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# Introducing Colorimetric Analysis with Document Scanner for High School Students

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**Abstract.** Spectrophotometer is modern analytical instrument for analysis the amount of substance based on the color change. However, for high school laboratory practice, only few schools had this instrument for chemical analysis. There are many studies reported the use of daily life electronic for colorimetric analysis such as smartphone, scanner and digital camera. This work demonstrated the used of document scanner for colorimetric glucose determination for easy learn the daily life instrument as analytical device. The color detection was based on the reaction of glucose with glucose oxidase enzyme to produce  $H_2O_2$ . The  $H_2O_2$  was then reacted with  $TiOSO_4$  to produce yellow color. The color formation was captured with a document scanner and the digital image was analyzed using ImageJ software. The result of the used of document scanner showed Horwitz Ratio of 0.40, recovery of 98.4%, linear responses from 0.5 mM to 3.0 mM ( $r = 0.9989$ ), limit of detection of 0.15 mM and limit of quantification of 0.40 mM.

## 1. Introduction

The higher technologies growth was also linear with the modern measurement methods development. The visual color observation would further could be measured or detected, electronically. When using the naked eye, similar colors can be difficult to distinguish, for example between blue-greenish green with the youngest green, and in general the colors are arranged from the basic colors of red, green and blue (RGB). These colors have different wavelengths of light. Quantitative analysis techniques commonly used in laboratories to identify the color of the solution are colorimetric analysis. Colorimetry is a quantitative analysis technique for colored samples, which is used to determine the concentration of a substance based on the color light intensity of the solution [1].

The rapid advancement of technology has led to the development of increasingly sophisticated instrumentation for colorimetric analysis. Instruments could be used in colorimetric analysis include UV-Vis spectrophotometers, colorimeters, and photoelectric colorimetry. However, these instruments are relatively expensive, that could not be accessed by some users, for example equipment for high school students. Colorimetric analysis is one of the interesting topics to study [2], it contains concepts that can be explained in colorimetric analysis, including: stoichiometric calculations, complementary colors, acid-base reactions, and the formation of complex compounds [3].



The development of colorimetric analysis techniques using simple and relatively easy tools such as smartphone camera [4,5], digital camera and document scanners [6] have been reported. One example is the colorimetric method using a scanner and digital imaging technique for food coloring measurement [7]. The research has succeeded in making a standard curve of a yellow food coloring solution. Where the digital imaging results obtained the standard curve for one of the RGB (Red, Green, Blue) color components of the Blue (B) as a color component of the yellow food coloring measurement. The RGB value indicates the quantity of compounds that are in the solution because basically, the color variation of a system changes with changing the concentration of a component. The ability of the scanner as a detector for recording digital image is the main idea for the colorimetric method application [6]. The colors recorded are complementary colors.

In this research, a colorimetric method will be demonstrated using a scanner with imaging techniques to determine the hydrogen peroxide. Scanners are used as image capture tools because they are relatively easy and widely used, and the amount of material used for research is less and more economical. This technique would be easily applied and learned in the high school student that modern instrumentation of colorimetric may be absent.

## 2. Materials and Methods

### 2.1. Materials and instrumentations

Instruments used in this research including document scanner (Canon LiDE 120), Laptop with ImageJ software (<http://imagej.nih.gov/>), spectrophotometer UV-Vis (Shimadzu UV-1800), microplate (IWAKI), and laboratorial glassware.

The materials used in this work such as enzyme glucose oxidase (Sigma-Aldrick, USA), hydrogen peroxide 30% (Merck, Jerman), sulfuric acid (Merck, Jerman), titanium dioxide (Sigma-Aldrick, USA), glucose (Merck, Jerman) and L-ascorbate (Merck, Jerman).

### 2.2. Instrument setting

Document scanner was used to record the digital image of solutions. The sample solutions were placed in a flat bottom 96 well microplate, that placed in the center of scanner windows. Outside the microplate protected with white styrofoam. The scanner was then connected to the laptop, record the image using the scanner software. The digital images obtained were then analyzed using the ImageJ software to extract the color intensity of Red, Green and Blue (RGB).

