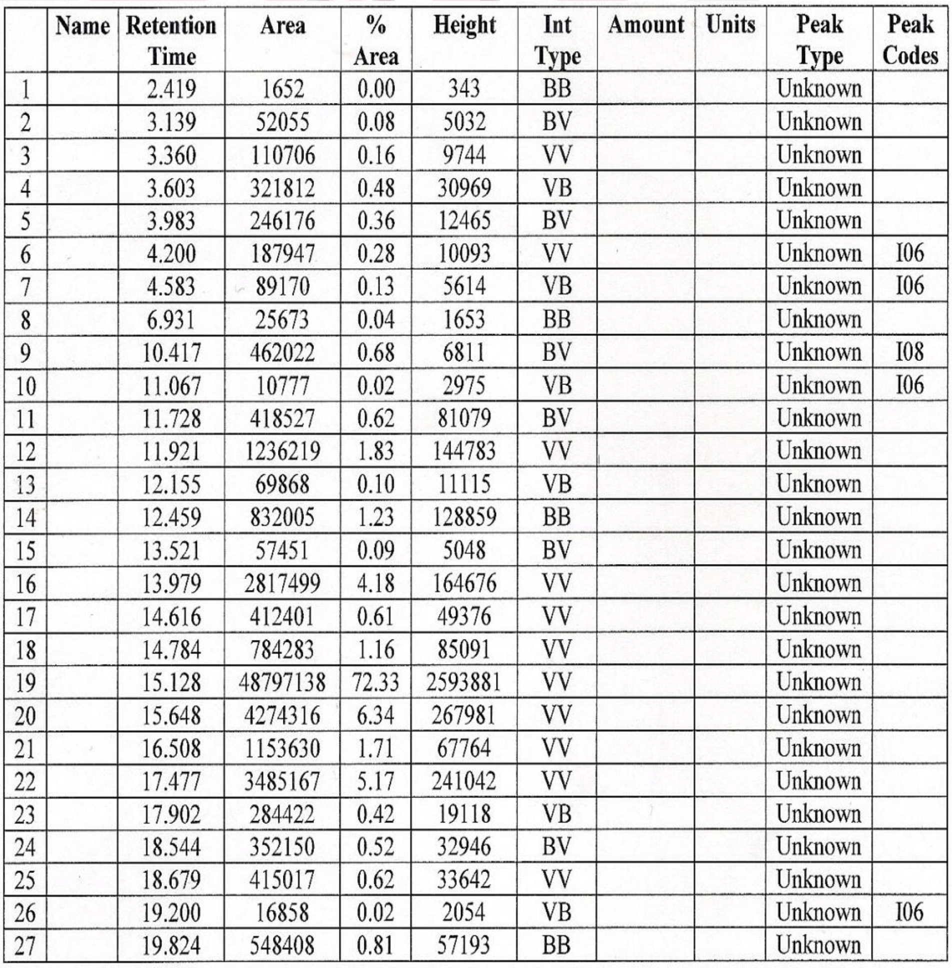
APPENDIXS

A

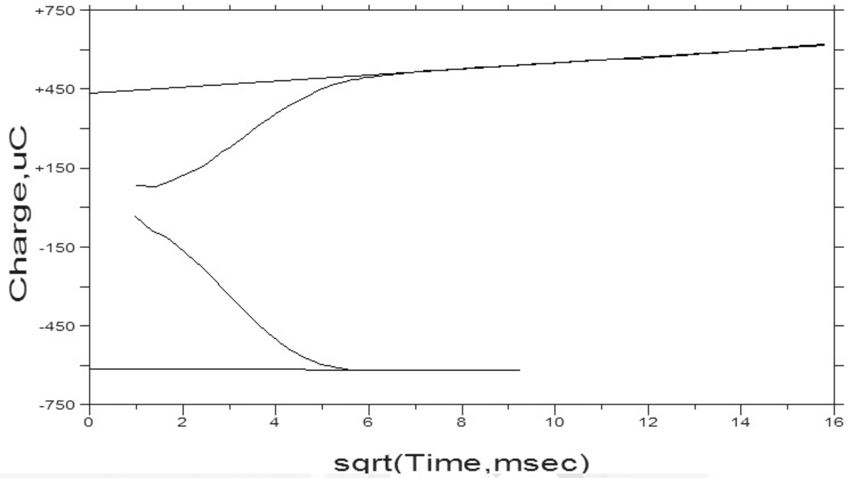




	Name	Retention Time	Area	% Area	Height	Int Type	Amount	Units	Peak Type	Peak Codes
1		2.419	1652	0.00	343	BB			Unknown	
2		3.139	52055	0.08	5032	BV			Unknown	
3		3.360	110706	0.16	9744	VV			Unknown	
4		3.603	321812	0.48	30969	VB			Unknown	
5		3.983	246176	0.36	12465	BV			Unknown	
6		4.200	187947	0.28	10093	VV			Unknown	I06
7		4.583	89170	0.13	5614	VB			Unknown	I06
8		6.931	25673	0.04	1653	BB			Unknown	
9		10.417	462022	0.68	6811	BV			Unknown	I08
10		11.067	10777	0.02	2975	VB			Unknown	I06
11		11.728	418527	0.62	81079	BV			Unknown	
12		11.921	1236219	1.83	144783	VV			Unknown	
13		12.155	69868	0.10	11115	VB			Unknown	
14		12.459	832005	1.23	128859	BB			Unknown	
15		13.521	57451	0.09	5048	BV			Unknown	
16		13.979	2817499	4.18	164676	VV			Unknown	
17		14.616	412401	0.61	49376	VV			Unknown	
