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Cyclic voltammetry readout circuitry for DNA biosensor application

I. H. Hamzah[1], A. A. Malik[2], A. F. A. Rahim[3], Z. H. C. Soh[4]
[1,3,4]Faculty of Electrical Engineering, Universiti Teknologi MARA, Cawangan Pulau Pinang, Malaysia
[2]School of Engineering, Penang Skills Development Centre, Penang, Malaysia

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ABSTRACT

Cyclic voltammetry electrochemical biosensors reported a wide usage and applications for its fast response, able to be miniaturized and its sensitivity. However, the bulky, expensive and laboratory-based readout circuitry made it impossible to be used in the field-based environment. A miniaturized and portable readout circuitry for the DNA detection using hybridization technique had been design and developed in this work. It embedded with fabricated FR4 based sensor and produced respective current when the applied voltage was within the range of 0 to 0.5 V. The readout circuitry had been verified with five analysis environments. Bare Au with distilled water (dH_2O), bare Au with ferricyanide reagent solution, DNA immobilization, DNA non-hybridization and DNA hybridization. All the results performed produced peak cathodic current when the applied input voltage is within 0.5 V to 3 V and hence proved that the miniaturized and portable readout circuitry is suitable to be implemented for cyclic voltammetry electrochemical biosensor.

Corresponding Author:

I. H. Hamzah
Faculty of Electrical Engineering
Universiti Teknologi MARA, Cawangan Pulau Pinang
13500, Permatang Pauh, Malaysia
Email: irnihami@uitm.edu.my

1. INTRODUCTION

Majority of the reported biosensor technologies were based on electrochemical biosensors [1, 2]. Electrochemical-based has been reported in literature [3-8] reported that electrochemical-based biosensor was the most commonly method cited not only in the research literature but also in the application of clinical analysis. Wangrecommended the application of electrochemical-based device for future large-scale generic testing [9]. Goodingand Nur Athilah et al. stated the advantages of electrochemical based devices as low cost; high sensitivity; independence from solution turbidity; able for miniaturisation; portable and low power consumption [10, 11]. Guilbault et al. and Ahmed et al. added the advantage of electrochemical in terms of response time which is almost the same as optical but faster than piezoelectric biosensors [12, 13].

Electrochemical biosensors methodology has been the focused in this work due to its fast response time compared to piezoelectric biosensors [14] and the sensitivity is better when miniaturized [15] compared to optical biosensors [16]. Electrochemical biosensors can be classified into amperometric (current measured); potentiometric (voltage measured); impedance (resistance and capacitance measured) and conductometric (conductivity measured). An amperometric biosensor is the leading, extensively used in the current research development, the most popular applications in biosensor systems due to its high sensitivity and wide linear range [17, 18]. 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 [19]. Potentiostats are widely used in electrochemistry techniques to identify, quantify, and characterize redox active species including inorganic, organic, biochemical species [20-22] and in evaluating kinetic parameters of electron transfer events [23]. Cyclic voltammetry (CV) is capable of determining thermodynamic and kinetic parameters of electron-transfer events [24], including such events in proteins [25].

Commercially available potentiostats tend to be bulky, expensive, laboratory-based and not field-portable with a few exceptions such as the PalmSens. Thus, they are not readily available to be used on-site for measurements. For these reasons, it was desirable to design and build a small, rugged, inexpensive potentiostat to interface with various types of electrochemical probes [23]. Therefore, the objective on this work is 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.

2. RESEARCH METHOD

Figure 1 shows a block diagram and schematic flows on the functionality of the fabricated readout circuitry in the current work. It started with the dc supply battery of 9 Volts. Then this value of voltage was fed to the voltage inverter circuit to produce negative voltage value. Subsequently, a voltage divider circuit has been applied to these negative value voltages to supply low negative input voltage to OP-AMP1. The OP-AMPS SYTEM acts as an CV electrochemical measurement system by measuring the output current via OP-AMP2 by varying input voltage value via OP-AMP1.

