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Evaluating of a Super Bright LED as a Spectrophotometer Light Source at The Clinical Laboratory

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ABSTRACT Spectrophotometers generally use a halogen lamp as a light source that passes through a filter (wavelength) according to the material to be analyzed. This study aims to analyze the ability of the LED as a light source on a spectrophotometer. In this study, the authors have determined blood sugar parameters as the test material. So that the determination of the wavelength of the LED as a light source must be adjusted to the specifications of the wavelength in the reagent manual procedure used. In the BAV Greiner Glucose Reagent procedure, the allowable wavelength is between 500 - 570 nm with a cuvette thickness of 1 cm. They were measured against the reagent blank by the endpoint method. From this reference, the author uses an LED light source of 530 nm, Epistar brand green. The module in this study consisted of a 530 nm LED lamp as a light source. Then a lens was added to focus the light beam from the 530 nm LED. The author also adds an aperture so that the light passing through is focused at one point of the circle. Further, the light will pass the cuvette. The results of the absorption of light will be received by the light sensor (photoresistor), and the data is processed by Arduino, and the results are displayed on display. From the results of this study, the value ranges *error* from 1% to 3% when a comparative test is carried out with the Analytical type Biolyzer100 spectrophotometer with six different samples and is repeated five times each. From these data, it is found that the LED with a wavelength of 530 nm is effective as a light source for checking blood sugar.

INDEX TERMS 530 nm LED, Lens, Cuvette, Photoresistors, Arduino, Absorption, Reagent, Biolyzer 100

I. INTRODUCTION

Spectrophotometry is a method for measuring how much a chemical substance absorbs light by measuring the intensity of the light as a beam of light passes through a sample solution. The basic principle is that each compound absorbs or transmits light in a certain range of wavelengths. This measurement can also be used to measure the amount of a known chemical [1][2]. Spectrophotometry is a tool that relies on the quantitative analysis of molecules depending on how much light is absorbed by the colored compound. Important features of a spectrophotometer are the spectral bandwidth (range of colors that can be transmitted through the test sample), percentage of sample transmission, logarithmic range of sample absorption, and sometimes the percentage of reflectance measurements. Spectrophotometers are usually used to measure the transmittance or reflectance of solutions, transparent or opaque solids, such as polished glass or gases[3]. Although many biochemicals are colored, they absorb visible light and can therefore be measured by colorimetric procedures, and even colorless biochemicals can often be converted into colored compounds suitable for chromogenic coloration reactions to produce compounds suitable for colorimetric analysis [4]. The basic concept of a spectrophotometer is to use the Photometric Method. Beer Lambert's law states that the energy absorbed depends on the thickness of the material through which the light passes, and the amount of light absorbed (absorbed) is directly proportional to the concentration of the substance that absorbs or absorbs it

[5][6]. In its application, spectrophotometry is widely used in laboratories, whether environmental laboratories, pharmacy laboratories, or health clinic laboratories. In the field of health clinical laboratories, a spectrophotometer or commonly known as a photometer is used to analyze the concentration of a solution from a patient sample using certain reagents according to the parameters to be analyzed, for example, blood sugar, uric acid, cholesterol, triglycerides, and so on. In general, spectrophotometry has important parts including, a lamp as a light source, a filter/wavelength selected according to the examination/measurement, and a light sensor as a receiver of the transmitted light [7]. Generally, spectrophotometers use halogen lamps as light sources with a lifetime of certain. In addition, there are filters/wavelengths that can be selected according to the desired examination [8][9][10]. Of course, such a spectrophotometric tool has a fairly high price, with a limited lifetime of halogen lamps. With the development of technology and knowledge, LEDs have various specifications, one of which is an LED that has a certain wavelength so that it can be selected according to needs [11][12][13]. The development of the era also makes it easier for us to know the amount of light intensity, with many choices of light sensors with certain types and specifications. Therefore, in this study, we will use LED as a spectrophotometer light source to analyze the solution according to the actual concentration.