18		14.784	784283	1.16	85091	VV			Unknown	
19		15.128	48797138	72.33	2593881	VV			Unknown	
20		15.648	4274316	6.34	267981	VV			Unknown	
21		16.508	1153630	1.71	67764	VV			Unknown	
22		17.477	3485167	5.17	241042	VV			Unknown	
23		17.902	284422	0.42	19118	VB			Unknown	
24		18.544	352150	0.52	32946	BV			Unknown	
25		18.679	415017	0.62	33642	VV			Unknown	
26		19.200	16858	0.02	2054	VB			Unknown	I06
27		19.824	548408	0.81	57193	BB			Unknown	

B

i) Chronocoulometry of 4 ×10^{-3} M $K_3Fe(CN)_6$ in 1 M KNO_3 using unmodified Pt electrode

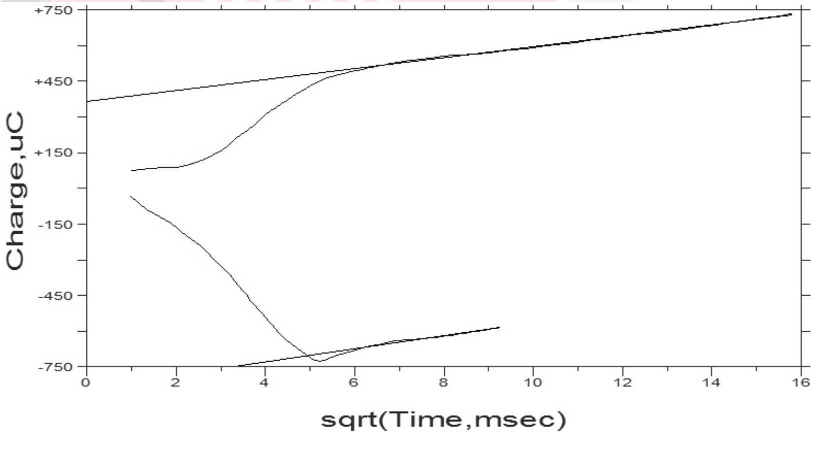


$$D^{1/2} = \frac{\text{slope x } \pi^{1/2}}{nFAC}$$

$$D^{1/2} = \frac{1.17\ e^{-5} \text{ x } \pi^{1/2}}{(1 \text{ x } 96500 \text{ x } 0.03\ cm^2 \text{ x } 4\ e^{-3}\ M)}$$

$$D^{1/2} = 1.79\ e^{-5}$$

ii) Chronocoulometry of 4 ×10^{-3} M $K_3Fe(CN)_6$ in 1 M KNO_3 using CNTs/leucine/Nafion modified Pt electrode

