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Developing an Automated Spectrophotometer with RGB LED and Light Sensor Using Arduino Microcontroller

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

This research used RGB LED (Red Green Blue Light Emission Diode), LDR (Light Dependent Resistant) sensors, 3D printed sampling cartridge, and Arduino Mega to create a spectrophotometer with automation software to select wavelength, calculate calibration parameters, and report the concentration of the materials being studied. Spectrophotometers are finding applications across many fields, including clinical laboratories, pharmaceuticals, molecular biology, biochemistry, and even the medical realm. This study assembled an Arduino mega system coupled with software for the automatic selection of best-fit color light, calibration curve, and actual concentration interpolation processes. Dilution solutions from multiple chemical materials were evaluated to examine their linearity of calibration curves. Our study demonstrated that the tested chemical samples revealed a significant regression coefficient of up to 0.9945 for serial dilution solutions. Relative concentrations were measured with remarkable precision, exhibiting less than

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5.0 % error compared to a commercial spectrophotometer. These findings highlight the viability of the RGB spectrophotometer and emphasize its potential with the promise of further refinement in development.

Keywords: Analytical instrument; automatic wavelength selection; LDR light sensing system; RGB spectrophotometry.

1. INTRODUCTION

A spectrophotometer is the most popular analytical instrument used to quantitatively evaluate the absorption or reflection of visible light, UV light, or infrared light with biomedical and pharmaceutical samples [1,2]. A spectrophotometer quantifies the amount of light transmitted through a substance and becomes an essential analytical device in various science fields. Determining the concentrations of chemical compounds would be easy now due to the development of the spectrophotometer [3,4]. Spectrophotometers boast the most applications in clinical laboratories and other multidisciplinary fields such as pharmaceuticals, molecular biology, biochemistry, and medicine [5,6]. Most biomedical companies maintain great spectrophotometers for quantitative measurement in diverse research. Scientists can measure the effect of metabolic intermediates on a cell by measuring the dye color in each section [7,8]. Even the degree of antigen-antibody reaction by the COVID-19 virus might be determined when it is appropriately configured [9].

Monitoring pollution in sewer networks can be at different levels, from merely taking periodic samples to be analyzed in the lab to continuously monitoring pollutants [10,11]. The measurement of comprehensive values of turbidity, pH, nutrients, conductivity, temperature, and organic compounds through spectrophotometric probes today constitutes robust techniques that facilitate the mobilization of pollutants to be contacted throughout the day, online, and continuously [12]. Furthermore, online monitoring helps plan actions and infrastructures and is required to comply with existing legislation on wastewater treatment [13,14]. Nowadays, to express the CSOs' pollutant dynamics, time-continuous transmittance measurements have been utilized with satisfactory results. Measurements are taken at several wavelengths, like visible and 254 nm UV wavelengths [15]. Spectroscopy devices that work highly linearly in the visible spectrum, such as those made of RGB-LED, should support increased knowledge of pollutants' movement and water quality monitoring during CSOs [16]. Cost-effective spectrophotometers with LEDs are being investigated and spread to determine wastewater pollution with high precision [17], and their comparison with transmittance calculated from classical devices compared to the incandescent lamps shows good agreement [18].

Low-cost RGB light-emitting diodes (RGB-LEDs) are used in constructing simple and low-cost spectrophotometers for molecular absorbance measurements in instrumental analysis. For instance, RGB-LED-based sensors have been examined to measure different parameters online, such as the microalgae and biomass concentration, within a photo-bioreactor, with a 2% error [19]. The use of RGB allowed the study of online chromatic values without the cost spiraling throughout the winemaking process [20]. An RGB sensor was also used to obtain information about the sample's color, detecting phytoplankton's movement [21]. In the case of tap water, RGB sensors through a webcam were also adopted to monitor the concentration of parameters, such as ortho-phosphate and other metal ion solutions [22]. A portable RGB diode might be employed for the on-site evaluation of nitrite and iron in river waters nutrients [23]. The results of visible spectrophotometry in the near-infrared region (NIR) with an RGB diode and a 360- to 740-nm spectrophotometer were used as a standard value when characterizing parameters, such as the ammonia concentration and electrical conductivity values [24].

RGB-LED has also been established to calculate the dense packing of bacterial cells in standard and sample solutions in well plates, for high accuracy [25]. The RGB-LED may play a role in the superposition of different wavelengths, unlike conventional spectrophotometers that can be applied for reduced wavelengths for each measurement. This assembly introduces an increased feasibility to interference. However, several authors have shown RGB to be practical, simple, compact, and at a low cost. Its performance suggests that it could be a good candidate as a replacement for typical commercial spectrophotometers used in photometric analytical procedures. A decreased sensitivity will often be possible and users will have to determine if this is not significant since the accuracy continues to be high enough [26,27].

