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by 2. Low-cost And Real-time Color Detector Developme Fatoni

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Low-cost and real-time color detector developments for glucose biosensor

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ABSTRACT

One of the analytical methods widely developed is a biosensor, which has several advantages. We reported the development of a real-time colorimetric detector for glucose biosensor using low-cost electrical components of LDR, TCS230 and webcam. The detection was based on the color recognition from the devices resulted in RGB color intensity of the yellow color of hydrogen peroxide and titanium oxysulfate reaction. The comparison of three developed low-cost methods showed that the detector based on TCS230 had the best sensitivity. The real-time colorimetric glucose biosensor using TCS230 showed a good linearity, in the glucose detection of 0.1 to 2.5 mM with the regression equation of $y = 27.89x + 35.31$ ($R^2 = 0.993$). Furthermore, the calculated limit of detection of 0.14 mM and calculated limit of quantification of 0.58 mM. The glucose biosensor was also showed high selectivity to detect glucose in the blood sample with good agreement compare to commercial glucose biosensor.

1. Introduction

The medical costs could be reduced by early detection and intervention of the diseases. The numerous methods of early detection have been developed and applied in the medical, but require expert analyst skill, long analysis time or require relatively expensive equipment. The development of novel efficient and reliable devices is required continuously as one of the strategies to reduce medical costs. An interesting analysis device widely developed is a biosensor, an analytical tool that combines biological sensing elements (enzyme, cell and antibody) with a transducer to convert biological interactions into easily readable signals [1].

The main advantages of biosensors are high selectivity, fast analysis time, simple preparation and manipulation, high sensitivity [2] and allowing real-time measurement [3]. Biosensor improvement strategies have been reported to increase sensitivity, selectivity, stability and effectiveness or to reduce production costs. The biosensor improvement could be performed on the biological sensing exploration, biological supporting material or on the detection system.

Numerous study of glucose biosensors have been reported using several detectors such as amperometric [4], electrochemical impedance spectroscopy [5],

The development of low-cost detection systems has been widely reported, such as the fabrication of cheap electrochemical detectors (CheapStat of < 80 USD) [6], color recognition devices using a smartphone [7], scanners [8], webcam [9] and digital cameras [10]. Colorimetric has several advantages as an analytical tool such as simple equipment needed, ease of standard preparation and color change visually observed [11]. Numerous simple color detection methods have been previously reported need series procedures of digital image capturing, color intensity analyzing using image processing software such as Matlab [12], Kylix [13], ImageJ [14] or Adobe Photoshop [15]. The separate processing of image capture and analyzing would difficult to perform real-time analysis.

In this work, the development of a low-cost and real-time color detection of glucose biosensor uses simple electronics components, LDR (light dependent resistor), the commercial photodetector of TCS230 and webcam have been studied. The color sensors fabrication was comprehensive from the data reading source code, color sensors assembly in the flow system and computer software to monitor data capturing the real-time measurements. This comprehensive study would allow further simple application and portable device fabrication. The best color sensors among the three color recognition devices have also been comparing to select the best performance color sensor which finally used to detect glucose.

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2. Experimental

2.1. Reagents and chemicals

Reagents and chemicals used were Hydrogen peroxide (30%, Merck Millipore), sodium tripolyphosphate (Sigma-Aldrich), acetic acid (glacial 100%, Merck Millipore), pH indicator solution (Merck Millipore), sodium dihydrogen phosphate (Merck Millipore), disodium hydrogen phosphate (Merck Millipore), D-(+)-glucose anhydrous (Merck Millipore), Glucose oxidase (Type II-S, 15–50 unit mg^{-1}, Sigma-Aldrich), chitosan from crab shell (highly viscous, Sigma-Aldrich and titanium (IV) oxysulfate (Sigma-Aldrich).

2.2. Apparatus

Electronic components used were LDR (light dependent resistor, GL 5528, Lida Optical and Electronic, co.ltd, China), color sensor TCS230 (TAOS Inc., USA), USB webcam (Logitech, Switzerland) were used as color recognition component. Arduino uno microcontroller (HK Shan Hai, China) was used to control, generate and send data of color recognition components connected to a notebook computer (HP1000, Hewlett-Packard, USA). Peristaltic pump (RZ1030, Runze Fluid Control Equipment, China) was used to driving the carrier solution of flow injection analysis.

