



**UNIVERSITI PUTRA MALAYSIA**

***DEVELOPMENT OF DNA ELECTROCHEMICAL SENSOR BASED ON  
SILICON NANOWIRES/GOLD NANOPARTICLES-MODIFIED  
ELECTRODE  
FOR EARLY DETECTION OF DENGUE VIRUS***

**JAHWARHAR IZUAN BIN ABDUL RASHID**

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By

**JAHWARHAR IZUAN BIN ABDUL RASHID**

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

**August 2016**

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

**DEVELOPMENT OF DNA ELECTROCHEMICAL SENSOR BASED ON SILICON NANOWIRES/GOLD NANOPARTICLES-MODIFIED ELECTRODE FOR EARLY DETECTION OF DENGUE VIRUS**

By

**JAHWARHAR IZUAN ABDUL RASHID**

**August 2016**

**Chairman : Professor Nor Azah Yusof, PhD**  
**Faculty : Institute of Advance Technology**

A new DNA electrochemical sensor based on silicon nanowires (SiNWs) and gold nanoparticles (AuNPs) modified electrode was developed for dengue virus detection. In this study, two different fabricated electrodes; SiNWs/AuNPs-modified Indium tin oxide (ITO) and SiNWs/AuNPs-modified screen printed gold electrode (SPGE) have been fabricated. Field Emission Scanning Electron Microscope (FE-SEM) and Energy Dispersive X-ray Spectroscopy (EDX) analysis confirmed that the SiNWs/AuNPs-nanocomposite was deposited and uniformly distribution on the surface of ITO and SPGE. Based on cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) characterization, the fabricated SiNWs/AuNPs-ITO and SiNWs/AuNPs-SPGE have shown a good electrical conductivity compared to unmodified electrode. SiNWs/AuNPs nanocomposite was further explored as DNA matrix for DNA probe immobilization where dengue virus oligonucleotide was used as bio-sensing model to evaluate the performances of DNA electrochemical sensor. Electrochemical detection of hybridization events between immobilized DNA probe and complementary sequences of dengue virus were monitored by Different pulse voltammetry (DPV) technique using methylene blue (MB) as a redox indicator. The decrease of MB peak current was obtained after hybridization detection by both fabricated electrodes. The optimal performance of SiNWs/AuNPs-ITO and SiNWs/AuNPs-SPGE for electrochemical detection of dengue virus were obtained using response surface methodology (RSM): SiNWs volume (10.8  $\mu\text{L}$  and 6  $\mu\text{L}$ ), dithiopropionic acid (DTPA) (0.52 mM and 0.45  $\mu\text{L}$ ), AuNPs volume (83  $\mu\text{L}$  and 29  $\mu\text{L}$ ), DNA probe concentration (5.8  $\mu\text{M}$  and 5  $\mu\text{M}$ ), immobilization time (14 hours and 10 hours), pH buffer (7.5 and 7.8), NaCl concentration (1.45 M and 0.80 M), hybridization temperature (45  $^{\circ}\text{C}$  and 42  $^{\circ}\text{C}$ ) and incubation time (12 min and 10 min), respectively. Under optimized condition, developed DNA sensor showed a higher sensitivity of oligonucleotide detection as compared to the non-optimized condition. It was shown that the developed DNA sensors; SiNWs/AuNPs-ITO and SiNWs/AuNPs-SPGE were able to detect complementary oligonucleotide dengue virus as low as 0.0891 ng/ $\mu\text{L}$  (10 pM) and 0.0000891 ng/ $\mu\text{L}$  (10 fM), respectively. The stability studies also have shown that fabricated ssDNA/AuNPs/SiNWs-ITO and the

