INVESTIGATION OF PAPER-BASED SENSOR FOR SWEAT-BASED GLUCOSE DETECTION USING D-GLUCOSE SOLUTION

BY

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A thesis submitted in fulfilment of the requirement for the degree of Master of Science (Biosciences)

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ABSTRACT

Diabetes is known as one of the chronic diseases caused by the insufficient insulin produced by the pancreas to convert glucose into energy. Monitoring glucose level using glucometer is very crucial for every diabetic patient in order to avoid other health problems. However, this procedure is painful and can cause finger pricking anxiety for some of the diabetic patient. As a replacement for this uncomfortable glucose monitoring procedure, researchers have introduced an inexpensive and user-friendly non-invasive paper-based sensor. This research aimed to investigate the application of paper-based glucose detection using sweat, by utilizing D-glucose solution. Approximately, 50 µL of glucose oxidase-peroxidase (GOD-POD) enzyme and 1.67 µL of glucose sample had been utilized for small scale detection. The limit of detection for this kit was recorded at 15.63 mg/L concentration of D-glucose. A specific microfluidic design with 1 cm diameter sensor area was printed on filter paper using Xerox wax printer. Glucose oxidase-peroxidase (GOD-POD) enzyme was immobilized on filter paper in order to react with 1.67 µL different concentrations of D-glucose solution to mimic the proportion of glucose in human sweat. The intensity of the colour changes resulted from the reaction between D-glucose solution and GOD-POD enzyme was measured using spectrometer. The wavelength shift range in between 4.12 nm to 34.9 nm were observed. The linear graph showed a clear correlation between D-glucose concentration and the wavelength shift.

خلاصة البحث

يُعد مرض السكري من الأمراض المزمنة التي يسببها نقص الأنسولين الذي ينتجه البنكرياس لتحويل الجلوكوز إلى طاقة. تعتبر مراقبة مستوى الجلوكوز، باستخدام مقياس الجلوكوز، أمرًا بالغ الأهمية لكل مريض مصاب بداء السكري من أجل تجنب المشاكل الصحية الأخرى. ومع ذلك، فإن هذا الإجراء مؤلم ويمكن أن يسبب وخز الإصبع قلقا بالنسبة لبعض المرضى. وقدم الباحثون مستشعرًا ورقيًا غير مكلف، سهل الاستخدام، وغير جراحي كبديل لهذا الإجراء غير المريح لمراقبة الجلوكوز. يهدف هذا البحث إلى التحقق من تطبيق الكشف الورقي للجلوكوز باستخدام العرق من حيث الاستخدام المحلول الجلوكوز D. وتم استخدام 50 ميكرولتر من إنزيم الجلوكوز أوكسيديز بيروكسيديز (GOD-POD) و 1.67 ميكرولتر من عينة الجلوكوز للكشف تقريبًا على نطاق صغير، وتم تسجيل حد الكشف عن هذه المجموعة عند تركيز 15.63 مجم / لتر من الجلوكوز D. وتمت طباعة تصميم موانع جزيئية محدد بمساحة مستشعر قطرها 1 سم على ورق ترشيح باستخدام طابعة الشمع من زيروكس. وتم تجميد إنزيم GOD-POD على ورق الترشيح من أجل التفاعل مع تركيزات مختلفة من محلول الجلوكوز D 1.67 ميكرولتر لتقليد نسبة الجلوكوز في العرق البشري. وتم قياس شدة التغيرات اللونية الناتجة عن التفاعل بين محلول الجلوكوز D وإنزيم GOD-POD باستخدام مقياس الطيف. ولوحظ مدى إزاحة الطول الموجي بين 4.12 نانومتر إلى 34.9 نانومتر. وأظهر الرسم البياني الخطي ارتباطًا واضحًا بين تركيز الجلوكوز D وانحراف الطول الموجي.

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APPROVAL PAGE

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DECLARATION

I hereby declare that this thesis is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

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Mama ayah, this is for you.

Proud Eri.

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Miracle in December do exist.

