Development of Arduino Uno-Based TCS3200 Color Sensor and Its Application on the Determination of Rhodamine B Level in Syrup

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Abstract: *The use of the notorious synthetic dye, rhodamine B, in food and beverage products has been widely reported. This application urges the need to develop an analytical method that can provide reliable rhodamine B data with an easy operational technique. Therefore, this research is aimed to develop an Arduino Uno-based TCS3200 color sensor and study its application to determine rhodamine B levels in syrup. The design of the analytical instrument included TCS3200, an Arduino Uno microcomputer, an Integrated Development Environment (IDE) software, a black box container, and a 24 × 2 matrix display screen, where samples were prepared via absorption using wool thread. With a linear range of 1–20 mg/L, our proposed colorimetric sensor had recoveries of 96.25–110.3%, which was better compared to that was obtained from the UV-vis (81.8–100.6%) method. The detection and quantification limits of the sensor were 2.766 and 8.383 mg/L, respectively. The syrup samples used in this study were purchased from the local stores in Banda Aceh. Based on the proposed TCS3200 color sensor, the highest rhodamine B concentration from the syrup sample was 16.74 mg/L. The t-test analysis in this study revealed that the Rhodamine B levels quantified using the newly developed TCS3200 color sensor were not statistically or significantly different from the UV-Vis spectrophotometer method.*

Keywords: *color sensor; TCS3200; rhodamine B; Arduino Uno; Zn(CNS)$_2$; IDE software*

INTRODUCTION

As a form of consumer protection efforts, sensor technology for food or beverage products has been developed intensely [1-3]. For example, Fourier Transform Infrared (FTIR) spectroscopy has been employed to separate halal and non-halal meatballs [4]. The color spectroscopy method has also reported the detection and analysis of synthetic dye rhodamine B in chili powder [5]. Dyes used in food products are derived from natural and artificial chemicals. Synthetic dyes are widely used because it is more cost-efficient and available. Additionally, the synthetic dye may give a brighter color to the food or beverage product [6].

As one of 30 synthetic dyes available, Rhodamine B is considered as a dangerous dye, where its use in food or beverage products has been prohibited. Nevertheless, Rhodamine B is often used in processing industries, papers, and fabrics [7-8]. Moreover, it could be employed as a ligand to bind metal ions [9]. However, in Indonesia, rhodamine B is still very popular as a food coloring agent, including in iced syrup. The syrup is intentionally added with rhodamine B, so the products obtain a more attractive appearance [10].

Methods that have been previously developed to identify rhodamine B in food ingredients include thin-layer chromatography (TLC) [11], voltammetry [12], and the standard method using UV-Vis spectrophotometer (the best option for identifying compounds with color). However, UV-Vis spectrophotometry has several drawbacks; not portable, complicated, and expensive [13]. Hence, this study tried to overcome the stated drawbacks by developing a simple measurement method using a portable sensor.

The sensor system developed in the present work was based on the TCS3200 color sensor constructed by the console to overcome external noise and program library modification [14]. The TCS3200 color sensor has been widely reported for different applications, including measuring levels of cyanide [14], nitrogen [15], and heavy metals [16]. In the case of colorimetric sensors, analytes should first be reacted with a complexing agent to cause a color change [17-18]. In this study, the sensor detects color degradation from tissue paper that has been spiked with reagents, so its sensitivity is specifically improved for rhodamine B analysis. The reagent used was $Zn(CNS)_2$, which can cause a color change from red to purple due to the formation of the rhodamine B-Zn-thiocyanate ((RhB) complex $2Zn(CNS)_4$) [19].

The color intensity contributed by the presence of rhodamine B was converted through the sensor output pin in the form of a square signal in which its frequency depends on the concentration. The box's signal with varied frequency was then processed using a microcontroller on Arduino Uno. In this processing, four filters were used, namely green, blue, red, and *no filter*. In this case, *no filter* was excluded because the three parameters were sufficient to represent the color degradation of rhodamine B in the sample [20]. Filter settings were performed by providing low and high logic in the Arduino IDE program, following the reported study [21].

