Naked Eye Colorimetric Glucose Detection Using Microplate Reader

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Abstract. Diabetes in general can be classified as Type 1, Type 2, gestational diabetes mellitus that occur during pregnancy, and specific types of diabetes due to certain health condition. Monitoring glucose level is crucial in order to maintain a healthy lifestyle, especially among diabetic patients. Today, researchers looking for a less painful, cheap, and user-friendly non-invasive method to monitor the body glucose level. One of the promising approaches is sweat based glucose detection. In order to calibrate the sensing device, we determined the limit of detection of the glucose concentration in sweat liquid by the well-established enzymatic reaction. This paper will discuss the utilisation of a microplate reader to measure the sensitivity of the chromogenic change in reaction. 50µL of glucose oxidase-peroxidase (GOD-POD) enzyme and 1.67µL glucose sample with different concentrations were mixed in 96 well flat bottom microplate, and the absorbance were detected by using a microplate reader. The absorbance value was used to calculate the concentration. The calculated concentration was compared with the known concentration. From the experimental procedure, the results showed the consistency of calculated and known concentration and $R^2 = 0.9995$

Keywords: enzymatic, glucose oxidase-peroxidase, sweat based glucose detection

1. Introduction

Diabetes is a condition where the content of sugar in the blood is peculiarly high. 10% of grown-ups have diabetes, and by 2045, the total diabetes-related health disbursement was likely to reach USD 185 billion compared to USD 162 billion in 2019 [1]. The current situation has prompted researches to seek for cheaper, easy to use, and user-friendly glucose monitoring device.

Other than blood, sweat, saliva, ocular fluid, and urine are among promising body fluid to replace blood as an indicator to monitor glucose level. Human perspirations are known as sweating, are available on human skin most of the time, can act like a chip for someone's body condition [2]. The sweat glucose concentration range in human is between 0.01-1mM [3]. The concentration of glucose level in sweat is reported to be 1-2% compared to the glucose level in blood [4].

Colorimetric method on the sweat specimen approach, act as a non-invasive glucose detector using glucose oxidase-peroxidase (GOD-POD) enzyme was introduced as an alternative to the invasive, blood drawing method [5]. In this study, we discussed the application of microplate reader in order to determine the limit of detection of GOD-POD enzymatic glucose detection. The amount of enzyme and D-glucose sample was reduced to a minimal amount which is between the range of 50µL and 1.67µL.

2. Methodology

2.1. Reagents

Glucose oxidase-peroxidase (GOD-POD) reagent buffer (pH 7.4, 0.42M potassium phosphate, 0.35M p-hydroxybenzoic acid, 0.64% w/v sodium azide) and GOD-POD reagent enzymes (freeze dried, GOD; > 12 000 U, POD; > 650 U, 80mg 4-aminoantipyrine) were purchased from NZYTech. GOD-POD reagent buffer was diluted in 1L distilled water. Freeze dried GOD-POD reagent enzymes was dissolved in 20mL of diluted reagent buffer and then transferred into the remaining buffer solution. The bottle of the enzyme solution was wrapped with aluminium foil and stored at 4℃. The solution was prepared at room temperature.

2.2. D-glucose solution

D-glucose standard solution (1.0 mg/mL) in 0.2% (w/v) benzoic acid was purchased from NZYTech. Different concentrations of D-glucose solutions were prepared by simple dilution technique using distilled water. The concentrations were from 1000 mg/L to 0.5 mg/L. 1.67µL of D-glucose solutions were mixed with 50µL of GOD-POD enzyme solution in a 96 well flat bottom plate. The experiment was done at room temperature.

2.3. Instrument and measurement

Tecan Microplate reader was used to measure the absorbance of the colour changes of the reacted enzyme in 96 well flat bottom plate. The microplate temperature was set at 40℃ with an incubation time of 20 minutes. The wavelength measurement was 510nm, with 25 number of flashes. The absorbance value will be used to calculate the glucose concentration in the sample. These calculated concentrations will be compared to the real concentration in order to determine the limit of detection for this kit.

