DESIGN, FABRICATION AND CHARACTERIZATION OF FR4-BASED DNA LABEL FREE SENSOR INTEGRATED WITH POCKET-SIZED READOUT CIRCUITRY

IRNI HAMIZA BINTI HAMZAH

UNIVERSITI SAINS MALAYSIA

2013

DESIGN, FABRICATION AND CHARACTERIZATION OF FR4-BASED DNA LABEL FREE SENSOR INTEGRATED WITH POCKET-SIZED READOUT CIRCUITRY

by

IRNI HAMIZA BINTI HAMZAH

Thesis submitted in fulfilment of the requirements
for the degree of
Doctor of Philosophy

June 2013

ACKNOWLEDGEMENTS

First of all, praise is due to Almighty ALLAH with His compassion and mercifulness to allow me finalizing this PhD work.

I would like to acknowledge the Ministry of Higher Education (MOHE) for the financial support through Academic Training Scheme for Institutions of Higher Education (SLAI), which gave me opportunity to obtain the PhD in Universiti Sains Malaysia (USM), Engineering Campus, Transkrian, Malaysia.

I wish to express my sincere gratitude to my main supervisor, PROF. DR. OTHMAN SIDEK, director of Collaborative microElectronic Design Excellence Centre (CEDEC) for providing me an opportunity, supervision and grant to do my work. I would also like to thanks DR. ASRULNIZAM ABD MANAF, senior lecturer from School of Electrical and Electronic Engineering, USM, for his idea, patience, advice, assistance and grant in keeping my progress on schedule. My grateful thanks are also extended to DR. ZAINIHARYATI MOHD ZAIN for her help in doing analytical chemistry analysis, to MR. YU CHOO YEE, who helped me with DNA handling practice and biochemical data collection, to DR. CHAN YEAN YEAN for the continuous support of DNA supply and for allowing me to use the microbiology facilities in School of Medical Sciences, USM Kubang Kerian and to MR. SHUKRI KORAKKOTTIL KUNHI MOHD for his support in sensor measurements and development.

I would also like to extend my thanks to the technicians of Nano-Optoelectronics Research and Technology Lab, School of Physics, USM and CEDEC, USM for their support and kind help in offering me the resources available in the lab.

Apart from the effort of myself, the success of this work depends largely to my supportive husband AZLAN HISSHAM MOHAMAD SAAD, who have made me feel special even when times were tough. Much of what I have learned over the years came

as the result of being a mother to 2 wonderful and delightful daughters, WANI NADHIRAH and WANI HUMAIRA, all of whom, in their own ways inspired me.

Finally, I wish to thank my sisters, NOOR ZURINA HAMZAH and SITI NADIA NORDIN and my mother, WAN ZAINAB WAN ISHAK for their support and encouragement throughout my study.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Au	Aurum
ASTM	American Standard Test Measurement
CPU	Central Processing Unit
CE	Counter Electrode
Cu	Copper
CV	Cyclic Voltammetry
DC	Direct Current
DNA	Deoxyribonucleic Acid
DRIE	Dry Reactive Ion Etching
FR4	Flame Retardant 4
$K_3Fe(CN)_6$	Kalium Ferricyanide
KCl	Kalium Klorida
kPa	Kilo Pascal
MEMS	Microelectromechanical systems
PCB	Printed Circuit Board
PDMS	Polydimethylsiloxane
PMMA	Polymethylmethacrylate
RE	Reference Electrode
RF	Radio Frequency
SEM	Scanning Electron Micrograph
TEC	Thermal Expansion Coefficient
Ti	Titanium
USB	Universal Serial Bus
WE	Working Electrode

REKA BENTUK, FABRIKASI DAN PENCIRIAN TANPA LABEL PENDERIA DNA BERASASKAN FR4 BERSAMA LITAR BACAAN BERSEPADU BERSAIZ POKET

ABSTRAK

Silikon, kaca dan seramik adalah substrat yang biasa digunakan sebagai bahan asas dalam fabrikasi filem SU8 dan bidang penderia bio asid deoksiribonukleik (DNA). Semua substrat ini adalah bahan bukan serasi bio, mahal, keras dan rapuh, sekali gus sukar untuk penggerudian dan pemotongan, memerlukan peralatan yang mahal dan teknik fabrikasi yang kompleks. Retakan mikro sering ditemui pada filem SU8 kerana jurang besar pada nilai pekali pengembangan haba (TEC) antara silikon/kaca dan SU8. Di samping itu, peralatan dan aksesori bersaiz besar yang memerlukan antara muka dengan perkakasan dan perisian melalui 'central processing unit' (CPU) menghadkan kemudahalihan keseluruhan sistem pengesan bio. Oleh itu, matlamat kajian ini adalah untuk menyelidiki kesesuaian 'polymethylmethacrylate' (PMMA) sebagai substrat bahan asas untuk pembikinan filem SU8 dan aplikasi bahan bukan serasi bio, 'flame retardant 4' (FR4) sebagai substrat bahan asas untuk pengesan bio DNA tanpa label. Satu litar bacaan mudah alih, dua pemuka papan litar bercetak dan bersaiz poket untuk pengesanan DNA melalui kaedah pengukuran arus voltammetri berkitar (CV) telah dibangun untuk disepadukan dengan pengesan berasaskan FR4. Emas (Au) telah difabrikasi kepada seluruh permukaan FR4 dan pencirian pengesan berasaskan FR4 untuk melaksanakan proses berbalik CV telah disahkan oleh beberapa siri analisis. Kumpulan thiol pada akhir 3'-ssDNA digunakan untuk memegunkan DNA dengan permukaan Au dan bertindak sebagai DNA prob. Dua jenis DNA sasaran yang terdiri daripada urutan asas nukleo pelengkap dan urutan asas nukleo bukan pelengkap telah digunakan untuk menyiasat mekanisme hibridisasi dengan DNA prob. Hasilan daripada kajian ini mendapati kejayaan penggunaan PMMA sebagai bahan asas untuk fabrikasi acuan SU8 apabila dibakar di dalam oven pada

suhu 90°C dan 70°C. Ianya juga mendapati bahawa fabrikasi Au boleh dilakukan dengan penggunaan Cu yang bebas oksida sebagai lapisan lekatan pada FR4. Saiz kawasan elektrod kaunter (CE), elektrod kerja (WE) dan elektrod rujukan (RE) masing-masing perlulah berkeluasan 6.25 mm^2, 0.581 mm^2 dan 1.04 mm^2 demi mencapai hubungan pasangan redoks berbalik bersamaan dengan satu dan memastikan keutuhan pengesan ini untuk digunakan dalam cecair 10 mM $K_3Fe(CN)_6$ yang dilarutkan dalam cecair 0.1 M KCl. Bahan berasaskan FR4 untuk pelekatan dengan PDMS didapati menghasilkan kekuatan yang paling kuat iaitu 55 kPa, apabila dibiarkan kering dalam suhu bilik pada 25°C selama 6 jam. Fabrikasi bahan pengesan berasaskan FR4 menunjukkan perbezaan pada nilai arus puncak bagi hanya permukaan Au, pemegunan DNA dan penghibridan DNA dengan menggunakan peralatan CV komersil dan litar bacaan mudah alih dan bersaiz poket yang telah dibangunkan didalam kajian ini. Oleh itu, seluruh sistem yang lengkap terdiri daripada pengesan barasaskan FR4 dan litar bacaan bersaiz poket telah berjaya dibangunkan dalam kajian ini untuk tujuan pengesanan DNA.