2.3. Color recognition devices design

Color recognition devices (color sensors) have been prepared using three different components of LDR, TCS230 and USB webcam. The LDR based color sensor was prepared using LDR equipped with three LED (red, green and blue) lights as in the previous reported [16]. The resistance change of the LDR was measured by an Arduino microcontroller (source code: Supplementary S1) connected to the computer. The Arduino board would send the data using the USB port to the computer including reading number, red, green and blue (RGB) color intensity. The computer software used to record the real-time measurement was pre-made software (Color Sensor Reader, Supplementary S2) build using Microsoft Visual Studio that record the incoming RGB color intensity on a real-time line graph and a log RGB value for further save and analyze.

The second color recognition was using the TCS230 commercial sensor. The TCS230 used a chip would recognize the color intensity of the object (RGB) assisted by four white LED lights and controlled by Arduino microcontrollers (Supplementary S3) resulted in an RGB value which could be transmitted to the computer using the USB port. The software used to capture real-time data was the same as LDR based software of "Color sensor reader."

The third color recognition devices were assembled using USB webcam, with the main principle was capturing an image at a specific time (every 3 s) and analyzed the color intensity of the center of the image to get the RGB value. The capturing and analyzing was performed by pre-made software (real-time webcam colorimeter) build using Microsoft Visual Studio (Supplementary S4).

2.4. Color sensor assembly and study in flow injection system

The color sensors using LDR, TCS230 and webcam, were first tested in a batch system to record the color intensity of the samples. The flow injection system was used a peristaltic pump to drive the solution and an injection tube to add the sample (Fig. 1). The color sensor would read the solution color at a defined period (every 3 s) and send the data to the computer to analyze and record.

2.5. Color sensors test

The first study of the built color sensors was to analyze the sample in various colors. The samples were prepare using buffer pH of 2.0 to 11.0 and added a universal pH indicator solution. The pH range of 2.0 to 11.0 was chosen to cover wide range of enzymatic reaction [17] for biosensor application. The different color solutions were analyze using the three developed color sensors in the batch system and flow injection analyses system. Each sample has been recorded for ten sampling readings, with a reading interval of 3 s.

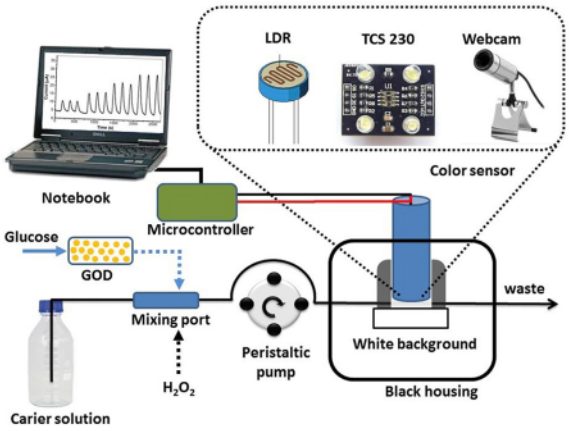

Fig. 1. The design of the color sensor in the flow analysis system.

2.6. Color sensor testing for the detection of hydrogen peroxide

Glucose biosensor proposed was used glucose oxidase enzyme to convert glucose into hydrogen peroxide and gluconate. Thus, the color sensors were tested to detect hydrogen peroxide reacted with titanium oxysulfate to produce a yellow color in flow injection analysis. Hydrogen peroxide solution of 0.1–1.0 mM of 100 µL color sensor flowing system, with titanium oxysulfate as a carrier solution. The titanium solution was prepared by dissolving 2 g titanium oxysulfate with 2 M HCl to get the 100 mL of solution. The batch system indicator ratio was 5 mL of hydrogen peroxide sample solution added with 100 µL titanium solution. The flow injection system where the hydrogen peroxide and titanium solution equally with the same flow rate, using the diluted titanium solution of 2% (*v*/v in pure water) from the original titanium solution. The color changes with the concentration were then recorded and analyzed. Each color intensity (red, green and blue) were plotted to the hydrogen peroxide concentration. The best color intensity calibration curve was then selected. The three fabricated color sensors (LDR, TCS230, and webcam) were then compared to their sensitivities to detect hydrogen peroxide from the slope of a calibration curve obtained. Some parameters such as sample volume and flow rate were also studied on the hydrogen peroxide detection in FIA to achieve the best responses and fast analysis time.