The present work includes an experimental protocol in which transmittance is obtained from an RGB-LED light through various water samples with different dyes and compounds that are fallen under a wide spectrum of transmittance. The results should be compared and discussed with those data acquired from a typical commercial spectrophotometer. An RGB-LED is supported with different regression equations to vary the emitted superposition of wavelengths to cover the broadest possible range within the visible spectrum of light, using the combination of the three tiny individual LEDs imbedded in the RGB-LED.

The contributions of this work might be explained as follows: First, we developed a novelty calibration process to measure transmittance values between 510 and 645 nm with a single RGB-LED, removing complicate optical devices, and with a high level of accuracy that could be confirmed. As indicated in the study, 18 individual LEDs are needed to analyze water samples from 510 to 645 nm; therefore, a single RGB-LED enables us to reduce the cost of the equipment significantly. Secondly, we establish a unique way to define the red, green, and blue RGB-LED light intensity combinations to measure the transmittance values that agree with those obtained using the typical spectrophotometer. The novelty lies in using the RGB light combination to generate a chemical response in various chemical compounds; this should be correlated with that obtained by equipment based on Vis-IR spectrophotometry lamps, where a single wavelength directly beams through the sample. Despite the importance of the spectrophotometer as an analytical instrument, it has yet to be possible to access many STEM science laboratories because of their monetary limitations at institutional administration. Creating an automated spectrophotometer with an Arduino microcontroller inspired us to understand the feasibility of low-cost assembling procedures, which will give us an in-depth comprehension of spectrophotometry and accessibility for our educational purposes.

Our system will have three unique properties that most other spectrophotometers don't offer. We will try our automated RGB light sources to find the most optimal wavelength color for passing through the samples. Secondly, the system will automatically calculate its calibration curve and regression coefficients and report. Thirdly, it will report the final concentration report for the users.

2. EXPERIMENTAL METHODS

2.1 Specifications of Each Electric Parts

2.1.1 Arduino mega 2560 for core system controls

The Arduino MEGA 2560 is a microcontroller for projects requiring more I/O lines, sketch memory, and RAM. With 54 digital I/O digital pins, 16 analog inputs, and a larger feature for your sketch, it was the recommended board applied for 3D printers and AI projects. This facilitates your projects to develop with plenty of room and opportunities to maintain the effectiveness of the Arduino platform. This document explains connecting your Mega2560 board to the computer and uploading your first sketch.

The Arduino Mega 2560 is programmed using the Arduino Software (IDE), our Integrated Development Environment common to all our boards and running both online and offline.

2.1.2 RGB LED for light sources with variable wavelength

An RGB LED module can theoretically generate 6 million colors from these three primary additive colors: Red, Green, and Blue. The primary combination of an RGB LED has a harmony of 3 separate light-emitting diodes in a single module, housed under a transparent protective plastic. This LED package has 4 pins, one for each of the three colored diodes, and generally with a cathode (-). The 3 primary color LEDs use the additive color mixing principle we discussed above to make more colors than imagined. LEDs are dimmable using PWM functionality that allows each red, green, and blue color to create all the different tones of the color. In the specification, each base-colored LED can produce 256 shades. It takes a high-quality and premium DMX controller to get every shade possible, but we will review that more in the controller's section.

2.1.3 LDR functions & sensitivity for sample transmittance reading

Photoresistors, also popularly called light-dependent resistors (LDR), are light-sensitive devices most often applied to reflect proportionally for the presence or absence of light or to measure the light intensity. In the dark, their resistance is extremely high, often up to 1 MΩ, but when the LDR sensor is sensed to light, the resistance drops exponentially, even down to a few ohms, according to the light intensity. LDRs have a sensitivity that follows with the wavelength of the light used and are nonlinear devices. They are used in many applications, but other devices, such as photodiodes and phototransistors, often perform this light-sensing function. Some countries have banned LDRs made of lead or cadmium over environmental safety concerns.

2.1.4 Stepper motor for sample cartridge control

A stepper motor is an electric motor. Its primary specification is that its shaft rotates by accurately calculated steps by rotating with a commanded amount of degrees. This capability is obtained thanks to the internal structure of the motor and allows one to know the accurate angular position of the shaft by calculating how many steps have been moved around, with no need for a sensor. This ability also makes it fit for a wide range of applications.

2.1.5 Keypad for dilution factor input

A keypad for Arduino is a user-input device that provides numerical or alphanumeric input to an Arduino microcontroller. It typically consists of a set of buttons arranged in a grid, similar to a telephone keypad, with each button representing a specific character or function.

Keypads for Arduino usually come in various configurations, such as 3x3, 4x4, or even larger matrices, depending on the number of buttons required for your project. These keypads are interfaced with the Arduino using digital input/output pins or specialized libraries and modules to simplify the process.

When a button on the keypad is pressed, it makes an electrical connection, allowing the Arduino to detect which button was pressed based on the change in the electrical signal. The Arduino can then interpret this input and perform the desired actions based on the programmed logic.