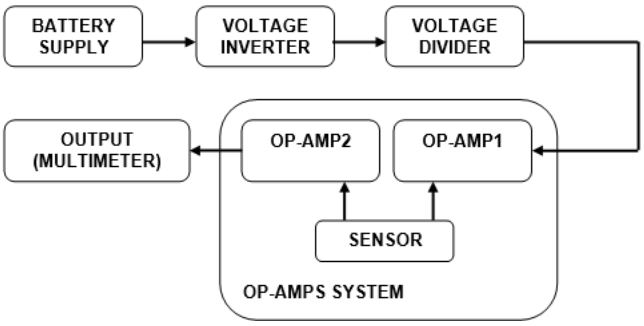

Figure 1. Block diagram on the flow of the readout circuitry

Figure 2 highlights all the detail circuitry on each of the block diagram as mentioned in Figure 1. Voltage inverter system is needed in this work to inverse the polarity of thepositive voltage in battery supply. The negative voltage supply is needed for two reasons, to invert the value of VIN as to produce the positive value and to produce negative voltage supply (-V) for the OP-AMPS. The implementation of 470 kΩ potentiometer at R3 for the voltage divider system made the voltage value possible to be varied. In this work, the input voltage has been varied from -800 mV to 0 V. R2 is set to 1.2 kΩ.

A voltage inverter system consists of three parts: an oscillator (CA555 timer IC) that converts DC in AC, a rectifier (diodes D1 and D2 1N1183) that converts AC to DC in negative output value and a voltage regulator (capacitor C3 220 µF) as a smoothing capacitor. The high period of the cycle takes 0.693×(R1+R2)×C1 seconds and the low period takes 0.693×R2×C1 seconds. With the values of R1=1.2 kΩ, R2=3.9 kΩ and C1=50 nF, this produces a nearly square wave at roughly 3.2 kHz at pin OUT IC CA555.

The output at $-V_{OUT}$ produced a negative output value ranging from -8 Volts to -7 Volts due to the voltage dropped in the voltage inverter system via diodes, resistors, charging and discharging process in the capacitors. The LM741 OP-AMP is used for OP-AMP1 and OP-AMP2 in which two types of +V and -V are needed to power up the OP-AMPS. In this work, both pin 7 of OP-AMPS are connected to the +V battery supply and both pin 4 of OP-AMPS are connected to the -VOUT. The output current is limited to 200 mA.

A sensor system is integrated with the USB port so that it can be plug-in with the designed FR4 based sensor. It contains three connectors to be adapted with the three electrodes of CE, RE and WE. OP-AMP1 and OP-AMP2 act as a main system for the readout circuitry to generate output current measurements due to the offset current of OP-AMPs by varying these low negative input voltages via OP-AMP1 as implemented in the CV method. R1 in OP-AMP2 block diagram is the potentiometer of 1kΩ value.