DESIGN, FABRICATION AND CHARACTERIZATION OF FR4-BASED DNA LABEL FREE SENSOR INTEGRATED WITH POCKET-SIZED READOUT CIRCUITRY

ABSTRACT

Silicon, glass and ceramic are commonly base substrates used in SU8 film fabrication and DNA biosensor. All these substrates are biocompatible, expensive, hard and brittle, difficult for drilling and dicing, requires expensive equipments and complex methodology of fabrication. Microcracks are often found on SU8 film due to the large gap of thermal expansion coefficient (TEC) between silicon/glass and SU8. In addition, the use of bulky equipments and accessories that must be interfaced with hardware and software through a central processing unit (CPU) limits the portability of the whole biosensor system. Hence, the aim of this work is to investigate on the suitability of polymethylmethacrylate (PMMA) to be used as a base substrate for SU8 film fabrication and the application of a non-biocompatible material, flame retardant 4 (FR4) as a base substrate for a label free DNA biosensor. A portable and handy readout circuitry with double-sided pocket-sized of PCB for DNA detection through current measurement cyclic voltammetry (CV) method has been developed and integrated with the FR4-based fabricated sensor. The Au is fabricated throughout all conducting tracks on the FR4 and the characterization of the fabricated FR4-based sensor to perform CV reversible process is confirmed by a series of analysis. The thiol group at the 3'-end of ssDNA is used to link the DNA with the Au surface and act as probe DNAs. Two types of target DNAs which consist of complementary nucleobase sequence and noncomplementary nucleobase sequence are used to investigate on the hybridization mechanism with the probe DNAs. The outcome of this work found that the use of PMMA as a base substrate for SU8 mold fabrication is successfully achieved when it is hard-baked and soft-baked in the oven at 90°C and 70°C, respectively. It is found that the fabrication of Au has been made possible by the use of oxide-free Cu as an

adhesion layer on the FR4 substrate. The area sizes of CE , WE and RE are found to be 6.25 mm^2, 0.581 mm^2 and 1.04 mm^2, respectively, in order to achieve the unity reversible redox relationship and to ensure the sensor's reliability for 10 mM $K_3Fe(CN)_6$ solution in 0.1 M KCl. The FR4-based PDMS adhesive bonding is revealed to produce the strongest strength, 55 kPa, when left to dry in room temperature of 25°C for 6 hours. A fabricated FR4-based sensor denoted differences in the values of peak currents for bare Au, DNA immobilization and DNA hybridization using commercialized CV apparatus and a portable, pocket-sized readout circuitry that is developed in this work. Hence, the whole complete system consists of fabricated FR4-based sensor and the pocket-sized readout circuitry, which is successfully developed in this work for the DNA detection purpose.

CHAPTER 1

INTRODUCTION

1 Background

Cancer and cardiovascular diseases rank as the top three conditions of hospitalisation worldwide (Anderson & Chu, 2007). Globally, breast cancer is the most common cancer among women, comprising of 23% of all female cancers that are newly diagnosed in more than 1.1 million women each year (Parkin et al., 2005). More than 411,000 deaths result from breast cancer annually (Stewart & Kleihues, 2003). In Malaysia, the statistical data for the year 2006 reflects that breast cancer is a major disease that leads to fatality and affected 39.3 per 100,000 populations (Female Breast Cancer, 2006). Diet, stress, lack of exercise, obesity, high blood pressure, diabetes and high cholesterol are among factors attributed to this disease (Chong, 2010). Unfortunately, many patients are unaware that they are suffering from these diseases until it is too late. Thus, the best solution is through early detection. Early detection mechanism can be used to greatly reduce the cost of patient care associated with advanced stages of many diseases (Morrison et al., 2008). As an example, early detection of cancer will increase the rate of 5-year relative survival to 95 percent (American Cancer Society, 2013).

Early detection mechanism involves a routine of laboratory tests on small samples of urine and blood (Guller, 2006). Currently, cancer can be detected by monitoring the concentration of certain antigens present in the bloodstream other than bodily fluids or through tissue examinations (Morrison et al., 2008). This can reassure if there are any early signs of diseases such as kidney problems, diabetes and cardiovascular problems. Early detection enables the patient to be given the correct and optimal treatment which includes determining and avoiding substances that are causing the diseases to become more severe (The Star, 2012).

Conventional diagnostic methods are expensive, burdensome to patients and time consuming in which they require separated steps, highly trained personnel for laboratory analysis, costly laboratory equipments and circumscription to state-of-the-art laboratories (Belluzo et al., 2007). The emergence of biosensors abolished all these drawbacks of conventional diagnostics. A biosensor is a device that utilizes specific biochemical reactions mediated by isolated enzymes, immunosystems, tissues, organelles or whole cells to detect chemical compounds usually by electrical, thermal or optical signals.

1.1 Problems and Motivations

The use of silicon or glass as a base substrate for SU8 film fabrication has been widely reported (Wong et al., 2005) and (Edwards et al., 2000). However, it has been reported that microcracks are often found on fabricated SU8 film (Truong & Nguyen, 2003) due to large gaps of thermal expansion coefficients (TEC) between silicon/glass and SU8. The TEC of silicon is 3.2 ppm/°C (Watanabe et al., 2004), glass plate is 8.6 ppm/°C (Makishima & Mackenzie, 1976) and SU8 is 52 ppm/°C (Chronis & Lee, 2005).

Silicon, glass and ceramic are common materials used as a base substrate for gold (Au) fabrication by the means of immobilization with thiol group at the 5'-end of probe DNAs (Choi et al., 2005, 2006a), (Lee & Lee, 2004), (Choi, et al., 2006b) and (Cho & Pak, 2002). These substrates are biocompatible and not readily available in the market. Other than that, silicon and glass are hard and brittle, thus difficult for drilling and dicing. It requires complex methodology and equipments such as deep reactive ion etching (DRIE) and diamond-coated cutter for dicing.

Commercialized biosensor screen printed electrode (SPE) i.e; model 220AT produced by DropSens® utilizes different types of metals between sensing electrodes and terminal electrodes. DropSens® utilizes silver at the exposed terminal layer and Au at the sensing electrodes. The Material International Anneal Copper Standard (IACS) in

Metal Statistics claimed that the silver conductivity is 105%, whereas the conductivity of Au is 70%. The Chemical Rubber Company (CRC) Handbook has listed the electrical resistivity for silver and Au to be 15.87 nW.m and 22.14 nW.m, respectively. The differences in this conductivity and resistivity data proved that there is a possibility of a correlation of current measurement for different metals fabricated on the same electrode from the sensing chamber to the terminal.

The DNA-based biosensor system incorporates the sensor device and measurement readout circuitry. Biosensor companies are selling their parts separately and therefore, users have to purchase extra accessories to integrate with the sensor strips. Thus, it will cause an increase in the budget for the biosensor system. Moreover, the use of bulky accessories that must be interfaced with the computer limits the portability and in-situ measurement on the biorecognition elements (Esteban et al., 2011).

.

1.2 Research Objectives

Based on the problem statements that have been identified, it is clear that there are gaps in the development of biosensor technologies. Therefore, the objectives of this work are:

(i) To study on the suitability of polymethylmethacrylate (PMMA) to be used as a base substrate to replace silicon and glass for SU8-10 mold and polydimethylsiloxane (PDMS) fabrication.

The PMMA characterization via different grayscale values ranging from 0% (solid black) to 100% (solid white) is used to evaluate the percentage on the square structure formed through the mask and mold perimeter and area measurement.

(ii) To evaluate the suitability of non-biocompatible material, Flame Retardant 4 (FR4) for gold (Au) fabrication using thermal evaporator and

wet etching techniques throughout all conducting tracks from the sensing layer to the terminal layer.

The analysis is conducted in relation to the CV reversible redox analysis and Randles-Sevcik equation.

(iii) To develop a portable, pocket-sized of double-sided printed circuit board (PCB) readout circuitry.