ssDNA/AuNPs/SiNWs-SPGE could be stored at 4 °C for 10 weeks and 7 weeks, respectively. It was found that the MB current signal of both developed DNA sensors have increased after the hybridization of immobilized DNA probe with genomic dengue virus from cell culture samples. However, this finding was unclear to justify the ability of both developed DNA sensor for direct detection of genomic dengue virus because of MB binding interaction issue and high non-specific hybridization for long genomic sequences. Hence, the preparation of specific and amplified target genomic dengue virus using reverse-transcribe-polymerase chain reaction (RT-PCR) were investigated. The parameters of annealing temperature, sonication time and reverse-forward (R/F) primer ratio using RT-PCR methods have been studied. Both developed DNA sensors are capable to discriminate the MB signal of blank electrode, negative serum samples, dengue 1 and 2 –spiked serum, cell culture and negative control. The LOD obtained for RT-PCR products value were 5.6 ng/μL and 2.8 ng/μL for SiNWs/AuNPs-ITO and SiNWs/AuNPs-SPGE, respectively. Furthermore, the developed DNA sensors; SiNWs/AuNPs-ITO and SiNWs/AuNPs-SPGE showed good reproducibility for nine measurements where the RSD value of 9.34 % and 8.23 % were obtained, respectively.

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

**PEMBANGUNAN ELEKTROKIMIA SENSOR DNA BERDASARKAN ELEKTROD MODIFIKASI NANOWAYAR SILIKON/NANOPARTIKEL EMAS UNTUK PENGESANAN AWAL VIRUS DENGGI**

Oleh

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**Ogos 2016**

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Sensor elektrokimia DNA baru berdasarkan modifikasi elektrod nanowayar silikon (SiNWs) dan nanopartikel emas (AuNPs) telah dibangunkan untuk pengesanan virus denggi. Dalam kajian ini, dua fabrikasi elektrod berbeza; ITO modifikasi SiNWs/AuNPs dan SPGE modifikasi SiNWs/AuNPs telah difabrikasikan. Analisis FE-SEM dan EDX mengesahkan nanokomposit SiNWs/AuNPs terapan dan disebarkan dengan sekata atas permukaan ITO dan SPGE. Berdasarkan pencirian CV dan EIS, fabrikasi ITO-SiNWs/AuNPs and SPGE-SiNWs/AuNPs mempamerkan konduktiviti elektrik yang baik berbanding dengan elektrod yang tidak dimodifikasikan. Nanokomposit SiNWs/AuNPs kemudian diuji sebagai matrik DNA untuk imobilisasi prob DNA di mana oligonukleotida virus denggi digunakan sebagai model bio-pengesanan untuk menilai kemampuan sensor elektrokimia DNA. Pengesanan peristiwa penghibridan secara elektrokimia antara prob DNA dan pelengkap jujukan virus denggi dipantau oleh teknik DPV menggunakan metilena biru (MB) sebagai petunjuk redoks. Penurunan puncak arus MB diperoleh selepas pengesanan penghibridan oleh kedua-dua fabrikasi elektrod. Kemampuan optimum bagi SiNWs/AuNPs-ITO and SiNWs/AuNPs-SPGE untuk pengesanan virus denggi menggunakan kaedah gerak balas permukaan (RSM); isipadu SiNWs (10.8  $\mu\text{L}$  dan 6  $\mu\text{L}$ ), dithiopropionic acid (DTPA) (0.52 mM dan 0.45  $\mu\text{L}$ ), isipadu AuNPs (83  $\mu\text{L}$  dan 29  $\mu\text{L}$ ), kepekatan prob DNA (5.8  $\mu\text{M}$  dan 5  $\mu\text{M}$ ), masa immobilisasi (14 hours dan 10 hours), pH penampakan (7.5 dan 7.8), NaCl concentration (1.45 M dan 0.80 M), suhu penghibridan (45  $^{\circ}\text{C}$  dan 42  $^{\circ}\text{C}$ ) and masa eraman (12 min dan 10 min), masing-masing. Di bawah keadaan optimum, sensor DNA yang dibangunkan menunjukkan sensitiviti tinggi dalam pengesanan oligonukleotida virus denggi berbanding keadaan tidak optimum. Ia telah ditunjukkan bahawa sensor DNA yang dibangunkan; ITO-SiNWs/AuNPs and SPGE-SiNWs/AuNPs mampu mengesan virus denggi pelengkap oligonukleotida pada serendah masing-masing 0.0891 ng/ $\mu\text{L}$  (10 pM) dan 0.0000891 ng/ $\mu\text{L}$  (10 fM). Kajian kestabilan menunjukkan fabrikasi ITO- SiNWs/AuNPs/ssDNA dan SPGE- SiNWs/AuNPs/ssDNA boleh masing-masing disimpan pada 4  $^{\circ}\text{C}$  selama 10 minggu dan 7 minggu. Ia telah didapati bahawa isyarat arus MB untuk kedua-dua sensor DNA yang dibangunkan meningkat selepas penghibridan antara prob DNA yang diimobilisasi dengan virus denggi genomik dari sampel kultur sel. Bagaimanapun,

