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Compact Spectrophotometer Using TEMT600 Sensor for Protein Concentration Measurement

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Abstract: The development of simple and cost-effective spectrophotometers is essential to meet the demand for more affordable protein analysis tools. Conventional spectrophotometers often face challenges such as high costs and complexity, limiting their widespread use. This study introduces a compact and low-cost spectrophotometer. The spectrophotometer features a white LED light source (520 nm) and a TEMT600 light sensor to accurately measure the concentration and absorbance of protein solutions. Measurements were performed by analyzing light absorbance using a simple optical system and evaluating protein samples prepared under controlled conditions. The device demonstrated an average accuracy of 96.26% for concentration measurements and 95.97% for absorbance, providing reliable performance for protein analysis. Despite minor deviations, averaging 3.74% for concentration and 4.25% for absorbance, mainly due to environmental factors and sample preparation inconsistencies, the results remained within acceptable accuracy limits. Its simplicity, cost-effectiveness, and high accuracy make this spectrophotometer a valuable tool for laboratory and educational applications. Further development could enhance its performance, making it more reliable under various conditions.

Keywords: Spectrophotometer, TEMT600, Concentration

1. Introduction

Proteins are macromolecules composed of a number of L-amino acids bound by peptide bonds and consist of a number of molecules. Each protein molecule consists of a number of amino acids with a special arrangement [1]. Proteins are essential to the human body [2] because they make up the structure and function of the body. Due to the importance of their role, proteins are essential for human life [3]. Without proteins, creatures cannot live. Some of the functions of protein are as the formation and repair of body tissues [4], as a key component in the immune system [5], as transportation of substances in the blood, and maintenance of fluid and electrolyte balance in cells and tissues [6]. About 19% of body weight is protein, and 45% of

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total body protein is muscle. An adult needs 1 gram of protein per kilogram of body weight every day, while growing children need 3 grams of protein per kilogram of body weight. Adults should get one-fifth of the necessary protein, and children should get one-third of the necessary protein [7]. Therefore, it is very important to know the level of protein consumed.

To measure protein levels, there are several methods that can be used. These include the modified Dumas method, the Kjeldahl method [8], and the Spectrophotometry method [9]. The Kjeldahl method does not provide a true measurement of protein because not all nitrogen in food comes from protein, and it takes a long time, and using sulfuric acid at high temperatures is dangerous. The modified Dumas method, on the other hand, entails high costs for the materials and tools used, and produces results that are not Consequently, a simpler method-the biuret method with UV-Vis spectrophotometry-is needed. This method focuses only on proteins and does not contain nitrogen from nonprotein materials. It is also faster than other methods [10]. However, the use of UV-Vis spectrophotometer also requires large funds and is limited to research groups with large funds only. Therefore, spectrophotometers are starting to be developed using low-cost microcontrollers so that measurements can be accessed by all groups.

Uv-Vis spectrophotometer was developed to use photodiode sensor with RGB LED as an application for food coloring determination [11]. A spectrophotometer was also developed using a Hamamatsu C12880MA miniaturized spectrometer chip and an LED light source [12]. A low-cost spectrophotometer was developed to detect mercury ions (Hg2+) with the help of CMOS/CCD images [13]. A simple UV-Vis spectrophotometer design based on the Arduino Uno microcontroller was also developed to measure anthocyanin levels in brown rice [14]. The development of a portable Arduino-based spectrophotometer called APD UV-Vis SpectBT has been carried out to measure light in the wavelength range of 190-1100 nm [15]. A spectrophotometer was also developed using an RGB LED light source and a TCS34725 color sensor to analyze color and measure the intensity of emitted light [16].