The readout circuitry is able to be used for current measurement and acts as a voltage controller to be interfaced with FR4-based sensor for the application of cyclic voltammetry (CV) method.

(iv) To test and validate the integrated system for DNA detection through DNA immobilization and hybridization process.

1.3 Originality of the Research Works

A total of 3 main contributions have been carried out in this work. First, the use of polymethylmethacrylate (PMMA) as a base substrate for the SU8-10 mold fabrication. The PMMA has been chosen due to its properties that can be easily cut or drilled, biocompatible, transparent and its TEC value which is near to SU8-10 compared to silicon wafer or glass slides. The fabrication technique of SU8 mold on PMMA substrate has been carried out and demonstrated in details in this work. Secondly, the use of FR4 as a base substrate in the biosensor development has been demonstrated in this work. None of the published works reported on the application of FR4 in the biosensor fabrication. A standard fabrication of Au throughout the FR4 surface from the sensor layer to the terminal layer has been implemented by using a cost effective technique of thermal evaporation and wet etching. The characterization of fabricated FR4-based biosensor was further investigated using the CV analysis via the commercialized micro(μ)AutolabIII from Metrohm, KM Utrecht, The Netherlands, using the software package NOVA 1.4. Finally, the pocket-sized readout circuitry is to

be integrated with the fabricated FR4-based biosensor that has been developed in this work. A complete biosensor system from the sensor device to the readout circuitry system is the final outcome of this research work. The incorporation of a stable, efficient and portable biosensor system encourages the possibility of commercial applications significantly.

1.4 Thesis Organization

This thesis is organized in 7 chapters. Chapter 1 presents an overview of flame retardant and their applications. A review on the DNA labelling and label free sensor is presented in section 1.1. It is then followed by problem statements, research objectives and ends with the highlights on the originality of the current work.

Chapter 2 reviews in detail the fundamental concepts, current works that have been carried out and comparisons among biosensor techniques specifically in electrochemical biosensors. Then, section 2.3 discusses on the basic biochemical terms of redox and ferricyanide mediator. In this section, the theoretical interpretation of electrons' movements towards the Au surface reaction during bare Au, DNA immobilization and DNA hybridization is explained. Section 2.4 reviews on the materials that have been employed in biosensor fabrication; i.e., glass, silicon, ceramic, PMMA, SU8 and PDMS. The following sections outline the current development of PDMS adhesive bonding methodology, materials that have been used in electrode fabrication and screen printing technique that has been applied in the commercialized screen printed electrodes from previous works. Section 2.8 signifies on the readout circuitry which is designed and developed to be interfaced with the fabricated biosensor. The electronic component functionalities and circuit operation are also explained and presented.

Chapter 3 identifies the design methodology that will be carried out in this work. This includes apparatus and techniques for sensor fabrication which will utilize FR4 as

a base substrate, apparatus and chemical analysis techniques for the DNA, fabrication of portable readout circuitry and ends with the research flowchart.

Chapter 4 briefly discusses on the materials and methods for common reported techniques and substrates, glass and the proposed FR4-based fabrication technique that has been implemented in this work. The sensor fabrication technique begins with the mask design, mask preparation, substrate preparation and sensor patterning. This technique includes software that will be used in film processing, substrate cleaning procedures and soft lithography methodology on the sensor patterning. Then, the mold preparation technique will be elaborated. A technique on the use of PMMA as a based substrate for SU8 fabrication will be presented. The procedures include the PMMA preparation, SU8 coating and soft lithography on ultra violet (UV) exposure. A fabrication on PDMS relief and adhesive bonding technique performed in this work is straightforward and simple, yet produces a strong irreversible seal. The fabrication techniques of various adhesion metals such as the Ti/Au, Cu/Au with oxide/sulphide layer, Cu/Au without oxide/sulphide layer and Cu/Ni/Au have also been investigated for its suitability with FR4-based.

Chapter 5 studies on the adhesive bonding on PDMS relief in terms of the quality and shear strength test for 3 types of substrates; i.e., glass, FR4 and FR4 coated with a solder mask. The American Standard Test Measurement (ASTM) will be conducted in order to determine the stress value. The discussion on the results will be presented in detail. The effect of surface roughness will be concluded in the last section. The experiments will be carried out for 3 types of surface to prove the influence on the surface roughness towards PDMS to trap air bubbles. Section 5.2 reports on the CV analysis that has been performed on the well known DNA label free sensor of glass/Titanium (Ti) which has been reported in many works and journals. The analysis includes a review on the fabrication technique, effect on the area size of electrodes and effect of the insulated layer towards bare Au, DNA immobilization and DNA hybridization. All the results obtained from these analyses will be utilized for the

proposed FR4-based substrate sensor. The reliability test has been performed on the proposed FR4-based sensor. 3 types of CV characterization; i.e., peak potential separation, peak current ratio and peak current function in relation to the Randles-Sevcik equation which have been conducted and presented in this chapter. Chapter 5 ends with the application of fabricated FR4-based sensor for DNA detection analysis in which the oxidation peak current and reduction peak current have been performed in 4 conditions; i.e., bare Au, after DNA immobilization, after DNA complementary hybridization and after DNA non-complementary hybridization.

Chapter 6 explains on the built-in op-amps that will be utilized as a potentiostat to be able to integrate with the fabricated FR4 sensor and will be applied as an electrochemical measurement for the CV analysis. The flow on the proposed portable readout circuit operation and all the block diagrams will be inferred in this chapter. The ORCAD version 9.1 software is applied for the circuit design and fabrication. Comparisons between the fabricated FR4 based sensor and the commercialized model 220AT DropSens® on CV analysis by using the fabricated portable circuit will be presented. The comparisons include the mediator of distilled water (dH_2O) and ferricyanide redox reagent. In the last section, the experimental analysis and results of the proposed FR4 based sensor integrated with the portable readout circuitry towards all the conditions of DNA immobilization, the control analysis by using non-complementary DNA target of DNA non-hybridization and finally, the complementary DNA target of DNA hybridization.

Finally, conclusions are drawn and contributions of this work are highlighted in Chapter 7. A number of recommendations are listed for future research in improving this current work at the end of this chapter.

CHAPTER 2

THEORY AND LITERATURE REVIEW

2 Introduction

This chapter reviews on the previous works and research activities that have been conducted in the biosensor field. The focuses are mainly on the theoretical DNA, DNA detection methods, the growth of biosensor technologies, the properties and applications of common materials used for biosensor fabrication, polydimethylsiloxane (PDMS) adhesive bonding methods, other common substrates used for biosensor electrodes, measurement methods, types of transducer and readout circuitry. Sections 2.1 and 2.2 describe the DNA theory and detection methods. Section 2.3 elaborates on the property of flame retardant 4 (FR4) and its application whereas Section 2.4 explains in detail the biosensor and its history from as early as 1960s to date. Meanwhile, Section 2.5 views the definition of biosensor technology and its classification and types of system while Section 2.6 demonstrates the chemical reaction on ferricyanide redox reaction which focuses on the electron transfer mechanism and its mediator, DNA affixation method on the transducer biolayer and surface barrier morphology. Section 2.7 highlights on the materials that have been utilized in sensor fabrication mainly on the reported materials that have been used for base substrate. Section 2.8 explores the well-known method for polydimethylsiloxane (PDMS) sealing that required the use of oxygen plasma to change the PDMS surface characteristic from hydrophobic to hydrophilic. Recently, the use of oxygen plasma has been eliminated. The trend of using the adhesive bonding has changed to the use of chemical substances. Section 2.9 views on the materials that are commonly used as an electrode surface. Section 2.10 lists a few names of commercialized companies that produced sensor electrodes based on the screen printing technology. These screen

printing electrodes have been utilized as non-biological materials for their electrode. Section 2.11 deals with the electronic circuit used for the current measurement by using CV method known as potentiostat. The function of the potentiostat circuit diagram is explained in details in order to comply with the concept of redox reaction. Section 2.12 ends with discussion.