penemuan ini tidak jelas untuk mengesahkan keupayaan kedua-dua sensor DNA yang dibangunkan mampu mengesan virus denggi genomik secara langsung kerana isu hubungan pelekatan MB dan penghibridan tidak spesifik yang tinggi pada jujukan genomik panjang. Oleh itu, penyediaan pengandaan dan spesifik sasaran denggi virus menggunakan transcriptase berbalik-reaksi rantai polimerase (RT-PCR) dikaji. Parameter seperti suhu penyepulih-indapan, masa sonikasi dan nisbah primer depan-belakang (R/F) menggunakan kaedah RT-PCR telah dikaji. Kedua-dua sensor DNA yang dibangunkan mampu untuk membezakan isyarat MB antara electrode kosong, sampel serum negatif, serum yang disuntik denggi jenis 1 dan 2, kultur sel denggi virus dan kawalan negatif. LOD untuk nilai produk RT-PCR adalah masing masing 5.6 ng/ $\mu$ L and 2.8 ng/ $\mu$ L untuk ITO- SiNWs/AuNPs dan SPGE- SiNWs/AuNPs. Tambahan pula, DNA sensor yang dibangunkan menunjukkan kebolehlulangan yang baik dengan sembilan pengiraan di mana nilai RSD yang diperoleh masing-masing adalah 9.34% dan 8.23%.

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I certify that a Thesis Examination Committee has met on 2 August 2016 to conduct the final examination of Jahwarhar Izuan Abdul Rashid on his thesis entitled "Development of DNA Electrochemical Sensor Based on Silicon Nanowires/Gold Nanoparticles-Modified Electrode for Early Detection of Dengue Virus" 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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## LIST OF ABBREVIATIONS

APTES	Aminopropyl-triethoxysilane
AuNPs	Gold nanoparticles
BLAST	Basic local alignment search tool
bp	Base pairs
CCD	Central composite design
CE	Counter electrode
CV	Cyclic voltammetry
DNA	Deoxyribonucleic acid
DPV	Different pulse voltammetry
DPTA	3,3 dithioproprionic acid
dsDNA	Double stranded DNA
EIS	Electrochemical impedance spectroscopy
FESEM	Field-emission Scanning electron microscopy
F/R ratio	Forward to reverse primer concentration ratio
FTIR	Fourier transform infrared spectroscopy
GPES	General purpose electrochemical system
ITO	Indium tin oxide
LOD	Limit of detection
MB	Methylene blue
NCBI	National center for biotechnology information
PCR	Polymerase chain reaction
RNA	Ribonucleic acid
RT-PCR	Reverse transcribe-polymerase chain reaction
SiNWs	Silicon nanowires
RSM	Response surface Methodology
RSD	Relative standard deviation
SAM	Self-assembly monolayer
SEM	Scanning electron microscopy
SPGE	Screen printed gold electrode
ssDNA	Single stranded DNA