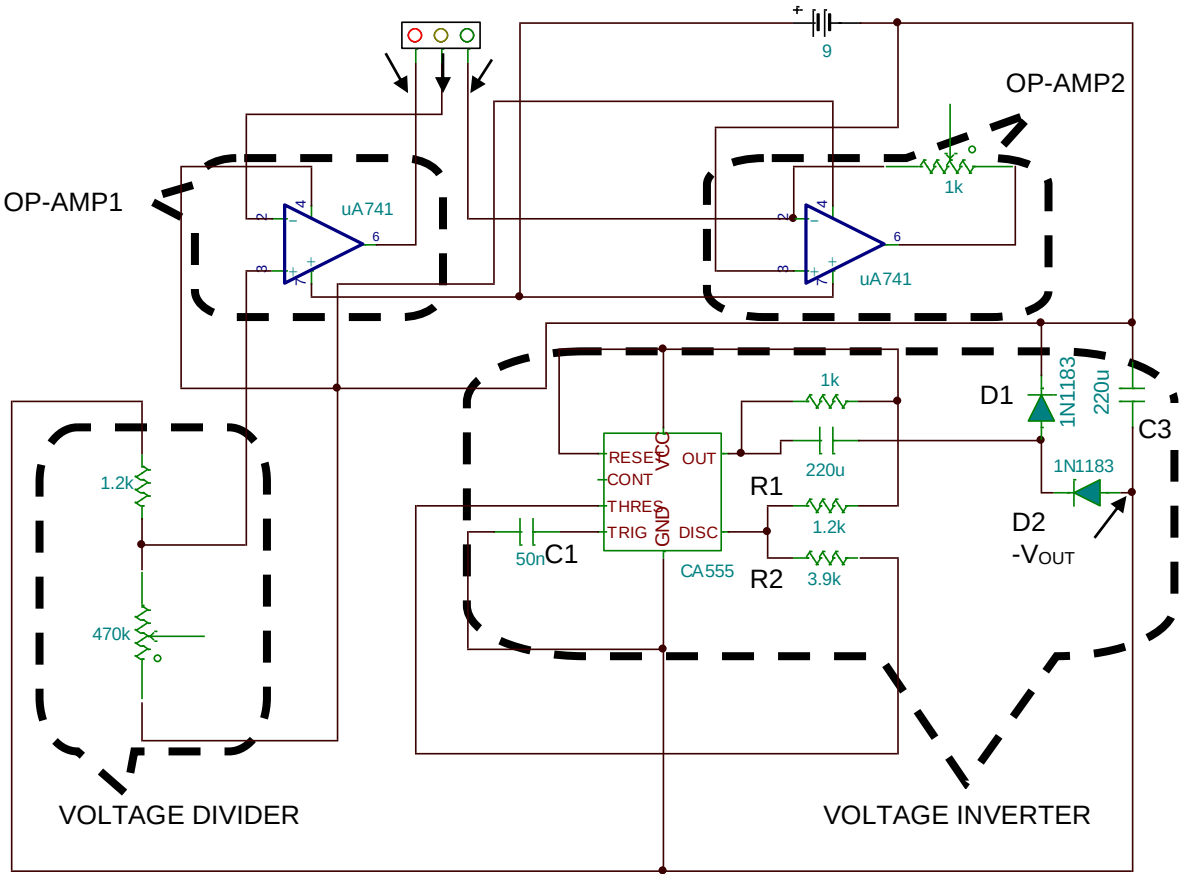

Figure 2. Detailed circuit on the block diagram as mentioned in Figure 1

3. RESULTS AND DISCUSSION

The detailed CV analysis and results performed on the fabricated FR4 based sensor as being produced in the work of Irni Hamiza et al., [26] is discussed in this section. The analysis is carried out in the medium of distilled water (dH_2O), ferricyanide redox reagent, DNA immobilization, a control analysis of non-complementary of the target DNA non-hybridization and ended with the complementary target DNA to ensure that the hybridization occurred in order to compare these two graphs obtained for the DNA non-hybridization and DNA hybridization The fabricated FR4 based sensor was plugged in to the readout circuitry as being developed and fabricated in this work.

3.1. Bare Au with distilled water (dH_2O)

The results from the sensor were described in Table 1. The analysis is done using 20 µl of dH_2O being pipette onto fabricated FR4 based sensor. The input voltage in the range of -0.1 V to -0.5 V is applied to the non-inverting input OP-AMP1. The objective on this analysis using dH_2O is to ensure that minimum redox activity occurred in the electrodes and therefore should produce nearly zero measurement in current readings.

Table 1. Analysis on sensors using dH_2O

Types of sensor	Input voltage	Output current
Fabricated FR4 based sensor	V_{IN} = -0.1 V	+0.01 µA
	V_{IN} = -0.2 V	+0.01 µA
	V_{IN} = -0.3 V	+0.01 µA
	V_{IN} = -0.4 V	+0.01 µA
	V_{IN} = -0.5 V	+0.01 µA

3.2. Bare Au with ferricyanide reagent solution

The results obtained from the sensor were described in Table 2. The analysis is done using 20 µl of ferricyanide redox reagent solution of potassium ferricyanide ($K_3[Fe(CN)_6]$) being pipette onto fabricated

FR4 based sensor. The input voltage in the range of 0 V to -0.5 V is applied to the non-inverting input OP-AMP1.

Table 2. Analysis on sensors using $K_3[Fe(CN)_6]$

Type of sensor	Input voltage	Output current
Fabricated FR4 based sensor	V_{IN} = 0.0 V	+1.5 µA
	V_{IN} = -0.1 V	+2.30 µA
	V_{IN} = -0.2 V	+1.40 µA
	V_{IN} = -0.3 V	+1.30 µA
	V_{IN} = -0.4 V	+0.90 µA
	V_{IN} = -0.5 V	+0.45 µA

3.3. DNA immobilization

Table 3 tabulates the data obtained for the experiment that is carried out for DNA probe immobilization process on FR4 based substrate. The process begins after the bare Au is pipette with ferricyanide redox reagent solution. DNA probe is left in the room temperature for 1.5 hours before it is pipette again with ferricyanide redox mediator and the results is recorded. Two samples of DNA probe immobilization is prepared in order to conduct the next experiment on the non-complementary DNA target and complementary DNA target.