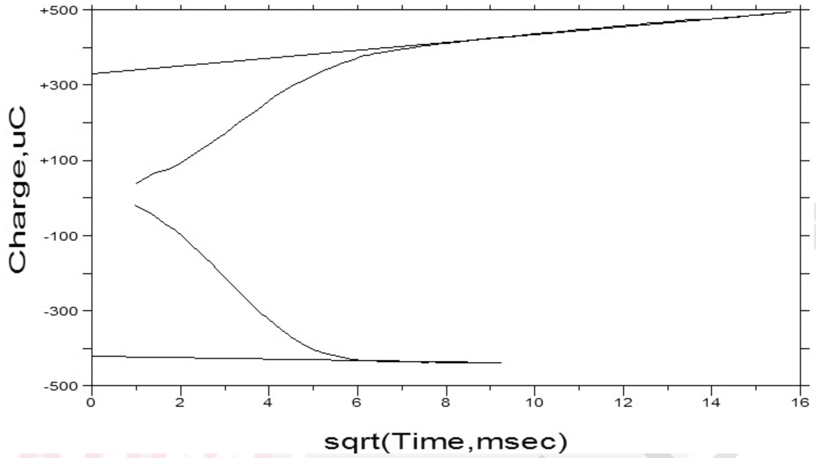


$$D^{1/2} = \frac{\text{slope x } \pi^{1/2}}{nFAC}$$

$$D^{1/2} = \frac{2{,}29\ e^{-5} \text{ x } \pi^{1/2}}{(1 \text{ x } 96500 \text{ x A x } 4\ e^{-3}\ M)}$$

A (real electrochemical surface area) = 0.06 cm^2

iii) Chronocoulometry of 4 ×10^{-3} M $K_3Fe(CN)_6$ in 1 M KNO_3 using CNTs/glutamine/Nafion modified Pt electrode

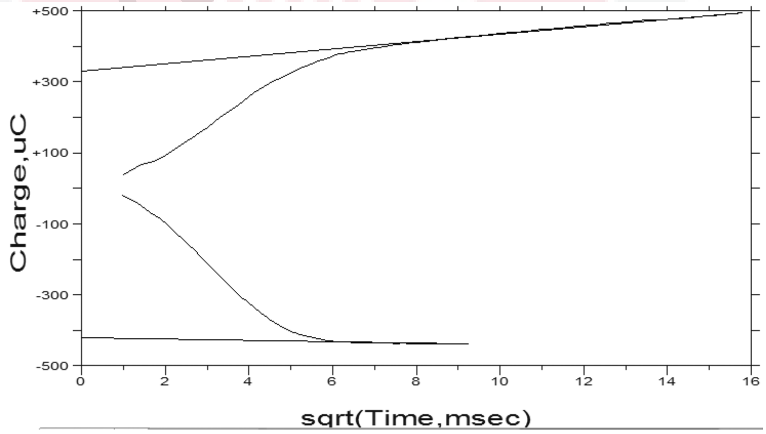


$$D^{1/2} = \frac{\text{slope x } \pi^{1/2}}{nFAC}$$

$$1.79\ e^{-5} = \frac{2.05\ e^{-5} \text{ x } \pi^{1/2}}{(1 \text{ x } 96500 \text{ x A x } 4\ e^{-3}\text{ M})}$$

A (real electrochemical surface area) = 0.05 cm^2

iv) Chronocoulometry of 4 ×10^{-3} M $K_3Fe(CN)_6$ in 1 M KNO_3 using unmodified gold electrode

