# A Study on the Relation Between Voltage and Bilirubin Concentration in Artificial Blood Solution by Bilirubin Detector

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**Abstracts:** This research project focuses on investigating the correlation between electric potential difference and bilirubin concentration within an artificial blood solution, utilizing the principles of light absorption. The bilirubin detector's design and construction incorporate a blue light source with a 460 nm wavelength and a green light source with a 560 nm wavelength, enabling interaction with bilirubin concentrations ranging from 2 to 14 mg/dl. Analysis of the project's outcomes revealed a direct relationship between the bilirubin detector's output voltage and the concentration of the bilirubin solution. Notably, an interaction with bilirubin was observed at the 460 nm wavelength, influencing light absorption. In cases of higher bilirubin concentration, light transmittance was diminished, leading to a heightened voltage difference. Conversely, lower bilirubin concentrations facilitated increased light transmission, resulting in a reduced voltage difference. This study contributes to a comprehensive understanding of bilirubin detection methodologies and their potential applications in medical diagnostics.

**Keywords:** Bilirubin, Light Absorbance, Spectrophotometry.

## 1. INTRODUCTION

Jaundice is a significant concern among newborns. According to statistics from the Ministry of Public Health, jaundice is a prevalent issue affecting 25-50 percent of newborns [1]. This condition arises due to an excess of the normal quantity of a yellow substance known as bilirubin. This substance accumulates within various organs, leading to various side effects, including the manifestation of yellowed skin and eyes [2]. This yellowness results from the presence of bilirubin, stemming from the breakdown of red blood cells and its contamination within the bloodstream. Jaundice can be triggered by a range of factors, with the primary contributor being an excessive production of bilirubin beyond regular levels [3]. Simultaneously, there might be a reduction in the excretion of bilirubin. In cases involving newborns with jaundice, determining the overall bilirubin content (Total Bilirubin) necessitates venipuncture. The normal range for total bilirubin levels in infants falls between 2.0 and 12.0 mg/dl, while a critical value surpassing 12.0 mg/dl requires attention [1, 4]. Jaundice treatment options encompass phototherapy and blood transfusion. In cases of blood transfusion, blood is extracted through the skin to access a vein, which could potentially induce discomfort at the puncture site. This process incurs costs, demands an extended testing period, and results in the utilization of diverse equipment for blood extraction [5-8].

Currently, following up on treatment requires diagnosing the cause of jaundice through a combination of history-taking, physical examinations, and laboratory tests. During the physical examination, the baby's skin is carefully observed. If the baby exhibits significantly high bilirubin levels, the doctor may recommend a blood transfusion, which can cause discomfort to the baby. While laboratory tests can yield highly accurate results, they also take time to generate results and quantify bilirubin using a bilirubin detector [9, 10].

According to 2021 data, there were approximately 540,000 births in Thailand, with as many as 130,000 to 270,000 newborns at risk of jaundice [11, 12]. Therefore, the estimated demand for bilirubin meter can be derived from hospitals with neonatal units across the country, as indicated by data from the Healthcare Accreditation Institute. There are around 1,400 hospitals that have met the HA standard, and they are required to have at least 1,000 bilirubin

meters. The price range for these devices varies widely based on functionality, ranging from thousands of baht to hundreds of thousands of baht. Furthermore, these devices often need to be imported from abroad, which can result in longer import times. Additionally, if the device requires repairs, it must be sent abroad, further prolonging the repair process compared to devices produced and distributed within the country [13].

The principle used to test bilirubin levels in newborns is the absorption principle and opacity comparison, also known as spectrophotometry [14]. Spectrophotometry is a measurement technique that determines the concentration of a substance by using light to measure the absorption of light at different wavelengths. It is an optical technique employed to analyze the physical properties of substances, both qualitatively and quantitatively, based on their light absorption characteristics. This technique is crucial in biochemistry due to its ability to provide rapid and highly accurate analyses, even for substances in microgram quantities, even when they are present in mixed solutions [15, 16].

The absorbance of a sample can be measured by allowing light to pass through the sample. Substances absorb some of the light and some of the remaining light is reflected and some is transmitted. Light transmission follows the Beer and Lambert's law states that for a single beam of parallel light passing through a sample of a homogeneous solution, The proportion of light intensity absorbed by the medium is directly proportional to the concentration of the substance and the distance that light transmission [17]. The expression of the Beer–Lambert law was shown in equation 1.

$$\log(I_0/I) = A = \varepsilon \ell c \quad (1)$$

Where $A$ is the absorbance, $\varepsilon$ is the molar attenuation coefficient or absorptivity of the attenuating species, $\ell$ is the optical path length, $c$ is the concentration of the attenuating species.

From a research, Noninvasive detection of bilirubin using pulsatile absorption [18], In this research, a spectrometer, the absorption spectrum of hemoglobin and bilirubin was measured in the range of 400-650 nm, revealing that bilirubin exhibits its maximum absorption at 460 nm and the green light with a wavelength of 560 nanometers cannot absorb bilirubin as shown in Figure 1. Therefore, it does not interfere with the absorption of blue light.