Tris

Tris(hydrometyl) amino methane

UV-Vis

Ultraviolet-visible





# CHAPTER 1

## INTRODUCTION

### 1.1 Background of study

Malaysia is among the seriously affected countries with a rapid increase in the number of dengue fever cases from 7103 in 2000 to 108,698 including 215 deaths in 2014 (Lee et al., 2015a). As of 5 December 2015, the number of dengue cases which has been recorded were 111,285 including 301 deaths that is 16.3% higher than 95,693 cases obtained in 2014 for the same period (WHO, 2015). A few factors that contribute to the dengue incidence in Malaysia are due to the massive urbanization, climate changes, poor environmental cleanliness, and increase human travel (Haliza and Eva, 2015; Mudin, 2015). Dengue fever is caused by the dengue virus, the genus of Flaviviridae virus family, which is transmitted to the human through the bite of female mosquitoes, *Aedes aegypti* (Lo et al., 2007).

The dengue virus genome consists of a single-stranded RNA virus and grouped into four antigenically serotypes (type-1, type -2, type-3, type-4). Any of these serotypes can cause a mild febrile disease at first primary infection and have high potential to develop into a more severe dengue shock syndrome (DSS) hemorrhagic fever (DHF) that can lead to fatality (Rai et al., 2012b). Until now, there is no specific medication or antibiotic to kill the dengue virus and patients just solely depend on the treatment of the dengue infection symptoms (Wright and Pritt, 2012). To date, reducing and destroying the population of mosquito dengue vector is the effective way to control the dengue virus transmission (Lee et al., 2015a).

There are several diagnostic tool which have been established for dengue virus detection including virus isolation (Gurukumar et al., 2009; Jyothi and Metri, 2015), Serology test (Wu et al., 2001; Guey Chuen et al., 2011; Zhang et al., 2015), NS1-capture ELISA (Hang et al., 2009; Blacksell, 2012; Jyothi and Metri, 2015) and reverse transcriptase polymerase chain reaction (RT-PCR) assay combined agarose gel electrophoresis technique (Najioullah et al., 2014; Decker et al., 2015). However, these existing diagnostic assays possess certain drawbacks such as time-consuming, expensive, laborious, low selectivity and sensitivity and the results will usually obtained after 5 -7 days upon dengue infection (Souza et al., 2011). Due to virulence of dengue virus, the development of sensitive, high selectivity, rapid detection of dengue virus at early diagnosis has seemed to be highly desirable to prevent the spread of dengue infection. The use of nucleic acid (DNA/RNA) biosensor based on DNA hybridization events has become a promising diagnostic tool for nucleic acid detection due to its ability to shorten the assay time, high specificity and allowing detection almost in real time (Hejazi et al., 2008; Karamollaoğlu et al., 2009; Wipawakarn et al., 2012). DNA biosensors are sensing devices consist of single-stranded DNA probe as biological recognition elements incorporated with the sensor transducer for converting hybridization DNA events into a useful analytical signal (Taufik et al., 2011). Several transducers hybridization events detection have been reported such as the

electrochemical transducer (Mandong et al., 2007; Wu et al., 2013), optical transducer (Lai et al., 2011; Delpont et al., 2012), piezoelectric transducer (Rodaree et al., 2011) and electrical transducer (Sorgenfrei et al., 2011).

For the past few years, the application of nucleic acid-based electrochemical sensor has tremendously increased in the microbial pathogen, which provides a rapid detection, high sensitivity, cost-effective portability and ease miniaturization (Lee et al., 2003; Feng et al., 2006; Yang et al., 2009; Girousi and Kinigopoulou, 2010; Wu et al., 2013; Cui et al., 2014). Generally, the electrochemical methods are based on the DNA hybridization events where a single-stranded DNA (ssDNA) known as DNA probe was immobilized on the electrode surface to capture specific DNA target (Li et al., 2008). This hybridization event was converted into a measurable signal based on the redox activities of either direct detection (guanine or adenine bases oxidation) or indirect detection (redox indicator, enzyme and nanoparticle label). Methylene blue (MB) is commonly employed as the redox indicator in DNA electrochemical detection (Bolat et al., 2013; Lin et al., 2014; Zhang et al., 2014). This is due the fact that MB shows a different affinities while binding to the surfaces of ssDNA and dsDNA, resulting in the variation of the electrochemical signal with or without the presence of DNA target (Sassolas et al., 2008; Bolat et al., 2013; Lin et al., 2014).