Building on previous research that successfully developed a UV-Vis spectrophotometer capable of measuring various solution concentrations, this study focuses on creating a simple, low-cost spectrophotometer specifically designed for measuring protein concentration. The device utilizes a white LED light source with a wavelength of 520 nm, paired with a NodeMCU ESP8266 microcontroller for efficient data processing and a TEMT600 sensor as the light detector. This combination ensures accurate detection of light intensity changes due to protein absorption. To enhance user convenience, the measurement results are displayed directly on an integrated LCD screen, making the device user-friendly and suitable for rapid analysis. This approach not only reduces production costs but also ensures a targeted and reliable solution for protein concentration measurement. The simplicity and affordability of this spectrophotometer make it a valuable tool for educational, research, and small-scale laboratory applications..

2. Materials and Method

The components of the designed system are geometrically arranged according to their specific functions. The design process includes creating block diagrams, hardware design, and software design as part of the overall tool development. The first step in building the system is to create a block diagram, which serves as a guide for ensuring that the designed circuit aligns with the intended functionality. Developing a block diagram helps in explaining both the system

structure and the working principle of the system being developed. Below is the block diagram of a simple spectrophotometer system designed for measuring protein concentration using a TEMT600 sensor.

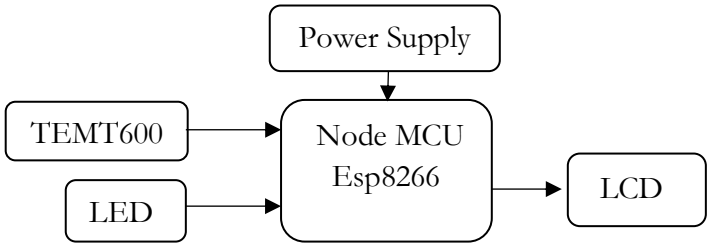

Figure 1. Block diagram of the system

Figure 1 illustrates a simple spectrophotometer system designed to measure protein concentration using a TEMT600 sensor. The system consists of several key components: a power supply serving as the voltage source, a white LED functioning as the light source, and a TEMT600 sensor acting as the detector for light emitted by the white LED. The NodeMCU ESP8266 serves as the central microcontroller, managing the operations of all components and processing the measurement data. Finally, an LCD is used to display the measurement results in real-time, providing users with accessible and straightforward data visualization.

The software design of the device focuses on the implementation of microcontroller programming. It involves creating an efficient and structured algorithm to control the device's functionality. The microcontroller is programmed to handle tasks such as collecting data from the TEMT600 sensor, processing measurements, and displaying the results on the LCD screen. The programming flowchart, as shown in Figure 2, illustrates the logical flow and sequential steps of the microcontroller's operations. This flowchart visually represents the process, including initializing components, reading sensor data, performing calculations for absorbance or concentration, and updating the display. By integrating these steps, the device ensures smooth operation, accurate real-time measurements, and user-friendly functionality.