The distance between the sample and the 8 × 8 diode array was set at 3 cm, following the sensor system's geometry. The console's color was made black so the color could be absorbed fully, and influence from the degradation of colors that enter the diode array could be avoided. After obtaining the concentration of rhodamine B using the Arduino Uno-based TCS3200 color sensor, the results were compared with the standard UV-Vis spectrophotometric method. Finally, the analysis results were compared to obtain the data on sensitivity and accuracy of the newly developed sensor [22].

■ EXPERIMENTAL SECTION

Materials

The materials used were a UV-Vis spectrophotometer (Thermo Fisher Scientific, Selangor Malaysia), a color sensor TCS3200 (ICTAOS/AMS), a console, and an Arduino Uno (wavgat). Syrup samples tested for rhodamine B levels were procured from local stores in Banda Aceh. The standard rhodamine B was purchased from The National Agency of Drug and Food Control of Indonesia (BPOM RI). All other chemicals used, i.e. NH_4OH, NaOH, HCl, C_2H_5OH, CH_3CO_2Na, $ZnCl_2$, CH_3CO_2H, and KCNS, were obtained from Merck (Selangor, Malaysia) in analytical grade.

Hardware Design

The hardware design was initiated by developing a console for the TCS3200 color sensor, then connecting the output port of the color sensor via a jumper cable to the Arduino Uno microcomputer to process frequency data and convert it into 8-bit RGB digital data. There were 256 color digit variations for each RGB color component that was sortable and distinguishable by the processing. These color digit variations were displayed on the computer screen and converted to reduce color variations. These color variations were also recorded in .xls format (MS Excel) (Fig. 1).

Development of the TCS3200 Sensor Console

The TCS3200 console sensor was designed in black to absorb all color wavelengths. The distance between the diode array and the color object was 3 cm. The console was arranged in such a way that light from outside could not enter. The TCS3200 sensor was positioned opposite the color sample, which was absorbed into a filter paper. Four LED units with white

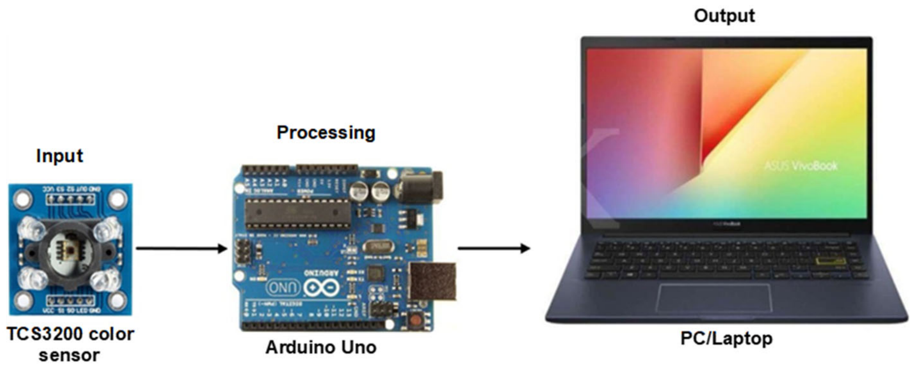

Fig 1. Schematic diagram of hardware design

wavelengths would hit the filter paper, and the intensity light reflected the diode array following the color intensity of the sample.

Software Design

Construction of the software design was initiated with a blink test on the Arduino Uno system to determine the response and performance of the microcomputer. The software used was Arduino IDE with available open-source libraries – C programming language. The program library was modified to enable the required color filters, Arduino Uno pins, the required display format, and data storage mode (Fig. 2).