Keypads are commonly used in projects such as security systems, access control systems, password entry systems, and other applications requiring user input. They support a convenient and intuitive way for users to interact with Arduino-based projects without needing more complex input devices.

2.1.6 LCD display for processing report

LCD stands for Liquid Crystal Display. It's a flat-panel display technology commonly used in electronic devices such as television screens, computer monitors, and digital watches. In the context of Arduino or other microcontroller projects, an LCD refers to a specific type of display module that utilizes liquid crystal technology for displaying alphanumeric characters or graphics.

Arduino-compatible LCD modules typically consist of a liquid crystal display panel, a backlight for visibility in low light conditions, and a driver circuitry that allows the microcontroller to control what is displayed on the screen. These modules often come in various sizes, such as 16x2, 20x4, etc., indicating the number of characters each line can display and the number of lines. LCDs are famous for displaying text-based information in Arduino projects due to their simplicity, low power consumption, and ease of use. They provide a convenient way to output data, sensor readings, messages, and other information in a format easily readable by users. Interfacing an LCD with an Arduino typically involves connecting it to the microcontroller using digital input/output pins and utilizing a library (such as the LiquidCrystal library for Arduino) to control the display. This allows programmers to write code to display text, numbers, and symbols on the LCD screen, making it a versatile tool for creating user interfaces in various projects.

2.2 Assembly of Our Electric Parts

As illustrated in Diagram 1 below, the Arduino mega worked as a core supervising function while connecting to RGB LED (B) and LDR light sensor (C). Between the two parts of B and C, sample cuvettes should be exactly aligned in the cartridge that had the capacity of 16 holders. A stepping motor rotated the cartridge. And the. Connect obstacle sensor to D2, stepper motor to D14, D15, D16, D17, and Keypad connection to D3 to D10, light sensor LDR to A3, buzzer to D46, RGB LEd to D50, D15, D52.

2.3 Our Hardware and Software Harmonized Coworking Procedures

Our software and algorithm have been developed with Arduino software development environment and C++ programming language. All the processes were printed out to the serial monitor and LCD, making it easy to debug malfunctioning codes and outputs.

The LCD displayed a greeting and asked the user to place the cartridge fit at the mark of cuvette #1 on the cartridge in which cuvettes# 1 to 6 were filled with serially diluted standard samples for calibration. Once the cartridge was placed with all the standard solutions and samples and set into the system, the microcontroller turned on a red light on the RGB LED and read the light intensity from the LDR light sensor for standard #1. It changed the light to green light and read from LDR. It continued to blue light. After the system read the light through standard #1 with the 3 colors, it turned the cartridge to standard #2 and did the identical reading procedures as before to standard#6. At this point, the system calculated the regression slope, intersection, and regression coefficient R squared for each color. Then, it chose the most outstanding R squared for the next sample color to be read.

Picture 1. Keypad for Arduino microcontroller

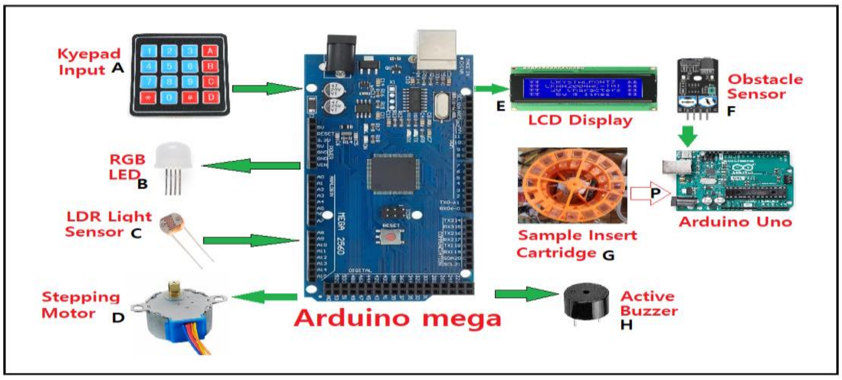

Diagram 1. Presents the arrangement of our Arduino microcontroller with the sensors and actuators used in this project

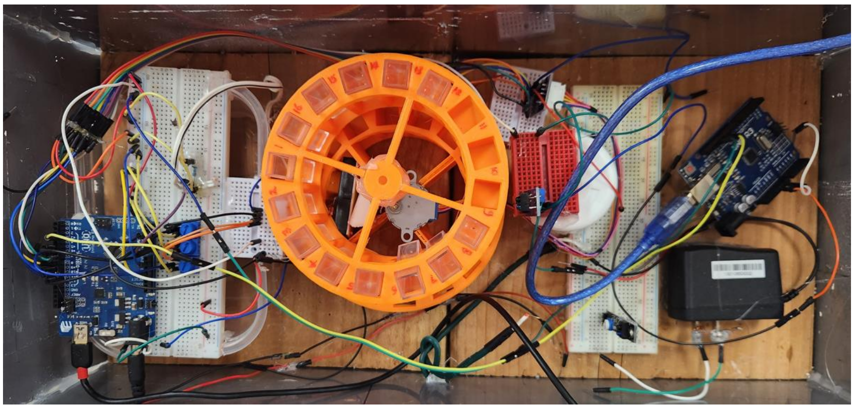

Fig. 1. Presents the pictorial representation of our completed project assembly as described above