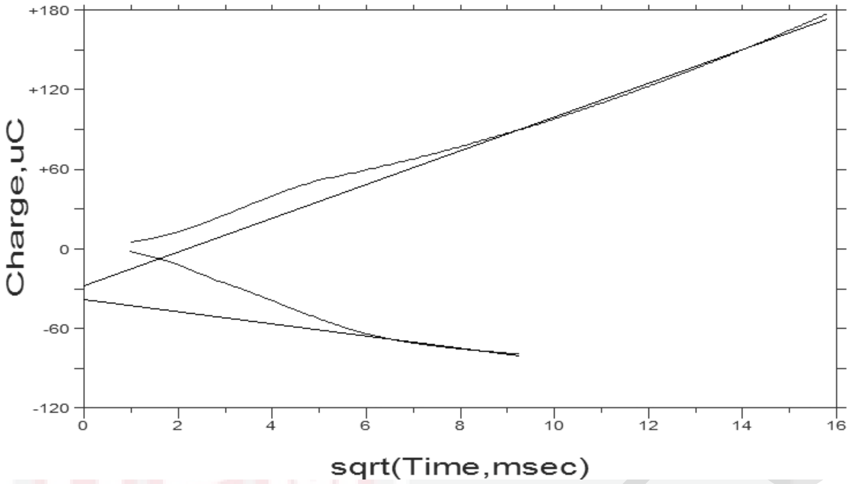


$$D^{1/2} = \frac{\text{slope x } \pi^{1/2}}{nFAC}$$

$$D^{1/2} = \frac{1.05\ e^{-5} \text{ x } \pi^{1/2}}{(1 \text{ x } 96500 \text{ x } 0.03\text{ cm}^2 \text{ x } 4\ e^{-3}\text{ M})}$$

$D^{1/2} = 6.912\ e^{-5}$

v) Chronocoulometry of 4 ×10^{-3} M $K_3Fe(CN)_6$ in 1 M KNO_3 using MPA-HFHAHFAF-OH modified gold electrode

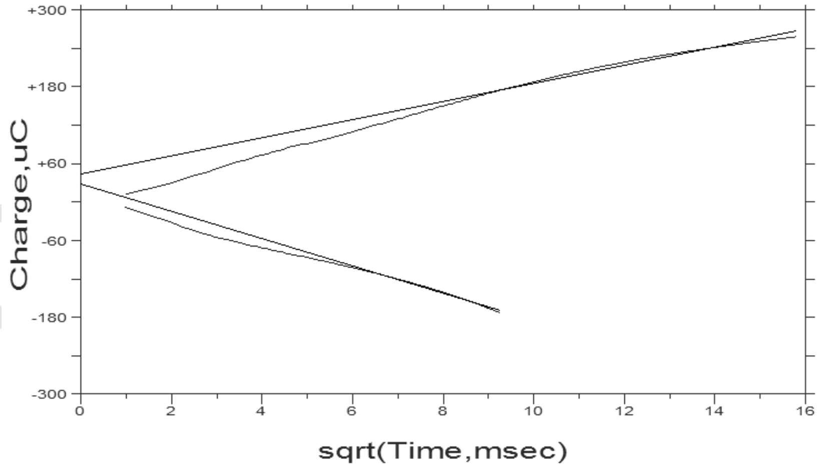


$$D^{1/2} = \frac{\text{slope x } \pi^{1/2}}{\text{nFAC}}$$

$$6.912\ e^{-5} = \frac{1.27\ e^{-5} \text{ x } \pi^{1/2}}{(1 \text{ x } 96500 \text{ x A x } 4\ e^{-3} \text{ M})}$$

A (real electrochemical surface area) = 0.08 cm^2

vi) Chronocoulometry of 4 ×10^{-3} M $K_3Fe(CN)_6$ in 1 M KNO_3 using Biotinyl Somatostatin-14/Nafion modified gold electrode



$$D^{1/2} = \frac{\text{slope x } \pi^{1/2}}{\text{nFAC}}$$

$$6.912\ e^{-5} = \frac{1.41\ e^{-5} \text{ x } \pi^{1/2}}{(1 \text{ x } 96500 \text{ x A x } 4\ e^{-3} \text{ M})}$$