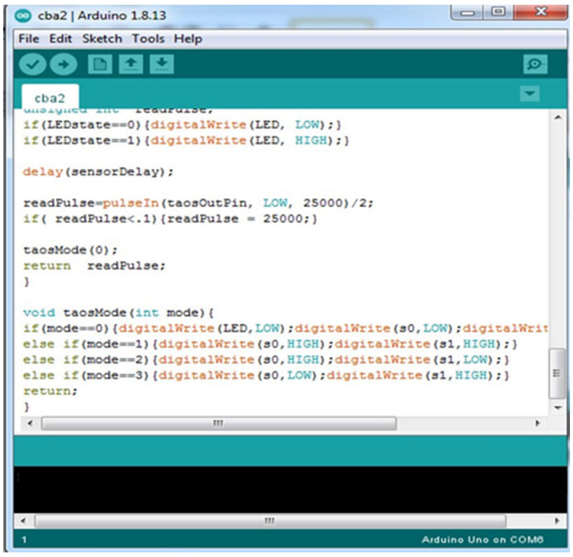

Fig 2. Display of the Arduino IDE Software main menu

Rhodamine Analysis Using TCS3200 Color Sensor

Construction of the calibration curve for rhodamine B

Briefly, the rhodamine B solution was added with 3.0 mL of Zn-thiocyanate. Then, the standard solution $(RhB)_2$-$Zn(CNS)_4$ with different concentrations measured the RGB value with the TCS3200 color sensor and absorbance with UV-Vis at the maximum wavelength obtained. The solution was prepared with 100 mg/L rhodamine B as the stock solution, which was then diluted using distilled water into standard solutions with varying concentrations ranging from 1 to 20 mg/L. These solutions were prepared to determine the maximum wavelength of rhodamine B and as a database for the TCS3200 color sensor. Following that, a solution of 1 mL $ZnCl_2$ 2 M and 2 mL KCNS 2 M as a reagent was made to detect the presence of rhodamine B, as suggested by a previous report [23].

Determination of rhodamine B level using the TCS3200 color sensor

The standard curve of $(RhB)_2$-$Zn(CNS)_4$ was obtained by measuring the RGB values of the standard solution $(RhB)_2$-$Zn(CNS)_4$ using the TCS3200 sensor. The concentration of rhodamine B used was 1 to 20 mg/L, which were priorly reacted with reagents. Measurements were carried out three times, and the concentration was averaged. Thereafter, RGB values were converted into a color index, namely Hue, Intensity, and Saturation (HIS). Conversion of RGB values to HIS values was carried out using the following Equations.

$$\text{Red color index } (I_R) = \frac{R}{R+G+B} \quad (1)$$

$$\text{Green color index } (I_G) = \frac{G}{R+G+B} \quad (2)$$

$$\text{Blue color index } (I_B) = \frac{B}{R+G+B} \quad (3)$$

The HIS color model was designed to resemble the perception of human vision, while the RGB values resembled the image of the display system [20]. The results of the calculation of the HIS value were then plotted as the dependent variable (y-axis) to the variation of concentration $(RhB)_2$-$Zn(CNS)_4$ (x-axis).

TCS3200 color sensor method validation

Method validation included accuracy, precision, sensitivity, and linearity, which were conducted based on the suggestion from a previous report [22].

Syrup sample preparation

Samples of commercial red syrup were purchased from local stores in Banda Aceh. Each sample (10 mL) was taken and put into an Erlenmeyer which was subsequently mixed in 20 mL of 25% ammonia solution (dissolved in 70% ethanol) for 24 h and evaporated on a hot plate. The evaporation residue was dissolved in 10 mL distilled water containing acid (10 mL distilled water and 5 mL acetic acid 10%). Wool thread (15 cm) was dipped into the acid solution and simmered for 10 min until the dye colors appeared on the wool thread, then lifted. The wool thread was then washed with distilled water, and the wool thread was dissolved in ethanol 70% and heated to a boil (Fig. 3). This solution was used as the sample, per suggestion by a published work [24]. The wool thread was used to extract rhodamine B-containing samples in an acidic environment. A comparative study has reported that wool thread has the highest dye adsorption as compared with silk and nylon [25]. Adsorption of dye analyte in wool thread is determined by its O- and N-containing functional groups, which has been reported in many published papers [26-28]. The dyed wool was then immersed in ethanol 70% and boiled until its original color returned. The obtained solution was analyzed for its rhodamine B levels using the TCS3200 color sensor and a reference method – UV-Vis spectrophotometry.

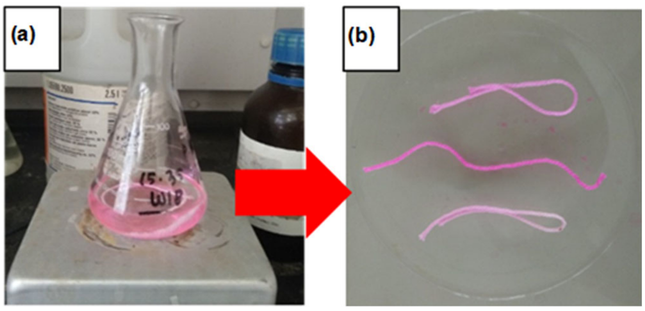

Fig 3. The extraction of rhodamine B from commercial red syrups using wool thread. Wool thread was dipped into the dissolved syrup residue for 10 min (a). Rhodamine B-containing wool thread before re-immersed to ethanol 70% and boiled (b)

Quantitative Analysis

The prepared sample was added with Zn-thiocyanate and then dipped in filter paper. Rhodamine B levels were measured using the TCS3200 color sensor [29]. The concentration was obtained based on the linear equation obtained from the calibration curve.

