

Glucose Detection in the Visible Spectrum Utilizing a Spectroscopy Method

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ABSTRACT: This paper investigates the direct detection of glucose in the visible light spectrum using a spectroscopy method. The method, which detects glucose without the need for additional enzymes or reagents, is developed using a light-emitting diode, a photodetector, and a data acquisition card (DAQ) to form a simple spectroscopy system. The system, operating at wavelengths of 505 nm, 700 nm, and 840 nm, has been experimentally tested for performance characterization. The experimental results show that the 505 nm operating wavelength produces optimal performance, with a linearity range of 20–45 mg/dL, a linearity value of 0.8812, sensitivity of $1.1 \text{ mV (mg/dL)}^{-1}$, stability precision greater than 99%, and a limit of detection (LOD) of 12.72 mg/dL. At this wavelength, the developed system improves sensitivity performance by about 69% compared to previous reports. This enhancement provides an alternative operating wavelength for glucose detection with better sensitivity performance.

1. INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by chronic high blood sugar and disruptions in the metabolism of carbohydrates, fats, and proteins due to issues with insulin secretion, insulin action, or both. The damaging effects of high blood sugar are classified into macrovascular complications, such as coronary artery disease, peripheral arterial disease, stroke, and microvascular complications, including diabetic nephropathy, neuropathy, and retinopathy [1]. Approximately 463 million individuals globally suffer from diabetes, while in Malaysia, it is estimated that 3.9 million (18.3%) of the adult population had elevated blood sugar in 2019, and these numbers are expected to grow [2]. The normal blood glucose level during fasting is below 100 mg/dL and should generally stay below 140 mg/dL after food consumption [3]. Meanwhile, the normal urine glucose concentration is about 8% of the plasma concentration, ranging from 0.3 to 1.1 moles or 100–300 mg/day/1.73 m² [4]. The presence of glucose exceeding 25 mg/dL, known as glucosuria, is a sign of uncontrolled diabetes or kidney dysfunction, as glucose is typically reabsorbed by kidneys [5]. Renal glucosuria is a condition in which glucose is excreted in the urine without the presence of diabetes and is significantly associated with atherosclerotic cardiovascular disease [3]. In essence, it is critical to monitor glucose levels for health assessment.

Various analytical methods have been employed for glucose detection, including spectrometry [6, 7], electrochemistry [8, 9], chromatography [10], and chemiluminescence [11]. Each method has its advantages and disadvantages. Spectrometry provides noninvasive methods that can monitor blood

glucose concentration continuously in real-time and can utilize different regions of the electromagnetic spectrum, such as near-infrared and mid-infrared [6]. However, spectrometry may have a low signal-to-noise ratio and can be sensitive to motion, which may cause variations in readings [7]. Electrochemical methods are highly sensitive and selective [8], but they require calibration and optimization of the electrode surface and electrolyte solution. Electrochemical methods also suffer from low selectivity and specificity, as other electroactive species can interfere with glucose detection [9]. Chromatographic methods, such as High-Performance Liquid Chromatography (HPLC), use separation as the first step in glucose analysis before proceeding to detection. However, the selection of detectors must match the separation process [10]. Meanwhile, chemiluminescence methods are highly sensitive and selective, but they require complex preparation, complicated operation, and are time-consuming [11]. Overall, spectrometry is chosen as the preferred method for glucose detection, as optical methods have been extensively researched due to their advantages, including the potential for minimal invasiveness or non-invasiveness, portability, cost-effectiveness, and selectivity [6, 12, 13].

A few progresses have been reported regarding the use of spectroscopy techniques in glucose detection for concentrations below 300 mg/dL [13–16]. The work reported in [13] and [14] has successfully sensed glucose in the near-infrared (NIR) spectrum, with operating wavelengths of 1550 nm and 940 nm, respectively. At 1550 nm wavelength, the spectroscopy system exhibits a linearity of 0.734 with a sensitivity of $0.1 \text{ mV (mg/dL)}^{-1}$ for concentration ranging from 0 to 200 mg/dL [13]. This wavelength demonstrates a moderate

