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Uric acid detection in visible spectrum

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ABSTRACT

The measurement of uric acid based on the optical absorption at visible light spectrum is investigated and tested. Sensing in the visible region was conducted for determination of suitable wavelength that produces high sensitivity and accuracy performance based on the Beer-Lambert law calculation. In this work, the uric acid is detected by detecting sodium urate as a product of chemical reaction between uric acid with sodium hydroxide buffer. The setup has been tested for uric acid concentration ranging from 15 mg/dL to 85 mg/dL. Three wavelengths have been analyzed which are 460 nm, 525 nm and 630 nm. Measured data at 460nm wavelength exhibits the highest sensitivity, which is 0.0012 (mg/dL)-with 86.51% accuracy. Detection of uric acid at visible light spectrum offers a low-cost sensor based on visible LEDs and photodiode is possible to be realized.

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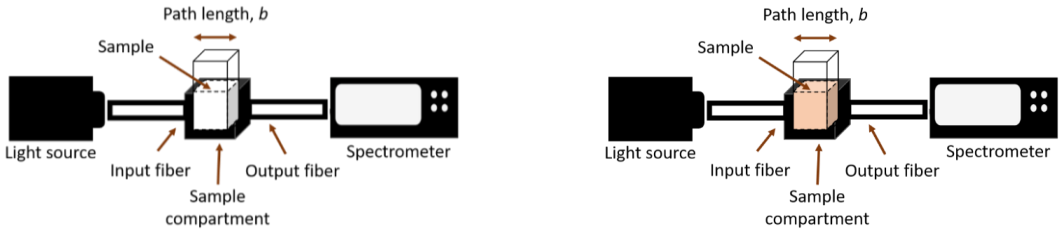
1. INTRODUCTION

Uric acid sensor has received much attention in recent years due to the increasing number of gout patients worldwide. Gout is a disease developed from the excess amount of uric acid present in blood and urine. Uric acid dissolves in blood and travels to the kidney to be eliminated through urine. Excess amount of uric acid will not only lead to gout, but it can also lead to kidney failure, heart disease, high cholesterol, diabetes and even hypertension [1, 2]. It is important to regulate the amount uric acid in maintaining the health of the bloodstream, since low amount of uric acid may lead to atherosclerosis and stroke [2]. Uric acid can be monitored using two methods; blood test measurement [3-6] and urine test measurement [1, 7-15]. A healthy range for uric acid levels in blood serum is from 3.5 to 7.2 mg/dL in men and from 2.6 to 6 mg/dL for women [14]. Meanwhile, the healthy range of uric acid in human urine is ten times greater than the uric acid levels in blood where it is ranging from 25 to 74 mg/dL [1, 12, 14]. In recent years, there has been extensive research on uric acid analytical techniques to detect uric acid in human urine such as electro-analytical, luminescence, chromatography and spectroscopy. As for spectroscopy system [1, 7-10, 16-21], the system detects uric acid by using chemical reagent or buffer solution to the sample to be tested by analyzing light absorbance or transmittance value. Spectroscopy shows a promising linear uric acid detection in a broad range which is from 0.58 to 58.84 mg/dL [14] and 58.84 to 218.56 mg/dL [13]. Thus, make it suitable for uric acid detection in both blood and urine.

As afore mentioned, interference cause by other substance in the sample in the spectroscopy system is minimize by adding a chemical reagent or buffer solution. In 2007, Yamaguchi has developed a simple and highly sensitive spectrophotometric method for the determination of uric acid based on fading of the o-hydroxyhydroquinonephthalein (QP)-palladium (II)-hexadecyltrimethylammonium complex in human urine. The absorbance measurement has been conducted using a Shimadzu spectrophotometer with deuterium and tungsten halogen lamp as the light source. This technique however has limitation in linearity range, where the linearity occurs in between 0.001 mg/dL to 0.02 mg/dL at 635 nm visible operating wavelength [18]. Later, a simple spectrophotometric method based-uricase enzyme for the detection of uric acid in normal urine and gout patient's urine samples has been developed based on the reaction of hydrogen peroxide (H_2O_2) with yellow color of 4-Aminodiphenylamine Diazonium Sulfate (variamine blue RT salt) to yield a pale yellow-green coloured solution at 269 nm wavelength im ultraviolet (UV) spectrum [8]. The calibration plot of different concentrations of uric acid was found to be linear between 9 mg/dL to 234 mg/dL with 20 minutes response time. Although broad sensing range has been achieved, the sensor requires very long response time. Besides of using chemical reagent, enzymes or buffer, uric acid also can be detected directly from the solution using spectrophotometer at 294.46 nm wavelength [19]. Although the method is simple, the effect of interefence by other substance is not studied since there is no chemical reagent was added. In this work, the uric acid detection at visible region was conducted by using NaOH buffer solution without any additional chemical reagent. Detection of the uric acid in this work is carried out by detecting sodium urate as a product of uric acid and NaOH mixing process.