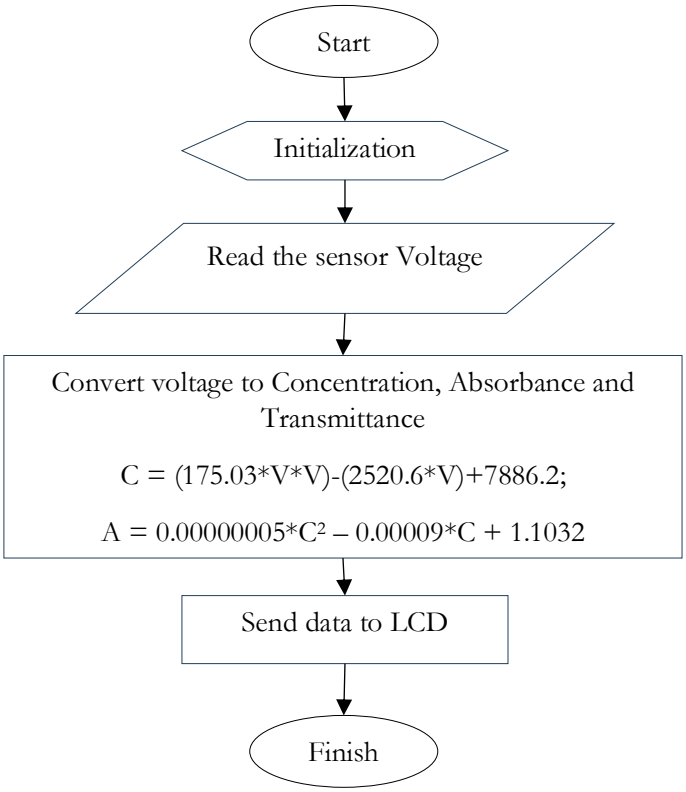

Figure 2. Flowchart of microcontroller device design

Figure 2 illustrates the flowchart of the microcontroller device design. The design process begins with the use of Arduino IDE software to create a program that will be uploaded to the NodeMCU board. The first step involves initializing the NodeMCU system to define the pins used for input and output operations. Once the device is powered on, the TEMT600 sensor starts measuring the light intensity that reaches it, providing a corresponding voltage value. This voltage value is then processed by the microcontroller to calculate the concentration and absorbance of the solution. Finally, the calculated results are displayed on the LCD20x4 screen, allowing users to view the data in real-time.

This tool features a hardware design composed of various electronic components, including a TEMT600 sensor, a white LED (520 nm), a NodeMCU ESP8266 microcontroller, and a 16x2 LCD. When power is supplied to the microcontroller, the device is activated. Upon startup, the white LED emits light directed toward the sample cuvette, and the TEMT600 sensor detects the light intensity. The sensor measures the values in terms of concentration, absorbance, and transmittance. These measurement results are then transmitted to the NodeMCU ESP8266, which processes the input data. Once processed, the calculated data is displayed on the 16x2 LCD screen, providing users with real-time measurement results.

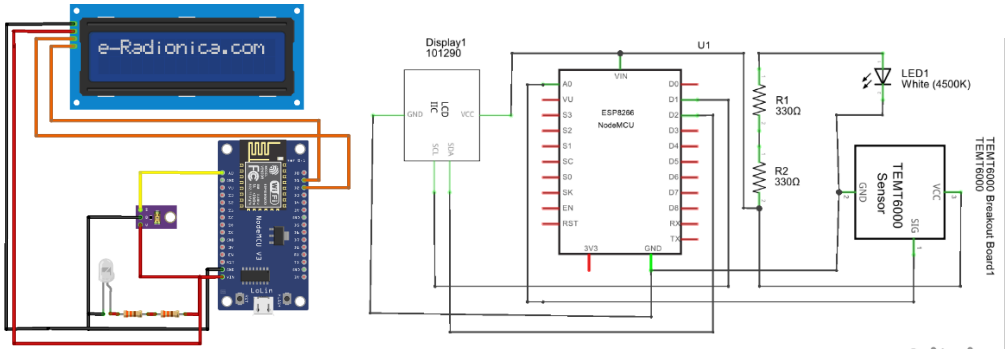

Figure 3. Electronic circuit of the system

Figure 3 illustrates the detailed system circuit where the NodeMCU ESP8266 is utilized as the central microcontroller for controlling and processing data from various components. In the circuit, the NodeMCU ESP8266 pin connections are established to integrate the required functionalities. The A0 pin on the NodeMCU is connected to the output pin of the TEMT600 sensor, which is responsible for detecting the intensity of light passing through the sample. This pin is crucial for reading the sensor's analog output voltage, which will later be processed into concentration, absorbance, and transmittance values.

The SDA (Serial Data) pin of the I2C module is connected to pin D2 of the NodeMCU, while the SCL (Serial Clock) pin is connected to pin D1. These connections facilitate the I2C communication protocol, enabling seamless data exchange between the NodeMCU and the LCD module. This protocol is essential for displaying real-time measurement results clearly and efficiently on the 16x2 LCD.