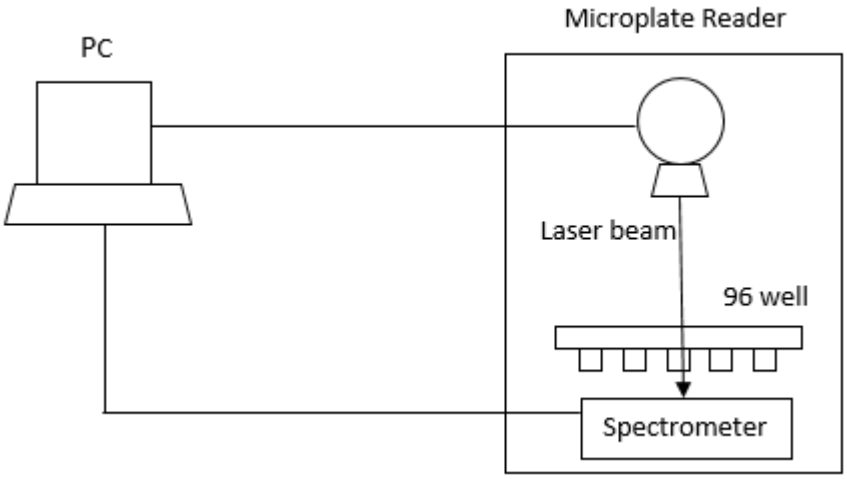

Figure 1. Microplate reader schematic diagram

3. Results and Discussions

3.1. Principle of GOD-POD enzyme

GOD-POD had been widely used to detect glucose. The principle for glucose detection is as follows:

$$\text{D-glucose} + O_2 + H_2O \xrightarrow{GOD} \text{D-gluconate} + H_2O_2$$

$$2H_2O_2 + p\text{-hydroxybenzoic} + \text{4-aminoantipirine} \xrightarrow{POD} \text{Quinoneimine dye} + 4H_2O_2$$

D-gluconate and hydrogen peroxidase (H_2O_2) were produced from the oxidation process in the presence of GOD enzyme. Quinoneimine dye was produced from the reaction between hydrogen peroxide, *p*-hydroxybenzoic, and 4-aminoantipirine in the presence of POD enzyme. The presence of red quinoneimine dye shows the presence of glucose. Different glucose concentration shows a different gradient of the red colour [6].

Figure 2. Colour changes occurred due to enzymatic reaction

3.2. Limit of detection

Small amounts of enzymes and samples were used in order to produce a point-of-care (POC) device in accordance with the ASSURED (affordable, sensitive, specific, user-friendly, robust and rapid, equipment free, and deliverable to those who need them) criteria by World Health Organization (WHO) [7]. In this experiment, in order to get the calculated concentration, the following equation was used:

$$C(D-glucose) = \frac{\Delta Sample\ Absorbance}{\Delta D-glucose\ Standard\ Absorbance} \times 1000$$

The absorbance value of the samples was measured at 510nm wavelength.

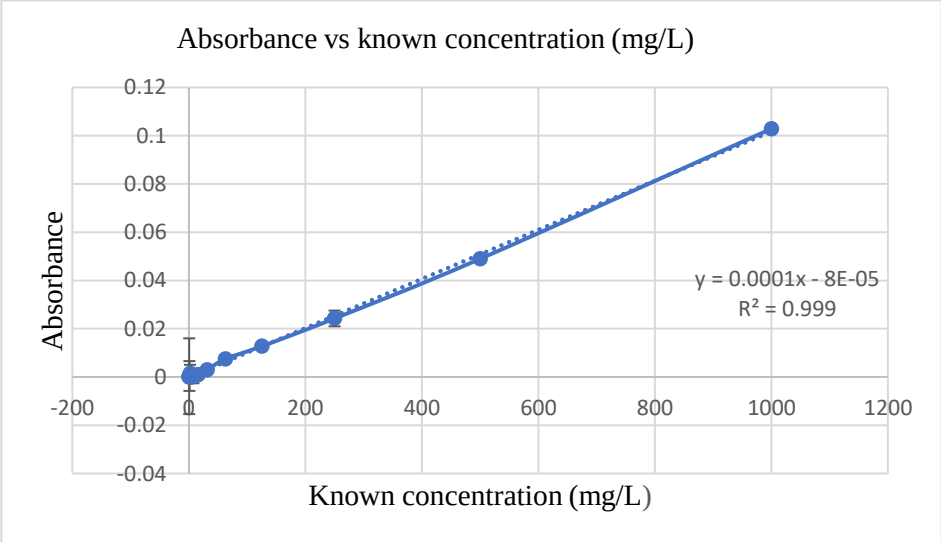

Figure 3. Absorbance vs known concentration graph

Table 1. Values of known concentration and calculated concentration

Known Concentration (mg/L)	Calculated Concentration (mg/L)
1000	1000
500	490.0411
250	241.0684
125	127.8561
62.5	75.08218
31.3	29.26105
15.6	10.49132
7.8	3.586157
4	-1.94623
2	13.42057
0.9	3.753811
0.5	3.307889
0	0