2. THEORY

Analysis of sensitivity and accuracy in this work was calculated using Beer Lambert law. The Beer-Lambert law defines the attenuation of light to the properties of the sample in terms of transmittance, *T* and absorbance, *A* as stated in (1) and (2-5). In this work, the output light intensity after passing through cuvette without sample, I_{ref}, and with sample, I_o, is measured by using spectrometer. Configurations of a basic spectrophotometer system with the addition of input and output fiber is illustrated in Figure 1.



(a) (b)

Figure 1. Spectrophotometer system (a) without sample and (b) with sample

The transmittance value based on (1) can be used to define the absorbance, *A*, of the sample. The relationship between transmittance, *T* and absorbance, *A* is shown in (2) [20-22].

$$T = \frac{I_o}{I_{ref}} \quad (1)$$

$$A = \log \frac{1}{T} = \varepsilon bc \quad (2)$$

The (2) also relates absorbance, *A*, concentration of sample, c, sample path length, b, and the absorptivity of the sample, ε. This relationship is known as the Beer-Lambert law. Based on (2), the concentration of a substance is directly proportional to the amount of light absorbed or inversely proportional to the logarithm of the transmitted light [23, 24]. The relationship between transmittance, *T* and absorbance, *A* is illustrated in Figure 2.

Analytical analysis is done by analyzing the calibration curve which consists of a plot of absorbance versus concentration series of standard solution [20]. If the curve-fit is linear, the sensitivity of the curve fit is its slope [25]. Thus, sensitivity is given as in (3).

$$Sensitivity = \frac{\Delta A}{\Delta c} \quad [(\text{mg/dL})^{-1}] \tag{3}$$

ΔA is the absorbance difference and Δc is concentration difference. The (3) shows that for a fixed concentration difference, higher absorbance will contribute to a higher sensitivity of spectrophotometer [26]. The Standard unit for sensitivity of the tested sensor is dependent on the unit used for the sample concentration. Thus, in this paper, the unit used for sensitivity is $(\text{mg/dL})^{-1}$. As for calibration accuracy, this parameter refers to how close the measured value, $C_{measured}$ with the linear-fit real value, C_{real} [25]. The (4) shows equation used for accuracy calculation based on the data from absorbance plot in Figure 3. The following section will describe procedure for sample preparation and experiment that was conducted.

$$Accuracy = \left(1 - \left|\frac{C_{real} - C_{measured}}{C_{real}}\right|\right) \times 100 \ [\%] \tag{4}$$

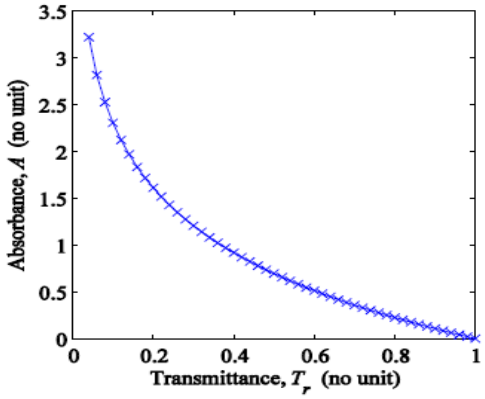


Figure 2. Graph of absorbance versus transmittance [22]