### 2.3. Color measurement using spectrophotometer

Glucose concentration analysis would be carried out using the enzyme glucose oxidase which produces  $\text{H}_2\text{O}_2$ . The amount of  $\text{H}_2\text{O}_2$  can be determined using indicator, in which color changes are easily observed, for example  $\text{TiOSO}_4$ . The  $\text{TiOSO}_4$  solution maximum wavelength was first determined.  $\text{H}_2\text{O}_2$  solution as an enzymatic reaction product, was added to the  $\text{TiOSO}_4$  solution with the concentration of  $\text{H}_2\text{O}_2$  of 0.6; 0.8; 1; 1.2 and 1.4 mM. Then all the absorbance solutions were measured using a UV-Vis spectrophotometer at the maximum wavelength. The absorbances were plotted to make the calibration curve between the absorbance and the concentration of  $\text{H}_2\text{O}_2$ . The sensitivity of the UV-Vis spectrophotometer was indicated by the slope of the resulting calibration curve.

### 2.4. Color measurement using document scanner

$\text{H}_2\text{O}_2$  standard solution of 0.6; 0.8; 1; 1.2 and 1.4 mM were placed into each of the microplate 96 wells. The microplate was then placed on the scanner connected to the laptop and then the scanning process was performed. The picture obtained is then determined the RGB value of each solution with the ImageJ Software. The RGB values of the  $\text{H}_2\text{O}_2$  standard solution are plotted into a calibration curve Red, Green, and Blue which related to the  $\text{H}_2\text{O}_2$  concentration. Scanner sensitivity was indicated by the slope of the resulting calibration curve. The highest sensitivity obtained was then compared with the sensitivity of the analysis results using the UV-Vis spectrophotometry method and was used for further procedures.

### 2.5. Precision study

The precision was determined by testing several different solutions with the same preparation conditions. This test was carried out by analyzing six of 1.4 mM H<sub>2</sub>O<sub>2</sub> standard solutions. Then, each solution was added the TiOSO<sub>4</sub> indicator. Precision was then determined by calculating standard deviation (SD), relative standard deviation (RSD) or coefficient of variation (KV). The acceptable value of the HORRAT (Horwitz Ratio) was less than 2.

### 2.6. Accuracy study

Accuracy was determined by adding a 1.4 mM H<sub>2</sub>O<sub>2</sub> standard solution to a known concentration of sample. The solutions were then added to the TiOSO<sub>4</sub> indicator. Percent recovery (% recovery) was calculated by the formula:

$$\% \text{ recovery} = (\text{Ch}-\text{Cb}) / \text{Cs} \times 100\%$$

Ch = calculated analytes concentration

Cb = concentration without analytes

Cs = theoretical analytic concentration

### 2.7. Linearity, limit of detection and limit of quantification

Linearity is the range (concentration) of specific analytes of the response related to the concentration that gives a linear equation, which is mean the increase in the responses are directly proportional to the increase in concentration. Linearity was performed by measuring the intensity of the red color standard solution H<sub>2</sub>O<sub>2</sub> concentration of 0.1; 0.5; 1; 1.5; 2; 2.5; 3 and 5 mM. Each solution was added with the TiOSO<sub>4</sub> indicator. The intensity of the red color of the standard H<sub>2</sub>O<sub>2</sub> solutions were then plotted into a calibration curve. The limit of detection and limit of quantification were then calculated using the following formula:

$$\text{LOD} = \frac{3 \times \text{SD}}{b}, \text{LOQ} = \frac{10 \times \text{SD}}{b}$$

Where:

SD: standard deviation of the intercept of linear equation

b : slope of the linear equation

### 2.8. Glucose measurement

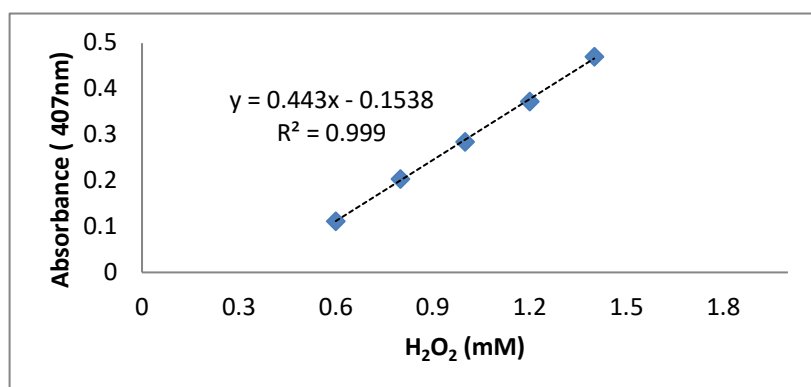
Glucose measurement was carried out enzymatically with the 2-unit glucose oxidase enzyme. One series of standard solutions of 0.5, 1.5 and 2.5 mM were reacted with the glucose oxidase enzyme, then allowed to stand for 20 minutes. Each solution was added with 1 mL of TiOSO<sub>4</sub> indicator and measured using a scanner and UV-Vis spectrophotometer. The glucose concentrations of the samples were then calculated based on the calibration curve of the glucose standard solution. Glucose analysis results obtained with a scanner and UV-Vis spectrophotometer were compared statistically with the Wilcoxon signed rank test.