In previous studies, many discussed LED Spectrophotometry with various methods, including the following. In 2015, Kim Ji Sun made a white LED spectrophotometer with a color sensor (TCS2300) as the receiver to analyze the color of a solution. As a result, the purity and wavelength can be detected with the CIE diagram, and the concentration can be estimated with the purity information. This method is more economical and efficient than the existing spectrophotometry [4]. Still, in the same year, Mohammad Karim Aly compared the LED spectrophotometer with a general spectrophotometer. The result was that the LED spectrophotometer had an *error of* -2.33%, which means the results are acceptable [14]. The LED (white color) as a light source in a spectrophotometer was made by P. Visconti in 2017; the concept resembles a Spectro in general, only the light source is different. As a result, confirming the correct functioning and interaction of the system, via the PC terminal, between the user and the realized control unit [15]. In a Chaianantakul study, Natpasit made an LED-based spectrophotometer for blood sugar detection. As a result, the instrument showed good reproducibility and high sensitivity and specificity. Mini-spectrophotometers are promising to be used instead of conventional spectrophotometers for screening and monitoring glucose concentrations in patients with diabetes mellitus [16]. From the literature study above, it shows that the use of LEDs can be used as an alternative to a light source in a simple, economical spectrophotometer, but there is a need for studies or research that discusses the sensitivity and or effectiveness of light sensors in responding or accepting any existing conditions. In the literature above, it has not been explained in detail about the ability of the light sensor as an important element in determining the absorbance value of a solution being analyzed. In addition to the light sensor, the research method test results are still dominantly carried out on dyes whose concentrations are not stated in detail. Therefore, a study on LED analysis as a light source on a spectrophotometer needs to be carried out. In addition to the ability of the LEDs and light sensors to be analyzed, the test method will also use comparisons with parameters in clinical laboratories in hospitals that have calibration standards, QC (*Quality Control*) and reagent concentrations that have clear values. So it is hoped that it will provide detailed information

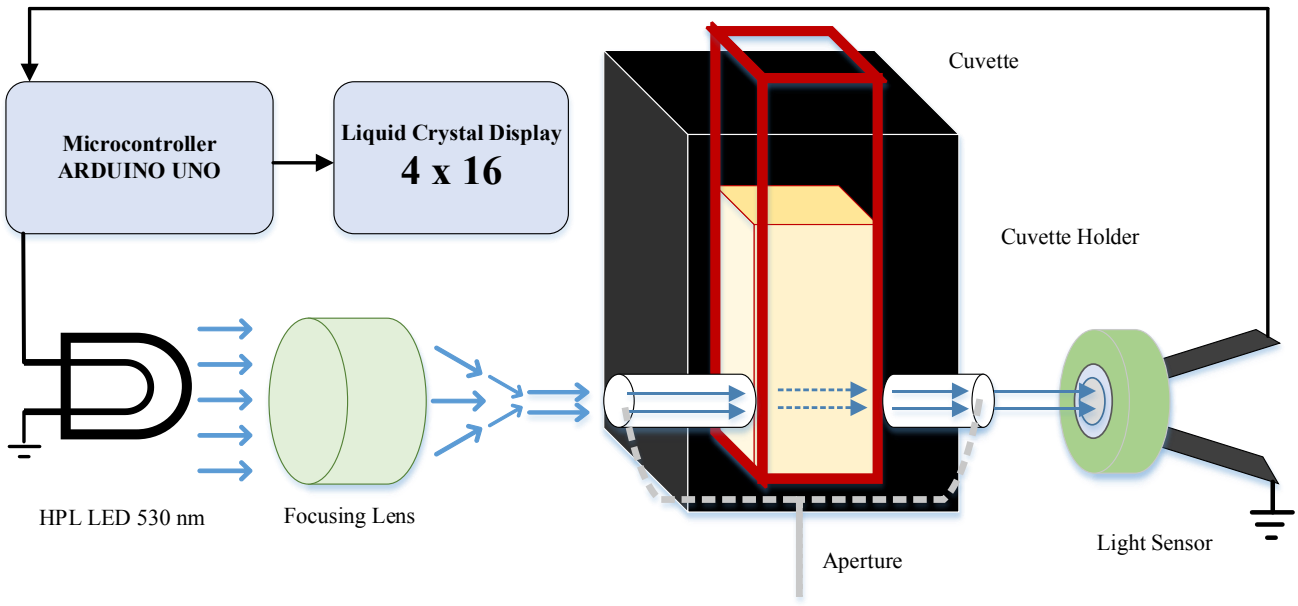

FIGURE 1. The diagram Block

about the effectiveness of the LED's ability as a light source on a spectrophotometer.

II. MATERIAL AND METHODS

A. EXPERIMENTAL SETUP

In this study, the light sources used are LED HPL with Wavelength 530 nm. The LED voltage input is given different conditions to get the best intensity. The light source will pass through the cuvette which has been filled with Glucose Working Reagent, which is reacted with the patient's sample (serum). Furthermore, the voltage value of the output on the light sensor is measured. The data analysis process is carried out by measuring the voltage value from the sensor output, then proceeding to analyze the results of the sample concentration, which is compared with the Analytical Biolyzer100 brand photometer.

B. MATERIALS AND TOOL

This module consists of LED with 530 nm as the light source, the lens to focus the light, cuvette, light sensor, Arduino Uno, and also the 4x16 LCD Display. Other materials there are Glucose Reagent, Calibrator/standard, Quality Control (QC), and the Analyzer Biolyzer100.