A (real electrochemical surface area) = 0.09 cm^2

B

Glutamine

$$\acute{i}/\sqrt{T} = nFAD^{1/2}C\pi 1^{/2}$$

$$D^{1/2} = \frac{\text{slope x } \pi^{1/2}}{nFAC}$$

$$D^{1/2} = \frac{7.81\ e^{-5} \text{ x } \pi^{1/2}}{(2 \text{ x } 96500 \text{ x } 0.05\ cm^{2} \text{ x } 1\ e^{-3}\ M)}$$

$$D^{1/2} = 1.43\ e^{-5}$$

Diffusion coefficient, D $= (1.43\ e^{-5})^{2}$

$= 2.05\ e^{-10}\ cm^{2}/s$

CNTs/glutamine/Nafion modified Pt electrode	**CNTs /Nafion modified Pt electrode**
Surface charge, Q = |intercept of $Q_{forward}$ - intercept of $Q_{reverse}$| $= \lvert 7.563\ e^{-7} – 5.843\ e^{-8} \rvert$ $= 1.720\ e^{-7}$ $=0.172\ \mu C$ Density charge = Q/A $= \frac{0.17\ \mu C}{(0.05\ cm^{2})}$ $= 3.40\ \mu C/cm^{2}$ Surface coverage, Γ $= Q/nFA$ $= \frac{0.17\ \mu C}{2(96500)(0.05)}$ $= 1.76\ e^{-11}\ molcm^{-2}$	Surface charge, Q = |intercept of $Q_{forward}$ - intercept of $Q_{reverse}$| $= \lvert 6.578\ e^{-8} – 8.406\ e^{-8} \rvert$ $= 1.828\ e^{-8}$ $=0.018\ \mu C$ Density charge = Q/A $= \frac{0.018\ \mu C}{(0.05\ cm^{2})}$ $= 0.36\ \mu C/cm^{2}$ Surface coverage, Γ $= Q/nFA$ $= \frac{0.018\ \mu C}{2(96500)(0.05\ cm^{2})}$ $= 1.87\ e^{-12}\ molcm^{-2}$

Leucine

$$\acute{i}/\sqrt{T} = nFAD^{1/2}C\pi 1^{/2}$$

$$D^{1/2} = \frac{\text{slope x } \pi^{1/2}}{nFAC}$$

$$D^{1/2} = \frac{5.687\ e^{-5} \text{ x } \pi^{1/2}}{(2 \text{ x } 96500 \text{ x } 0.06\ cm^2 \text{ x } 1\ e^{-3}\ M)}$$

$$D^{1/2} = 1.04\ e^{-5}$$

$$\text{Diffusion coefficient, } D = (\ 1.04\ e^{-5})^2$$

$$= 1.09\ e^{-10}\ cm^2/s$$

CNTs/leucine/Nafion modified Pt electrode	**CNTs /Nafion modified Pt electrode**
Surface charge, Q = |intercept of $Q_{forward}$ - intercept of $Q_{reverse}$| = |$4.267\ e^{-7} - 8.694\ e^{-8}$| = $3.398\ e^{-7}$ =0.34 μC Density charge = Q/A = $\frac{0.34\ \mu C}{(0.06\ cm^2)}$ = 5.67 $\mu C/cm^2$ Surface coverage, Γ = Q/nFA = $\frac{0.39\ \mu C}{2(96500)(0.06\ cm^2)}$ = $3.37\ e^{-11}\ molcm^{-2}$	Surface charge, Q = |intercept of $Q_{forward}$ - intercept of $Q_{reverse}$| = |$4.376\ e^{-8} – 7.911\ e^{-8}$| = $3.535\ e^{-8}$ =0.04 μC Density charge = Q/A = $\frac{0.04\ \mu C}{(0.06\ cm^2)}$ = 0.66 $\mu C/cm^2$ Surface coverage, Γ= Q/nFA = $\frac{0.04\ \mu C}{2(96500)(0.06\ cm^2)}$ =$3.45\ e^{-12}\ molcm^{-2}$