2.1 DeoxyriboNucleic Acid (DNA)

Nucleic acids interaction of DNA strands or known as DNA detection has been a major scientific interest due to its accuracy (Schiffman et al., 1995) and (Park et al., 2011), cheap (Hoogendoorn et al., 2000), fast (Ansorge, 1985) and reliability (Quint et al., 1995) and (Schiffman et al., 1995). DNA detection has been widely applied in the field of paleontology (Pereira, 2008) and (Kapitonov & Jurka, 2004), archaeology (Suzuki et al., 2010), molecular biology (Brown, 2001) and (Saghatelian et al., 2003), medical diagnostics (Li et al., 2011) and (Mathur et al., 2008) and forensic analysis (Heller, 2002).

Advances in DNA detection technology allow these nucleotides sequence to be investigated through the hybridization on base-pairing method of DNA probing and target. DNA sample contains white blood cells which are synthesized using detergent and all the useable DNA is separated from the extra cellular material (Luftig & Richey, 2001). These DNA must be denatured using heat or chemicals. Denaturing is a process by which the hydrogen bonds of the original double-stranded DNA are broken, leaving a lot of DNA stranded in which their bases are available for hydrogen bonding (Basu, 1968).

Scientists use this stranded DNA as probes or known as markers DNA to investigate on the other single stranded DNA as targets and hydrogen binding which will be formed to a complementary DNA sequence in the sample. Human DNA is

different from other living organisms in terms of sequence and characteristics. Another useful application on DNA profiling is the genetic fingerprints of parents and children. A child's genetic fingerprint is made of 50% of the father's genetic information and 50% of the mother's genetic information (Balding, 1995).

Another field that had attracted researchers to enhance the biosensor device system is in terms of readout circuitry and system identification for the fabricated sensor. Ayers et al. (2007) developed a complementary metal oxide semiconductor (CMOS) silicon based potentiostat circuit and Lee et al. (2010) applied DNA sensor chip with capacitive readout circuitry. However, all these previous works (Ayers et al., 2007) and (Lee et al., 2010) required the use of micromachining process and complexity of fabrication which are not typically available in the low-cost standard microelectronic processes. This work aims to develop a simple structure of Flame Retardant 4 (FR4) based substrate DNA label free sensor and integrated with the pocket-sized readout circuitry, in which the current output reading will be able to be detected via multimeter.

2.2 DNA Detection Methods

DNA detection method can be classified into 2 types, DNA labelling and DNA label free. The differences and details on these DNA are described in Sections 2.2.1 and 2.2.2.

2.2.1 DNA Labelling

DNA labelling enables the location of a particular DNA molecule i.e., on a nitrocellulose or nylon membrane, in a chromosome or in a gel to be determined by detecting the signal emitted by the marker. Some of the examples for DNA labelling are:

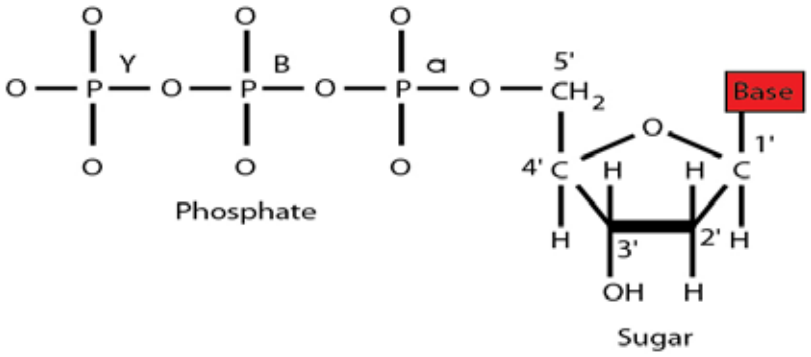
(i) southern hybridization,

(ii) fluorescent in situ hybridization (FISH),

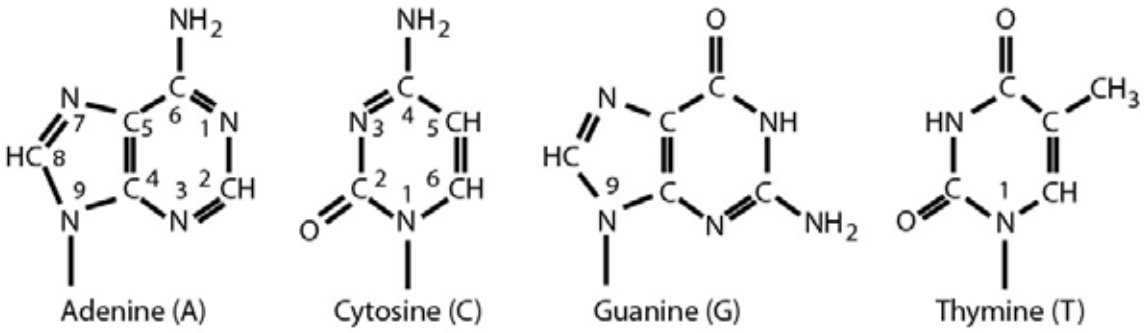
(iii) DNA sequencing and

(iv) chemiluminescent.

Radioactive markers are frequently used for labelling DNA molecules. Nucleotides are synthesized when one of their phosphorus atoms is replaced by ^{32}P or ^{33}P, one of the oxygen atoms in the phosphate group is replaced with ^{35}S and one or more of the hydrogen atoms are replaced with ^{3}H (Brown, 2002) as shown in Figure 2.1. However, radioactive markers are hazardous to the human health and environment. Therefore, fluorescent markers such as FISH, DNA sequencing and chemiluminescence have become popular as alternatives for DNA labelling. One major disadvantage of chemiluminescence is that the signal must be formed by treatment chemical substances i.e., the use of dioxetane towards enzyme alkaline phosphatise to produce chemiluminescent emission (Dodeigne et al., 2000).



(a)



(b)

Figure 2.1: The structure of radioactive markers displacement and four bases in DNA for nucleotides arrangement (Brown, 2002). (a) A structure of radioactive markers displacement in nucleotides. (b) The original structure of four bases nucleotides in DNA.

2.2.2 DNA Label Free

On the contrary, label free DNA requires no tagging or labelling in the nucleotides arrangement. Recently, a lot of reported works in DNA detection are focusing on the development of “label-free” or “self-labelled” in which the detection readouts can be measured directly from the analyte without additional target manipulation. Some of the reported works carried out by using these methods were optical sensors employing the molecular beacons (Tyagi & Kramer, 1996), (Dubertret et al., 2001), (Steemers et al., 2000), (Tsourkas et al., 2002) and (Fang et al., 1999), electrical (Fan et al., 2003), (Pan & Rothberg, 2005), (Drummond et al., 2003) and (Erdem et al., 2006), surface plasmon-based (Mannelli et al., 2005) and (Yao et al., 2006) and microgravimetric DNA detection systems (Janshoff et al., 2000) and (Su et al., 2005). This technique of label free DNA immobilization and hybridization has been utilized in this work.

2.3 Flame Retardant 4 (FR4) and Their Applications

FR4 is a composite material comprises of woven fiberglass cloth with an epoxy resin binder that is flame resistant. FR4 glass epoxy is a versatile with a high-pressure thermoset plastic laminate. FR4 glass epoxy possesses a good dielectric strength with a value of 20 MV/m (Colotti, 2005) with nearly zero water absorption (0.15%) (Colotti, 2005). FR4 is an electrical insulator with mechanical shear strength of 22k psi (Colotti, 2005).