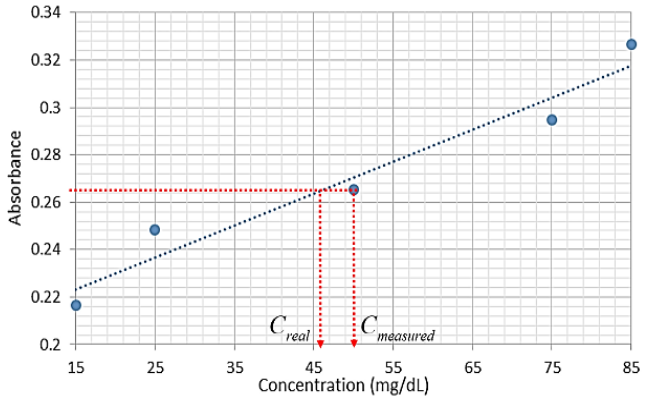


Figure 3. Accuracy measurement from absorbance plot

3. EXPERIMENT

Uric acid solution is prepared by mixing uric acid powder into sodium hydroxide (NaOH) solution [4, 7, 24]. In this research, uric acid and sodium hydroxide solution was purchased from Axon Scientific. The sample is prepared by mixing uric acid powder in 0.1 M NaOH solution as shown in Figure 4 (a) and (b). Table 1 shows the amount of uric acid powder that needs to be added to 80 mL of NaOH solution for producing uric acid sample with 15 mg/dL, 25 mg/dL, 50 mg/dL, 75 mg/dL and 85 mg/dL concentration. The sample preparation process was carried out at room temperature. Mixture of uric acid powder and NaOH solution will produce sodium urate and H_2O [24]. Higher concentration of uric acid in NaOH solution will form a solid sodium urate as shown in Figure 4 (c). Therefore, the mixture needs to be stirred for about 3 minutes before being transferred into the cuvette for fully dilution process as visualized in Figure 4 (d). In this measurement, 1.5 mL of sample were transferred into each cuvette for the characterization process as in Figure 4 (e). Figure 4 illustrates the steps taken for standard uric acid sample preparation.

Experiment was carried out by using tungsten-halogen as the light source (1) and spectrometer as optical detector. In this experiment, variable optical attenuator (2) is used to ensure intensity of light is not saturated at the spectrometer. The maximum photon count is limit to a maximum peak of 14000 photon count. Light from optical fibre is collimated into parallel beam using Ocean Optics 74-UV collimating lens (3) to interact with solution in cuvette (4) as shown in Figure 5. Then, light from cuvette is collected using the collimating lens and delivered to Ocean Optics HR4000CG-UV-NIR high resolution spectrometer (5). Ocean Optics OceanView spectrometer operating software is used to obtain and process data from spectrometer. Uric acid concentration is calculated by comparing intensity of light that passes through sample, I_o and does not pass through sample, $I_{ref.}$ Wavelengths selected to sample uric acid are 460 nm, 525 nm and 630 nm based on available visible LED in the market.

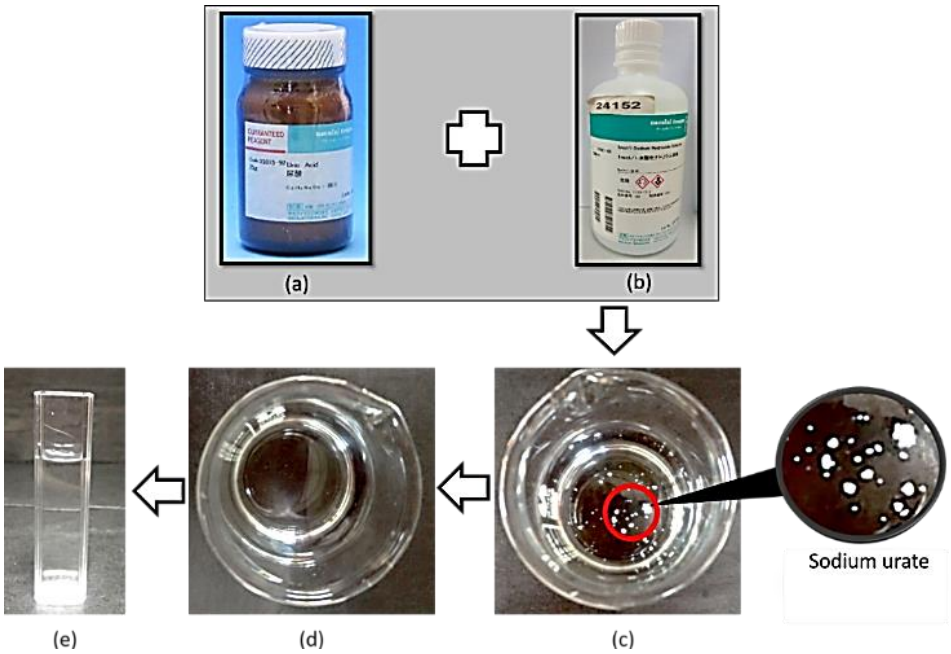


Figure 4. Preparation of standard uric acid sample; (a) uric acid powder (b) NaOH solution (c) sample after adding NaOH with uric acid producing particles of sodium urate (d) sample after stirred using glass rod (e) transfer 1.5 ml into cuvette for measurement