Method Comparison using Two-Way t-Test

Results of the samples between the TCS3200 color sensor and the UV-Vis spectrophotometry method were compared [30]. In addition, a two-way t-test was carried out to see the significance between the newly studied TCS3200 color sensor method and the reference method using UV-Vis spectroscopy by calculating the t value for each method and then comparing it with the $t_{theoretical}$.

■ RESULTS AND DISCUSSION

Maximum λ of Rhodamine B Complex

The complex $(RhB)_2$-$Zn(CNS)_4$ was produced to give rhodamine B a specific color, allowing easier analysis. The solution of rhodamine B, which was initially red, turned to purple and was then measured using a UV-Vis spectrophotometer at a wavelength ranging from 574 to 600 nm. The UV-vis absorbance corresponding to the $(RhB)_2$-$Zn(CNS)_4$ complex scanned from 574 to 600 nm is presented in Fig. 4.

Based on the measurement results, the UV-Vis spectrometer spectrum of $(RhB)_2$-$Zn(CNS)_4$ showed a maximum absorption (0.442 au) at a wavelength of 590 nm. The difference in wavelength between rhodamine B and $(RhB)_2$-$Zn(CNS)_4$ is due to a shift in

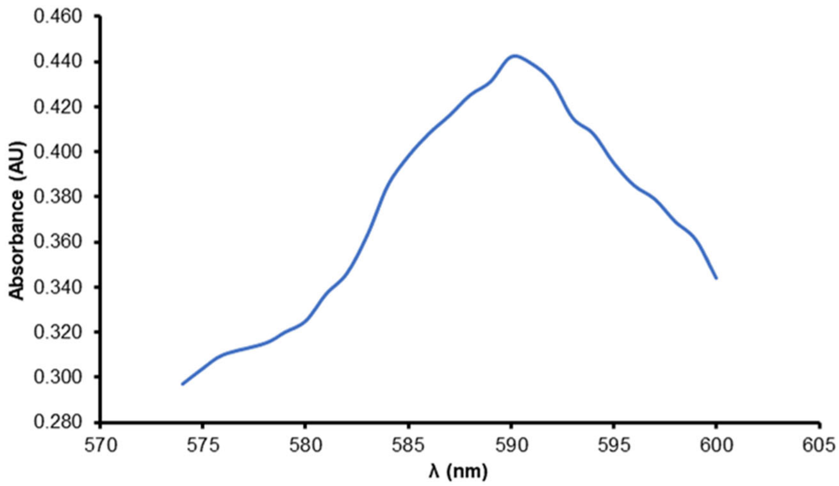

Fig 4. UV-Vis spectrometer spectrum of $(RhB)_2$-$Zn(CNS)_4$ showing a maximum wavelength at 590 nm

wavelength towards the bathochromic direction caused by substitution, solvent effects, and the influence of the chromophore group [31]. The successful formation of the $(RhB)_2$-$Zn(CNS)_4$ complex was indicated by a color change from red to purple and a shift in wavelength. The equation for the reaction between rhodamine B and $Zn(CNS)_2$ can be seen in Fig. 5.

Based on the graph, we can see three regression equations obtained from each RGB index value, namely I_R y = 0.0028x + 0.3411; I_G y = 0.0032x + 0.3513 and I_B y = -0.0058x + 0.3059. The values of the determination coefficient (R^2) were 0.9792,0.9700, and 0.9729 respectively. The R index had the best determination coefficient (R^2) of 0.9792. Therefore, the regression equation for the R index was used to determine the concentration of rhodamine B in the sample.