FR4 is the most commonly used as a printed circuit board (PCB) material. The FR4 has also been used as electromagnetic energy harvesting for body-worn sensor (Hatipoglu & Urey, 2010) or body implantable devices (Fischell et al., 2003) due to its low Young's modulus (15-20 GPa) which is ten times smaller than silicon (130-188 GPa) (Hopcroft et al., 2010). FR4 is also a broadband due to its high intrinsic damping and these criteria make it suitable for energy scavengers to operate in a broadband environment (Hatipoglu & Urey, 2010). Figure 2.2 depicts a scanning electron micrograph (SEM) of FR4 board cross-section.

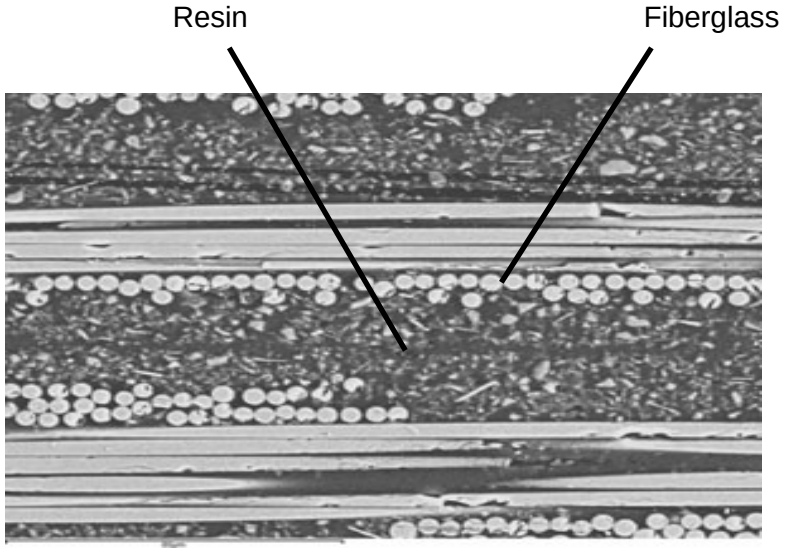


Figure 2.2: SEM of FR4 board cross-section ('The secret life of FR4 boards', 2011).

2.4 Biosensor

Early definition of the biosensor refers to any device that uses specific biochemical reactions to detect chemical compounds in biological samples. One of such examples is the first biosensor which is used to monitor glucose concentration in blood samples by using an enzyme-coated oxygen electrode by Clark and Lyons (1962). The diagram on this biosensor is illustrated in Figure 2.3.

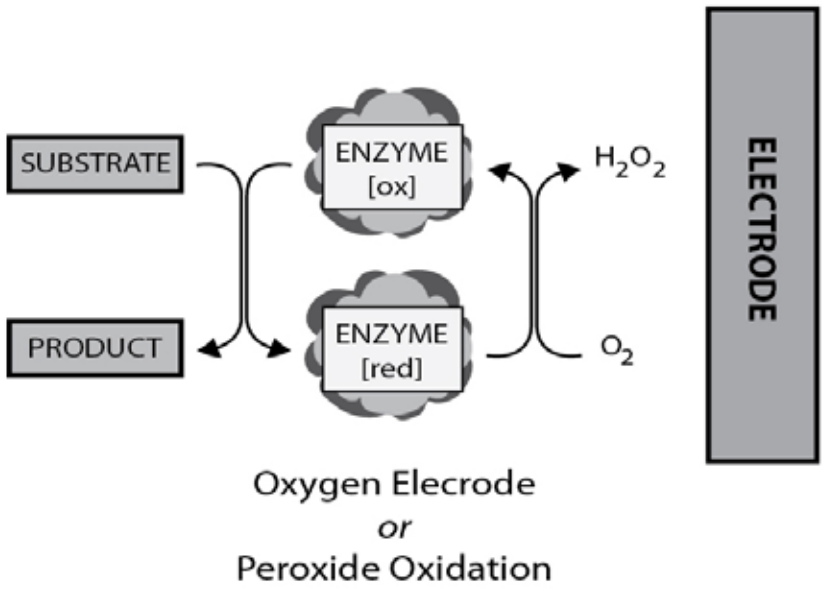


Figure 2.3: Biosensor schematic diagram on the first biosensor invented by Clark and Lyons (Newman & Turner, 2005).

According to the International Union of Pure and Applied Chemistry (IUPAC) in 1999, a biosensor is a self-contained integrated device that is capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element which is in direct spatial contact with a transduction element as shown in Figure 2.4. In other words, a 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.

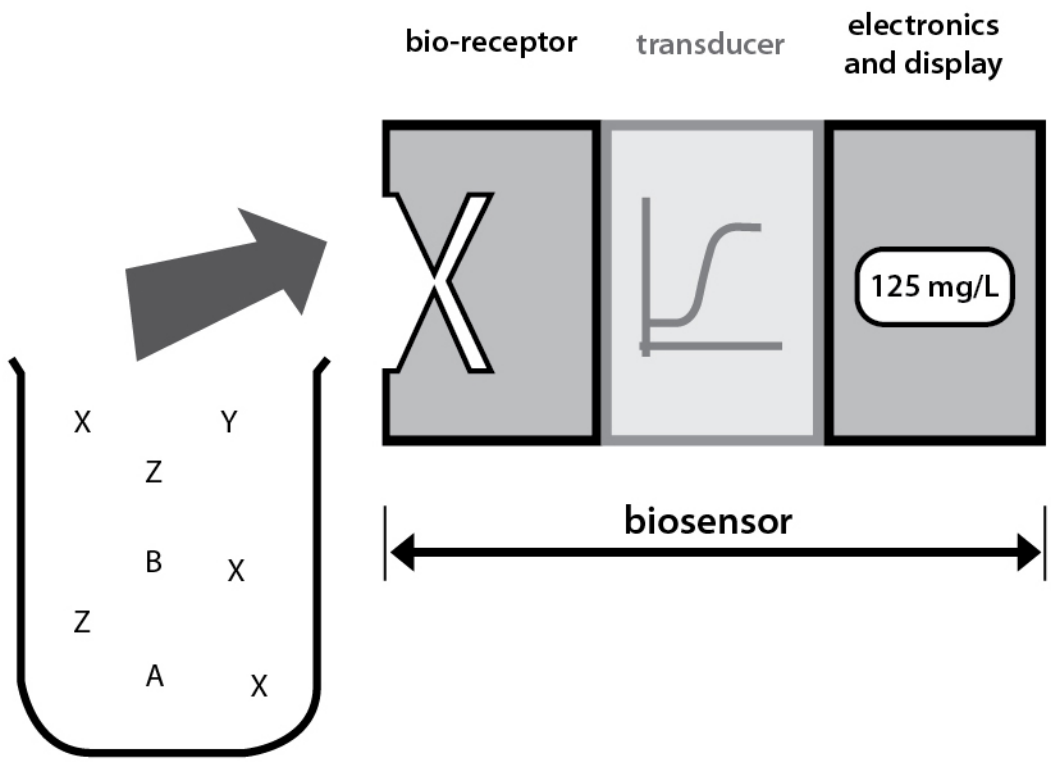


Figure 2.4: Principle of biosensor as defined by IUPAC (IUPAC, 1999).

The biosensor component defined by IUPAC is illustrated in Figure 2.5 (Maheshwari et al., 2010). A transducer (b) is the key part of a biosensor which makes a physical change accompanying the reaction from either one of the followings:

1. the heat output (or absorbed) by the reaction; such as thermistor biosensors,
2. changes in the distribution of charges causing an electrical potential to be produced; such as potentiometric biosensors,
3. movement of electrons produced in a redox reaction; such as amperometric biosensors,

4. light output during the reaction or a light absorbance difference between the reactants and products; such as optical biosensors,
5. effects due to the mass of the reactants or products; such as piezo-electric biosensors.