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Concentration

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LIST OF SYMBOLS

mmol/L	Milimoles per liter
mM	Milimolar
O_2	Oxygen
H_2O_2	Hydrogen peroxidase
℃	Degree celcius
E_{photon}	Photon energy
h	Planck constant
v	Frequency
c	Speed of light
λ	Wavelength
A	Absorbance
I_0	Intensity of incident light
I	Transmitted light
ε	Molar absorption coefficient
l	Optical path length
c	Concentration of the sample
OH	Hydroxyl
NH_2	Amino
CHO	Aldehyde
SCH_3	Methyl Mercaptan
U	Unit of enzyme
µL	Microliter

M	Molarity
w/v	Weight per volume
L	Liter
mL	Mililiter
mg	Miligram
AU	Arbitrary unit
cm	Centimeter
Δλ	Wavelength shift
nm	Nanometer

LIST OF ABBREVIATIONS

IDF	International Diabetes Federation
PET	Polyethylene Terephthalate
FPCB	Flexible Printed Circuit Board
GOD	Glucose Oxidase
GOx-HRP	Glucose Oxidase-Horseradish Peroxidase
TBHBA	2,4,6-Tribomo-3-Hydroxy Benzoic Acid
GOD-POD	Glucose Oxidase-Peroxidase
WHO	World Health Organization
ASSURED	Affordable, Sensitive, Specific, User friendly, Robust and rapid, Equipment free, and Deliverable to those who need them
PBS	Paper-Based Sensor
HRP	Horseradish Peroxidase
UV	Ultraviolet
IR	Infrared
Cover SA	Cover Sample Area

CHAPTER ONE

INTRODUCTION

1.1 GENERAL BACKGROUND

Diabetes is known as one of the killer diseases alongside with heart attack and high blood pressure. According to International Diabetes Federation (IDF) - Diabetes Atlas (2019), 1 out of 11 adults have diabetes, 20% diabetic patients are above 65 years old, and 16.7% of birth rate are affected by hyperglycaemia during pregnancy.

Diabetes can be classified into three categories which are type 1, type 2 and gestational diabetes. Type 1 diabetes happened due to autoimmune destruction of the beta cells in pancreas and lead to inability of the pancreas to produce insulin. Meanwhile, Type 2 diabetes is a disease cause by the insulin resistance due to high glucose level in blood. Mostly, Type 2 diabetes occurred due to unhealthy diet and inactive lifestyle. Gestational diabetes develops during pregnancy, usually in second or third trimester. If not treated properly, diabetes can lead to chronic health problems such as cardiovascular disease, nerve, kidney, and eye damage.

Glucose is a type of sugar resulted from the breakdown of carbohydrate in stomach can be used immediately as energy once in bloodstream. Glucose also can be stored in our body to be used later. In order to be stored, insulin plays very important role. However, for diabetic patient, the amount of insulin is insufficient and can lead to high glucose level remained in bloodstream (hyperglycemia). Therefore, it is crucial for them, to monitor their blood glucose level in order to avoid any health complications. The most famous way to monitor it is by pricking the finger to get a

drop of blood and touch the edge of the strip test on the blood as shown in Figure 1.1. The result will be shown on glucose meter. However, this method is invasive, painful, and not user friendly especially among elderly and pediatric patients.