With the growing of nanotechnology fields, it has been proven that the utilization of nanomaterials in DNA biosensors can enhance the DNA immobilization on the transducer surface and can be acted as the signal amplifier for the hybridization events, thus increasing the electrochemical responses of DNA biosensor (Kerman et al., 2004; LaGier et al., 2005; Girousi and Kinigopoulou, 2010). Numerous kinds of nanomaterials have been explored in the construction of DNA sensors based nanomaterials including gold nanoparticles (AuNPs) (Zhang et al., 2010a; Thirupathiraja et al., 2011; Li et al., 2012; Gao et al., 2013), nanowires (Wu et al., 2012; Ramulu et al., 2013), carbon nanotubes (Elahi et al., 2012), quantum dots (Qds) (Li et al., 2011a; Sharma et al., 2012) and nanoporous substrates (Li et al., 2011b; Ahangar and Mehrgardi, 2012).

In recent years, silicon nanowires (SiNWs), the one-dimensional nanostructures has been frequently utilized as the sensing materials for chemical and biological detections (Zhang et al., 2008; Singh et al., 2010; Zhang et al., 2011; Kulkarni et al., 2012; Oh et al., 2012; Yang et al., 2013). This is due to possessing some unique characteristics such as high surface area, excellent mechanical and electrical properties and good favorable biocompatibility characteristic (Zhang et al., 2009; He et al., 2010; Singh et al., 2010). However, most of the previous studies were based on SiNWs-Field-Effect Transistor (FET) sensor for ultrasensitive and selective DNA detection. Zhang et al. (2010b) had successfully employed a single SiNWs based on FET sensor to detect RT-PCR product of dengue virus below 10 fM within 30 minutes. Nevertheless, the high cost generated for the fabrication of SiNWs-FET sensor has become the main barrier to develop a low-cost sensor since it involved advanced lithography method such as E-beam, AFM or deep UV lithography (Wenga et al., 2013).

However, there are some reports on the utilization of SiNWs as sensing material for fabrication of electrochemical biosensors. For example Yan et al., (2012) reported the utilization of Ni(OH)<sub>2</sub>-SiNWs as working electrode for the detection of hydrogen

peroxide ( $H_2O_2$ ). The sensitivity of an electrochemical sensor for the detection of acetylcholinesterase was greatly enhanced after the modification of working electrode with SiNWs coated with AuNPs (Su et al., 2008). Similar research have also found that the modified electrodes based on SiNWs could enhance electron transfer and conductivity of sensors for electrochemical detection of bovine serum albumin protein (Kwon et al., 2011), glucose (Chen et al., 2006), ascorbic acid (Su et al., 2013), pesticide (Su et al., 2008), ethanol (Tao et al., 2009) and glutamate (Yang et al., 2006).

## 1.2 Problem statements and research motivation

Current clinical diagnostic for early dengue virus detection relies on the application of molecular biology diagnostic based on nucleic acid amplification via reverse-transcriptase polymerase chain reaction (RT-PCR) combined with agarose electrophoresis (Poloni et al., 2010; Najioullah et al., 2014; Decker et al., 2015). Since the molecular size of amplified RT-PCR product becomes the main analytical for nucleic acid detection through agarose gel electrophoresis, there are a few limitations observed. Firstly, agarose gel electrophoresis does not provide quantitative results and has low specificity which cannot differentiate the size of amplified nucleic acid between non-complementary and DNA target that can lead to the false-positive results (Giakoumaki et al., 2003; Pedrero et al., 2011). Secondly, the analysis procedures of gel agarose electrophoresis are time-consuming, include tedious steps and require hazardous elements such as ethidium bromide for gel staining and ultraviolet for band visualization (Wang, 2002; Chua et al., 2011; Bora et al., 2013).