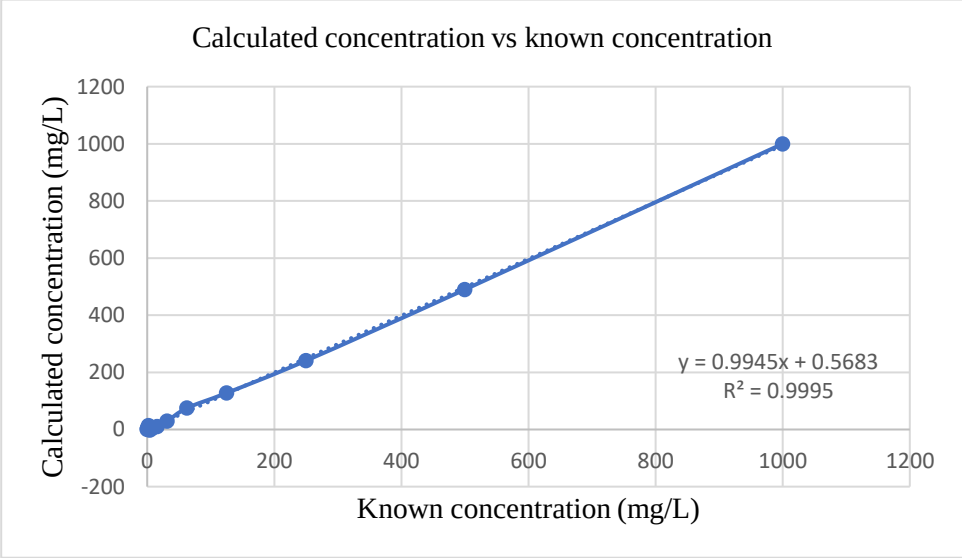

Figure 4. Calculated concentration vs known concentration graph

Results in Table 1 and Figure 4 show the consistent values from 1000mg/L until 15.6mg/L for both known and calculated values. However, starting 7.8mg/L until 0.5mg/L, the concentration values are inconsistent. Beer-Lambert Law that used in absorption spectroscopy, mentioned that the absorbance is proportional to the distance that light passes via the sample [8]. The equation is as follow:

$$A = log_{10}\frac{I_0}{I} = \varepsilon lc$$

Where: A = absorbance
I_0 = intensities of incident
I = transmitted light
ε = molar absorption coefficient
l = optical path length
c = concentration of the sample

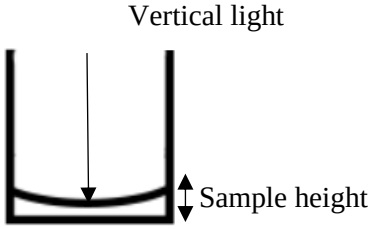

Figure 5. Flat bottom microwell light path sketch

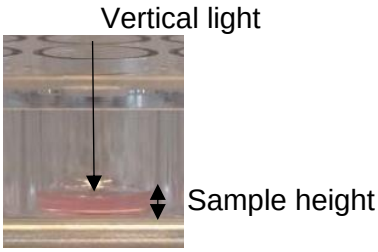

Figure 6. Flat bottom microwell light path

Vertical light was used in microplate reader, where the pathlength is dependent on the liquid volume. Since the sample volume used in this experiment is very small (51.67μL), the sample height is short, and this might affect the pathlength and the absorbance reading, especially for the low concentration sample. In order to overcome this problem, we are suggesting to use a u-shape plate instead, since this can increase the sample height with the same amount of sample volume, as well as improve the limit of detection of glucose.

4. Conclusion

In conclusion, the concentration of glucose can be measured using microplate reader. However, inaccuracy might occur if the volume is small. In future work, the u-shape microplate might help to overcome this problem. This experiment also is an early step for the development of non-invasive paper-based detector for sweat-based glucose detection.

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