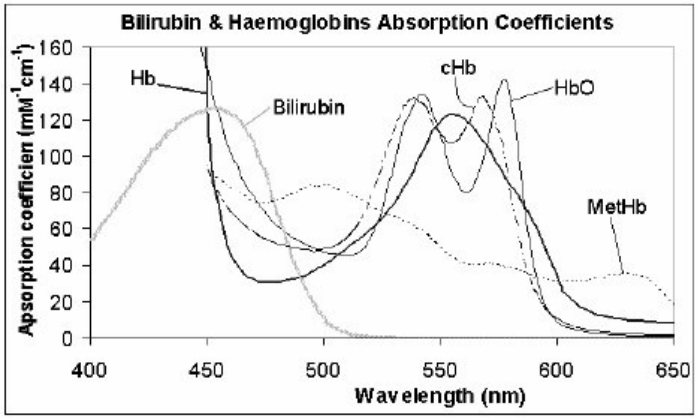


**Figure 1.** Absorption spectra for Haemoglobins & Bilirubin in the 400-650 nm range [18].

The researchers, therefore, studied the relationship between voltage and bilirubin in an artificial blood solution using a bilirubin detector. This detector employed LED bulbs with a wavelength of 460 nm as light sources and green light with a wavelength of 560 nm as light sources reference and operated on the principle of light absorption and spectrophotometry. The objective was to compare the optical absorption of these two light wavelengths by bilirubin in an artificial blood solution and establish an equation that correlates voltage readings with bilirubin concentration. This research aimed to provide guidelines for developing a non-invasive bilirubin measuring device for infants.

## 2. MATERIEL AND METHODS

The basic method of the study of the design and construction of a bilirubin meter in artificial blood solution was based on the principles of spectrophotometry. The study consisted of 3 main parts: 1) Designing and constructing a bilirubin detector. 2) Establishing an equation to correlate bilirubin concentration (in mg/dl) with the voltage of the sample substance in the test tube. 3)Testing the functionality and performance of the designed bilirubin meter.

### 2.1. Designing and constructing a bilirubin detector

A bilirubin detector was composed of 4 sectors: 1) Input sector 2) signal conditioning sector 3) signal processing and control sector and 4) display sector that were shown as figure 2.

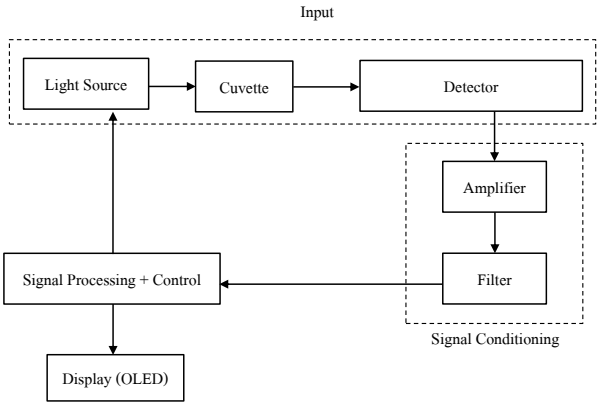


**Figure 2.** Diagram of Bilirubin detector in artificial blood solution.

1) Input sector were composed of 2 light sources, a cuvette tube containing with bilirubin in artificial blood solution in the prepared cavity and a phototransistor. 2 LEDS were used as two light sources, a blue LED with a wavelength of 460 nm, which can be absorbed by bilirubin and a green LED with a wavelength of 560 nm as the background that is in the part of a bilirubin detector in the next work. a phototransistor is used to detect blue light and green light. When blue light and green light pass through the bilirubin, some of the two lights are absorbed by bilirubin and the transmitted light were detected by the phototransistor and converted to electric current. In design, a direct current supply provided by a 3.3 volts of the ESP-WROOM-32 of microcontroller [19]. Therefore, resistance was required to limit the current in order not to exceed the flow of current that will cause damage to two light sources. The resistance can be calculated by use Equation 2.

$$R_{LED} = \frac{V_{DD} - V_{FLED}}{I_{FLED}} \quad (2)$$

For a green LED, $V_{DD}$ represents a direct current source from the ESP-WROOM-32 that is equal to 3.3 volts. $V_{FLED560}$ represents the forward bias of green light 560 nm which is equal to 2 volts. $I_{LED560}$ represents the forward bias current of green light which is equal to 20milliamperes. And if $R_1 = R_{LED}$ then $R_1$ = 65 Ω which is used R = 68 Ω to connect with the green LED.

For a blue LED, $V_{FLED460}$ represents the forward bias voltage of blue light which is equal to 3.2 volts, $I_{LED460}$ represents the forward bias current of blue light which is equal to 30 milliamperes. According to the data, there is no need for a resistor in the circuit because the blue LED can receive enough voltage from the supply. Therefore, it can be connected directly from the power source to the LED bulb.

When the light from a source pass through a cuvette containing bilirubin, a transmissive light from the cuvette was measured with two phototransistors were used as a detector. One of phototransistor was used to detect the blue light

and another one was used to detect green light. By the principle of the phototransistor detect the light, it converted light energy into electric current. The current depends on the intensity of the transmissive light. A current to voltage circuit was used to convert electric current into voltage.

For the current to voltage circuit, it requires an output voltage ($\mathbf{V_{out}}$) equal to 1 volt and the current flowing in the circuit ($I_{C(sat)}$) equal to 20 milliamperes (mA) and $R_{(Photoresistor)} = R_2 = R_6$ . The resistance value can be calculated from Ohm's Law then the resistance of a phototransistors was $R_2 = R_6 = 50\ \Omega$. Therefore, the resistance $R_1 = 68\ \Omega$, $R_2 = 50\ \Omega$ and $R_6 = 50\ \Omega$ were used in the input sector.