C. EXPERIMENT

In this study, an analysis will be carried out on the ability of the LED by giving different treatments on the LED input voltage. Each treatment will be used to perform Glucose Reagent with Quality Control (QC) as a validation Result whether each result is within QC range or not. The best result means the value is almost in the mean of the glucose value limit on the QC kit insert. So the best result will be used to analyze the blood sugar of the patient and will compare with the analyzer Biolyzer100.

D. THE DIAGRAM BLOCK

The light source uses an LED with a wavelength of 530 nm (FIGURE 1). Will be forwarded to a cuvette that has been filled by a mixture of working reagents with patient samples (serum). The absorbed light is then received by the light detector/sensor. The light sensor will respond in the form of analog data that is sent to the processor to be processed into digital data. The data is converted into absorbance values. Then it is changed again to the concentration value. The results of the concentration value will be shown on display. This research will begin with the design of an LED Spectrophotometer as a device for testing. Then the next research step is to measure the absorbance and concentration of the output readings of the two sensors that will be compared. The technical implementation is to change the sensor position alternately while still paying attention to the correct sensor position. The sample testing mechanism uses patient samples (serum), STD from BAV Greiner brand Glucose reagent, and *Quality Control* (Unitrol) BAV Greiner brand. Each of these samples will take data for comparison with the Analyticon brand Biolyzer 100 comparison tool, Germany. Glucose reagent is prepared for sample measurement. Read aquadest for initial standardization on unit tests. Then read the reagent blank (R1). This serves as the starting point for reading the Endpoint method. Then confirm whether it is necessary or not to do the standard. If the standard is done, the liquid that is inserted into the device is glucose reagent (R1) added to the standard. The reading results will be in the form of coefficient values, the data will be saved. Then proceed with the reading of the sample or QC (*Quality Control*). The result of reading the absorbance sample / QC is multiplied by the standard coefficient that has been formed earlier, and the result is the concentration value of the sample / QC.

E. Analog

The main circuit in this study has three circuit blocks: a light sensor circuit, a microcontroller circuit, and an LCD circuit. The light sensor circuit is a circuit that functions to receive light from the absorption of sample readings. The light sensor will detect changes in the light from the sample reading. The intensity received by the light sensor will be converted into resistance. Then there will be a voltage division. The voltage division will enter the Arduino A0 pin to be converted into ADC (Analog Digital Converter) data. This circuit has the characteristic that if the intensity received by the light sensor is large, the result of the voltage division will also be large. And vice versa, if the light sensor detects a low intensity, the resulting voltage will also be below.

Arduino circuit functions to process all information from the light sensor. In Arduino, the author has compiled a program starting from converting data from analog to digital data, and then the data is entered into the formula for calculating the absorbance value. After obtaining the absorbance value, it is continued with calculations to find the concentration of the readable solution. In addition, the author has also created a storage system to store absorbance data for blanks and factor values. Then the concentration results will be displayed on the LCD

III. RESULT

A. MODUL DESIGN

The final result of the block diagram is shown in FIGURE 2. An additional lens is used to focus the light and add an aperture before and after the cuvette. It is important to make the light beam pass the cuvette, and the sensor can detect well without any interferences. The final design, FIGURE 2, consists of 3 buttons, they are Yes, Next, and Reset. There is a display LCD with 4x16 and a hole to take the cuvette.

(a) (b)

FIGURE 2. The spectrophotometer based on super bright LED (a) the panel side, (b) inside the module

B. RESULT OF MEASUREMENT

To analyze the capability of LED in this study to perform reading the absorbance of the glucose quality control and

evaluate the result value, whether within range or not (TABLE 1).

TABLE 1
LED Test with Glucose Quality Control (QC)

TREATMENT	LED Condition	Sensor Output (mV)	ABS	QC (mg/dl)	QC Range (mg/dl)
Treatment1 Vin LED **5.9V**	Dim	0.475	0.029	-	
Treatment 2 Vin LED **6.3 V**	Medium	0.625	0.276	120.1	**75.9**
Treatment 3 Vin LED **6.5V**	Bright	0.692	0.216	89.2	**to**
Treatment 4 Vin LED **12V**	Bright	0.855	0.208	85.2	**103**

Based on the results of Quality Control readings in Treatment 3 with a power range of 5 watts and Treatment 4 with a range of 10 watts of power indicates that the concentration is within the allowable limits. While in treatment 2, with a power range of 4 watts, the concentration results were above the allowable limit. So that Treatments 3 and 4 can be used for testing blood sugar concentrations. However, the author uses Treatment 4 for testing blood sugar concentrations because it maximizes the ability of the LED to work optimally at a voltage of 12 Volts with a maximum power of 10 watts.