Table 3. V_{IN} vs current generated for DNA probe immobilization

Input voltage	Output current
V_{IN} = 0.0 V	+1.20 µA
V_{IN} = -0.1 V	+1.90 µA
V_{IN} = -0.2 V	+1.30 µA
V_{IN} = -0.3 V	+1.10 µA
V_{IN} = -0.4 V	+0.80 µA

3.4. DNA non-hybridization

Table 4 tabulates the data obtained for the experiment that is carried out for non-complementary DNA target process on FR4 based substrate. The process begins after the previous process of DNA probe which is immobilized on FR4 based substrate sensor and the value of current generated is recorded for the dropped ferricyanide redox mediator. Non-complementary DNA target is pipette and left in the room temperature for 1 hour before it is pipette again with ferricyanide redox mediator and the results is recorded. This experiment is carried out as a control procedure in order to compare the results obtained for DNA non-hybridization and DNA hybridization process.

Table 4.V_{IN} vs current generated for non-complementary DNA target

Input voltage	Output current
V_{IN} = 0.0 V	+1.30 µA
V_{IN} = -0.1 V	+1.82 µA
V_{IN} = -0.2 V	+1.40 µA
V_{IN} = -0.3 V	+1.20 µA
V_{IN} = -0.4 V	+1.1 µA

3.5. DNA hybridization

Table 5 tabulates the data obtained for the experiment that is carried out for complementary DNA target process on FR4 based substrate. The process begins after the process of DNA probe is immobilized on FR4 based substrate sensor and the value of current generated is recorded for the dropped ferricyanide redox mediator. Complementary DNA target is pipette and left in the room temperature for 1 hour before it is pipette again with ferricyanide redox mediator and value of current generated is recorded.

Table 5.V_{IN} vs current generated for complementary DNA target

Input voltage	Output current
V_{IN} = 0.0 V	+0.83 µA
V_{IN} = -0.1 V	+0.80 µA
V_{IN} = -0.2 V	+0.75 µA
V_{IN} = -0.3 V	+0.80 µA
V_{IN} = -0.4 V	+1.00 µA

All these data obtained from Table 3 until Table 5 were compiled as a scattered graph in Figure 3. It shown that all the graphs for bare Au, DNA immobilization and DNA hybridization produced a clear and prominent gap in between of the peak oxidation current and thus proves that FR4-based integrated with pocket-sized readout circuitry is suitable to be used as a complete DNA sensor system. Graphs obtained from Figure 3 reflected that non-hybridization DNA data produced the results of peak current CV analysis close to the DNA immobilization as the size of the DNA formed on the surface sensor was nearly the same to the single stranded DNA probe. This is because non-complementary DNA target does not hybrid with the DNA probe and therefore the helical structure which is bigger than the single stranded DNA will not be formed and the surface barrier is nearly the same as the single stranded DNA probe.