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linearity value, which is below 0.85 [17]. Saad et al. demonstrated better linearity in glucose detection (50–300 mg/dL) using an LED and photodiode at 800 nm, 940 nm, and 950 nm. At 940 nm, the system showed a high linearity of 0.965 and sensitivity of $0.34 \text{ mV (mg/dL)}^{-1}$ [14]. Both studies in [13] and [14] mixed glucose with distilled water, but no interference studies were conducted. These NIR detection components are costly, prompting research into glucose detection at 660 nm and 780 nm [15]. In [15], glucose in human serum was detected at the human finger with a concentration range of 80–180 mg/dL. At 660 nm, the system had linearity of 0.7014 and sensitivity of $0.34 \text{ mV (mg/dL)}^{-1}$, though the performance was still low.

This paper investigates additional wavelengths in the visible spectrum to improve performance. In theory, glucose absorbance varies with wavelength [18], motivating the exploration of other potential wavelengths. This paper uses an LED at 505 nm, 700 nm, and 840 nm, with a photodiode connected to a National Instruments Data Acquisition Card (NI-DAQ) to capture light absorbance. Experimental details are provided in the following section.

2. PRINCIPLE OF ANALYSIS

2.1. Beer-Lambert Law

The Beer-Lambert law is the theory referenced to study glucose detection behaviour in this work. This theory states that absorbance, A , is directly related to the sample concentration, c , the path length of the sample, b , and the molar absorption coefficient, ε , of the sample, as stated in Equation (1) [19].

$$A = \varepsilon bc \quad (1)$$

where A is the measure of the amount of light absorbed by the solution, ε a constant that describes how strongly a substance absorbs light at a particular wavelength, b the length of the sample through which the light passes, typically the width of the cuvette or container holding the solution, and c the concentration of the solute in the solution. In this paper, detection was performed using a photodetector. Therefore, the absorbance value is represented by the voltage decrement, ΔV . To investigate the optimal operating wavelength for the system, linearity, sensitivity, and stability performances are analyzed. The theoretical concepts of each performance are discussed in the following section.

2.2. Linearity Performance

Linear calibration offers a reliable and versatile method for quantifying analytes using spectroscopy systems. It establishes a straightforward relationship between the analyte concentration and the measured signal, enabling accurate estimation of concentrations based on the calibration curve [20]. Linear regression, a well-established statistical method, is used to determine the best-fit line, simplifying the calibration process and minimizing the need for complex mathematical models. Linear calibration also reduces the impact of constant determinate errors. Thus, linear calibration is applied in this study based on the coefficient of determination, R^2 . The linear calibration

equation is presented in Equation (2) [21].

$$R^2 = \left\{ \frac{\sum_{i=1}^n (x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum_{i=1}^n (x_i - \bar{x})^2 \sum_{i=1}^n (y_i - \bar{y})^2}} \right\}^2 \quad (2)$$

where n is the maximum number of solutions, x_i the concentration of the solutions, \bar{x} the mean concentration, y_i is the light absorbance, and \bar{y} the mean light absorbance. The value of R^2 in Equation (2) ranges from 0 to 1, where 1 indicates the best linear relationship between glucose concentration and sensing output voltage, while a value of 0 represents the opposite condition.

2.3. Sensitivity Performance

In this paper, the sensitivity performance of the developed system is analyzed by examining the output voltage response to the sample concentration. The sensitivity of the system is represented by the slope of its voltage response. Mathematically, the system sensitivity can be calculated using the following formulation [22].

$$\text{Sensitivity} = \frac{|V_{\max} - V_{\min}|}{|C_{\max} - C_{\min}|} \text{V(mg dL}^{-1}\text{)}^{-1} \quad (3)$$

where V_{\max} is the maximum output voltage, V_{\min} the minimum output voltage, C_{\max} the maximum concentration, and C_{\min} the minimum concentration value. In general, a high-sensitivity spectroscopy system improves the system's ability to detect small changes in concentration, thus providing precise measurements [23]. For sensor systems that use reagents, a high-sensitivity system also reduces the amount of reagents required, thereby lowering the overall cost per analysis [22].