MPA-HFHAHFAF-OH peptide

$$\acute{\imath}/\sqrt{T} = nFAD^{1/2}C\pi 1^{/2}$$

$$D^{1/2} = \frac{\text{slope x } \pi^{1/2}}{nFAC}$$

$$D^{1/2} = \frac{1.375\ e^{-5} \text{ x } \pi^{1/2}}{(1 \text{ x } 96500 \text{ x } 0.08\ cm^2 \text{ x } 2.49\ e^{-5}\ M)}$$

$$D^{1/2} = 1.27\ e^{-4}$$

Diffusion coefficient, D $= (1.27\ e^{-4})^2$

$= 1.61\ e^{-8}\ cm^2/s$

MPA-HFHAHFAF-OH modified gold electrode	**MPA modified gold electrode**
Surface charge, Q = |intercept of $Q_{forward}$ - intercept of $Q_{reverse}$| $= \lvert 5.339\ e^{-8} – 9.129\ e^{-8} \rvert$ $= 3.79\ e^{-8}$ $=0.038\ \mu C$ Density charge = Q/A $= \frac{0.038\ \mu C}{(0.08\ cm^2)}$ $= 0.48\ \mu C/cm^2$ Surface coverage, $\Gamma = Q/nFA$ $= \frac{3.79\ e^{-8}}{1(96500)(0.08)}$ $= 4.91e^{-12}\ molcm^{-2}$	Surface charge, Q = |intercept of $Q_{forward}$ - intercept of $Q_{reverse}$| $= \lvert 7.145\ e^{-9} – 6.145\ e^{-9} \rvert$ $= 1.00\ e^{-9}$ $=0.001\mu C$ Density charge = Q/A $= \frac{0.001\mu C}{(0.08\ cm^2)}$ $= 0.01\ \mu C/cm^2$ Surface coverage, $\Gamma = Q/nFA$ $= \frac{1.00\ e^{-9}}{1(96500)(0.08\ cm^2)}$ $=1.29\ e^{-13}\ molcm^{-2}$

Biotinyl Somatostatin-14

<table>
<tr><td colspan="2">$\acute{i}/\sqrt{T} = nFAD^{1/2}C\pi 1^{/2}$
$D^{1/2} = \frac{\text{slope x } \pi^{1/2}}{nFAC}$
$D^{1/2} = \frac{3.662\ e^{-5} \text{ x } \pi^{1/2}}{(1 \text{ x } 96500 \text{ x } 0.09\ cm^{2} \text{ x } 2.49\ e^{-5}\ M)}$
$D^{1/2} = 3.00\ e^{-4}$
Diffusion coefficient, D $= (3.00\ e^{-4})^{2}$
$= 9.00\ e^{-8}\ cm^{2}/s$</td></tr>
<tr><td>Biotinyl Somatostatin-14/Nafion modified gold electrode</td><td>Nafion modified gold electrode</td></tr>
<tr><td>Surface charge, Q
= |intercept of $Q_{forward}$ - intercept of $Q_{reverse}$|
$= |1.721\ e^{-9} – 3.351\ e^{-7}|$
$= 3.333\ e^{-7}$
$=0.33\ \mu C$
Density charge = Q/A
$= \frac{0.33\ \mu C}{(0.09\ cm^{2})}$
$= 3.67\ \mu C/cm^{2}$
Surface coverage, $\Gamma = Q/nFA$
$= \frac{3.333\ e^{-7}}{1(96500)(0.09)}$
$= 3.83\ e^{-11}\ molcm^{-2}$</td><td>Surface charge, Q
= |intercept of $Q_{forward}$ - intercept of $Q_{reverse}$|
$= |7.109\ e^{-8} – 4.310\ e^{-8}|$
$= 2.799\ e^{-8}$
$=0.03\mu C$
Density charge = Q/A
$= \frac{0.03\mu C}{(0.09\ cm^{2})}$
$= 0.33\ \mu C/cm^{2}$
Surface coverage, $\Gamma = Q/nFA$
$= \frac{2.799\ e^{-8}}{1(96500)(0.09\ cm^{2})}$
$=3.22\ e^{-12}\ molcm^{-2}$</td></tr>
</table>

LIST OF PUBLICATION, PATENT AND CONFERENCES/WORKSHOPS ATTENDED

1. **N. Daud**, N.A. Yusof, W.T. Tan, and A.H. Abdullah (2012) Electrochemical Sensor for As(III) Utilizing MWNTs/Leucine Modified Electrode, *Int. J. Electrochem. Sci.*, 7:175-185.