Table 1. Amount of uric acid powder added into 80 mL of NaOH solution

Concentration (mg/dL)	Uric Acid powder (mg)
15	12
25	20
50	40
75	60
85	68

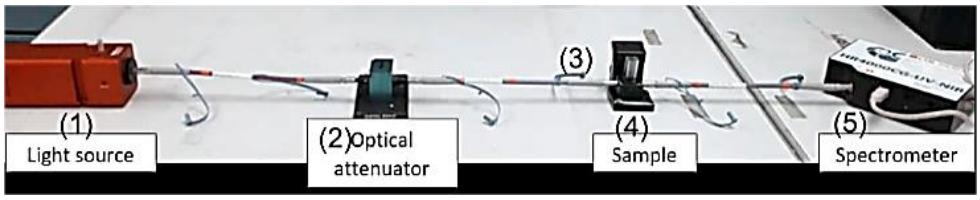


Figure 5. Experimental setup

4. RESULTS AND DISCUSSION

Figure 6 visualized the intensity spectra observed using HR4000CG-UV-VIS Ocean Optics spectrometer for SLS201 Stabilized Tungsten Halogen light operated at 400 nm to 700 nm wavelength range. This figure shows that light intensity will decrease as the concentration of uric acid increases, in which obey the Beer-Lambert law. The relationship between light intensity with absorbance is associated using (1) and (2). Calculated absorbance is plotted as in Figure 7 while calculated sensitivity and accuracy is tabulated in Table 2.

Table 2 shows that the highest sensitivity is at 460 nm wavelength which is 0.0012 $(mg/dL)^{-1}$. Although all tested wavelength demonstrate a similar pattern of absorbance due to substance in the sample, amount of the light absorb is difference, thus exhibit difference sensitivity performance. For accuracy performance, 525 nm wavelength spectrophotometer system produces accuracy above 90%. The result agrees well with what has been reported in the previous literature where less error will occur when the system has absorbance value within the recommended range, which are between 0.2 and 0.8 [22].

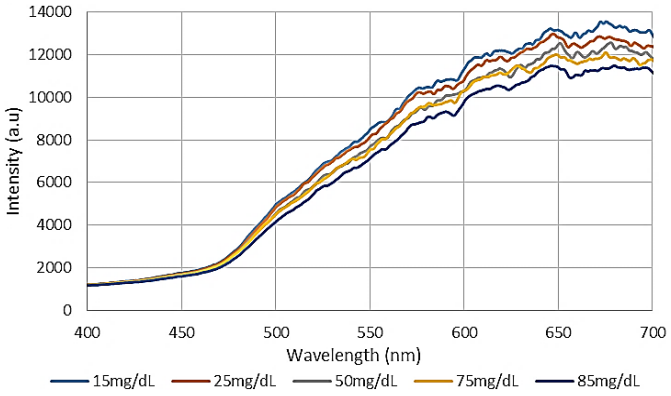


Figure 6. Intensity spectra of different uric acid concentration

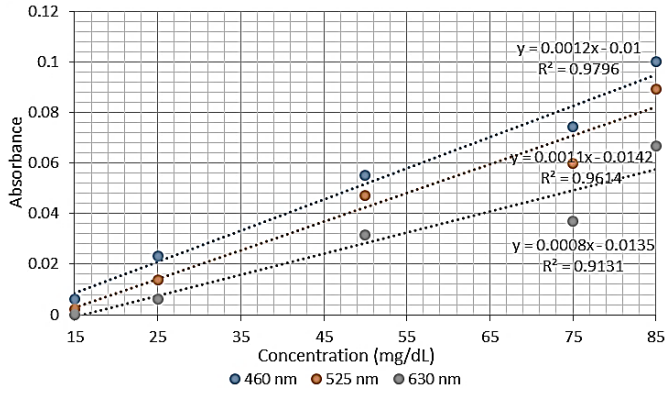


Figure 7. Absorbance analytical curve

Table 2. Spectrophotometer performance for selected tested wavelength

Wavelength (nm)	Sensitivity $(mg/dL)^{-1}$	Accuracy (%)
460	0.0012	86.51
525	0.0011	91.17
630	0.0008	84.27

5. CONCLUSION

The research study was carried out to analyze sensitivity and accuracy performance of spectrophotometer system at different sampled wavelength using absorbance analytical technique. The absorption was calculated using Beer's Lambert law formula. Through the analysis, the spectrometer was able to observe the current concentration of uric acid and its absorption wavelength at visible light region. In this work, spectrophotometer system operated at 460nm wavelength has the highest sensitivity while only system at 525 nm wavelength has accuracy higher than 90%. As discussed, the accuracy is influenced by limitation of Beer-Lambert law where absorbance value beyond 0.2 and 0.8 will have large error. The spectrophotometer system with visible light source will offer a simple and economical uric acid detection system and can be applied in biomedical application.

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