1. Greeting → Beep
2. Input Dilution Factor
3. Read Std Sample 1 as Red
4. Read Std Sample 1 as Green
5. Read Std Sample 1 as Blue
6. Read Std Sample 2 ~ 6 as RGB
7. Calculate Slope, Intersection, Rsquared
8. Choose light color as greatest Rsquared
9. Now, read Sample 1 ~ 8 with chosen light
10. Calculate actual concentration with the read value, slope and intersection

Diagram 2. Illustrates the flow diagram of our software and algorithm

2.4 Sample Preparation

Sixteen cuvettes were prepared, and among them, 6 cuvettes were used as a standard solution that was prepared first all the time. We could prepare the standard solution with a known dilution factor. Still, we mainly needed to know the actual concentration since we didn't use pure chemical compounds to develop this system. As a dilution factor, 1:2, 1:4, 1:6, or 1:10 was used for a serially diluted solution. After collecting the 6 test tubes and based on the calculation with dilution factor, 3 ml of solution was filled into each cuvette. All the dilution solutions, reverse osmosis (RO)-filtered water, were used.

2.5 Data Analysis

The LDR light sensor analog values were sent and stored in the series of arrays in the Arduino memory. After the 6th data from the standard solution, their calibration curves were calculated for slope a, intersection b, and regression coefficient R. At this point; our algorithm chose the color of greatest R squared. The remaining samples were read with only a single color, and their relative concentrations were reported on the LCD display while recorded into a memory card.

3. RESULTS AND DISCUSSION

3.1 Food Dye Measurement for RGB Functionality

3.1.1 Red color dye 1:10 serial dilution with 6-point calibration curve

With RGB light and an LDR sensor, serially diluted red dye solutions were measured in our preliminary prototype model. When the relative concentrations were 6, the regression coefficient was moderately dispersed from 0.42 to 0.58, as in Fig. 2.a. However, when the concentration points were reduced to 4, their regression coefficient from the calibration curve was satisfactorily high from 0.97 to 1.00, as seen in Fig. 2.b.

3.1.2 Green color dye 1:10 serial dilution with 6 & 4 point calibration curve

At this time, the blue dye was serially diluted in water and measured in our spectrophotometry. When the analog absorbance from the six standards was obtained, the regression coefficient was moderate to highly dispersed from 0.36 to 0.83, as in Fig. 3.a. However, when the concentration points were reduced to 4, their regression coefficient from the calibration curve was satisfactorily high from 0.73 to 0.98, as seen in Fig. 3.b.

3.1.3 Blue color dye 1:10 serial dilution with 6 & 4 point calibration curve

With RGB light and LDR sensor, serially diluted blue dye solutions were prepared and measured in our RGB spectrophotometry. Fig. 4 below shows that the analog absorbance with six standards moderately dispersed the regression coefficient from 0.31 to 0.99, as in Fig. 4.a. However, when the concentration points were reduced to 4, their regression coefficient from the calibration curve was satisfactorily high from 0.518 to 0.968, as seen in Fig. 4.b.

3.2 RGB Spectrophotometer Performance with Gold Colloidal Solutions

It was our curiosity to know the feasibility of measuring colloidal solutions. Results are shown in Fig. 5, which shows that it was a perfect candidate for accurate measurement using this RGB spectrophotometer, considering their R squared even with 6 data point graphs from 0.7221 to 0.9825.