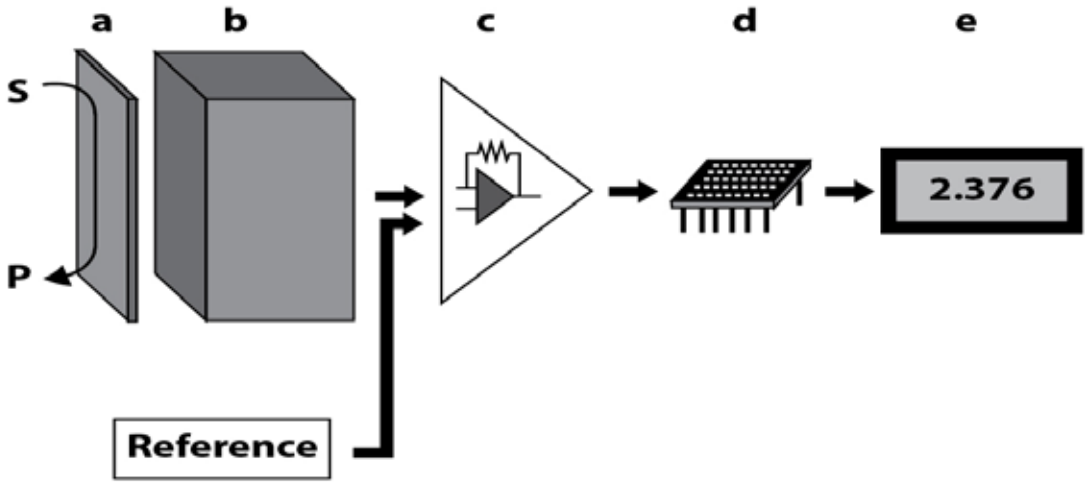


Figure 2.5: Schematic diagram showing the main components of a biosensor (Maheshwari et al., 2010). Biocatalyst (a) converts substrate (S) to product (P). Transducer (b) converts it to electrical signals. The output from the tranducer is amplified (c), processed by electronic microprocessor (d) and display (e).

An amperometric biosensor is the leading biosensor which is extensively used in the current research development and the most popular applications in biosensor systems due to its high sensitivity and wide linear range (Maheshwari et al., 2010). It combines the selectivity of the enzyme for the recognition of a given target analyte with the direct transduction of the rate of the biocatalytic reaction into a current signal, allowing a rapid, simple and direct determination of various compounds (Wang, 1999). Table 2.1 summarizes all the listed materials that have been implemented for the transducer.

Table 2.1: Materials and Substrate for Transducer Biolayer (Lee, 2000).

No	Types of Biosensor	Materials and substrate that have been used and reported in published journals
1	Amperometric	Electrode : Carbon, Platinum (Pt), Gold (Au), Nickel (Ni), Silver (Ag), Silver Chloride (AgCl) Receptor : Carbon paste, Electron Mediator, Lipid, Conducting Polymer Coated Wire : Pt, Cu, Fe, Polyvinyl chloride (PVC)
2	Potentiometric	Ion-selective Electrode : Glass, PVC, Ionopore Field Effect Transistor : Polymeric Encapsulant, p-n junction, Silicon Dioxide (SiO_2), Silicon Nitrate (Si_3N_4)
3	Piezo-electric	Surface Acoustic Wave/Gravimetric Detector : Silicon (Si), Platinum (Pt), Gold (Au), Quartz Surface Plasma Detector : Quartz, Glass, Silver (Ag), Gold (Au)
4	Optical	Optical Fiber Waveguide : Quartz, Glass
5	Calorimetric	Thermistor : Metal Couple, Oxide, Cantilever

2.4.1 Three Generations of Biosensors

Biosensors can be classified into three generations according to the attachment method of bio-receptor to the transducer. These three generations are illustrated in Figure 2.6. These three generations are described below:

(i) The first generation biosensor consists of the reaction diffuses to the transducer and causes an electrical response.

(ii) The second generation biosensor involves specific ‘mediators’ between the reaction and transducer in order to amplify response.

(iii) The third generation biosensor denotes the reaction itself causing the response and no product or mediator involved. This third generation is also known as direct electron transfer (DET) between the redox-active bio molecule and electrode surface.

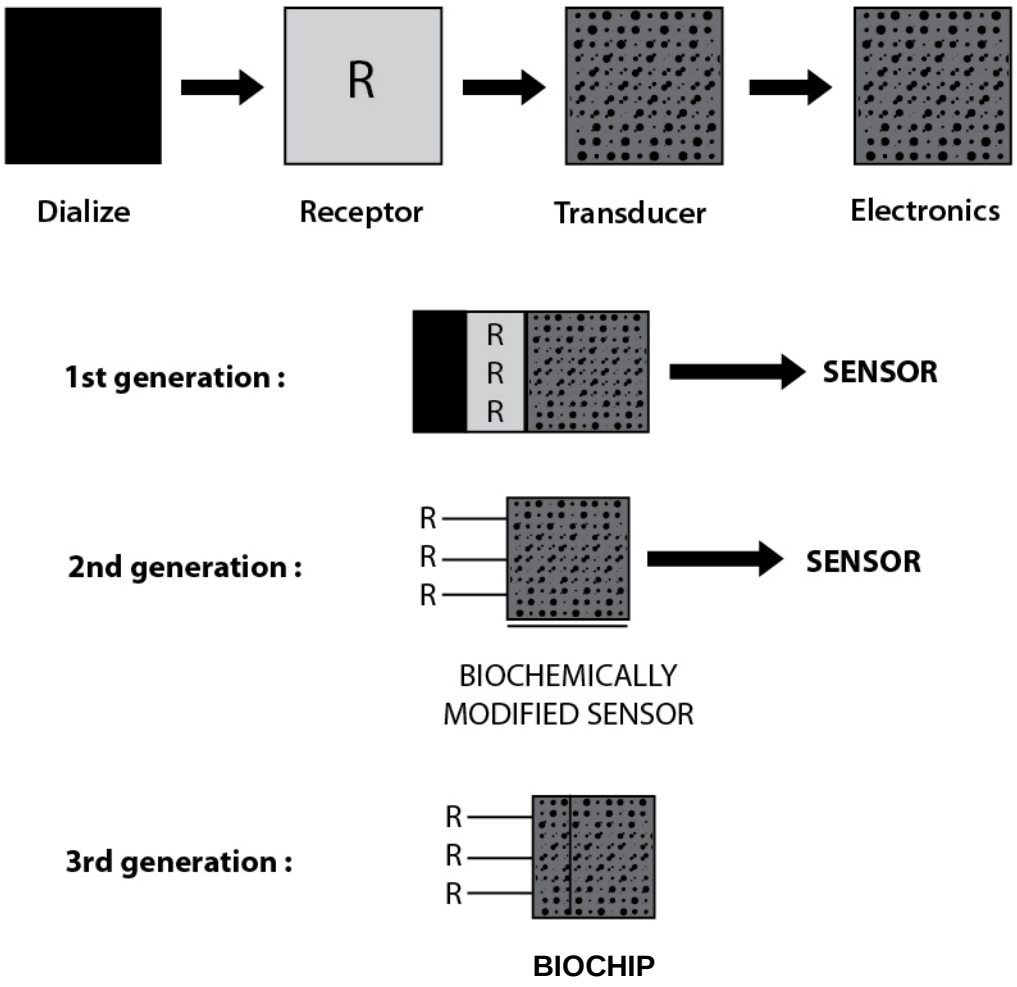
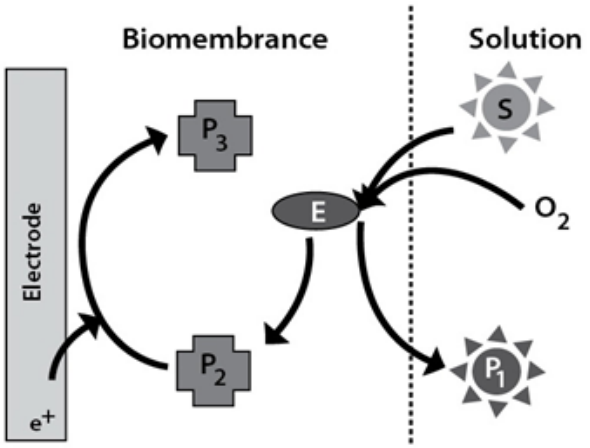
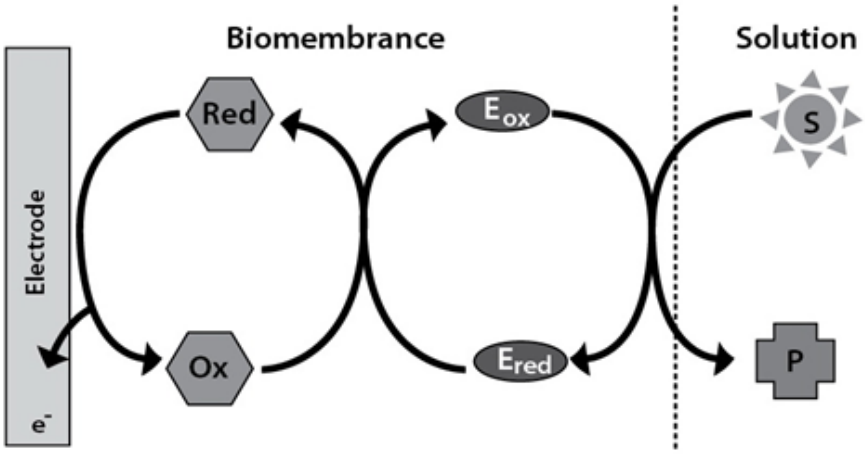