2.7. Biosensor preparation and glucose detection

Glucose oxidase was immobilized in the chitosan cryogel beads as a glucose biosensing element. The chitosan cryogel beads have been prepared using the previously reported procedure [18]. In brief, chitosan solution (2%, *w*/*v*) has been prepared using acetic acid (1%, *v*/v). The chitosan solution was then dropped in tripolyphosphate (1%, w/v) to get chitosan beads, allow at room temperature for 30 min and kept at −20 °C for 6 h. The glucose oxidase was then immobilized on the chitosan beads surface by glutaraldehyde crosslinking. The immobilization was performed by soaking the chitosan cryogel bead in 2% (*v*/v) of glutaraldehyde for 20 min. The beads were then rinsed with pure water and immersed in a glucose oxidase solution of 20 U/mL

chitosan beads source. The glucose oxidase enzyme was prepared in 50 mM phosphate buffer of pH 7.0. The immobilization process was carried out at 4 °C for 12 h. The immobilized glucose oxidase was mount in 2 mL mini reactor tube and install to the previously design FIA, before the mixing line of titanium oxysulfate carrier solution (Fig. 1). Series of glucose concentration was then tested to get linear responses.

2.8. Enzymatic optimization

The enzyme of glucose oxidase amount and reaction time were studied. The enzyme concentrations studied were 10 to 30 U/mL chitosan bead, such as described in the before, with a 0.5 mM glucose used to study. The enzymatic reaction durations were studied by adjusting the flow rate of flow injection analysis. The flow rate used was 0.2 to 4.0 mL/min to get the interaction duration of sample and enzyme of 0.5 to 10 min in the 2 mL reactor. The high response with a fast analysis time was then selected for further study.

2.9. Linear range, limit of detection and limit of quantification

Glucose standard solution in a series concentration was injected into the fabricated biosensor, with the optimal flow rate and enzyme amount. The resulted yellow color intensity from the best color sensor device (TCS230 based color sensor) was recorded. The glucose concentration versus the yellow color intensity change was plotted to get the calibration curve. Furthermore, the limit of detection and limit of quantification were calculated based on the linear range of the calibration curve obtained.

2.10. Interferences effect on the glucose detection

The principal reaction of the fabricated glucose biosensors was the reaction between the indicator of titanium oxysulfate with the resulted of hydrogen peroxide. Other oxidizing and reducing agents in the sample may result in false reading in glucose detection. Common interference in the glucose determination of blood samples such as ascorbic acid and uric acid were tested. The ascorbic acid of 0.25 mM and uric acid of 0.5 mM were added to the glucose standard solution of 2.5 mM. The glucose solution and glucose with interferences solution was diluted two times before analysis. The biosensor reading between glucose and glucose with the interferences compared to determine the biosensor selectivity in the glucose determination.

2.11. Glucose determination in the blood sample

The fabricated glucose biosensor was used to determine the glucose concentration of the blood sample. Six blood samples from the local hospital were diluted using 50 mM phosphate buffer with pH of 7.0 before analysis. The samples were analyzed using fabricated glucose biosensor under optimal conditions. A standard glucose solution of 0.2 to 1.0 mM was used to prepare the calibration curve. The results of blood glucose concentration were compared to the glucose concentration of the commercial glucose sensor.

3. Results and discussion

3.1. Color recognition devices design

Comprehensive real-time color sensors have been fabricated using three different types of electronic components of LDR, commercial color sensors of TCS230 and USB webcam. Color detector using LDR was based on the changes in LDR resistance values when exposed to light, assisted by three LED light of red, green and blue. The change in the LDR resistance values depends on the object in front of the LDR, when received the reflecting light from the LED. This LDR base measurement was also previously reported as a low-cost reflectometer [19], water quality colorimetry [20] and wireless colorimetry [21]. This resistance value was further recorded by the Arduino microcontroller, to be converted into a more simple number as the color intensity. The value of analog signal resistance ranges from 0 to 1025, whereas the intensity of color in digital images should be 0 to 255. The second color detection device was based on the use of TCS230, a programmable light to frequency photodiode based sensor with red, green and blue filter developed by TAOS. The output of the TCS230 was the frequency, which can then be converted to the color intensity of red, green and blue using the Arduino microcontroller. The generated color intensity (RGB) could be sent to a notebook computer for further analysis. The TCS230 was also reported previously to determine dangerous gases [22], leads [23] and ammonia gas [24]. The third low-cost color recognition device was based on the use of a webcam to capture an image at a specified time, continued by analyzing the captured image to get the color intensity of the middle of the picture (previously defined) to generated final data of series color intensity (RGB). The use of a webcam as a lab on chip detector [25], eye-gaze estimation [26] and blood flow monitoring [9] were reported.