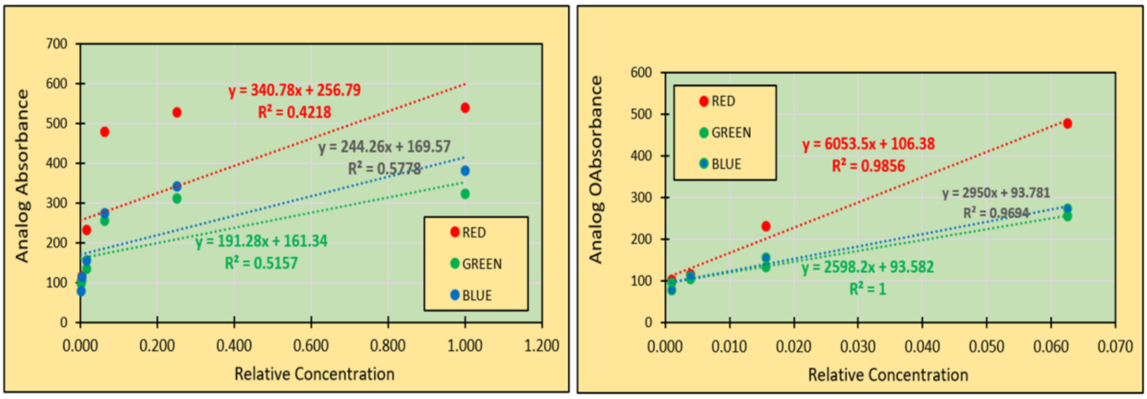

Fig. 2a. Red Color Dye 1:10 Serial Dilution with 6 Point Calibration Curve. R2 was moderately low at 6 points; 2b. Red Color Dye 1:10 Serial Dilution with 4-Point Calibration Curve. The 4-point calculation was far higher, with R2 near 1

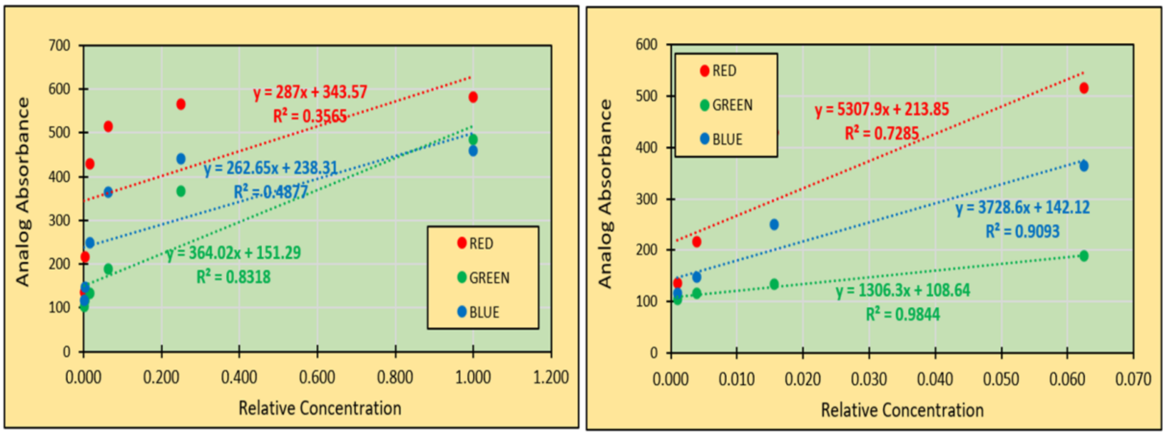

Fig. 3a. Green Color Dye 1:10 Serial Dilution with 6-Point Calibration Curve. R2 was moderately low at 6 points; 3b. Red Color Dye 1:10 Serial Dilution with 4-Point Calibration Curve.

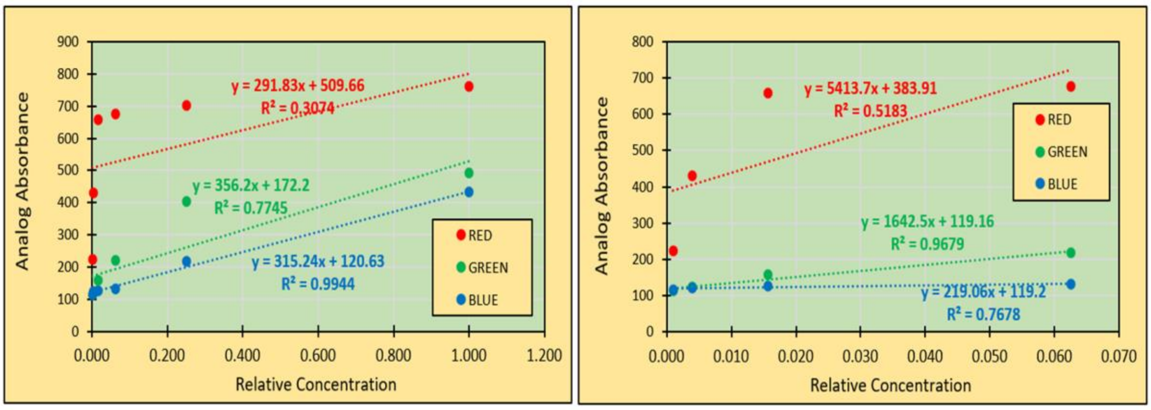

Fig. 4a. Blue Color Dye 1:10 Serial Dilution with 6 Point Calibration Curve. R2 was moderately low at 6 points; b Red Color Dye 1:10 Serial Dilution with 4 Point Calibration Curve. The 4-point calculation was far higher, with R2 near 1