Figure 2.6: Three biosensor generations (Lee, 2000).

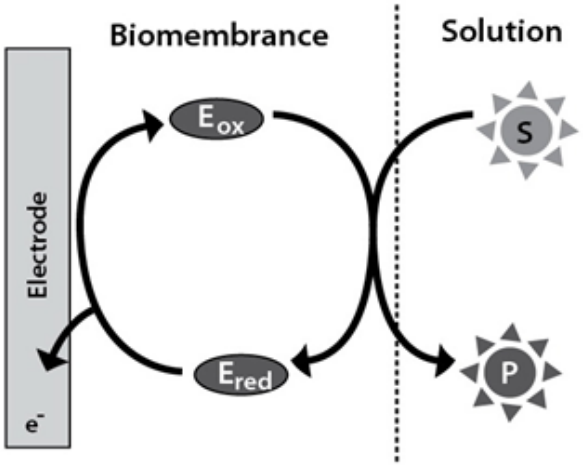
Three generations of amperometric biosensors are described in Figure 2.7. Figure 2.7(a) shows schematically the first generation of amperometric biosensor which utilizes the hydrogen peroxide (H_2O_2) and oxygen (O_2) to produce reaction. Figure 2.7(b) reflects the second generation in which the ferrocene acts as mediator to transfer electrons that have been produced to the electrode's surface. Finally, Figure 2.7(c) displays the third generation of direct electron transfer (DET) process that utilizes the electrons produced in the reaction.



(a)



(b)



(c)

Figure 2.7: Three generations of amperometric biosensors (Mousty, 2004).

2.5 Biosensor Technologies

Biosensor technologies comprise of four basic systems:

1. biological or physiological system in which refers to the analyte;
2. instrumentation or sensing system in which it refers to the instrument; highly accurate sensors;
3. electrical system in which it refers to the battery and circuitry;
4. electronic system in which it manages the conversion to an analog or digital display.

From this basic platform, different technologies have been constructed to develop biosensors for specific application. Figure 2.8 describes the key biosensor technologies that are currently in use. From Figure 2.8, the electrochemical, piezoelectric and optoelectronic biosensors have been proven to show growth in technology improvement.

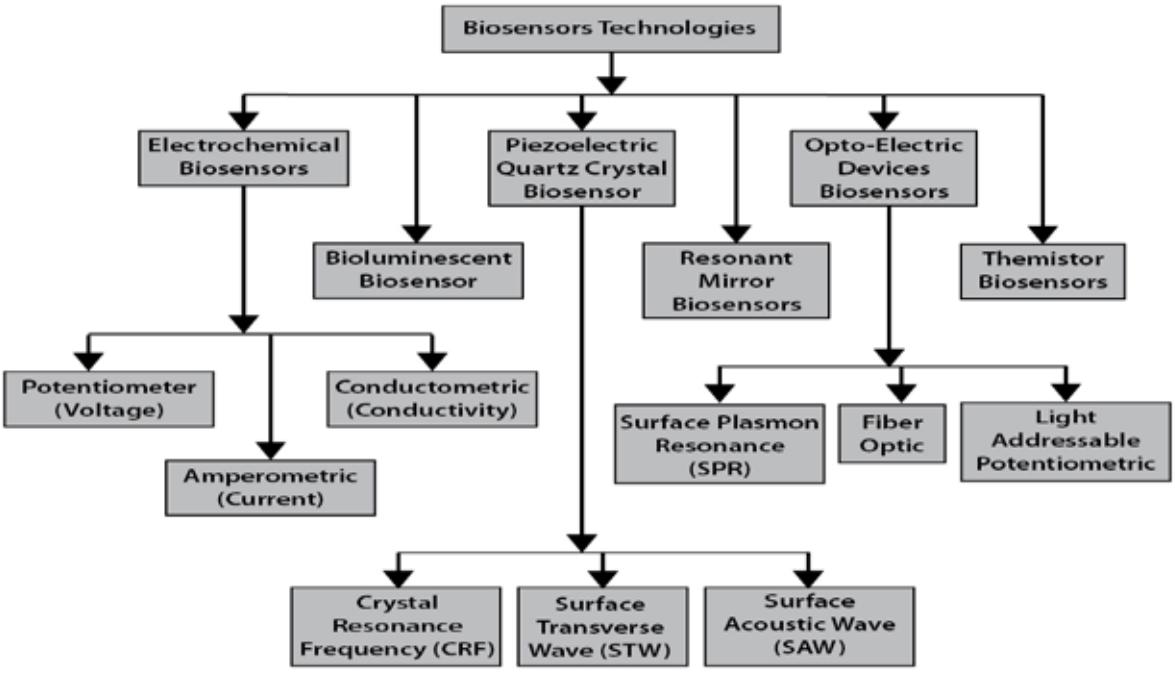


Figure 2.8: Different types of biosensor technologies (Thusu, 2010).

2.5.1 Electrochemical Biosensors

Majority of the reported biosensor technologies are based on electrochemical biosensors (Meadows, 1996). Electrochemical-based has been reported in literature by Tothill (2001), D'Orazio (2003), Bakker & Telting-Diaz (2002) and Bakker (2004). Stefan et al., (2000) and Warsinke et al. (2000) reported that the electrochemical-based biosensor was the most commonly cited method not only in the research literature but also in the application of clinical analysis. They claimed that the increasing application of electrochemical-based device was due to its improved design, stability and promising alternative as compared to the existing laboratory equipments. Wang (2002) recommended the application of electrochemical-based device for future large-scale generic testing. Gooding (2002) stated that the advantages of electrochemical based devices include low cost; high sensitivity; independence from solution turbidity; able for miniaturisation; portable and low power consumption. Guilbault et al. (2004) added the advantage of electrochemical in terms of response time which is almost the same as optical but faster than piezoelectric biosensors. Interestingly, Spichiger-Keller (1998) reported that electrochemical biosensor performance in terms of response and sensitivity is better when miniaturised (i.e., micro dimensions such as microelectrodes) which is contrary to the performance of optical biosensors. Electrochemical biosensors can be classified into amperometric (current measured); potentiometric (voltage measured); impedance (resistance and capacitance measured) and conductometric (conductivity measured).