Measurement using UV-Vis Spectrophotometer

The standard curve of $(RhB)_2$-$Zn(CNS)_4$ was measured at a wavelength of 590 nm by a UV-Vis spectrophotometer. The concentration of rhodamine B that was used ranged from 1 to 20 mg/L, which was priorly reacted with reagents. Measurements were carried out three times and averaged for each concentration. The absorbance measurements can be seen in Fig. 6. The regression equation y = 0.0023x + 0.0773 had a determination coefficient (R^2) of 0.9927. Hence, it can be concluded that the concentration was

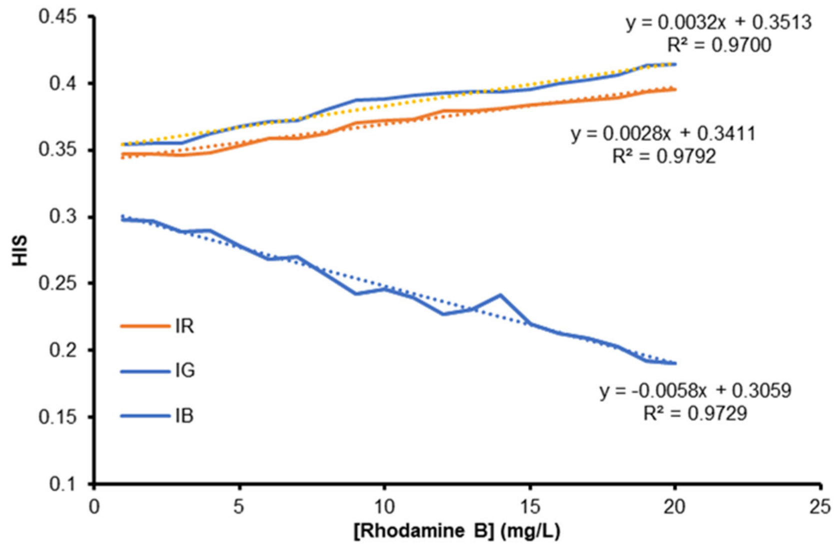

Fig 5. Calibration curve for $(RhB)_2$-$Zn(CNS)_4$

directly proportional to the absorbance, meaning that the absorbance for the complex $(RhB)_2$-$Zn(CNS)_4$ was dependent on rhodamine B concentration.

Method Validation

Accuracy

The accuracy of the proposed sensor method was based on the recovery (%), representing the value proximity of the standard concentration solution to the actual concentration. The concentrations of $(RhB)_2$-$Zn(CNS)_4$ used were 1, 10, and 20 mg/L for the analysis with TCS3200 and UV-Vis color sensors. The actual concentration values and the percent recovery values from each method can be seen in Table 1. The recovery % calculation for the TCS3200 color sensor was still within the allowable error range of 90–110% [32]. However, at a concentration of 1 mg/L, UV-Vis had a recovery value below the permissible range (81.8%). Therefore, our proposed method was suggested to have better accuracy for determining rhodamine B levels at a low concentration (1 mg/L).

Precision

The precision was determined to see the proximity of the value changes in the repetition process. The precision value was derived from the standard curve with a respective concentration of $(RhB)_2$-$Zn(CNS)_4$ (1, 10, and 20 mg/L), expressed by the variation coefficient (VC). The precision values for both methods based on intra-day and inter-day repetition are presented in Table 2. The variation coefficient value obtained by the two measurements increased with the decrement in the concentration of the standard solution. The method is accurate if it provides a variation coefficient value of less than 2% [32]. Nonetheless, inter-day repetition yielded higher variation coefficient, especially when rhodamine B concentration was 1 mg/L.

Linearity

Linearity is the functional area of sample measurement. The linearity of measurements using the TCS3200 color sensor and UV-Vis spectrophotometer for a concentration range of 1–20 mg/L is depicted in Fig. 5 and 6, respectively. Several studies used a