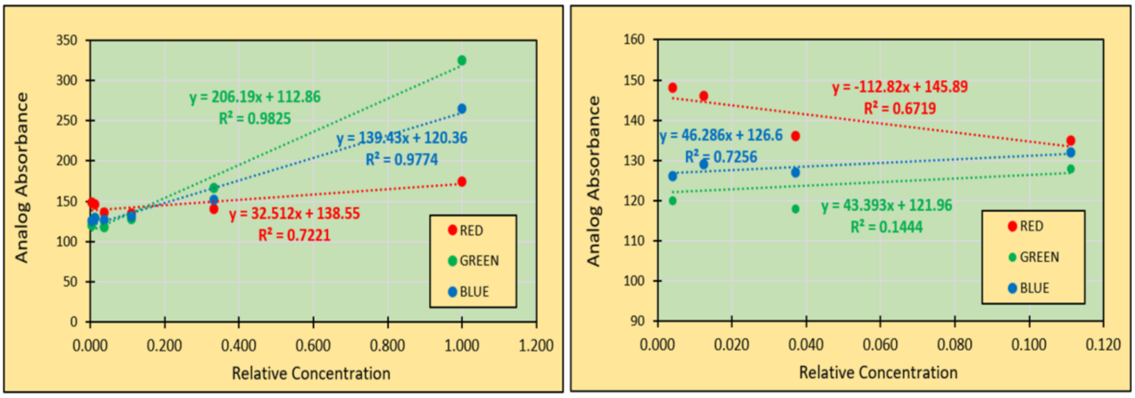

Fig. 5a. Presents the analog absorbance from the 6 Point Calibration Curve of the gold colloidal solution. R2 was higher at 6 points; b presents 4 point Calibration Curve. The 4-point calculation was less R2 compared to the 6 points

3.3 RGB Spectrophotometer Performance with Methylene Blue

Methylthioninium chloride, also known as methylene blue, is a salt for a dye and medication. As a medication, it is mainly consumed to treat methemoglobinemia by chemically reducing the ferric iron in hemoglobin to ferrous iron. As seen in Fig. 6, their R squared high as 0.926 in blue color at 6 points, as 0.9787 in the green collar at 4 points.

3.4 RGB Spectrophotometer Performance with Eosin Staining Dye

Hematoxylin and Eosin stain or hematoxylin-eosin stain; often called it short as H&E stain or HE stain for one of the principal tissue stains used in histology. This dye is the most widely used stain in medical diagnosis and is often the gold standard. In this study, the R squared in blue is 0.986 for 6 points and 0.978 for 4 points as illustrated in Fig. 7. The RGB spectrometer could accurately measure the eosin stain even with standard solutions.

3.5 Analog Absorbance through Turbid Water Dilution Solution

Biochemical analysis with antigen-antibody should be possible if turbid water could be measured with high linearity of analog absorbance with concentration. In this data set, the calibration curves from the RGB spectrophotometer were compared with those from a commercial visible-IR spectrophotometer. It was so impressive that the regression coefficients R squared were more significant than that from the commercial spectrophotometer in 6-point calibration seen in Fig. 8. At the same time, it was better for the commercial spectrophotometer in 4 4-point curves.

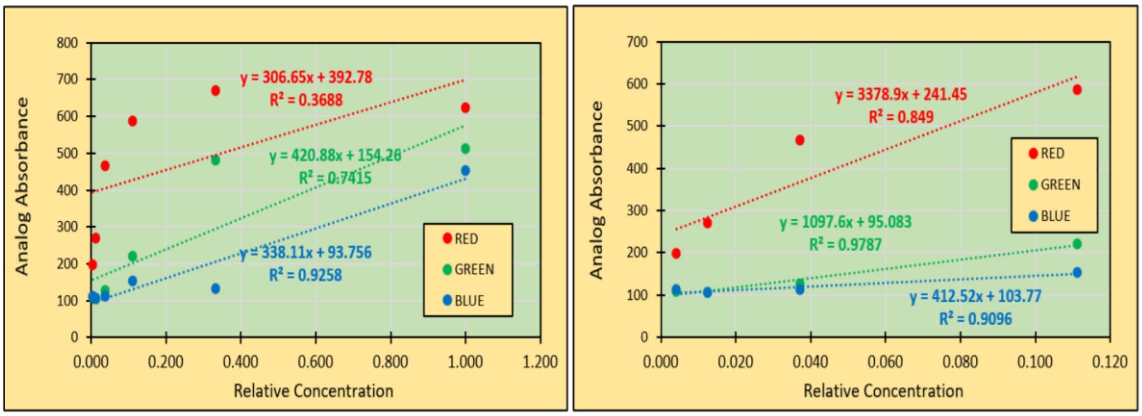

Fig. 6a. Presents the analog absorbance from the 6 Point Calibration Curve of methylene blue solution. R2 was lower at 6-points still with R squared 0.926 high; b presents 4-point Calibration Curve. The 4-point calculation had a higher R2 compared to the 6-point calculation