2) Signal conditioning sector was composed of high pass filter circuit, non-inverting amplifier circuit and low pass filter circuit. The signal output from the input sector is an electric current. Therefore, it converts the signal from the current to the analog voltage by using a current-to-voltage converter circuit. But the analog voltage signal was small and there was noise in signal then the signal was reduced noise by high pass, amplified by the Non-inverting amplifier circuit and low pass filter circuit to obtain the suitable analog voltage to the signal processing sector.

As for the high pass filter circuit, by setting the cutoff frequency ($f_c$) equal to 5 Hz and the capacitance was $C_1 = C_3$ is 0.1 μF and then the capacitive reactance $X_c = R_3 = R_7$ is 318.31 kΩ therefore which can be calculated according to equation 3.

$$C = \frac{1}{2\pi f_c X_c} \quad (3)$$

where in the research used $R_3 = R_7$ was 330 kΩ.

In design of the AC amplifier circuit used an MCP604-E/P op-amp because it was suitable for high voltage that can accept high voltages from the power supply and reduces signal loss from the sensor during Bilirubin measurement and increases the quality of signals entering the circuit [20].

The AC Amplifier circuit was composed of two circuits, A was used as the AC amplifier circuit and C was used as the voltage follower circuit to prevent the voltage drop. In the A1 circuit determine the input voltage ($V_{in}$) was equal to 0.4 volts and output voltage from the non-inverting amplifier ($V_{out}$) was set to 2 Volts, a gain of the signal ($A_v$) was 4.5. By setting $R_4$ and $R_5$ were the first amplifier circuit and $R_8$ and $R_9$ were the second amplifier circuit and the resistance $R_4 = R_8$ equal to 330 kΩ in the circuit, the resistance $R_5$ and $R_9$ can be found from the signal gain values which can be calculated according to equation 4.

$$A_v = 1 + \frac{R_f}{R_i} \quad (4)$$

Determine $R_f = R_4 = R_8$ and $R_i = R_5 = R_9$ therefore resistance was $R_5 = R_9 = 94.29$ kΩ but this research used $R_5$ = R9 = 100 kΩ in amplifier circuit.

As for the low pass filter of two circuits, by setting the cutoff frequency ($f_c$) equal to 5 Hz and use capacitive reactance $X_c = R_4 = R_8$ was 330 kΩ and find the capacitance by using equation 2 therefore the capacitance $C_2 = C_4$ was 0.096 μF but in the research has used 0.01 μF.

Design and construction of Bilirubin detector circuit in artificial blood solution, the resistance and capacitance was shown as follows: $R_1$ = 68 Ω, $R_2$ = 50 Ω, $R_3$ = 330 kΩ, $R_4$= 330 kΩ, $R_5$ = 100 kΩ, $R_6$ = 50 Ω, $R_7$ = 330 kΩ, $R_8$= 330 kΩ, $R_9$= 100 kΩ, $R_{10}$= 100 kΩ, $C_1$= 0.1 μF, $C_2$= 0.1 μF, $C_3$= 0.1 μF, $C_4$= 0.1 μF.

3) Signal processing and control sector, the circuit has chosen the ESP-WROOM-32 microcontroller because of its high resolution and accuracy. It can be used to process signals in the desired range well. It also has a maximum input voltage of 3.6 volts and supports Wi-Fi. This is suitable for storing data in the form of an application to be used for future development of bilirubin measurement research.

From the signal conditioning sector, the output signal from the MCP604-E/P op-amp is an electrical potential difference which is an analog signal. When the electric potential difference signal is input to the microcontroller board and this electric potential difference value is used with the bilirubin concentration value to create an equation of calibrate graph to find the relationship between the electric potential difference and the bilirubin concentration value in the artificial blood solution in milligrams per deciliter (mg/dl). This equation was used in a signal processing program by using the Arduino program.

From the design of the switch control circuit, a microswitch was chosen to connected with a pull-up resistor. The integrated circuit of design and construction of Bilirubin detector in artificial blood solution was shown in Figure 3.

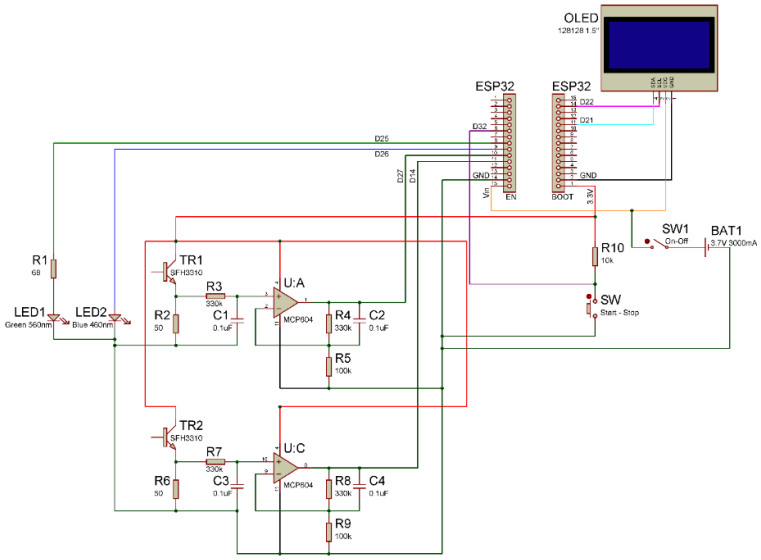


**Figure 3** Design and construction of Bilirubin detector circuit in artificial blood solution.

4) Display sector, the design of a display sector used a 1.5-inch OLED screen with a resolution of 128x128 to display the electric potential difference in volt. The value displayed on the OLED screen was the result of signal processing of microcontroller ESP-WROOM-32**.** The display was shown in 3 voltages output in millivolt (mV). The screen will show as follows: 1) Green is the voltage output value of the green light. 2) Blue is the voltage output value of the blue light and 3) DIFFERENCE is the voltage difference output of the LED between the green light and the blue LED which was shown in figure 4.