2.4. Stability Precision Performance

The stability precision performance of a spectroscopy system is examined by investigating the system's time response. The stability precision can be calculated using the following equation [24].

$$\text{Stability} = \left(1 - \frac{V_n - V_{\text{average}}}{V_{\text{average}}} \right) \times 100\% \quad (4)$$

where V_n is the voltage value of the n th measurement, and V_{average} is the average value of the stable set of n measurements. High stability and precision indicate superior operating stability due to chemical reactions throughout the detection process.

3. EXPERIMENT RESULTS AND DISCUSSION

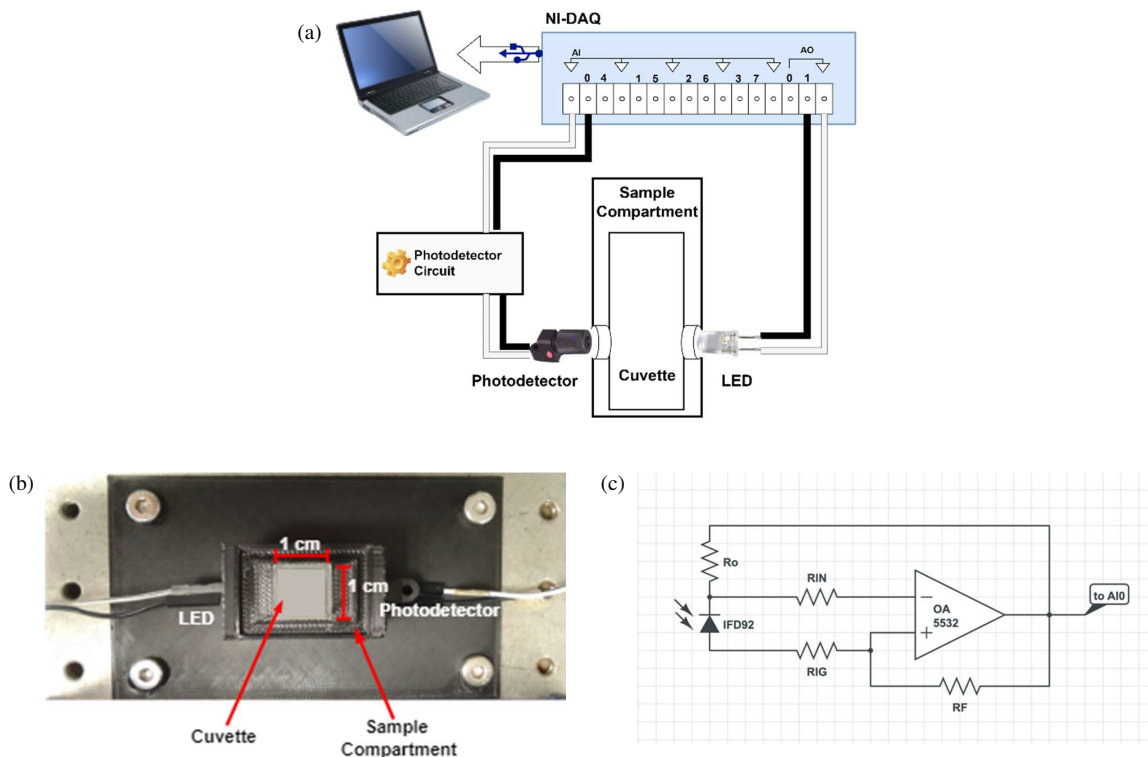
This section outlines the methodology for glucose sample preparation and the experimental setup used in this study.

3.1. Glucose Sample Preparation

The glucose stock solution used in this study was purchased from Axon Scientific. To prepare the glucose stock solution,

TABLE 1. Volume of stock solution and DI water for glucose sample preparation.

Sample Concentration (mg/dL)	Volume of Stock Solution (mL)	Volume of DI water (mL)	Volume of Sample (mL)
20	20	30	50
25	25	25	50
30	30	20	50
35	35	15	50
40	40	10	50
45	45	5	50

**FIGURE 1.** Experimental setup for glucose detection. (a) Block diagram. (b) Sample compartment. (c) Photodetector circuit.

a 20% glucose stock solution (molecular weight 180.16 g/mol) was diluted in deionized (DI) water to achieve the desired concentrations. Initially, a 50 mg/dL glucose solution was prepared by diluting 6.25 mL of the 20% glucose solution with 243.75 mL of DI water to produce approximately 250 mL of stock solution. During the dilution process, the sample was stirred for about 3 minutes to ensure uniformity. Then, sample solutions with concentrations of 20, 25, 30, 35, 40, and 45 mg/dL were prepared according to the molarity formula. All samples were prepared at room temperature. Table 1 shows the amount of glucose solution to be added to DI water to prepare each glucose sample.