2. N.A. Yusof, **N. Daud**, W.T. Tan and A.H. Abdullah (2011) Electrocatalytic Characteristic of Carbon Nanotubes/Glutamine/Nafion Modified Platinum Electrode in Development of Sensor for Determination of As(III), *Int. J. Electrochem. Sci.*, 6:2385-2397.

3. **N. Daud**, N.A. Yusof and W.T. Tan (2011) Development of Electrochemical Sensor for Detection of Mercury by Exploiting His-Phe-His-Ala-His-Phe-Ala-Phe Modified Electrode, *Int. J. Electrochem. Sci.*, 6:2798-2807.

4. N.A. Yusof, **N. Daud**, Nanoparticles/Amino Acid Nanohybrid Modified Electrode, Patent No. PI 20092843.

5. **Fundamental Science Congress**, UPM (5-6th July 2011) Development of Electrochemical Sensor for Detection of Mercury by Exploiting Peptide Modified Electrode (Oral Presentation).

6. **Pameran Reka Cipta,Penyelidikan dan Inovasi**, UPM (28th July 2011) **Bronze Medal-** Electrocatalytic Characteristic of Nanoparticles/Amino Acid Nanohybrid Modified Electrode In Development of Biosensor For Determination Of As(III).

7. **Fundamental Science Congress**, UPM (17-18th June 2009) Electrochemical Biosensor for As(III) Utilizing CNTs/ Leucine Modified Electrode (Poster).

8. **1st Regional Conference on Biosensor and Biodiagnostics**, Kuala Lumpur (21-22nd May 2008), Development of Novel Nanotransducer for As(III) Ion Biosensors Exploiting Amino Acids as Recognition Element (Poster).

9. **2nd International Conference for Young Chemist**, USM (18-20th June 2008), Development of Novel Nanotransducer for As(III) Ion Biosensors Exploiting Amino Acids as Recognition Element (Oral Presentation).

10. **NANO-SciTech,** UiTM (Nov 2008), Electrocatalytic Characteristic of Nanoparticles/ Glutamine Nanohybrid Modified Electrode in Development of Biosensor for Determination of As(III) (Oral Presentation).

11. **Workshop on Peptide Synthesis**, UPM/Malaysia Genome Institute, (11-15th July 2011).

12. **Workshop on Potentiometric Ion Sensor Fabrication**, UKM, (16-20 April 2007).

BIODATA OF STUDENT

Nurulhaidah Daud was born on 30 August 1985 in Melaka Hospital, Melaka. She received her primary education in Sekolah Kebangsaan Bandar Tenggara 2, Kulai, Johor, and secondary education in Sekolah Menengah Kebangsaan Agama Segamat, Johor. She completed her Bachelor of Science (Honours) majoring in Chemistry degree with Second Class Upper Honours from Faculty of Science, Universiti Putra Malaysia in 2007. In the same year, she enrolled for a full time Master of Science degree course majoring in Analytical Chemistry at Chemistry Department, Faculty of Science, Universiti Putra Malaysia. Then, in 2010, she successfully converted her direction of study by pursuing her Ph.D research majoring in Analytical Chemistry in the same faculty and university. During her studies, she was offered to serve as a laboratory demonstrator to assist undergraduates in practical classes since the semester of November, 2007/2008 to the semester of November 2010. She was also responsible for three undergraduate students' final projects (2010-2011); where she was dedicated in helping them to decide on their projects and ideas. She also worked closely with them to teach the routine techniques that we had been using in our work.