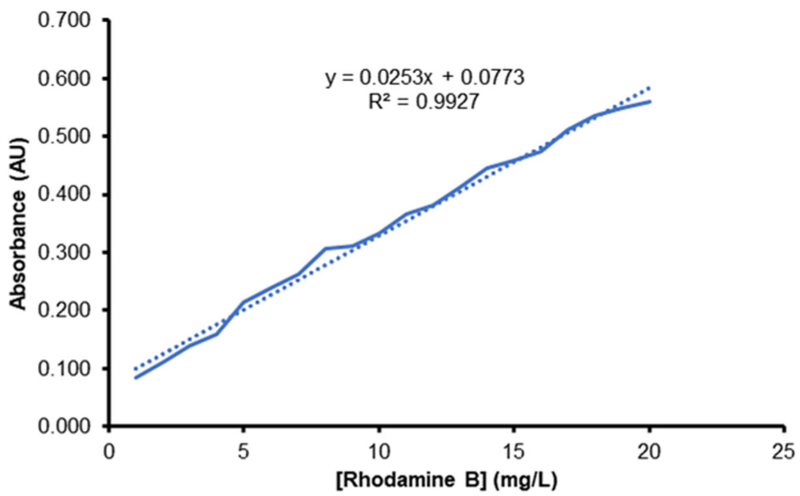

Fig 6. Calibration curve for $(RhB)_2$-$Zn(CNS)_4$

Table 1. Recovery percentages of TCS3200 sensor and UV-vis spectrophotometer

Concentration (mg/L)	Actual concentration (mg/L)		Recovery (%)	
	TCS3200	UV-Vis	TCS3200	UV-Vis
1	1.030	0.818	103.5	81.80
10	11.03	10.06	110.3	100.6
20	19.25	19.03	96.25	95.15

Table 2. VC values of TCS3200 sensor and UV-vis spectrophotometer obtained from intra-day and inter-day repetition

[Rhodamine B] (mg/L)	Intra-day variation coefficient (%)		Inter-day variation coefficient (%)	
	TCS3200	UV-Vis	TCS3200	UV-Vis
1	0.291	0.721	7.966	8.563
10	0.268	0.521	1.294	1.664
20	0.253	0.357	0.509	0.851

non-linear calibration curve because the sensor system formed an exponential response [33]. However, in the present study, the quantitative analysis was conducted based on linear regression.

Sensitivity

The sensitivity value is shown from the slope of the complex standard curve of $(RhB)_2$-$Zn(CNS)_4$ for each method. Based on the linear regression standard curve equation, the slope value for the TCS3200 color sensor measurement method was obtained from the regression equation $y = 0.0028x + 0.3411$, which was 0.0028. While the slope value for the UV-Vis spectrophotometer measurement method was obtained from the regression equation $y = 0.0253x + 0.0773$ is 0.0253. Based on the constructed standard curve, we calculated the limit of detection (LOD) by multiplying the standard deviation of response by 3.3 and dividing with the slope. Meanwhile, the limit of quantification (LOQ) could be obtained by multiplying the standard deviation of response by 10 and dividing it with the slope. The LOD obtained for the TCS3200 color sensor and UV-Vis spectrophotometer was 2.766 and 1.715 mg/L, respectively. These values explain why the inter-day precision for the 1 mg/L rhodamine B sample obtained for both methods exceeded the acceptable maximum variation coefficient (< 2%). As for the LOQ, the values reached 8.383 and 5.196 mg/L for the TCS3200 sensor and UV-Vis spectrophotometer, respectively. Lower LOD and LOQ in UV-Vis spectrophotometer suggest its superiority in comparison to the TCS3200 color sensor, in terms of sensitivity.

Quantitative Analysis Using the TCS3200 Color Sensor

Samples were measured using a series of tools that had been readily connected to the TCS3200 color sensor. The measurement was carried out by dipping the filter paper into the sample solution to which 3 mL of $Zn(CNS)_2$ reagent had been added, then dried and measured using the TCS3200 color sensor in dark conditions. Measurements were carried out three times on each sample with 3 cm-long distance between the sensor and the sample. Such distance was given to allow even distribution of the emitted light from four Light Emitting Diodes (LEDs) to the sample and the photodiode, in which the sample could emit a current proportional to the basic color of received light.

Table 3 shows that the RGB value obtained from each sample is a code to indicate a specific color. The HIS value in the table was obtained using Eq. (1-3). The I_R value was used to determine the concentration of rhodamine B in the sample because it had the best R^2 (0.9792) among the others (Fig. 6). The total concentration of rhodamine B obtained from the measurement using the TCS3200 color sensor based on the I_R value can be observed in Table 4, showing the concentration of each sample with five repetitions. The red index value obtained from Eq. (1) has the same function as the absorbance value, the dependent variable in determining the concentration. Therefore, the concentration of rhodamine B in the sample was calculated by substituting the red color index value of the sample into the standard curve regression equation $(RhB)_2$-$Zn(CNS)_4$ R index.