Those color sensing devices based have been reported as colorimetric sensors. However, most of them used separated image capturing and analyzing or commercial data capturing software to perform the analysis. Furthermore, only a few studies reported the use of color recognition devices in real-time analysis. In this study, the three low-cost color recognition devices have been prepared including the electronic driver and software to achieve comprehensive real-time and low-cost color sensors. Furthermore, the color sensor devices would be compared to select the best performance for further application.

3.2. Color sensor assembly and study in flow injection analysis

The design of the flow injection analysis of the color sensors was similar of fabricated color recognition devices. The color sensors were placed in front of 1 mL flow glass flow cell, with a white background. For the webcam sensor, the additional light was used of the white LED strip.

3.3. Color sensor testing with buffer and universal indicator

The color detector that has been designed is then tested to measure different color solutions. In this test, a solution of pH 2 to pH 11 was mixed with a universal indicator solution. The example of a sequence of solutions changes color with the pH measured using TCS230 based color sensor as shown in Fig. 2. The designed color recognition devices could distinguish the color intensity of the tested colored solution.

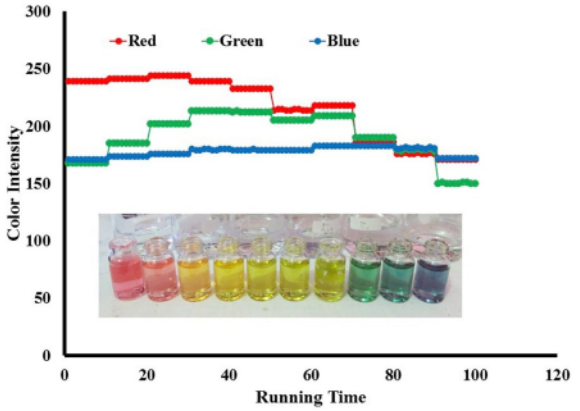

Fig. 2. Color recognition device tested using a series solution (inset) in different pH of 2.0 to 11.0 with the addition of universal pH solution.

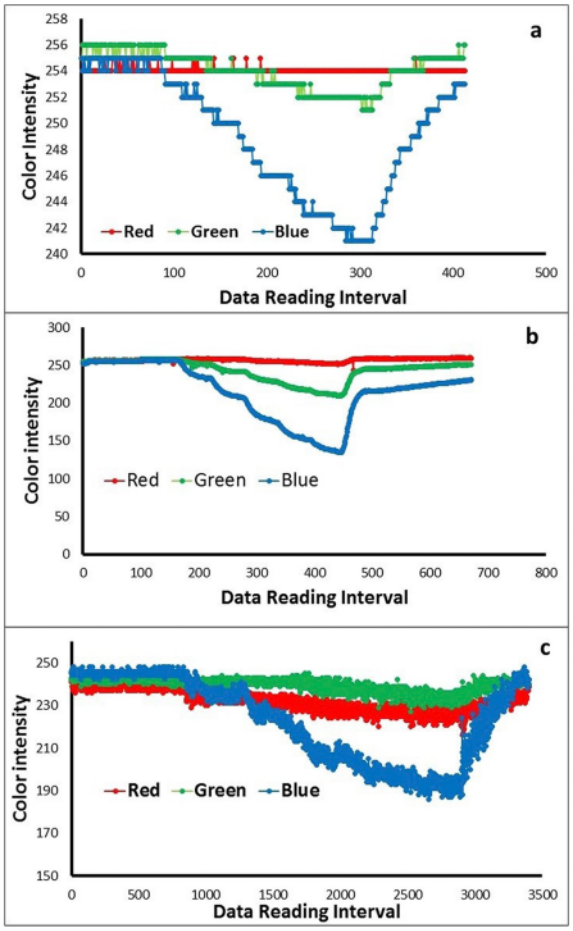

Fig. 3. Test results of hydrogen peroxide solution (0.1–5.0 mM) and titanium oxysulfate using color detection developed by LDR (a), TCS230 (b) and webcam (c).