3.2. Experimental Setup

The experimental setup to study glucose detection in the visible spectrum is shown in Figure 1. As illustrated in Figure 1(a),

the system consists of a National Instruments data acquisition card (NI-DAQ), model NI-USB-6003, with 16-bit ADC resolution and a maximum sampling rate of 100 kS/s, a light-emitting diode (LED) from Roithner Lasertechnik with operating wavelengths of 505 nm, 700 nm, and 840 nm, a 3D-printed sample compartment, an IF-D92 Phototransistor from Industrial Fiber Optics Inc., and a computer for data processing. In this setup, the LED is supplied with a stable input voltage from the NI-DAQ card. The LED is driven by a stable, programmable DC voltage from the NI-DAQ's analog output channel. The LED's positive terminal connects to the DAQ output AO1, while the negative terminal connects to the ground, forming a complete circuit.

The system, operating with the LED, is arranged to produce a constant output voltage, V_o , of 4.7 V as its initial output, V_{oi} . The LED is connected to the sample compartment, as shown in Figure 1(b). The other end of the sample compartment is con-

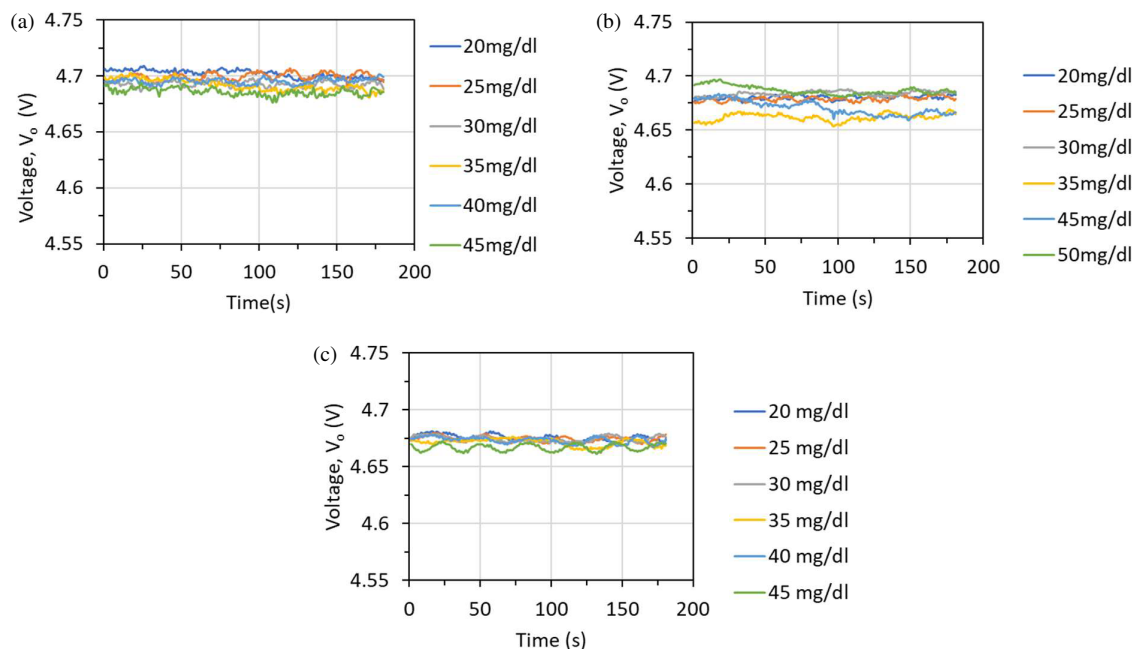


FIGURE 2. Spectrometer time response up to 180 seconds duration. (a) 505 nm wavelength, (b) 700 nm wavelength, (c) 840 nm wavelength.

nected to a photodetector that is aligned with the LED. The LED and photodiode are separated by approximately a 1 cm gap, creating a spectrometer path length for light-sample interaction in the compartment. The light interacting with the sample is detected by the photodiode and then processed by the computer via the NI-DAQ card. To enhance the linearity performance of the detector, an operational amplifier circuit is developed and inserted in between the photodiode and NI-DAQ card. The schematic diagram is illustrated in Figure 1(c).