## 3. Results and Discussion

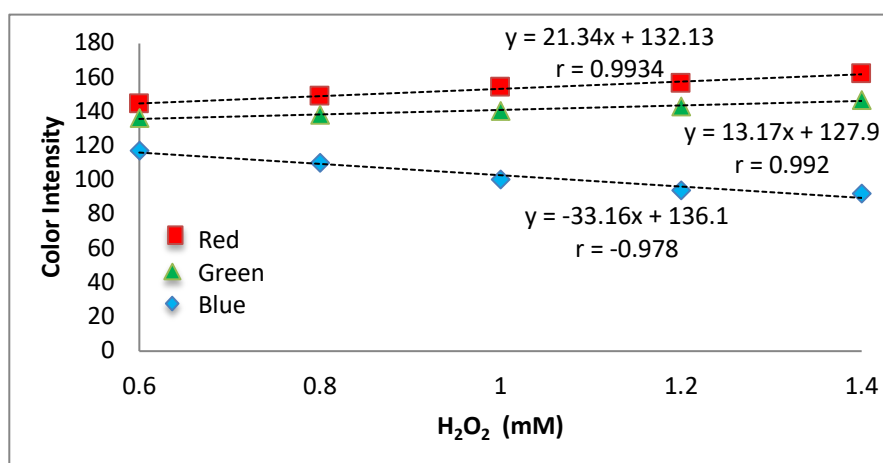
### 3.1. Color change measurement using spectrophotometer

The color change measurement was based on the color change of the redox indicator by hydrogen peroxide, as enzymatic reaction products in enzymatic determination of glucose determination. The maximum wavelength ( $\lambda_{\text{max}}$ ) obtained was 407 nm for the yellow color produced from the reaction of hydrogen peroxide and TiOSO<sub>4</sub>, according to the following equation:





**Figure 1.** Calibration curve of hydrogen peroxide with the addition of TiOSO<sub>4</sub>.



**Figure 2.** Calibration curve of hydrogen peroxide measured using document scanner

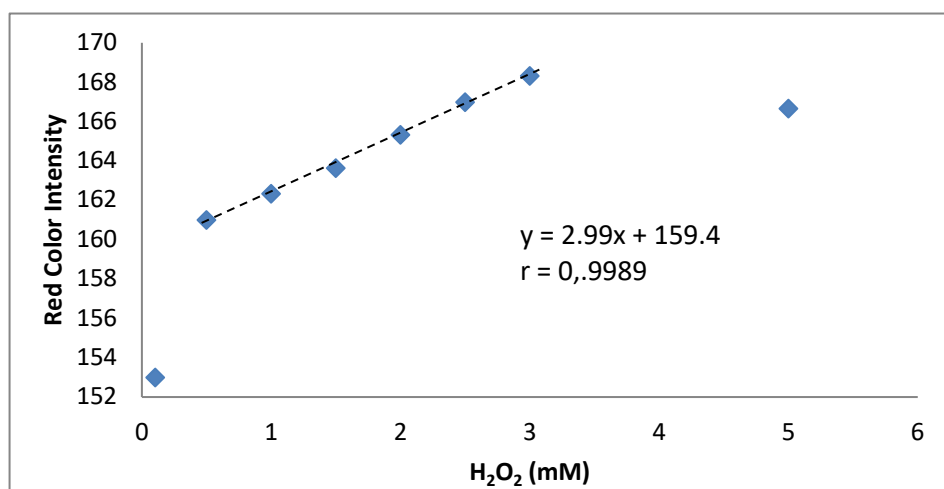
The color change with the hydrogen concentration showed a linear response with the regression equation of  $y = 0.44x - 0.15$  and a correlation coefficient ( $r$ ) of 0.9995 (**Fig. 1**).

### 3.2. Document scanner setting and color measurement

The color measurement using a document scanner was performed by scanning the hydrogen peroxide - TiOSO<sub>4</sub> solution in a 96 well microplate placed on the scanner window. The color intensity of the red, green, and blue was then obtained after the digital picture of document scanner was extracted using the ImageJ software. Hydrogen peroxide solution of 0.6; 0.8; 1; 1.2; and 1.4 mM showed a linear for both red, green and blue color intensity (**Fig. 2**). However, the Red color showed the highest sensitivity with the good linearity ( $r = 0.9934$ ). Thus, for further procedure, the red color intensity was selected.