3.4. Color sensor testing for the detection of hydrogen peroxide

The fabricated color sensors were used to determine the color intensity of the hydrogen peroxide solution in a series concentration of 0.1 to 1.0 mM. Fig. 3(a) showed the LDR based color sensor on hydrogen peroxide detection. In another side, the TCS230 based was showed a better resolution in the detection of hydrogen peroxide (Fig. 3b), with the ratio between color intensity change with the increasing hydrogen peroxide concentration was higher than LDR based color sensor. For the webcam-based color sensor, the hydrogen peroxide detection was a better resolution than that of LDR based color sensor, but it was lower resolution than TCS230 based color sensor (Fig. 3c). However, all developed real-time color sensors were showed a similar pattern in the hydrogen peroxide detection, with the blue color intensity showed higher changes with the increasing of hydrogen peroxide concentration. The changes in the blue color intensity (B) would use for further study comparison of hydrogen peroxide determination.

A comparison of the three developed color sensors on the color change with the concentration of hydrogen peroxide has been tested (Fig. 4). The linear regression equation obtained for LDR color sensor was $y = -12.55x + 253.26$ with r^2 of 0.99, webcam color sensor was $y = 47.91x + 236.24$ with r^2 of 0.98 and TCS230 color sensor was $y = -107.04x + 235.66$ with r^2 of 0.97. TCS 230 based color sensor showed the highest sensitivity (slope). Thus, the TCS230 color sensor

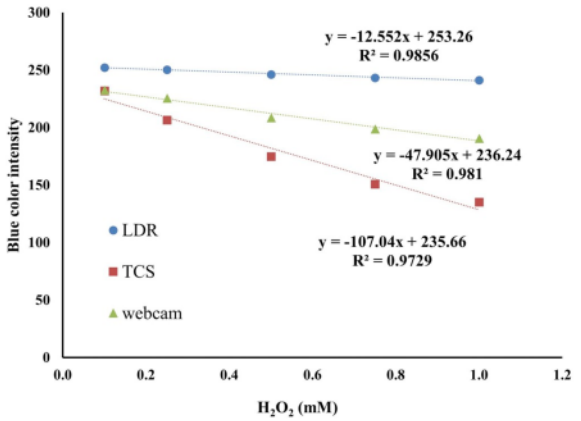

Fig. 4. Calibration curve comparison of fabricated color sensors based on LDR (blue), TCS230 (orange) and webcam (grey) to detect hydrogen peroxide (0.1–1 mM) using titanium oxysulfate to produce a yellow color. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

would be further studied as a glucose biosensor detector. The comparison of the color recognition devices was resumed in Table 1, including the simplicity of the assembling, software development, and cost spends to build the color sensors.

3.5. Glucose detection study

The immobilized glucose oxidase with chitosan cryogel bead was set up in flow injection analysis with the TCS230 based color detector at room temperature. The flow rate was adjusted to 200 μL/min, thus, the glucose solution would flow through the immobilized glucose oxidase of 1 mL reactor for 5 min. Standard glucose solution has been tested using the fabricated glucose biosensor. The response of the glucose detection showed a high correlation between glucose concentration and color intensity changes of both red, green and blue (Fig. 5). The color blue color intensity change showed the highest sensitivity with the regression equation of $y = 50.34(\pm 1.8)\,x + 29.74\,(\pm 0.78)$ with the r^2 of 0.998.

3.6. Enzymatic optimization

The enzyme of glucose oxidase immobilized in the chitosan cryogel beads was optimized. In the enzyme reactor, a low enzyme amount would result in low responses, whereas a large amount of enzyme made an ineffective biosensor system. In this study, the immobilized glucose oxidase was studied from 10 to 30 units enzyme in 1 mL chitosan cryogel bead. The result showed that the increase of immobilized glucose oxidase from 10 to 20 units would significantly increase the response of glucose detection (Fig. 6). The higher enzyme mount of > 20 units would not significantly increase glucose detection responses. In this situation, the cryogel bead as enzyme immobilization supporting material may have limited space of surface for enzyme immobilization, thus, the larger enzyme amount occupied a similar immobilization supporting material surface, resulting in similar responses.