**Figure 4** Voltage output value on OLED display.

The bilirubin detector was use used a 3.7 volts battery ($V_{DC}$) with a current of 3,000 milliamperes per hour (mAh) is enough for supplying each part of the circuit.

### 2.2. Bilirubin Detector Test

To design and construction a noninvasive bilirubin detector, it is necessary to create a calibration curve to establish an equation for the relationship between the voltage difference from the transmission light and the bilirubin concentration in the artificial blood solution. The artificial blood solution used Phosphate Buffered Saline (PBS) The light used in the test is blue light at a wavelength of 460 nanometers and green light at a wavelength of 570 nanometers so that the voltage difference measured from the artificial blood solution is as close to the value measured from real blood as possible. Therefore, albumin and glucose were added in PBS to the test [21]. The test uses bilirubin concentrations of 2, 5, 8, 11 and 14 mg/dl because they cover the range of bilirubin in infants' blood, from 2 mg/dl to 12 mg/dl The test used glucose to test because glucose is a very important substance that resides in the body and travels through the blood and can measure by spectrophotometry[22]. The glucose level used in the test ranges from 70 mg/dl to 200 mg/dl as it covers the baby's blood sugar level from 70 mg/dl to 140 mg/dl and albumin is used because albumin is the most abundant protein in infant serum. The serum albumin of infants in 0-4 days ranges from 2800 mg/dl to 4400. Therefore, albumin concentrations of 3500 mg/dl.

Preparing bilirubin, glucose, and albumin solutions by diluting the solution by adding the solution in the appropriate ratio with the formula for calculating the volume of the solution as shown in Equation 5.

$$C_1 V_1 = C_2 V_2 \quad (5)$$

where $C_1$ represents the concentration of the solution before dilution. in milligrams per deciliter (mg/dl)
$V_1$ represents the volume of the solution before dilution. In milliliters (mL)
$C_2$ represents the solution concentration after dilution. in milligrams per deciliter (mg/dl)
$V_2$ represents the volume of the solution after dilution. In milliliters (mL)

In functional test of the project, the transmission of light from bilirubin, glucose, and albumin will be determined. The multiple regression analysis methods were used to analyze data set. The test procedure was shown as follows:

1) Preparation of the artificial blood solution in 10 mL of each sample and placed in the cuvette. There are a total of 5 sample sets that were prepared as follows:

Set 1: Bilirubin at concentrations in the 2, 5, 8, 11 and 14 mg/dl, albumin solution at concentrations in the 3500 mg/dl, glucose solution concentrations at 70, 135 and 200 mg/dl.

Set 2 (B+A): Bilirubin at concentrations in the 2, 5, 8, 11 and 14 mg/dl mixed with albumin solution at concentrations in the 3500 mg/dl.

Set 3 (B+A+G70): Bilirubin at concentrations in the 2, 5, 8, 11 and 14 mg/dl mixed with glucose solution concentrations at 70 mg/dl and albumin solution at concentrations in the 3500 mg/dl.

Set 4 (B+A+G135): Bilirubin at concentrations in the 2, 5, 8, 11 and 14 mg/dl mixed with glucose solution concentrations at 135 mg/dl and albumin solution at concentrations in the 3500 mg/dl.

Set 5 (B+A+G200): Bilirubin at concentrations in the 2, 5, 8, 11 and 14 mg/dl mixed with glucose solution concentrations at 200 mg/dl and albumin solution at concentrations in the 3500 mg/dl.

2) Turn on the bilirubin detector and place the cuvette containing the sample into the chamber. The test of the absorption of green light and blue light that passes through a cuvette containing bilirubin, albumin, and glucose of set 1 which was tested 3 times per data set to determine the voltage and voltage difference of the three pure substances in artificial blood solution.

3) Calculate the average voltage, average voltage difference and the standard deviation of blue light and green light and record in the table 1.

4) The test of the absorption green light and blue light that passes through a cuvette containing bilirubin mixed with albumin, and glucose of set 2, 3, 4 and 5 which was tested 3 times per data set to determine the voltage and voltage difference of the mixture. Calculate the average voltage, average voltage difference and the standard deviation of blue light and green light and record in the table 2.

5) Create a graph of the relationship between the voltage difference and the concentration of bilirubin at 2, 5, 8, 11 and 14 mg/dl. Create a graph of the relationship between the voltage difference and the concentration of bilirubin at 2, 5, 8, 11 and 14 mg/dl mixed with albumin 3500 mg/dl. Create a graph of the relationship between the voltage difference and the concentration of bilirubin 2, 5, 8, 11 and 14 mg/dL mixed with albumin 3500 mg/dl and glucose 70 milligrams per deciliter. Create a graph of the relationship between the voltage difference and concentration of bilirubin mixed with albumin 3500 mg/dl and glucose 135 mg/dl. Create a graph of the relationship between the voltage difference and the concentration of bilirubin 2, 5, 8, 11 and 14 mg/dL mixed with albumin 3500 mg/dl and glucose at 200 mg/dl.