4. RESULTS AND DISCUSSION

This section discusses the results obtained from the experimental work. The performance of the system is analyzed in terms of its stability, precision, linearity, sensitivity, and limit of detection (LOD).

4.1. Analysis of System Stability Precision

Time response analysis of the system was conducted for glucose samples with concentrations of 20, 25, 30, 35, 40, and 45 mg/dL for 180 seconds. For each spectrometer operating wavelength, the output voltage, V_o , for all concentrations is plotted in Figure 2. As shown, all three operating wavelengths were able to quantify the concentration value, as the V_o differs for different glucose concentrations. From the graph, the voltage V_o begins to decrease from the initial voltage, V_{oi} , after about 5 seconds. This decrease in V_o represents the absorbance of photons due to glucose in the sample. However, this pattern is not perfectly stable, as there is a small fluctuation in the voltage V_o . For system performance analysis, the system is analyzed using an average output voltage, V_o , for all concentrations. The average value was calculated from three measurements, with a relative standard deviation (RSD) of less than 0.06%.

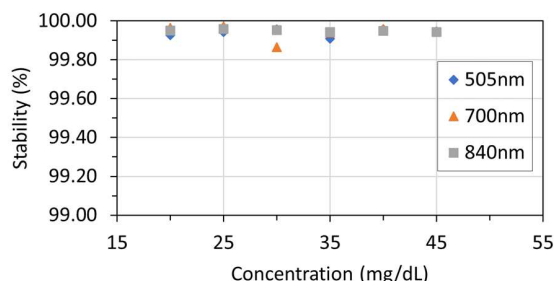


FIGURE 3. Stability precision of spectrometer at different operating wavelengths (for 180 seconds).

To better understand this pattern, the stability and precision of the system were calculated and plotted in Figure 3. As shown in the graph, all wavelengths exhibit good stability precision for all glucose concentrations, with values greater than 99.9%, except for the 30 mg/dL concentration at the 700 nm wavelength. However, the lowest stability and precision for this sample is still relatively high, at 99.87%.

4.2. Analysis of System Linearity

Next, the system is analyzed for its linearity over a 180-second duration. This linearity analysis is essential for determining the optimal response time for the system. Using the linear regression technique, the linearity coefficient, R^2 , for every 15 seconds of the sample is plotted in Figure 4. As shown in Figure 4(a), the R^2 for the spectrometer operating at the 505 nm wavelength exhibits an increasing trend from 15 seconds to 90 seconds of response time, after which it begins to decline. The highest R^2 of 0.8812 is observed at a 90-second response time. The second tested operating wavelength of 700 nm shows a low