Besides the enzyme amount, glucose detection was also influenced by the interaction duration between glucose and glucose oxidase enzyme. In this flow system glucose biosensor, the interaction duration was studied by adjusting the flow rate to get the interaction of enzyme and substrate of 30 s to 10 min. Since the volume of the enzyme reactor was 2 mL, thus the flow rate of the carrier buffer was adjusted from 200 to 4000 $\mu L \cdot min^{-1}$ to achieve the studied interaction time. The result showed that increasing interaction between glucose and enzyme from

Table 1
Comparison of fabricated real-time and low-cost color sensors.

No	Elements	Assembly	Software	Sensitivity	Cost ($US)	Power source and data transfer
1	LDR	Simple	Microcontroller + computer	Lowest	4	USB port
2	TCS230	Simple	Microcontroller + computer	Highest	5	USB port
3	Webcam	Very simple	Computer	Medium	5	USB port + external

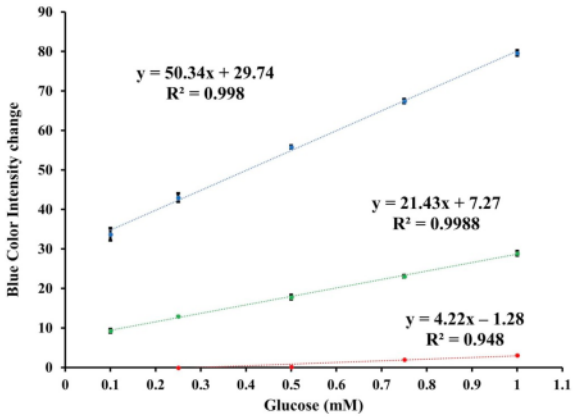

Fig. 5. Fabricated colorimetric real-time glucose biosensor showed linear responses on glucose detection at 0.1 to 1.0 mM.

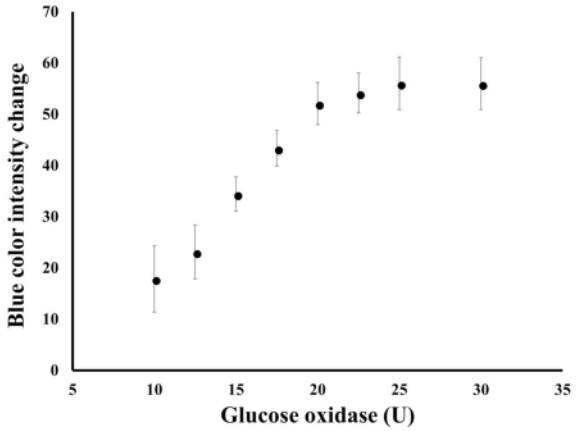

Fig. 6. The effect of glucose oxidase amount on the glucose biosensor detection.

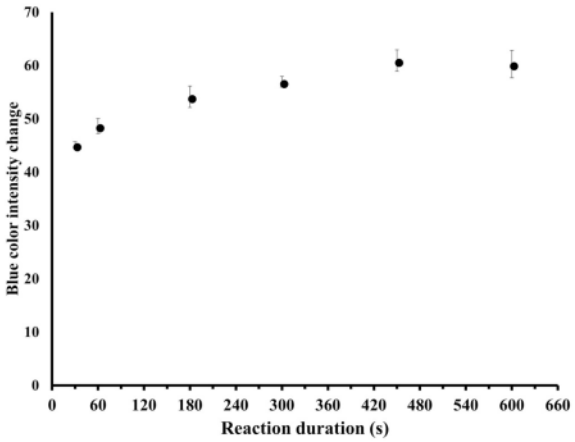

Fig. 7. The reaction duration effect to glucose detection responses.