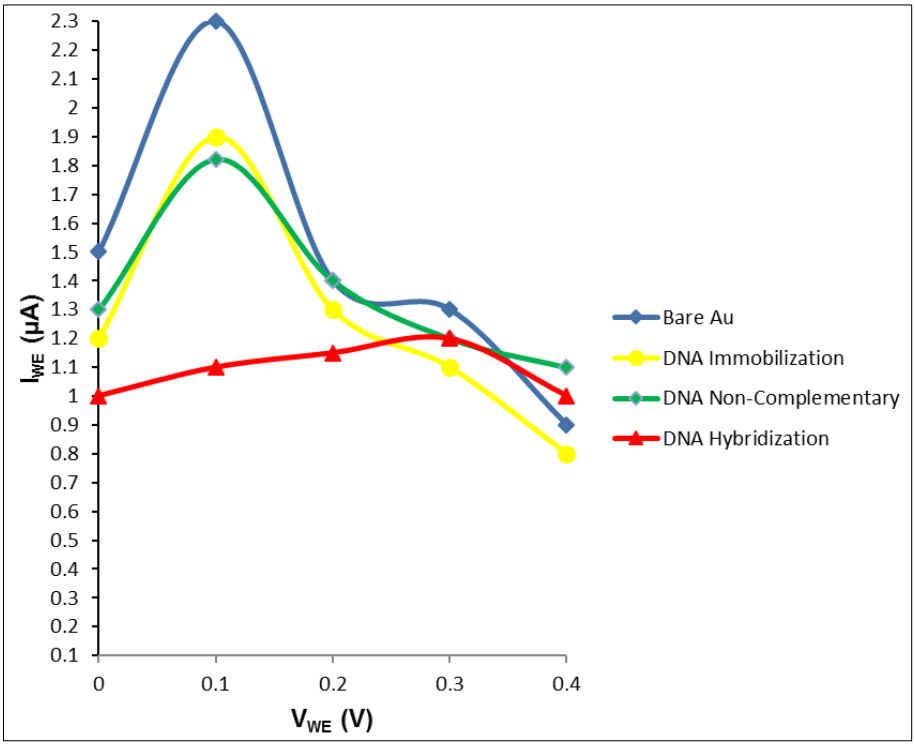

Figure 3. A CV analysis graph obtained from the portable readout circuitry for the DNA process

4. CONCLUSION

A fabricated FR4 based sensor generated a peak currents value for both anodic and cathodic in the range of 0 V to 0.5 V. Therefore, this suggested the novel fabricated FR4-based sensor utilized in this work is well-integrated with the portable and pocket-sized readout circuitry system as had been developed in this work due to the value of the input voltage needed to generate the peak currents laid within the small-scale voltage range.

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BIOGRAPHIES OF AUTHORS

Irni Hamiza Hamzah was born in Machang, Kelantan on 6th December 1974. She obtained her B. Eng (Hons) in Electrical and Electronic Engineering in 1998, MSc. Electronics System and Design Engineering in 2005 and PhD in BioMEMs Sensors in 2013, which all had been obtained from School of of Electrical and Electronic Engineering, UniversitiSains Malaysia, Malaysia. She is currently a Senior Lecturer in Electronic Engineering Department, Faculty of Electrical Engineering, UniversitiTeknologi MARA, Penang Branch Campus, Malaysia. Her research interests include Biosensors, BioMEMs, Neural Networks and Renewable Energy. She is a registered Board of Engineers Malaysia (BEM) Professional Engineer.

Azman Ab Malik Azman Ab Malik was born in Melaka on 12 October 2018. He obtained his Diploma in Electronic Technology from KKTM Pasir Mas in 2007, Bachelor in Electrical Engineering and Technology from UNIKL BMI, Master in Electrical and Electronic Engineering from USM and Phd in Electrical Engineering from UITM. His interest in innovation towards electrical and electronic and cross multi-disciplinary area to identify a new model or method in engineering. His research interest includes electrical power, power system, renewable energy, hybrid system, embedded system, wireless power transfer and energy storage. He is currently a Senior Lecturer in School of Engineering, Penang Skill Development Centre, Pulau Pinang, Malaysia.

Alhan Farhanah Abd Rahim obtained her B.Eng Hons in Electronics Engineering from University of Southampton in 1998, MSc and PhD in Solid State Physics from UniversitiSains Malaysia in 2003 and 2014 respectively. She is currently senior lecturer at the Faculty of Electrical Engineering, UniversitiTeknologi MARA, Malaysia. Her research interests are in synthesizing and fabricating advance semiconductor materials (group IV, III-V)) and devices utilizing low cost techniques. Her PhD research work entittle: Studies of Ge nanostructures Studies of Si and Ge Nanostructures Synthesized by Electrochemical and Plasma Assisted Techniques for Sensing Applications. She is author and co-author of over 20 scientific publications in this field. She is a companion member of Institute of Engineer's Malaysia (IEM) and a registered Board of Engineers Malaysia (BEM) Professional Engineer.

Zainal Hisham Che Soh was born in Machang, Kelantan on 16th March 1974. He obtained his B. Eng (Hons) in Electronic Engineering from University of Leeds, UK in 1997, MSc. in Computer Science in Real-Time Software Engineering from UTM in 2004 and PhD in Electrical and Electronic from USM in 2013. His research interest in Internet of Things, Big Data, Distributed/Parallel Computing, Artificial Intelligence, Microcontroller System and Wireless Sensor Network. He works in UiTMPulau Pinang under Faculty of Electrical Engineering, UiTM, Pulau Pinang. He is a member of IEE, IET, BEM and MySEIG.