Additionally, the circuit includes a white LED configured as the light source, which emits a stable 520 nm wavelength. The LED is connected in series with two 330-ohm resistors. These resistors play a critical role in regulating the current flowing through the LED, preventing damage due to excessive current and ensuring consistent light intensity for accurate measurements.The integration of these components and their proper connections ensures a reliable system for measuring protein concentration, with the NodeMCU acting as the central hub for data collection, processing, and output display. This circuit design balances functionality, simplicity, and cost-effectiveness, making it suitable for various laboratory and educational applications.

3. Results and Discussion

The results of the research demonstrate that the simple spectrophotometer effectively measures the parameters under investigation, such as sensor characterization, accuracy, and sensitivity of the TEMT600 sensor used, as well as the accuracy of the concentration and absorbance values. The system incorporates several key components, including the NodeMCU ESP8266 microcontroller, TEMT600 sensor, white LED, and LCD.

The NodeMCU ESP8266 serves as the core component of the system, responsible for processing both inputs and outputs. It collects data from the sensor and other components, processes it, and then displays the results on the Liquid Crystal Display (LCD). This allows the user to view real-time measurements of concentration, absorbance, and transmittance of the solution being analyzed.

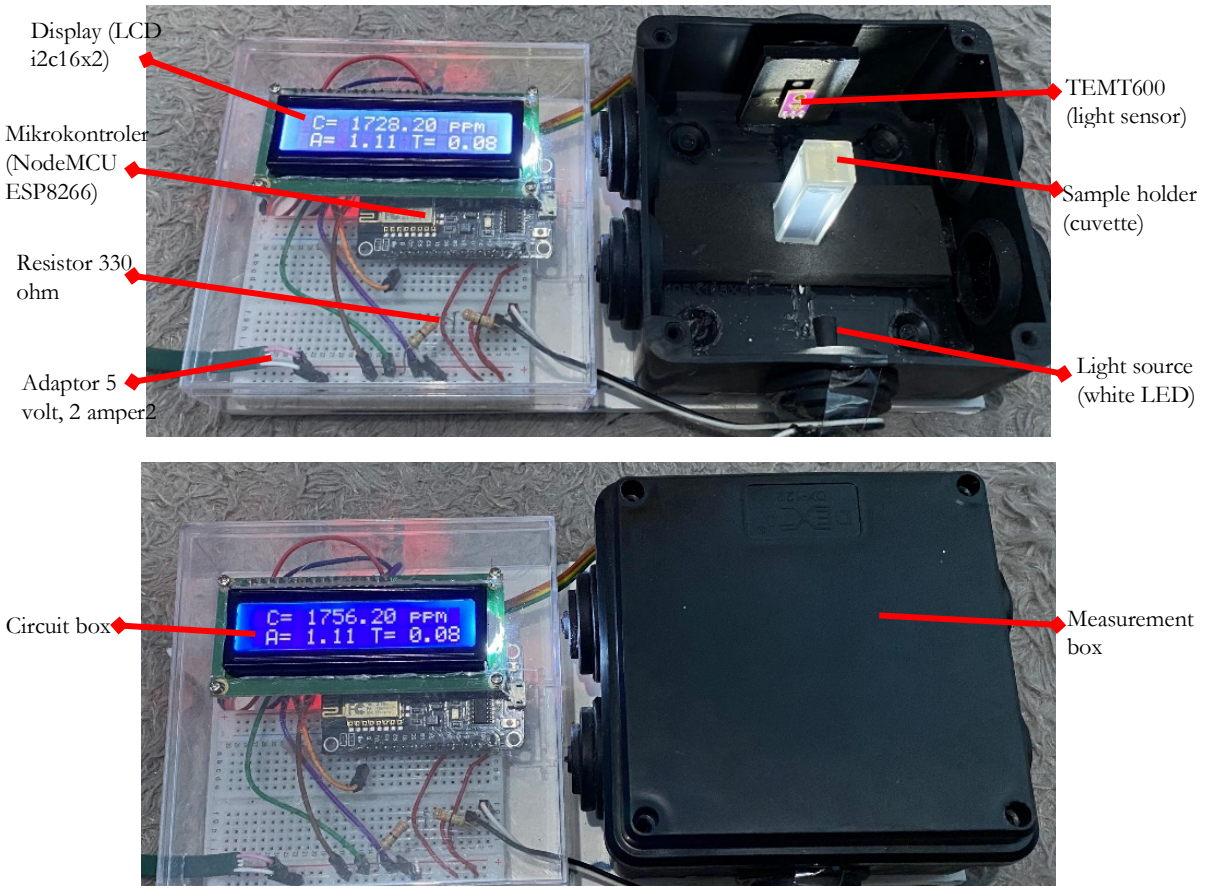

Figure 4. Results of circuit implementation to mechanics

Figure 4 shows the implementation of the spectrophotometer into the mechanical structure that has been carefully designed. In this setup, the sensor and all the components are arranged in optimal positions to ensure proper functionality. The TEMT600 sensor, the sample cuvette, and the white LED are aligned in a straight line. This arrangement maximizes the absorption of light by the sample, which in turn enhances the sensor's ability to measure the amount of light transmitted through the sample more accurately and effectively. By ensuring that the components are placed in the correct positions, the system can achieve better performance in measuring protein concentration and other parameters.