C. MODULE COMPARISON WITH BIOLYZER100

Data collection on the patient's blood sugar concentration in this study used random blood/serum samples. In addition, the blood sugar that is measured is random blood sugar (without fasting). The author took six samples of the patient's blood/serum, then reacted with reagents and read them in the *module* and also in the comparison device (Biolyzer100) at the same time. This is necessary and concern because the chemical reactions that occur between the reagents and the blood/serum samples will continue to run. So that the incubation time according to the reagent procedure needs to be considered, and the reading process in the module and comparison must be carried out simultaneously.

From the results of the measurements that have been carried out, it can be seen that the higher the absorbance value of the sample, the higher the concentration of the sample itself (fig. It can be seen from the comparative test that the author did as in the linear curve of the comparison of the concentration results with the sample absorbance on the module and comparison tool (Biolyzer100) as follows.

TABLE 2.
Reagent Blank and Factor Value

Parameter Measure	Module	Biolyzer100
Reagent Blank	0.076	0.091
Factor	301.205	346,021

TABLE 3
Measurement from Patients

PX	BIOLYZER 100		MODULE		Error%
	ABS	CONC.	ABS	CONC.	
1	0.248	85,808	0.293	88,279	3%
2	0.366	126.77	0.435	130,946	3%
3	0.697	242,902	0.799	240,690	1%
4	0.305	105.502	0.345	104.050	1%
5	0.381	131.754	0.431	128.768	2%
6	0.391	135.154	0.442	132.976	2%

Based on the linearity graph (FIGURE 3), there are two graphic results, namely the graph of the results of the comparison tool and the author's module. The result is the tool that the author made has a ratio of $R^2 = 0.9999$. At the same time, the comparison device (Biolyzer100) has a value of $R^2 = 1$. This means that the absorbance value is directly proportional to the concentration value. If the absorbance value is high, the concentration will also be high. Likewise, when compared with the Biolyzer 100 device, it has an R-value = 1.

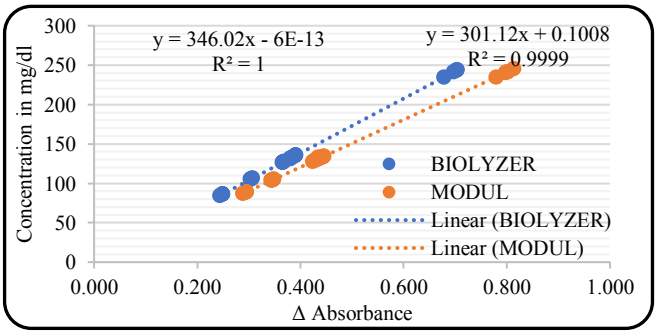

FIGURE 3. Linearity Graph of Absorbance and Concentration

IV. DISCUSSION

When the author's module was tested and compared using a patient sample, the results showed any value *error* ranging from 1% - 3%. The Value *error* can occur due to several things. Among them are material conditions (reagents, QC, and standards), incubation time, and stability of absorbance readings on the module. In addition, there are some differences in the hardware of the writer's tool and the comparison tool, such as the wavelength used. In the module, *an* LED with a wavelength of 530 nm is used, while the comparison tool uses a Filter/Wavelength of 500 nm. This is still allowed to be done referring to the procedure for using glucose reagents as in table 1.

The mechanical design that the author made for this research is to use an LED with a wavelength of 530 nm as a

light source that is directly forwarded and focused using a lens through a cuvette and detected by a light sensor. An LED with a wavelength of 530 nm has a green color. In contrast, the comparison device (Biolyzer100) uses a halogen lamp as a light source [32]. Also, equally, there is a lens that functions to focus the light from the halogen lamp, then it is forwarded to the cuvette and passed through a filter with a wavelength of 500 nm, which is then detected by the light sensor. This difference resulted in one of the deviations *errors* in this study.

V. CONCLUSION

Based on the purpose of this research that the author wants to analyze the ability of LEDs and light sensors, to read glucose samples concentration. So the conclusions can be drawn. The selection of a green LED with a wavelength of 530 nm can be used as a spectrophotometer light source for checking blood sugar, and the power is within ten Watt. The addition of a lens serves to focus the LED light beam so that it can enter the slit, helping to optimize the light intensity from the LED to the sensor. From the test results using a reagent blood sugar, we get an error of 1.6% on the results of the Quality Control and 1-3% in comparison with the results of the reading six patients looping five times respectively, with comparable commercial instrument Biolyzer 100.

In this study, there are still shortcomings, so improvements are needed. Among them, the need for sensor reading signal processing modules so that more stable data is obtained.

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Attachment :

1. Schematic : https://drive.google.com/file/d/1yKLU-kU7bIayQ4QRUVr-c7PnvJU7gviS/view?usp=sharing (File from Eagle)

2. Software : https://drive.google.com/file/d/1yXbrnGk_OrPeV6UpcAadYici_yCdkRxa/view?usp=sharing