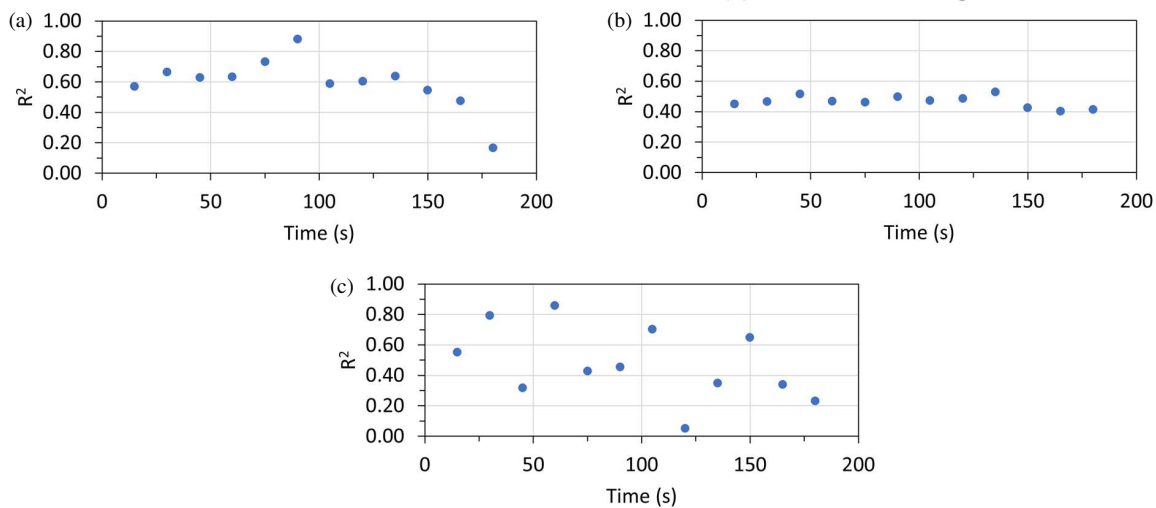


FIGURE 4. Linearity coefficient, R^2 at different operating wavelengths (for 180 seconds duration). (a) 505 nm wavelength, (b) 700 nm wavelength, (c) 840 nm wavelength.

R^2 value with fluctuations between 0.4 and 0.6. For the 840 nm wavelength, the R^2 follows a random trend, with a maximum value of 0.8580 recorded at a 60-second response time. This finding aligns with the results reported in [25], where the absorbance due to glucose varies at different wavelengths. Additionally, the unique pattern of the linearity performance over time is influenced by the mutarotation phenomenon, as reported by [26].

4.3. Analysis of System Sensitivity

In this section, the system's sensitivity performance is analyzed at a 90-second response time, as this period yielded the highest R^2 value from the linearity analysis. The system's sensitivity is determined by the relationship between the detected output voltage, V_o , and each glucose concentration, as shown in Figure 5. As illustrated in this figure, the highest sensitivity is achieved at the 700 nm wavelength, with a value of $1.3 \text{ mV}/(\text{mg}/\text{dL})^{-1}$. However, despite this highest sensitivity value, this wavelength suffers from very low linearity performance of 0.4901. Similarly, the system operating at the 840 nm wavelength also shows a relatively low linearity of 0.4286

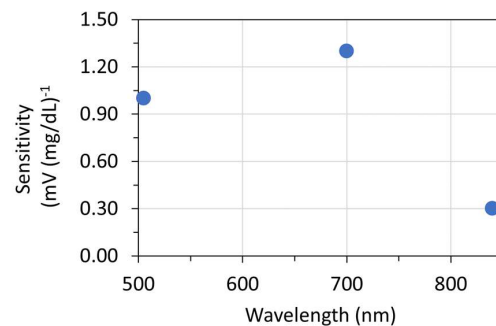


FIGURE 6. Sensitivity performances at different operating wavelengths (at 90 seconds response time).

and the lowest sensitivity of $0.2 \text{ mV}/(\text{mg}/\text{dL})^{-1}$. Among these wavelengths, 505 nm offers the best linearity performance of 0.8812 with a sensitivity of $1.1 \text{ mV}/(\text{mg}/\text{dL})^{-1}$. The sensitivity values are plotted in Figure 6. In general, a higher-sensitivity spectro-photometer is preferred because of its ability to detect smaller changes in the sample's absorbance [27].

Another performance parameter analyzed in this work is the limit of detection (LOD), which refers to the lowest glucose concentration that can be reliably detected by the developed system. The analytical method described in [24] was used to calculate the LOD. It was found that the smallest LOD value is $12.72 \text{ mg}/\text{dL}$ at the 505 nm wavelength. The other operating wavelengths, 700 nm and 840 nm, have LOD values of $34.55 \text{ mg}/\text{dL}$ and $34.16 \text{ mg}/\text{dL}$, respectively. A performance comparison of the developed system is summarized in Table 2.