The results of the three key parameters concentration (C), absorbance (A), and transmittance (T) are displayed on the Liquid Crystal Display (LCD). The concentration value indicates the amount of protein in the sample, while absorbance and transmittance give insight into how much light is absorbed or transmitted through the sample. The spectrophotometer developed in this research demonstrates the practicality and effectiveness of the system in delivering real-time measurements of protein concentration and other related parameters. Despite its simplicity and cost-effectiveness, the device can deliver reliable results suitable for educational and small-scale laboratory applications.

In this study, the characterization of the TEMT600 sensor is determined by comparing the theoretical concentration values of the solution with the output voltage from the TEMT600 sensor. Before measuring the voltage, the samples are prepared first. In this research, the sample

used is a protein solution with an initial concentration of 10,000 ppm, which is then diluted to create 8 different concentrations of protein solutions: 1100 ppm, 1700 ppm, 2300 ppm, 3000 ppm, 3600 ppm, 4200 ppm, 4800 ppm, and 5400 ppm. Biuret reagent is then added to each sample as an indicator to detect any color changes in the protein solution.

After preparing all the samples, the relationship between the output voltage and the concentration of the protein solution is plotted. This relationship is shown in Figure 5, illustrating how the sensor's output voltage corresponds to the concentration of the protein solution. The data obtained from this experiment allows for the evaluation of the sensor's sensitivity and accuracy, as well as its ability to measure varying concentrations of protein in solution.

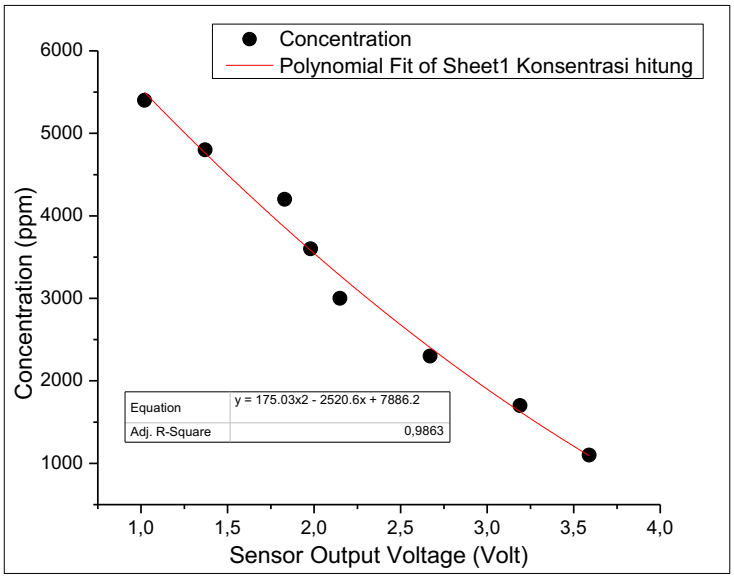

Figure 5. Relationship graph of sensor output voltage with calculated concentration