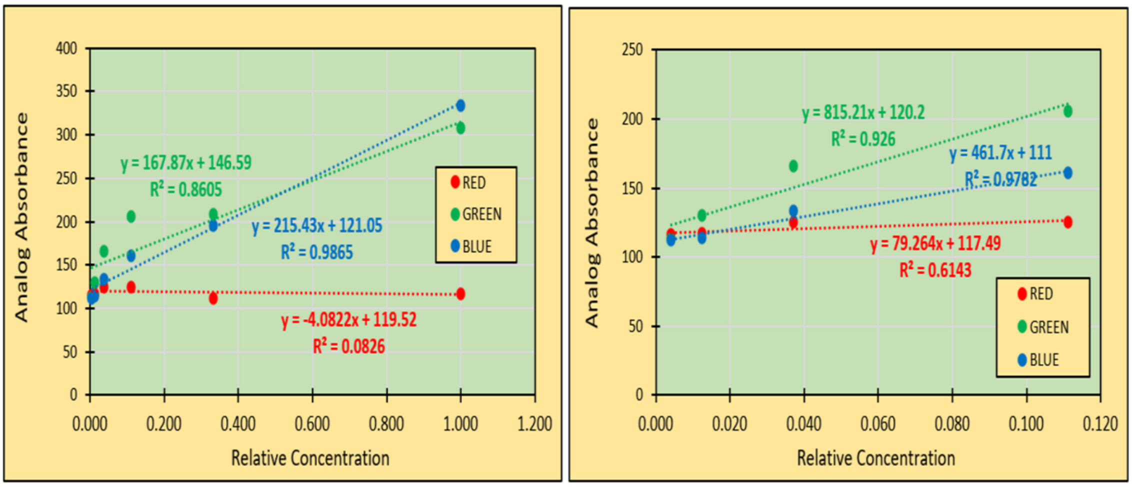

Fig. 7a. presents the analog absorbance from the 6 Point Calibration Curve of methylene blue solution. R2 was lower at 6-points still with R squared 0.987 high; b presents a 4-point Calibration Curve. The 4-point calculation had a lower R2 compared to the 6-point calculation

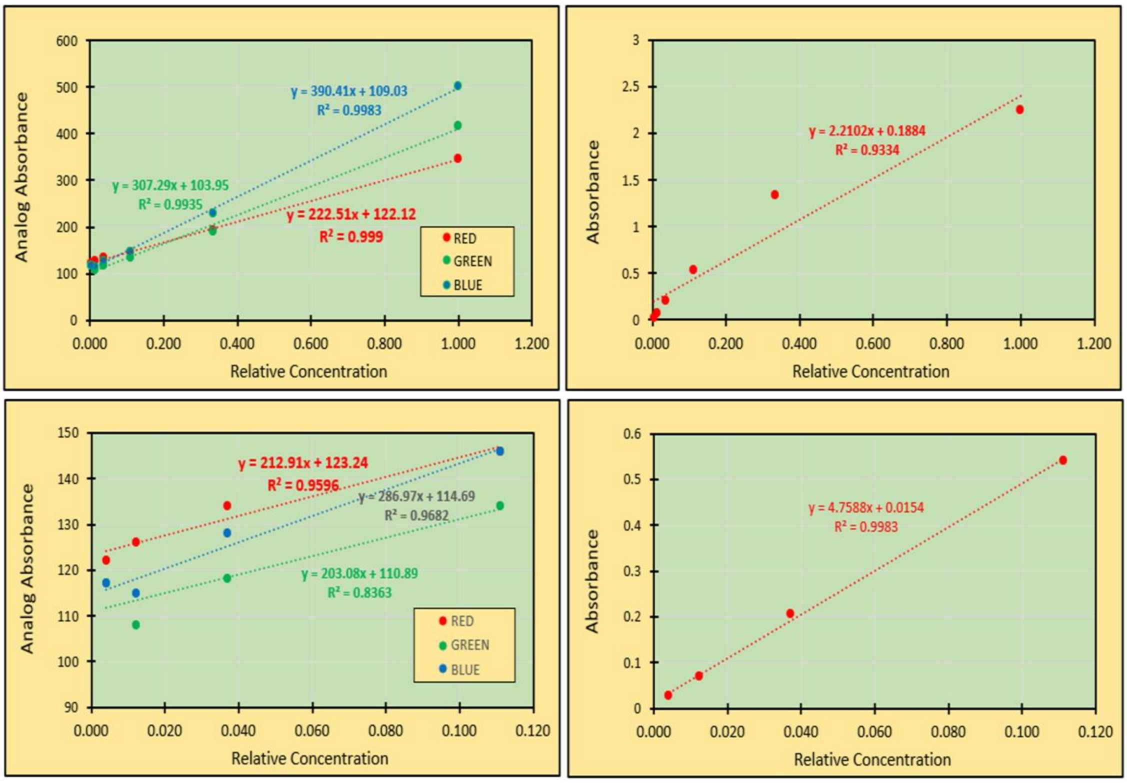

Fig. 8. Presents the comparison of RGB (a;left) and commercial vis-uv spectrophotometer (b;right) with six and four-point calibration curves (1:3 turbid water serially diluted)

3.6 Analog Absorbance through Multivitamin Dilution Solution

This study analyzed dissolved multivitamin solutions with our RGB and commercial spectrophotometer. The trend was similar to the previous data in that the regression coefficients R squared were more significant than that from the commercial spectrophotometer in 6-point calibration as in Fig. 9. At the same time, it looked better for the commercial spectrophotometer in 4 4-point curves.