## 3. RESULTS AND DISCUSSIONS

From a study on the relation between voltage and bilirubin concentration in artificial blood solution by bilirubin detector that used light absorption and spectrophotometry principles. The project was divided 2 parts: 1) Design and construction of a bilirubin detector circuit and 2) Functional test of the bilirubin detector circuit.

### 3.1. Design and construction of a bilirubin detector circuit

For the design and construction of a bilirubin detector circuit was used a circuit to measure the light transmission that passed through a cuvette that contained a solution of bilirubin, glucose, and albumin. The prototype used a 3.7 volts rechargeable battery (Vdc) of 3,000 milliamps per hour (mAh). The 2 LED bulbs which were the blue light at a wavelength of 460 nanometers and the green light with wavelength 570 nanometers was used as the light sources. The prototype used the principle of spectrophotometry and the principle of light absorption through a cuvette. The OLED screen to display voltage values in millivolt (mV) and showed that Green is the voltage output value of the Green LED light, Blue is the voltage output value of the blue LED light and DIFFERENCE is the voltage difference output of the LED between the green light and the blue LED. It was able to measure the voltage output value of the transmission light from the sample compared to the bilirubin concentration. The prototype of bilirubin detector was shown in figure 5.

**Figure 5** Prototype of bilirubin detector.

The prototype used a 3.7 volts rechargeable battery (Vdc) of 3,000 milliamps per hour (mAh). The 2 LED bulbs which were blue light at a wavelength of 460 nanometers and the green light with wavelength 570 nanometers was used as the light sources. The prototype used the principle of spectrophotometry and the principle of light absorption through a cuvette. The OLED screen to display voltage values in millivolt (mV) and showed that Green is the voltage output value of the Green LED light, Blue is the voltage output value of the blue LED light and DIFFERENCE is the voltage difference output of the LED between the green light and the blue LED. It was able to measure the voltage output value of the transmission light from the sample compared to the bilirubin concentration.

### 3.2. Functional Test of The Bilirubin Detector Circuit.

1) The functional test of the bilirubin detector circuit that consisted of a blue light at a wavelength of 460 nm and a green light at a wavelength of 560 nm to find the voltage values of light transmitted through bilirubin, albumin, and glucose in artificial blood solution.

**Table 1 Results of the functional testing of the bilirubin detector circuit from collecting sample set 1**

| Sample | Concentration of substance (mg/dl) | | | Average voltage of green light (mV) | Average voltage of blue light (mV) | Average voltage difference (mV) |
|---|---|---|---|---|---|---|
| Set | Bilirubin | Albumin | Glucose | | | |
| 1 | 2 | - | - | 2227.52 ± 0.93 | 2001.03 ± 2.46 | 226.49 ± 1.61 |
| | 5 | - | - | 2220.53 ± 2.42 | 1974.70 ± 0.81 | 245.83 ± 3.22 |
| | 8 | - | - | 2203.07 ± 2.46 | 1952.67 ± 2.03 | 250.40 ± 0.47 |
| | 11 | - | - | 2202.26 ± 2.03 | 1935.47 ± 2.03 | 266.79 ± 0.00 |
| | 14 | - | - | 2194.47 ± 1.68 | 1904.04 ± 2.83 | 290.43 ± 3.05 |
| | | 3500 | - | 1897.86 ± 1.23 | 1460.47 ± 1.61 | 437.39 ± 1.23 |
| | | - | 70 | 2255.46 ± 1.68 | 2055.57 ± 3.98 | 199.89 ± 2.91 |
| | | - | 135 | 2233.96 ± 1.23 | 2030.85 ± 1.23 | 203.11 ± 2.42 |
| | | - | 200 | 2230.74 ± 2.03 | 2021.72 ± 1.86 | 209.02 ± 1.23 |

Table 1 shows the results of measuring the voltage from bilirubin concentration by measuring 3 times in blue light and green light. The bilirubin concentrations were 2, 5, 8, 11, and 14 milligrams per deciliter (mg/dl). The average voltage of green light was 2227.52 ± 0.93, 2220.53 ± 2.42, 2203.07 ± 2.46, 2202.26 ± 2.03 and 2194.47 ± 1.68 mV respectively. For the blue light, the average voltage was 2001.03 ± 2.46, 1974.70 ± 0.81, 1952.67 ± 2.03, 1935.47 ± 2.03 and 1904.04 ± 2.83 mV respectively. It was also found that the average voltage difference of bilirubin concentration was 226.49 ± 1.61, 245.83 ± 3.22, 250.40 ± 0.47, 266.79 ± 0.00 and 290.43 ± 3.05 mV, respectively.

The results of measuring the voltage from concentration of albumin in artificial blood solution were 3500 mg/dl, measured 3 times in green and blue light. The average voltage of green light was 1897.86 ± 1.23 mV and the blue light was 1460.47 ± 1.61 mV, and the average electric potential difference was 437.39 ± 1.23 mV.