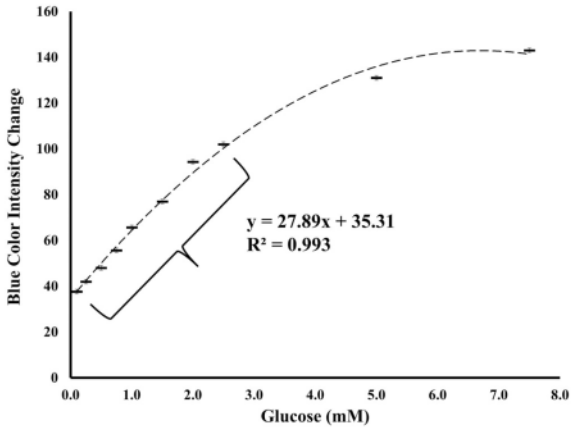

Fig. 8. The calibration curve of the glucose biosensor with the linear responses of 0.1 mM to 2.5 mM.

30 to 180 s increased the glucose detection responses, whereas the higher reaction time was showing a similar response (Fig. 7). The shorter reaction duration caused the glucose passed through the enzyme reaction too fast, which resulted in the complete enzymatic reaction of all glucose samples. The higher reaction duration above 180 s did not significantly increase the glucose detection response, limited by the amount of the enzyme in the reactor.

3.7. Linear range and limit of detection

Under the optimum, the glucose biosensor was used to detect the standard glucose solution from the lowest concentration (0.1 mM) to the highest concentration (7.5 mM) to get the linear range, the limit of detection and limit of quantification. The linear equation between blue color intensity change (y) and glucose concentration (x) was $y = 27.89x + 35.31$ ($R^2 = 0.993$) (Fig. 8), with the calculated limit of detection was 0.14 mM and limit of quantification was 0.58 mM.

3.8. Interference effect

The typical blood interfering species such as ascorbic acid (AA) and uric acid (UA) were used to study the selectivity of the fabricated biosensor. Glucose solution and interference solution as sample simulation used ascorbic acid of 0.25 mM and uric acid 0.5 mM. Those concentrations of ascorbic acid and uric acid were higher than that of normal blood ascorbic acid of about 50 μM [27] and uric acid of about 6.4–6.8 mg/dl (380–400 μM) [28]. There was no significant difference between glucose, glucose with ascorbic acid and glucose with uric acid addition responses, with the color intensity change of 70.7 ± 2.5, 71.0 ± 2.6 and 70.3 ± 1.5 respectively.

Table 2
The glucose concentration of blood samples measured by fabricated glucose biosensor compared to commercial glucose sensor.

Sample	Biosensor (mg/dL)	Commercial glucose sensor
	(3 replications)	(mg/dL)
1	89.1 ± 03	88
2	137.5 ± 0.9	135
3	176.9 ± 0.6	175
4	186.5 ± 1.2	188
5	216.6 ± 1.0	216
6	225.7 ± 0.9	224

3.9. Glucose determination of blood sample

The blood samples were diluted ten times before analyzing to reduce the matrix effect and to adjust the sample concentration match to the calibration curve of the glucose biosensor. Table 2 shows the glucose concentration in the blood samples measured using the fabricated glucose biosensor compared to the commercial glucose sensor. The different in glucose concentration between the fabricated glucose biosensor and commercial glucose sensors were not significant ($P > .05$) analyzed using the Wilcoxon-signed rank test. Those the fabricated glucose biosensor showed an excellent agreement to the commercial glucose biosensor.

4. Conclusions

Real-time color detectors have been successfully developed using economic devices LDR, TCS230, and webcam. Test results with test solutions of hydrogen peroxide and titanium oxysulfate showed that the detectors were able to test the color change depending on the concentration of hydrogen peroxide. Color detector with TCS230 has the best sensitivity on measurement of 0.1–1.0 mM test solution with regression equation of $y = -107.04x + 235.66$ with r^2 of 0.97. Furthermore, the TCS230 based color sensor has been assembled to build a real-time colorimetric glucose biosensor. The fabricated glucose biosensor showed good linearity to detect glucose with the regression equation $y = 27.89x + 35.31$ ($R^2 = 0.993$), the calculated limit of detection of 0.14 mM and calculated limit of quantification of 0.58 mM. The glucose biosensor was also showed high selectivity to detect glucose in the blood sample with good agreement compare to commercial glucose biosensor.

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Declaration of Competing Interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sbsr.2020.100325.

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