To overcome this issues, an ultrasensitive DNA electrochemical sensor is highly suggested as a promising way for a direct detection of nucleic acid dengue virus instead of using agarose gel electrophoresis. This is because DNA electrochemical sensor could display high specificity of amplified nucleic between nonspecific and specific DNA target and the level concentration of amplified nucleic acid can be quantified by monitoring the current response. However, electrochemical detection of nucleic acid dengue virus is challenging because of its low concentrations in the real samples. Chemically modified electrodes have been frequently used to overcome the problems of poor selectivity and sensitivity faced at bare electrodes.

In the present study, silicon nanowires decorated with gold nanoparticles (SiNWs/AuNPs)-modified electrode is employed as one of the strategies to improve the electrochemical detection of nucleic acid dengue virus. To date, the utilization of SiNWs in the electrochemical system for detection of DNA/RNA has not been explored. Thus, the suitability of SiNWs as DNA immobilization and hybridization layers in the electrochemical detection is still unknown. The suitability of the immobilization procedure and DNA layer on the electrochemical transducer (electrode) for DNA probe loading and hybridization are the main challenges in designing an ultrasensitive DNA sensor with a low detection limit. This is because an effective modifying layer on the electrode must be compatible with the DNA probe immobilization in terms of good orientation, reactivity and must be able to avoid nonspecific interaction as well as being sensitive enough to convert DNA hybridization events into a measurable signal. Hence, the performance of DNA electrochemical

sensor greatly depends on the optimum condition of DNA layer formation, DNA probe immobilization and hybridization. Without understanding the nature of DNA probe loading and hybridization process in the presence of SiNWs as sensing material, it is difficult to propose and develop high sensitive and selective of electrochemical DNA biosensor for rapid identification of dengue virus

### **1.3 Novelty of research**

The utilization of silicon nanowires (SiNWs) decorated gold nanoparticles (AuNPs) nanocomposites is the first being employed in the DNA electrochemical biosensor construction as DNA probe immobilization and hybridization matrix. In addition, the role of SiNWs/AuNPs nanocomposites in the current response for DNA hybridization detection based on electrochemical methods was first explored. By monitoring the redox current changes with or without DNA sequences dengue detection by SiNWs/AuNPs-based electrochemical sensor can be an alternative technique to the gold standard nucleic acid detection, which relies on the agarose gel electrophoresis and southern/northern blotting.

### **1.4 Objectives of the study**

The goal of this study is to develop novel, simple and cost-effective DNA electrochemical sensor based on SiNWs/AuNPs-modified electrodes using methylene blue as redox indicator for early detection of nucleic acid dengue virus. The following specific objectives were designed to achieve this goal;

- I. To prepare and characterize the SiNWs/AuNPs-modified ITO and SiNWs/AuNPs-modified SPGE for electrochemical detection of dengue virus.
- II. To enhance DNA probe loading on the surface of SiNWs/AuNPs-modified ITO and SiNWs/AuNPs-modified SPGE for electrochemical detection of dengue virus.
- III. To optimize the electrochemical detection of dengue virus and its analytical performance using SiNWs/AuNPs-modified ITO and SiNWs/AuNPs-modified SPGE.
- IV. To evaluate the analytical performance of optimized DNA electrochemical sensor for the detection of genomic dengue virus from real samples.

## 1.5 Scope and limitation

In this study, the immobilized DNA probe on the modified electrode are specific for biorecognition sensing for nucleic acid dengue virus serotype 1 and 2. Therefore, there is a limitation in this study when employing this DNA electrochemical sensor for the detection of different serotype of dengue virus such as serotype 3,4 and 5, Furthermore, this DNA electrochemical could play detect the samples dengue virus for the first 5 days. After 5 days, the nucleic acid dengue virus was degraded upon the formation of antibody igG and igM.





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