The results of measuring the voltage from the concentration of glucose in the artificial blood solution were measured 3 times. The voltage value of the green light, the blue light voltage and voltage difference of 70 mg/dl glucose solution were 2255.46 ± 1.68 mV, 2055.57 ± 3.98 mV and 199.89 ± 2.91 mV, in 135 mg/dl glucose solution were 2233.96 ± 1.23, 2030.85 ± 1.23 and 203.11 ± 2.42 mV and in 200 mg/dl glucose solution were 2230.74 ± 2.03 mV, 2021.72 ± 1.86 mV and 209.02 ± 1.23 mV, respectively.

From measuring the voltage from light transmission from bilirubin in green light and blue light, it was found that when the concentration value of bilirubin increases, the change in voltage difference tends to decrease accordingly because there is increased light absorption from the solution, causing the light transmitted to decrease which are consistent with Beer-Lambert's law, resulting in the voltage difference decreasing [17]. For the concentration of albumin affects the absorption of green and blue light. For the concentration of glucose, it was found that even if the concentration of the glucose solution was changed from 70 – 200 mg/dl, the voltage difference changes very little, with almost no significance of the changing. Therefore, glucose does not absorb blue light at a wavelength of 460 nm.

2) The functional testing of blue light at 460 nm and green light in the bilirubin detector circuit was tested in the sample set 2, 3, 4 and 5. The results of this project were shown in table 2.

**Table 2 Results of the functional testing of the bilirubin detector circuit from collecting sample set 2, 3, 4, 5.**

| Sample | Concentration of substance (mg/dl) | | | Average voltage of green light (mV) | Average voltage of blue light (mV) | Average voltage difference (mV) |
|---|---|---|---|---|---|---|
| Set | Bilirubin | Albumin | Glucose | | | |
| 2 (B+A) | 2 | 3500 | - | 2107.15 ± 0.47 | 1735.05 ± 2.59 | 372.10 ± 3.05 |
| | 5 | 3500 | - | 2103.39 ± 0.47 | 1720.54 ± 0.47 | 382.85 ± 0.81 |
| | 8 | 3500 | - | 2091.84 ± 1.23 | 1704.69 ± 3.22 | 387.15 ± 2.59 |
| | 11 | 3500 | - | 2072.49 ± 1.23 | 1677.29 ± 0.81 | 395.21 ± 0.47 |
| | 14 | 3500 | - | 2056.11 ± 1.61 | 1653.64 ± 1.23 | 402.46 ± 2.83 |
| 3 (B+A+G70) | 2 | 3500 | 70 | 2103.39 ± 1.23 | 1682.12 ± 1.61 | 421.27 ± 0.47 |
| | 5 | 3500 | 70 | 2085.39 ± 1.68 | 1655.79 ± 1.68 | 429.60 ± 2.91 |
| | 8 | 3500 | 70 | 2057.72 ± 2.42 | 1612.81 ± 2.91 | 444.91 ± 2.13 |
| | 11 | 3500 | 70 | 2050.73 ± 1.68 | 1575.73 ± 0.81 | 475.00 ± 2.46 |
| | 14 | 3500 | 70 | 2042.94 ± 1.68 | 1562.57 ± 0.93 | 480.38 ± 2.13 |
| 4 (B+A+G135) | 2 | 3500 | 135 | 2065.51 ± 1.68 | 1568.74 ± 1.68 | 496.76 ± 1.68 |
| | 5 | 3500 | 135 | 2050.46 ± 0.81 | 1542.15 ± 1.23 | 508.32 ± 0.47 |
| | 8 | 3500 | 135 | 2034.08 ± 0.93 | 1505.61 ± 2.13 | 528.47 ± 1.68 |
| | 11 | 3500 | 135 | 2030.05 ± 1.86 | 1492.44 ± 2.83 | 537.60 ± 1.40 |
| | 14 | 3500 | 135 | 2000.76 ± 2.03 | 1451.34 ± 1.68 | 549.42 ± 0.47 |
| 5 (B+A+G200) | 2 | 3500 | 200 | 2021.99 ± 1.23 | 1440.59 ± 1.68 | 581.39 ± 2.46 |
| | 5 | 3500 | 200 | 2021.45 ± 0.81 | 1430.38 ± 2.83 | 591.07 ± 2.03 |
| | 8 | 3500 | 200 | 2023.06 ± 0.81 | 1412.38 ± 1.68 | 610.68 ± 1.68 |
| | 11 | 3500 | 200 | 2006.13 ± 0.81 | 1393.84 ± 0.93 | 612.29 ± 1.23 |
| | 14 | 3500 | 200 | 1986.52 ± 3.36 | 1372.35 ± 2.46 | 614.17 ± 3.22 |

The functional testing results of set 2: the blue light absorbance of bilirubin concentrations of 2, 5, 8, 11 and 14 mg/dl mixed with albumin solution at a concentration of 3500 mg/dl, it was found that the difference voltage output was 372.10 ± 3.05, 382.85 ± 0.81, 387.15 ± 2.59 and 395.21 ± 0.47 mV, respectively. The functional testing results of set 3: the blue light absorbance of bilirubin concentrations of 2, 5, 8, 11 and 14 mg/dl mixed with albumin solution at a concentration of 3500 mg/dl and 70 mg/dl glucose solution, it was found that the difference voltage output were 421.27 ± 0.47, 429.60 ± 2.91, 444.91 ± 2.13, 475.00 ± 2.46 and 480.38 ± 2.13 mV, respectively. The functional testing results of set 4: the blue light absorbance of bilirubin concentrations of 2, 5, 8, 11 and 14 mg/dl mixed with albumin solution at a concentration of 3500 mg/dl and 135 mg/dl glucose solution, it was found that the difference voltage output were 496.76 ± 1.68, 508.32 ± 0.47, 528.47 ± 1.68, 537.60 ± 1.40 and 549.42 ± 0.47 mV, respectively. The functional testing results of set 5: the blue light absorbance of bilirubin concentrations of 2, 5, 8, 11 and 14 mg/dl mixed with albumin solution at a concentration of 3500 mg/dl and 200 mg/dl glucose solution, it was found that the difference voltage output were 581.39 ± 2.46, 591.07 ± 2.03, 610.68 ± 1.68, 612.29 ± 1.23 and 614.17 ± 3.22 mV, respectively.