Following the analysis, we found that samples A, B, and C contained rhodamine B with an average of 1.74, 16.74, and 5.10 mg/L, respectively. However, sample A had a rhodamine B concentration lower than the LOD of both the TCS3200 and UV-Vis spectrophotometer (2.766 and 1.715 mg/L, respectively). In this case, the response generated from sample A could not be differentiated from that of the blank standard. Hence, the presence of rhodamine B in sample A could not be

Table 3. RGB value samples

Repetition		RGB Measurement			HIS Value Measurement			Color
		R	G	B	I_R	I_G	I_B	
Sample A	1	232	213	224	0.346	0.317	0.334	
	2	233	213	224	0.347	0.317	0.334	
	3	233	212	224	0.348	0.317	0.334	
	4	231	211	222	0.343	0.312	0.330	
	5	230	210	223	0.346	0.316	0.336	
	$\bar{A}$	231.8	211.8	223.4	0.345	0.316	0.334	
Sample B	1	206	108	215	0.389	0.203	0.406	
	2	206	107	216	0.388	0.202	0.407	
	3	206	108	216	0.386	0.203	0.407	
	4	205	106	215	0.389	0.201	0.408	
	5	206	107	215	0.388	0.203	0.407	
	$\bar{D}$	205.2	107.2	215.4	0.388	0.203	0.407	
Sample C	1	222	173	228	0.356	0.278	0.366	
	2	221	173	227	0.355	0.278	0.365	
	3	221	172	226	0.357	0.276	0.366	
	4	220	171	227	0.355	0.276	0.367	
	5	220	172	228	0.354	0.277	0.367	
	$\bar{F}$	220.8	172.2	227.2	0.355	0.276	0.366	

Table 4. Sample concentration value of TCS3200 color sensor

Sample (X)	Repetition (mg/L)					$\bar{X}$ (mg/L)
	1	2	3	4	5	
A	1.75	2.10	2.46	0.67	1.75	1.74
B	17.10	16.75	16.03	17.10	16.75	16.74
C	5.32	4.96	5.67	4.96	4.61	5.10

confirmed by either method. As for sample C, the calculated concentration was lower than the LOQ of the TCS3200. Although its presence was confirmed, its quantitative concentration value was not reliable. Therefore, for the following analysis of comparing TCS3200 with UV-Vis spectrophotometer, samples A and C were excluded.

Comparing Methods Between the TCS3200 Color Sensor with UV-Vis Spectrophotometry Using the Two-Way t-Test

Method comparisons were carried out to see whether the TCS3200 color sensor had similar results to a UV-Vis spectrophotometer. The prepared samples were measured for five repetitions with UV-Vis at a wavelength of 590 nm and TCS 3200. Concentrations of rhodamine B in sample B were 16.74 and 17.26 mg/L for measurements using TCS3200 and UV-Vis spectrophotometer, respectively. *T*-test (a = 8.95%) performed on the obtained data revealed that the $t_{experimental}$ and $t_{theoretical}$ values were 1.21 and 2.31, respectively. Therefore, H_0 is accepted because the value of $t_{experimental} < t_{theoretical}$. H_0 states that differences of data obtained from TCS3200 and the UV-Vis spectrophotometer are not meaningful or significant. This analysis validates the high concentration of rhodamine B in sample B, calculated using TCS3200. It is worth mentioning that high concentrations of rhodamine B exposed to the human body could cause adverse health effects [34].

■ CONCLUSION

The analytical performance of the newly developed TCS3200 color sensor was satisfactory, considering that the analysis could be run *in situ* and available at an affordable cost, and the components were free to access. The results showed that the color gradation only occurred in the R (Red) component, while the other components, G (Green) and B (Blue), were not concentration-depended. The *t*-test results showed that $t_{experimental} < t_{theoretical}$ suggesting the absence of statistical significance between the results obtained from the TCS3200 color sensor and the UV-Vis spectrophotometric method. The syrup samples procured from the local stores in Banda Aceh were tested qualitatively and quantitatively and was found to contain rhodamine B with high concentrations.

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