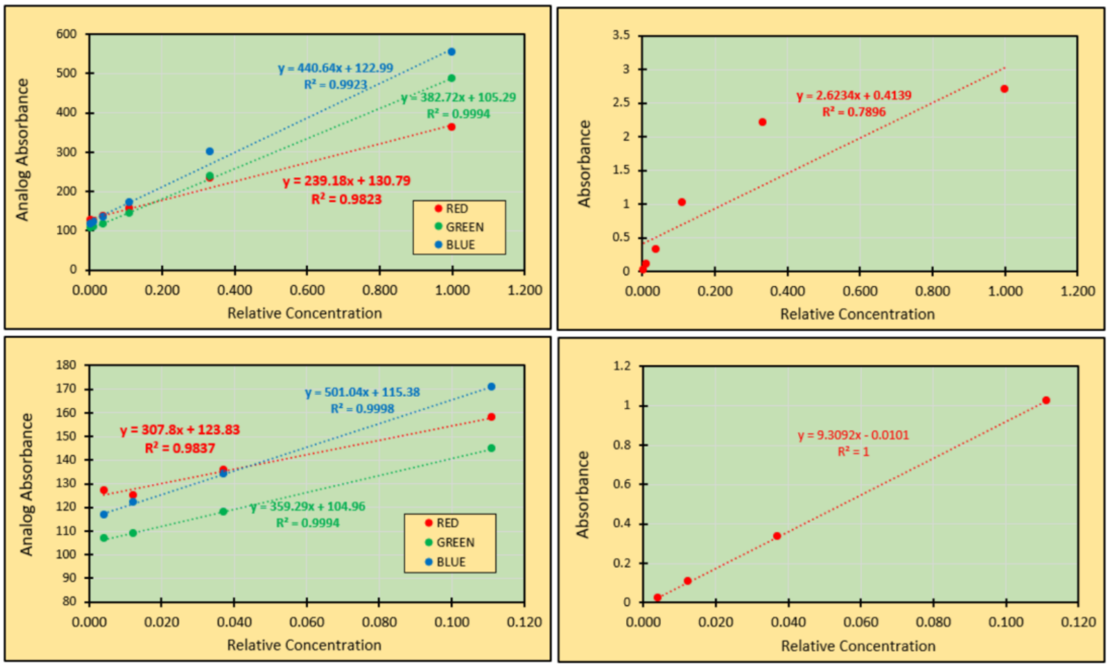

Fig. 9. Presents the comparison of RGB (a; left) and commercial vis-uv spectrophotometers (b; right) with the six and four-point calibration curve (1:3 multivitamin serially diluted solution)

Table 1. Presents measurements of unknown samples with our RGB spectrophotometer

Chemical Testing Articles	Red Food Dye		Methylene Blue	
	TRUE	Measure	TRUE	Measure
1	0.045	0.048	0.5	0.43
2		0.038		0.59
3		0.052		0.4
4		0.049		0.62
5		0.044		0.55
6		0.046		0.47
Mean		0.046		0.51
STD		0.00141		0.028284
Accuracy		2.60%		2.00%
Precision		3.05%		5.55%
Accuracy = Abs (True-Measure)*100/True				
Precision = STD*100/Mean				

3.7 LGB Spectrophotometry Performance Test Using Real Samples

Unknown samples from red food dye and methylene blue were measured with our RGB spectrophotometer. Table 1 shows 2.6% accuracy and 3.05% precision for red food dye and 2.0% accuracy and 5.55% precision for methylene blue, respectively.

4. CONCLUSIONS

Our RGB spectrophotometer was assembled with the RGB led, LDR sensors, 3D printed sampling cartridge, and Arduino mega with automation software to select wavelength, calculate calibration parameters, and report the concentration of the studied material. Spectrophotometers are finding applications across many fields, including clinical laboratories, pharmaceuticals, molecular biology, biochemistry, and even the medical realm.

The RGB spectrophotometry could measure, as seen in the figures above, from food dyes, gold colloids, methylene blue, Eosin, turbid water, and multivitamins. In most cases, the regression coefficient was higher than 0.995. At some point, the regression coefficient was even more significant than that from commercial spectrophotometry. We found that the samples were measured within 2.6% and 2.0% in accuracy and 3.05% and 5.55% in precision for red food dye and methylene blue, respectively. The study confirmed the feasibility of developing an RGB spectrophotometer with high accuracy and precision. More studies might be required to create further reliable data.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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