3) The graph of relation between the voltage output in millivolt (mV) with the bilirubin (B) at concentration 2, 5, 8, 11 and 14 in milligram per deciliter (mg/dl), the voltage output with the bilirubin mixed with albumin 3500 mg/dl (B+A), the voltage output with the bilirubin mixed with albumin 3500 mg/dl and glucose at a concentration of 70 mg/dl (B+A+G70), the voltage output with the bilirubin mixed with albumin 3500 mg/dl and glucose at a concentration of 135 mg/dl (B+A+G135) and the voltage output with the bilirubin mixed with albumin 3500 mg/dl and glucose at a concentration of 200 mg/dl (B+A+G200) were shown in figure 6, 7, 8, 9, 10.

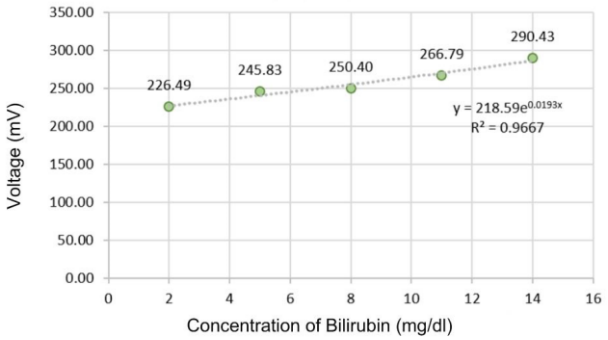


**Figure 6** Graph of the relation between the voltage output and concentration of bilirubin (B).

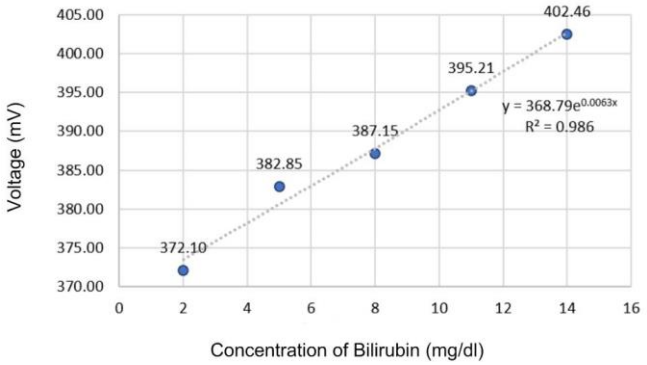


**Figure 7** Graph of the relation between the voltage output and concentration of bilirubin mixed with albumin (B+A).

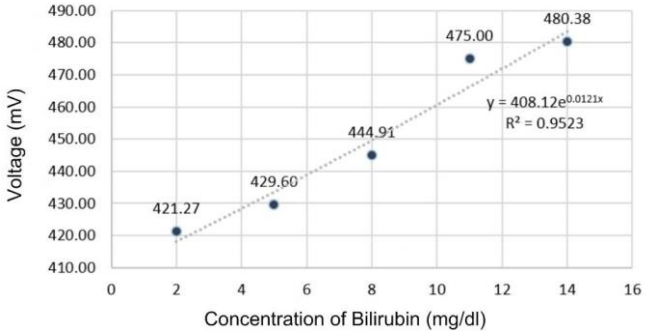


**Figure 8** Graph of the relation between the voltage output and concentration of bilirubin mixed with albumin and glucose 70 mg/dl (B+A+G70).

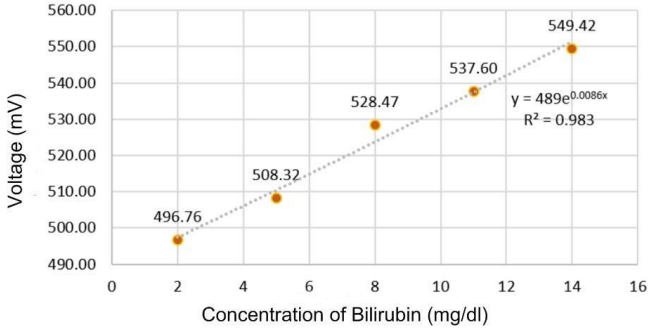


**Figure 9** Graph of the relation between the voltage output and concentration of bilirubin mixed with albumin and glucose 135 mg/dl (B+A+G135).