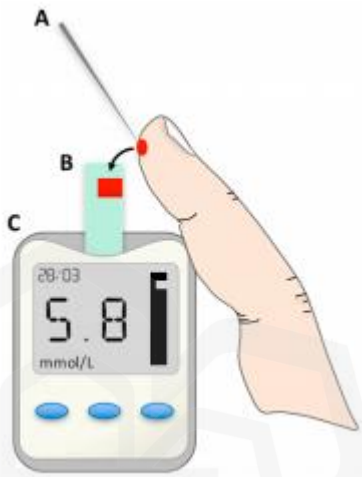


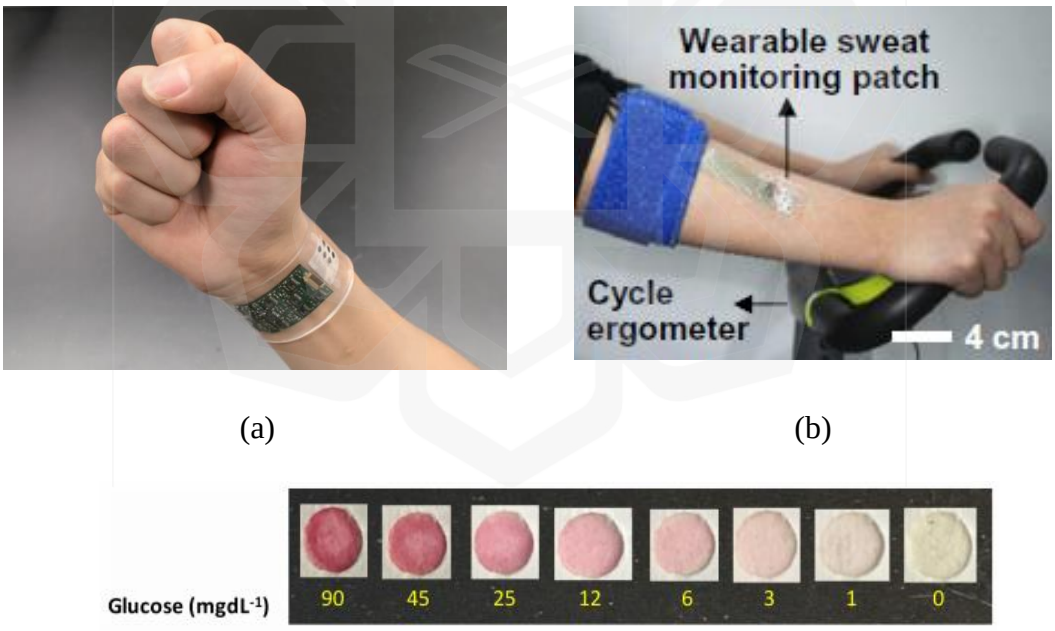
Figure 1.1 Conventional Glucose Monitoring (Bruen, Delaney, Florea, & Diamond, 2017)

Researchers had introduced non-invasive method as an alternative to invasive method in healthcare industry. Non-invasive is a procedure that no introduction of medical equipment into human body. This method is preferred since it is less pain and the patient's wound heal quickly. There were several non-invasive methods that had been introduced to detect the glucose level among diabetic patients by using body fluids other than blood. According to Bruen et al., (2017), urine, breath analysis, saliva, ocular fluid, and sweat can be used for glucose detection due to the presence of glucose and acetone as diabetes biomarker in them. The presence of glucose in urine or glycosuria happened when the kidney could not reabsorb the glucose back into the body due to high glucose level in blood. The concentration of glucose for diabetic patient in urine is more than 5.55 mM (Makaram, Owens, & Aceros, 2014). Saliva body fluid that plays an important role in digestive system and consist of glucose. If

the range of salivary glucose concentration are in between 0.55-1.77 mM, it is considered that the person has diabetes (Gupta, Sandhu, Bansal, & Sharma, 2014). Unlike other body fluids, for breath analysis, acetone is a biomarker for diabetes (Xing et al., 2015). For diabetic patient, the range concentration of acetone recorded is 0.1-103.7 ppm (Jiang et al., 2016). Sweat is a biofluid that can determine the glucose, alcohol, drugs and ion in blood (Jadoon et al., 2015). Glucose diffused into the sweat through the bloodstream can reflect the glucose level in blood. The glucose content in sweat is much smaller which is 1-2% compared to glucose in blood (Moyer, Wilson, Finkelshtein, Wong, & Potts, 2012). Compared to other body fluids, sweat can be access easily, easy to handle, no need sample preparation, and less likely to be contaminated since it come out to the skin surface.

There are many non-invasive glucose monitoring devices were invented with different types of materials and working principle. Polyethylene terephthalate (PET), patch, and paper are some of the materials had been used and tested in the manufacturing process of glucose monitoring device as in Figure 1.2. According to Gao et al. (2016), a sensor that can give *in situ* sweat analysis such as body temperature, ion, glucose, and lactate level was invented using PET. In this study, a flexible printed circuit board (FPCB) that consist of components of the sensor such as, transducer, amplifier, and wireless transmitter was embedded on PET. A wearable patch that can deliver diabetic medication (metformin or chlorpropamide) through skin and monitor glucose level simultaneously was developed (Lee et al., 2017). The advantage of transdermal drug delivery can prolong the period of drug released up to seven days (Prausnitz & Langer, 2008). This method can reduce the dose of medication to the patient, thus reducing the cost burden borne by the patient. However, since PET is a polymer and the patch use glue as adhesive, it can cause skin

problem to certain people. In addition, this material is not readily available. Unlike these two materials, paper is a material that has unique characteristics. It can transport liquid by using capillary action and able to hold liquid within the fiber web in it (Cate, Adkins, Mettakoonpitak, & Henry, 2014). Every type of paper has their own specifications such as pore size, thickness and weight. Filter paper is one of the materials that had been used widely since it is not treated with additives and most of the impurities are removed after the bleaching process (Yamada, Henares, Suzuki, & Citterio, 2015). Furthermore, filter paper is affordable, easily available anywhere, can be use directly, less or no modification needed, and since the colour is white, it is suitable for colorimetric glucose detection.