Based on the graph that has been plotted, the relationship between the TEMT600 sensor output voltage and the calculated concentration of the protein sample solution follows a polynomial trend. This relationship can be represented by the equation:

$$C = 175.03*V2 - 2520.6*V + 7886.2 \quad (1)$$

This polynomial equation describes the non-linear correlation between the concentration of the protein solution and the voltage reading from the sensor. It establishes an accurate relationship that allows for the precise prediction of the concentration based on the sensor output. This equation can be utilized as a calibration tool, enabling the spectrophotometer to measure the concentration of the protein solution reliably in future experiments. By integrating this equation into the device's software, the spectrophotometer can effectively quantify the concentration from the sensor's voltage readings. Moreover, this equation provides a foundation for further analysis, refinements, and enhancements to improve the accuracy and reliability of the spectrophotometer for future

Where C is the concentration of the protein sample and V is the output voltage from the sensor. With this equation, the concentration of the protein sample in the spectrophotometer device can be calculated by incorporating the equation into the code within the Arduino IDE. The code is then uploaded to the NodeMCU ESP8266 microcontroller, which processes the

input data from the TEMT600 sensor. The resulting concentration value is then displayed on the 16x2 LCD, allowing users to easily read the protein concentration in real-time. This integration between hardware and software ensures accurate and efficient measurement of protein concentrations using the simple spectrophotometer system.

To characterize the TEMT600 sensor and enable it to produce the absorbance value of the protein solution, the concentration value calculated by the spectrophotometer device is compared with the absorbance value of the protein solution, which is measured using the Agilent Technologies Cary 8454 spectrophotometer as the standard tool, at a wavelength of 520 nm. By comparing these values, the performance of the TEMT600 sensor can be validated. The relationship between the measured concentration and absorbance on the standard instrument is plotted in Figure 6. This comparison allows for the evaluation of the sensor's accuracy and its ability to match the absorbance readings provided by the standard spectrophotometer.

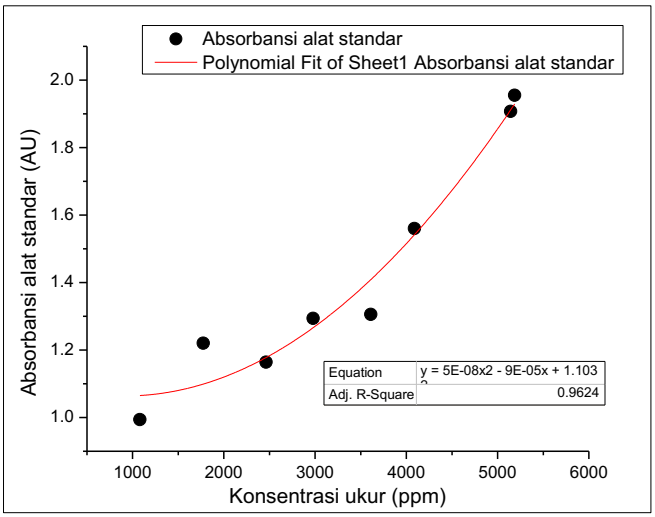

Figure 6 Relationship between measured concentration and absorbance

Based on the graph that has been plotted, it can be seen that the relationship between the sample concentration and absorbance is directly proportional. As the concentration level increases, the absorbance value also increases. This behavior is in accordance with the Beer-Lambert Law, which states that absorbance is directly proportional to concentration. From this relationship, a polynomial fit is applied, yielding an equation that can be used to measure absorbance on the simple spectrophotometer. The equation is:

$$A = 0.00000005*C2 - 0.00009*C + 1.1032 \quad (2)$$

This polynomial equation describes the non-linear correlation between the concentration of the protein solution and its absorbance. The equation provides an accurate relationship that allows for the prediction of the absorbance of the protein solution based on its concentration. This equation can be used as a calibration tool for the simple spectrophotometer, enabling more reliable and consistent measurements in future experiments. By incorporating this equation into the device's software, the spectrophotometer can effectively measure and quantify protein concentrations, making it a valuable tool for both educational and research purposes.

Additionally, this equation can serve as a foundation for further analysis and improvements in the spectrophotometer's design, ensuring greater accuracy in measuring a wide range of concentrations and absorbance values.

Where A is the absorbance, and C is the measured concentration. With this equation, a simple spectrophotometer can measure the absorbance value of the protein sample by entering this equation into the NodeMCU ESP8266 microcontroller. The microcontroller processes the concentration data obtained from the TEMT600 sensor and calculates the corresponding absorbance value using the equation. The calculated absorbance is then displayed on the 16x2 LCD, providing real-time feedback on the absorbance of the sample. This method ensures that the spectrophotometer can accurately measure and display the absorbance of protein solutions, even with a low-cost setup.

The concentration accuracy data for the simple spectrophotometer is obtained by comparing the theoretical concentration values with the concentration values measured by a near-infrared spectrophotometer. Data was collected 8 times, each with different concentrations for each experiment. The protein solution was used as the sample for measuring concentration accuracy in this study. The test results for the accuracy of the measured concentration on the simple spectrophotometer compared to the calculated concentration values can be seen in Table 1 and Figure 7. These results provide an assessment of the performance and accuracy of the simple spectrophotometer in determining the concentration of protein solutions, demonstrating how well the device can match the theoretical and calculated values.

Table 1. Precision of protein solution concentration measurement

Ch (ppm)	Cu (Ppm)	Accuracy	%Accuracy	Eror(%)
1100	1093.05	0.9937	99.37	0.63
1700	1626.61	0.9568	95.68	4.32
2300	2403.97	0.9548	95.48	4.52
3000	3275.99	0.9080	90.80	9.20
3600	3581.60	0.9949	99.49	0.51
4200	3859.66	0.9190	91.90	8.10
4800	4761.49	0.9920	99.20	0.80
5400	5497.29	0.9820	98.20	1.80
Average		0.9626	96.26	3.74

From the data, it can be seen that the spectrophotometer can measure the concentration of the protein solution very accurately, with an average accuracy value of 96.26%. Although the accuracy is high, there is still a measurement deviation, with an average of 3.74%. This deviation occurs due to factors that can interfere with the measurement results, such as unpredictable environmental conditions and poor sample calibration. These factors can cause slight inconsistencies in the measurements, leading to deviations from the expected values. Despite this, the spectrophotometer still provides reliable and precise results, with a high level of accuracy in measuring the concentration of protein solutions.

Absorption accuracy data was obtained by comparing the results of absorption measurements from a standard measuring instrument, namely the Agilent Technologies Cary 8454 spectrophotometer, with the absorption values measured on a simple spectrophotometer.

Data collection was carried out 8 times, each with a different concentration for each experiment. The samples were tested for absorption using standard instruments in the form of protein solutions. The results of the absorbance accuracy test comparing the near infrared spectrophotometer with standard instruments for measuring protein solutions can be seen in Table 2. These results help assess the accuracy and reliability of simple spectrophotometers in measuring absorbance values compared to standard instruments.