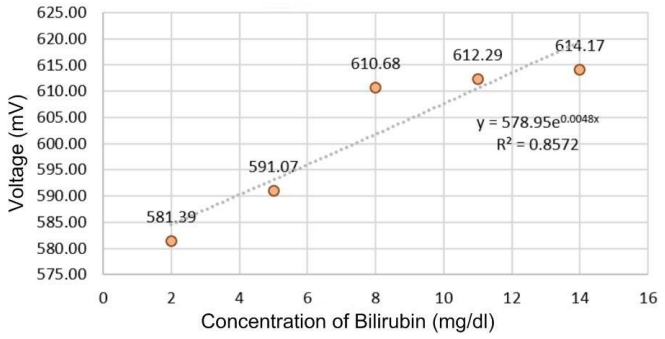


**Figure 10** Graph of the relation between the voltage output and concentration of bilirubin mixed with albumin and glucose 200 mg/dl (B+A+G200).

From figure 6-10, the equation of graph, the correlation coefficient (r) and the coefficient of determination ($R^2$) were shown in below:

B: $y = 218.59e^{0.0193x}$ , r = 0.9832, $R^2 = 0.9667$
B+A: $y = 368{,}79e^{0.0063x}$ , r = 0.9929, $R^2 = 0.9860$
B+A+G70: $y = 408.12e^{0.0121x}$ , r = 0.9759, $R^2 = 0.9523$
B+A+G135: $y = 489e^{0.0086x}$ , r = 0.9915, $R^2 = 0.9830$
B+A+G200: $y = 578.95e^{0.0048x}$ , r = 0.9259, $R^2 = 0.8572$

From the equation between the bilirubin concentration and the voltage difference, it was found that a high bilirubin concentration results in a large voltage difference. A low concentration of bilirubin will result in a low voltage difference and when mixed with albumin solution, it causes the voltage difference to increase. The high concentration of bilirubin mixed with albumin solution and glucose made in a large voltage difference. As for the low concentration of bilirubin mixed with albumin and glucose solutions, the voltage difference was also low as well. The bar graph showed the comparison shown in figure 11.

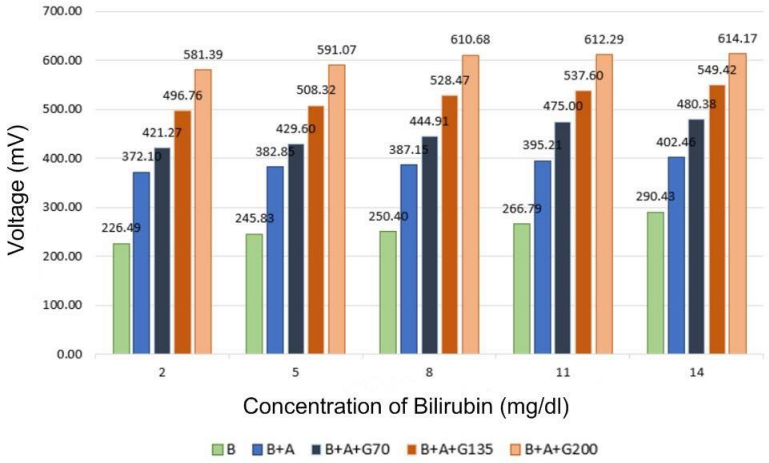


**Figure 11** Bar graph of the relation between the voltage output and concentration of bilirubin (mg/dl) of each sample

The bar graph shows a comparison of the electric potential difference values of each sample. It was found that when the concentration of bilirubin increased, the electric potential difference became more valuable. It shows that the concentration of bilirubin affects the transmission of light. Therefore, the blue light at 460 nm can be used as a light source for detecting bilirubin but this wavelength can detect albumin as well that was agreed with the previous of non -invasive of bilirubin.

When mixed with albumin solution, it causes the voltage difference to increase. The high concentration of bilirubin mixed with albumin solution and glucose made in a large voltage difference because green light with a wavelength of 560 nm and blue light with a wavelength of 460 nm have similar values, they affect the absorption of bilirubin. albumin and glucose Therefore, the wavelength of the green light used as a background should be adjusted to be different from that of the blue light. This will make the determination of the voltage difference more valuable and can create an equation for the relationship between the voltage difference with concentration of bilirubin and compare the difference between the 2 variables to be more stable and accurate in order to further develop a non-invasive device for measuring bilirubin in newborns.

## CONCLUSIONS

In conclusion, this study delved into the intricate relationship between voltage and bilirubin concentration within artificial blood solutions, utilizing a bilirubin detector grounded in the principle of light absorption. The bilirubin detector employed two distinct light sources, namely blue light at a wavelength of 460 nm and green light at a wavelength of 560 nm, integrated into the detector circuit. The bilirubin solutions were meticulously measured at concentrations spanning 2, 5, 8, 11, and 14 mg/dl, and they were further combined with albumin solutions at concentrations of 3500 mg/dl and glucose at varying concentrations of 70, 135, and 200 mg/dl.

By systematically collecting voltage output data through five distinct sample sets, our findings substantiate that bilirubin effectively absorbs the blue light at 460 nm. Moreover, a noteworthy correlation emerged between the concentration of bilirubin and the voltage output, where an increase in bilirubin concentration corresponded to a proportional increase in voltage. Conversely, lower bilirubin concentrations resulted in diminished voltage outputs. Notably, the presence of albumin in the solutions influenced the light absorption characteristics of both the blue light at 460 nm and the green light at 560 nm.

Our research culminated in the derivation of an empirical equation that encapsulates the intricate relationship between voltage values and bilirubin concentrations. This equation serves as a pivotal foundation for the future design and construction of a noninvasive bilirubin detector, underscoring the practical implications and potential advancements in clinical diagnostics and monitoring. Further this technology promise offering valuable insights into the health and well-being of individuals while minimizing invasive procedures.

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