### 3.3. Precision study

Precision was studied to show the degree of the fitness between individual test results repeatedly measured from a homogeneous mixture [8]. Precision was performed by measuring 6 standard solutions of H<sub>2</sub>O<sub>2</sub> 1.4 mM. The precision test results of the H<sub>2</sub>O<sub>2</sub> standard solution show the value of relative standard deviations or coefficient of variation greater than 2%. The precision is expressed as a percentage of Relative Standard Deviation (RSD) or Coefficient of Variation (CV) with an acceptable threshold based on its accuracy. The result showed the CV of the measurements was 2.14%, whereas the theoretical CV was 5.36%, therefore, the calculated HORRAT was 0.40 (valid for value less than 2).



**Figure 3.** Linearity study of hydrogen peroxide with  $\text{TiOSO}_4$  measured using a document scanner

#### 3.4. Accuracy study

Accuracy shows the degree of closeness of the analysis results with the actual analyte concentration. The results showed an average value of % recovery obtained from the samples were 98.4%. This reapproval value approves the agreed percent requirements between 90-107% [8]. This shows that the colorimetric method using the scanner showed accurate results.

#### 3.5. Linearity, limit of detection and limit of quantification

The linearity study showed the document scanner could detect hydrogen peroxide with the linear range of 0.5 to 3.0 mM (**Fig. 3**). Furthermore, the calculated limit of detection was 0.15 mM and limit of quantification was 0.49 mM.

#### 3.6. Glucose measurement

Glucose concentration measurement was performed as model of the use the document scanner for biosensor, using glucose oxidase enzyme as biorecognition. The glucose assay was performed by analyzing standard glucose solutions of 0.5, 1.5, and 2.5 mM. The yellow solution resulted was measured both using a UV-Vis spectrophotometer and a document scanner. The results showed there is no significant difference between the glucose levels from the analysis using the colorimetric method of a document scanner (0.37, 1.53 and 2.44 mM) and UV-Vis spectrophotometer (0.45, 1.45 and 2.45 mM).

### 4. Conclusion

Document scanner could be used to detect hydrogen peroxide concentration by analyzing the digital image of color changes related to the concentration. This simple colorimetric method showed linear range of 0.5 to 3.0 mM ( $r = 0.9985$ ), with limit of detection of 0.19 mM and limit of quantification of 0.64 mM. The precision study showed Horwitz Ratio of the document scanner for hydrogen peroxide detection of 0.40. Furthermore, the colorimetric analysis using the document scanner was not significantly different compare to spectrophotometric method.

### Acknowledgement

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Table of contents











Volume 1494

2020

◀ Previous issue      Next issue ▶

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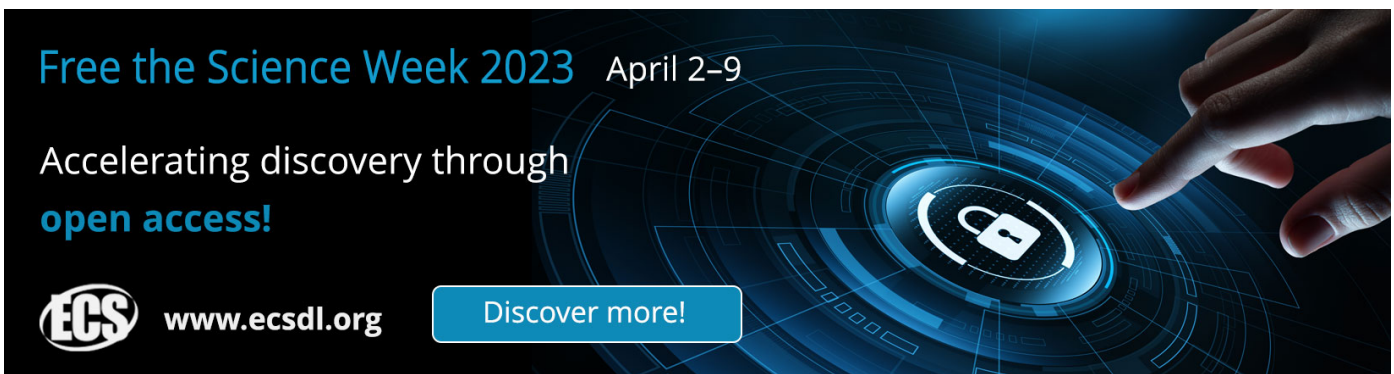
## Preface

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