Table 2. Measurement of the accuracy of absorbance of protein solution

Absorbance measurement with an Agilent Tecnologies Cary 8454 UV-Vis spectrophotometer	Measurement of Absorbance with a Spectrophotometer that has been made	Accuracy	%Accuracy	Error
0.993652	1.06	0.9332	93.32	6.68
1.22012	1.10	0.9016	90.16	9.84
1.16383	1.19	0.9775	97.75	2.25
1.29408	1.28	0.9891	98.91	1.09
1.30588	1.43	0.9050	90.50	9.50
1.56007	1.57	0.9936	99.36	0.64
1.90737	1.96	0.9724	97.24	2.76
1.95527	1.98	0.9874	98.74	1.26
Average		0.9575	95.75	4.25

From the data on the absorbance measurement results of the protein solution above, it is clear that the simple spectrophotometer can measure the absorbance value effectively and accurately, with an impressive average percentage accuracy of 95.97%. This demonstrates that the device is capable of providing reliable results in determining the absorbance of protein solutions. However, despite the high accuracy, there is still some deviation in the measurement values. This deviation can be attributed to various factors such as environmental fluctuations, such as changes in light intensity, temperature, and humidity, as well as inconsistencies in sample preparation. These factors can introduce slight variations in the measurement results. The average deviation recorded in the measurements is 4.25%, which is relatively minor, but still noteworthy. While the device performs well overall, these deviations highlight areas for potential improvement, particularly in stabilizing environmental conditions and ensuring more consistent sample preparation. Nevertheless, despite these minor challenges, the spectrophotometer remains a reliable and effective tool for measuring absorbance, with good accuracy and performance for practical use in protein analysis.

The developed spectrophotometer has demonstrated performance in accordance with the fundamental principles of spectrophotometry, specifically Lambert-Beer's Law. This law states that the intensity of light transmitted through a material is directly proportional to the thickness and concentration of the material [17,18]. By utilizing this principle, the spectrophotometer successfully delivers highly accurate results in measuring the concentration and absorbance of proteins, achieving an average accuracy rate of 96.26% for concentration and 95.75% for

absorbance. This high level of precision highlights the tool's potential for application in various protein-based research and analytical fields.

However, despite its excellent performance, the spectrophotometer's application is currently limited to protein solutions. It lacks the capability to measure other types of solutions, such as carbohydrate solutions, lipids, or complex compounds. This limitation presents both a challenge and an opportunity for further development. Future enhancements could focus on equipping the spectrophotometer with additional features that enable the measurement of diverse types of solutions, thereby increasing its flexibility and utility. Furthermore, integrating a more advanced data storage system could be a priority to support automatic recording of measurement results. This would enable the tool not only to display real-time results but also to store measurement data in an organized format for long-term analysis. Such developments are expected to enhance the efficiency, adaptability, and practical value of the spectrophotometer across various scientific and industrial applications.

4. Conclusion

A simple spectrophotometer equipped with a white LED light source at a wavelength of 520 nm and a TEMT600 light sensor has been successfully developed and tested. This device is capable of measuring the concentration and absorbance of protein solutions with impressive accuracy. The spectrophotometer achieved an average measurement accuracy of 96.26% for concentration measurements and 95.97% for absorbance measurements, indicating that it can effectively and reliably measure both parameters in protein solutions. These high levels of accuracy suggest that the system is performing well in detecting light intensity changes caused by the interaction between the light and the protein solution.However, while the device shows excellent performance, slight deviations from the expected values were observed. These deviations, averaging 3.74% for concentration measurements and 4.25% for absorbance measurements, are primarily caused by environmental fluctuations that can affect light intensity, such as variations in temperature, humidity, and ambient light. Additionally, poor sample preparation, such as inconsistencies in sample volumes or improper cuvettes, may contribute to these discrepancies. Despite these challenges, the deviations are relatively small and fall within acceptable limits, making the device suitable for practical use. The spectrophotometer is still considered reliable and effective for accurately measuring the concentration and absorbance of protein solutions. Its performance demonstrates that it can be a valuable tool for scientific experiments, particularly in educational and research settings where budget constraints require cost-effective yet accurate measurement tools..

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