(a) (b)

(c)

Figure 1.2 Non-invasive (a) PET Band (Gao et al., 2016), (b) Wearable Patch (Lee et al., 2017), and (c) Paper Based (Santana-Jiménez, Márquez-Lucero, Osuna, Estrada-Moreno, & Dominguez, 2018) Glucose Monitoring Device.

Enzyme is a catalyst that can speed up the reaction by lowering down the activation energy without effecting the chemical reaction. The usage of the enzyme can provide a calorimetric naked-eye glucose detection. Glucose oxidase is an enzyme that can used in glucose detection. Soni and Jha (2015) had done an experiment for glucose detection in saliva using the mixture of glucose oxidase (GOD), methyl red and dithiothreitol reducing agent. The result from RGB colour analysis, blue colour showed the highest sensitivity compared to red and green. Later in 2018, Santana-Jiménez et al. used glucose oxidase-horseradish peroxidase (GOx-HRP) coupled with 2,4,6-tribomo-3-hydroxy benzoic acid (TBHBA) that act as chromophore that give red colour if glucose detected in the sample. The intensity of red colour depends on the amount of glucose in it. Glucose oxidase-peroxidase (GOD-POD) are the enzymes that produce quinoneimine dye as an indicator to the presence of glucose in liquid sample. The intensity of the colour changes from colourless to pinkish red reflects the amount of the glucose in it (S. Sharma, A. Anjankar, & A. Kale, 2017). In this research, GOD-POD enzyme was used since less preparation needed. Enzyme immobilization is a process that immobilize enzyme on any material. Enzyme can be immobilized on the paper substrate via physical adsorption as the positively charge ion from the enzyme and the negatively charge ion from the paper surface form an electrostatic force (Kong & Hu, 2012). This method is easy to handle, straightforward, and no instrument required.

Today, people are looking for non-invasive and not harmful point-of-care (POC) device accordance with the ASSURED criteria by World Health Organization (WHO). ASSURED stands for Affordable, Sensitive, Specific, User friendly, Robust and rapid, Equipment free, and Deliverable to those who need them. Sweat-based

glucose sensor is one of the applications of microfluidic paper-based device that meet these ASSURED criteria.

1.2 PROBLEM STATEMENTS

Conventional method for glucose detection is invasive and inconvenient especially among paediatric and elderly patient (Moyer et al., 2012). According to Shlomowitz & Feher (2014) 30% diabetic patients was reported has finger prick anxiety that caused the patient to avoid finger prick blood glucose testing. Unlike invasive method, non-invasive method can be less harmful and more patient friendly. According to Hsu et.al (2012), low-income group are more likely to get diabetes and could not get an appropriate diabetes care compared to high-income group. There are many things need to be considered in diabetes care such as, glucose test, medications, hospital visits, and haemodialysis and these costs a lot of money (Ganasegeran et al., 2020). This research aimed to produce a low cost, user-friendly paper-based non-invasive glucose monitoring device.

1.3 RESEARCH QUESTIONS

Based on the problem statement, there are three research questions:

1. What is the suitable amount of GOD-POD enzyme needed in order to produce a small sized paper-based sensor (PBS)?
2. What is the suitable material, size, design, and fabrication method for this PBS?
3. How to immobilize the GOD-POD enzyme on the PBS?

1.4 RESEARCH OBJECTIVES

By the end of this research, there are three objectives to be achieved:

1. To optimize amount of GOD-POD enzyme for a small sized PBS.
2. To fabricate a PBS for glucose detection.
3. To immobilize the GOD-POD enzyme on PBS.

1.5 RESEARCH HYPOTHESIS

1. A suitable small amount of GOD-POD enzyme that can fit into designated small sized sensor can be optimized.
2. A paper-based detector that can hold the amount of the GOD-POD enzyme can be fabricated.
3. The GOD-POD enzyme can be immobilized on the paper and work accordingly.

1.6 THESIS STRUCTURE

This thesis is divided into six chapters. In Chapter 1, a brief introduction of this research along with problem statements, research questions, objectives, and