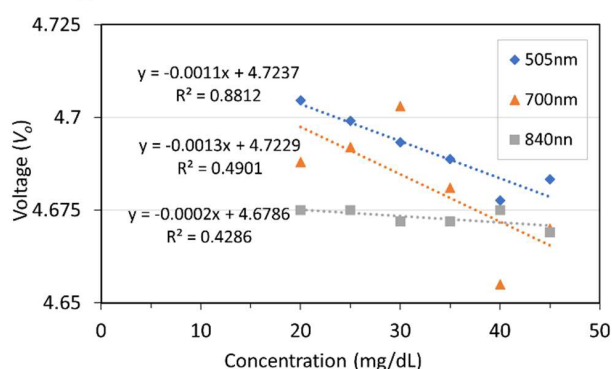


FIGURE 5. Detected output voltage for 20–45 mg/dL of glucose concentration (90 seconds response time).

4.4. Comparison with Previously Reported Work

Next, a performance comparison with previously reported works is presented to evaluate the improvements made by the proposed spectroscopy system. The comparison will focus on the operating wavelength and type of glucose sample used during testing, specifically concerning linearity and sensitivity

TABLE 2. Spectrometer performance.

Wavelength (nm)	R^2	Sensitivity (mV (mg/dL)^{-1})	Stability precision (%)	LOD (mg/dL)
505	0.8812	1.1	99.94	12.72
700	0.4901	1.3	99.94	34.55
840	0.4286	0.2	99.95	34.16

TABLE 3. Performance comparison between the proposed system and the previous spectrophotometer.

Wavelength (nm)	Linear range	R^2	Sensitivity (mV (mg/dL)^{-1})
1550 nm [13]	0–200 mg/dL (Glucose in distilled water)	0.734	0.1
940 nm [14]	50–300 mg/dL (Glucose in distilled water)	0.965	0.34
660 nm [15]	80–180 mg/dl (Blood glucose — human finger)	0.7014	0.3
505 nm [This work]	15–45 mg/dL (Glucose in deionized water)	0.8812	1.1

performances. The closest references are summarized in Table 3. Regarding the operating wavelength, [13] and [14] successfully detected glucose in the NIR spectrum, with operating wavelengths of 1550 nm and 940 nm, respectively. Another spectroscopy system was developed at a 660 nm operating wavelength, which falls within the visible spectrum [15]. This work introduces an additional potential wavelength in the visible spectrum for glucose detection. When comparing sample preparation methods, only the work in [15] tested the system using human blood serum, which considers interference from other substances. The other studies, including this work, prepared their samples by mixing glucose with distilled water. However, in this work, deionized water was used, ensuring no interference from other substances. Considering the differences in operating wavelength and sample preparation methods, the work in [14] achieved the highest linearity of 0.965, while the works in [13] and [15] achieved linearity values below 0.75. In comparison, the linearity performance in this work is 0.8812, which is better than the results at 1550 nm and 660 nm wavelengths. Despite the moderate linearity, this work achieved the highest sensitivity at 505 nm, with a value of $1.1 \text{ mV}/(\text{mg/dL})^{-1}$, which is approximately 10 times greater than that of other reported works in the lower glucose concentration range. The enhancement is influenced by the molar attenuation coefficient of glucose that varies at different operating wavelengths [18]. However, this work is limited to detecting glucose at low concentrations, specifically focusing on glycosuria. The system's performance can be further investigated over a broader range of glucose concentrations in future studies.

5. CONCLUSION

In conclusion, glucose sensing in the visible spectrum has been successfully demonstrated in this work. The method used is based on direct detection spectroscopy, without the need for enzymes or reagents in the sensing process. Experimental results show that sensor performance is optimized when the operating wavelength is set to 505 nm. At this wavelength,

the sensor achieves a linearity range of 20–45 mg/dL, with a linearity of 0.8812, sensitivity of $1.1 \text{ mV}/(\text{mg/dL})^{-1}$, LOD of 12.72 mg/dL, and stability precision of 99.94%. Compared to previous developments, the proposed system offers a significant improvement in sensitivity, with a value of $1.1 \text{ mV}/(\text{mg/dL})^{-1}$, compared to $0.34 \text{ mV}/(\text{mg/dL})^{-1}$ in earlier systems. The proposed sensor, operating in the visible spectrum, delivers enhanced sensitivity performance and a fast response time, owing to the absence of enzymes or reagents in the sensing operation.

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