2.5.1(a) Amperometric

Amperometric involves three electrodes system consisting of working electrode (WE), reference electrode (RE) and counter electrode (CE), which are well-known and extensively used in electrochemistry. An appropriate value of potential is applied at WE

to facilitate the transfer of electrons, RE measures and controls the WE potential whereas CE supplies the appropriate current needed by WE.

The fundamental principle of all electrochemical sensors is the transfer of electrons to or from the conduction band of an electronic conductor (usually metal or carbon) to or from a redox active species at the electrode surface (Patel et al., 2007). Oxidation involves the loss of electrons from the highest occupied molecular orbital whereas reduction involves electrons being injected into the lowest unoccupied molecular orbital. Figure 2.9 denotes the transfer of electrons to or from the gold (Au) surface as applied in this research.

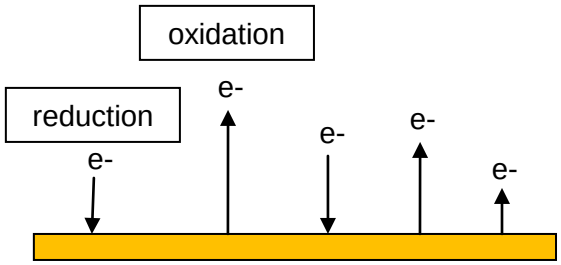


Figure 2.9: Oxidation and reduction of electrons from gold (Au) surface.

Au is used as a single layer for all three electrodes, i.e., WE, CE and RE. Gau et al. (2005) in his work reported on the use of Au as the reference electrode due to its low voltage difference which can be maintained for short periods of time and its properties of malleability and durability simplify fabrication and allow the use of extremely thin electrodes.

An electrochemical measurement method called cyclic voltammetry involves cycling the potential of an electrode and measuring the resulting current. The controlling potential applied across these two electrodes is an excitation signal.

Cost effective methods such as soft lithography by using thin film of photoresist mask expands the micro size design and Au fabrication technique on various material

substrates. Morita et al. (1988) reported on the use of Chromium (Cr) as an adhesive layer for Au evaporation on the silicon wafer substrate and Triroj et al. (2006) introduced the adhesion layer of Titanium (Ti) for Au evaporation on the glass substrate.

2.5.1(b) Comparison Among Electrochemical Biosensors Techniques

Table 2.2 summarizes the comparison of commonly used biosensor technologies as listed previously in Figure 2.8. As shown in Table 2.2, amperometric provides the best solution and approach compared to other methods of biosensors.

Table 2.2: Comparison on various biosensor technologies as described in Figure 2.8 (Parkinson & Pejcic, 2005).

Biosensors	Cost	Implantable	Selectivity	Sensitivity	Detection Strategy	Other Characteristics
Amperometric	Cheap	Yes	Very Good	Excellent	Indirect	Free from sample turbidity & versatile
Potentiometric	Cheap	Yes	Very Good	Very Good	Indirect	Free from sample turbidity
Fluorescence	Expensive	Difficult	Very Good	Excellent	Indirect	Time consuming
Electrochemical Impedance Spectroscopy	Expensive	No	Good	Good	Direct	Free from sample turbidity & versatile
Surface Plasmon Resonance	Expensive	No	Good	Good	Direct	Versatile
Chemilu minescence	Expensive	Difficult	Very Good	Excellent	Indirect	Limited to certain samples

Piezoelectric	Expensive	No	Good	Very Good	Direct	Free from sample turbidity
Thermometer / calorimeter	Cheap	Yes	Poor	Good	Direct	Limited capability

2.6 Redox Reaction

Redox reaction is the reaction that involved with electrons transfer between species. It is formed by the concepts of reduction and oxidation. An oxidation reaction and reduction reaction occur simultaneously to form a whole redox reaction. Figure 2.10 illustrates the process.

- **Oxidation** is the loss of electrons or an increase in oxidation state by a molecule, atom or ion.
- **Reduction** is the gain of electrons or a decrease in oxidation state by a molecule, atom or ion.

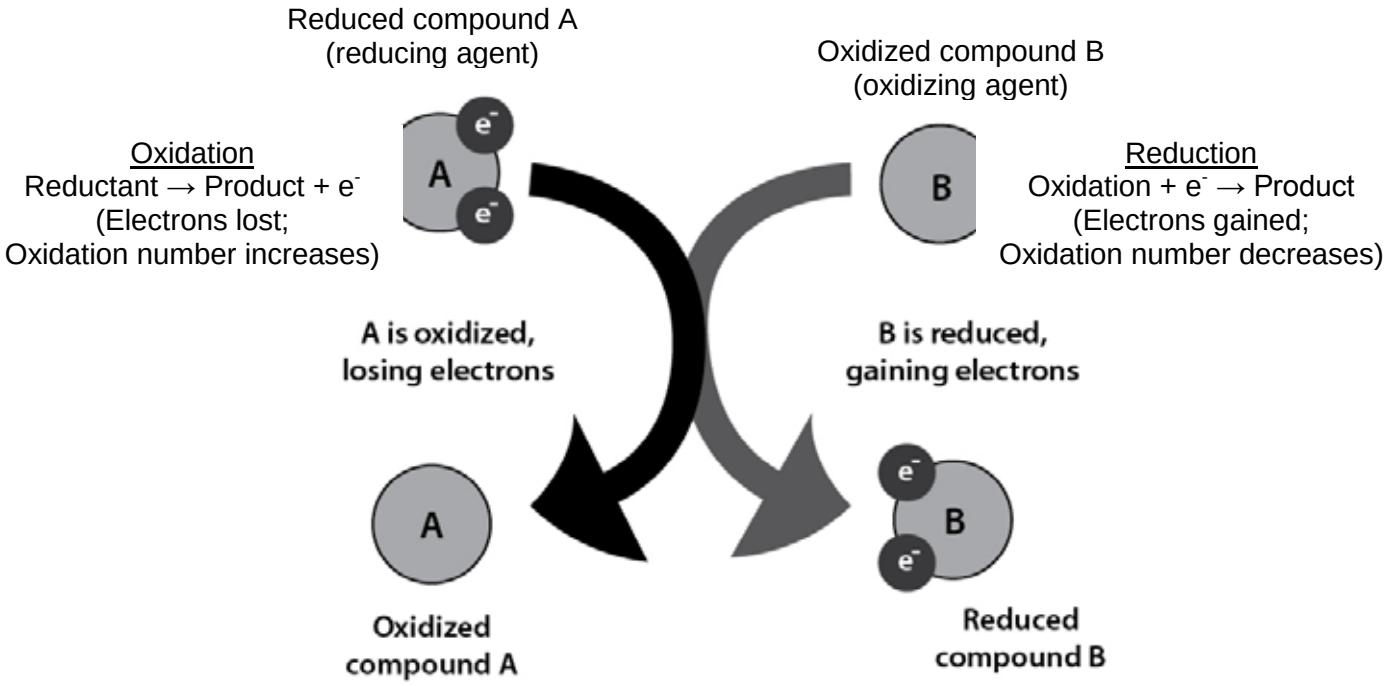


Figure 2.10: Passage of electrons from compound A to compound B (Purvis et al., 2003).