Spectrophotometer with enhanced sensitivity for uric acid detection

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A spectrophotometer with an LED as the light source for uric acid detection is proposed in this work. The mechanism of uric acid detection is based on energy absorbed by sodium urate, which is a chemical product of uric acid and sodium hydroxide solution. For the performance validation, comparison between the spectrophotometer with an LED and halogen lamp is carried out. Measurement results suggest that the spectrophotometer system with LED light has better sensitivity than that with halogen light. At a 460 nm wavelength, the sensitivity for the spectrophotometer with an LED is 0.0046 dL/mg, which is 73% higher than that with halogen light that records 0.0012 dL/mg. This enhanced sensitivity is attributed to the higher luminous efficacy of the LED light beam. As a result, a larger amount of flux interacts with the sample, leading to the sensitivity enhancement. The spectrophotometer with an LED is also applied for the detection of uric acid in a real human urine sample. Based on the experimental data at a 460 nm wavelength, the method manages to achieve the sensitivity of 0.0016 dL/mg, accuracy of 96.01%, limit of detection of 4.79 mg/dL, and limit of quantification of 14.52 mg/dL. These findings show that the use of LED as the input light source is promising for the spectrophotometer.

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Health monitoring has received tremendous attention by society in recent years. In general, health monitoring is carried out by measuring an amount of substance in the human body that causes diseases. Detection of the substance can be done by using invasive methods such as blood extraction. However, the invasive technique that requires blood extraction normally will cause pain, calluses, and scars to the patient, especially hemophiliacs, neonates, the elderly, and disabled people[1]. Therefore, the non-invasive method becomes an alternative to overcome the disadvantages. Besides blood, other human body fluids such as urine, tears, and saliva also have disease-signaling properties. These human body fluids that are categorized as human waste can be extracted for the non-invasive health monitoring method.

Uric acid is one of the substances that exist in human urine. Originally, uric acid comes from the consumption of protein such as meat, nuts, and seafood. As a result of human urine metabolism, uric acid is produced, and it dissolves in the blood before being sent to the kidney for elimination through urine. The healthy range for uric acid levels in blood serum is from 3.5 to 7.2 mg/dL for men and from 2.6 to 6 mg/dL for women[2]. Meanwhile, the healthy range of uric acid in human urine is ten times greater than the uric acid levels in blood, where it ranges from 25 to 74 mg/dL[3]. Therefore, the monitoring of uric acid is crucial, since an excess amount of uric acid will lead to gout, kidney failure, heart disease, high cholesterol, diabetes, and even hypertension[3–6]. On the other hand, lower amounts of the substance may lead to atherosclerosis and stroke[6]. In essence, it is important to scrutinize the amount of uric acid for health monitoring.

In recent years, there has been extensive research to detect uric acid in human urine using non-invasive methods such as electro-analytical techniques[6–9], chemiluminescence[10,11], chromatography[12], and spectroscopy[3,13,14]. Electro-analytical techniques such as capillary electrophoresis[6], electrochemical analysis[7], potentiometry[8], and voltammetry[9] offer simple detection methods using electrodes. Most of the electro-analytical techniques show non-linearity in the higher range of uric acid concentration, thus making it unsuitable for uric acid detection in human urine. For example, capillary electrophoresis shows instability when the uric acid concentration is larger than 500 μM (1 M = 1 mol/L)[6]. Meanwhile, chemiluminescence[10,11] and chromatography[12] have the capability to detect uric acid concentration in both blood and urine. However, they suffer from a complex chemical preparation process, narrow linear range, and slow response time. The spectroscopy technique on the other hand shows a promising linear uric acid detection in a broad range, which is from 9 to 234 mg/dL[3]. Moreover, the spectroscopy technique does not require disposal for any component, hence making it more eco-friendly.

A few developments have been reported regarding the use of the spectrophotometer technique for uric acid detection[3,13,14]. In 2007, Yamaguchi developed a spectrophotometric method for the determination of uric acid based on fading of the palladium(II)-based complex in human urine[13]. The absorbance measurement has been conducted using a Shimadzu spectrophotometer with a deuterium and tungsten halogen lamp as the light source. The new enzyme in Ref. [13] improves the sensitivity up to 45.071 $(\mathrm{mg/dL})^{-1}$, but the detection is for the lower sensing range from 0.01 to 0.20 μg/mL. In a different development, a simple spectrophotometric method for the detection of uric acid in a gout patient's urine sample has been proposed[3]. It is based on the enzymatic reaction of uricase in the presence of dye variamine blue RT salt that exhibits a very good relative standard deviation (RSD) of 0.7% with sensitivity of -0.0001 $(\mathrm{mg/dL})^{-1}$. The amount of uric acid is measured through a ultraviolet-visible (UV-Vis) spectrophotometer (Varian-Carry Win). In a more recent development, uric acid has been detected directly from the sample using a spectrophotometer at the 294.46 nm wavelength[14]. The direct detection method utilized a deuterium lamp as the input light source in the experiment. This method achieved 0.01 $(\mathrm{mg/dL})^{-1}$ sensitivity. However, interference caused by other substances has not been studied. Despite the achievements of the spectrophotometric method in uric acid detection, all of the aforementioned works have been conducted using a spectrophotometer system with a deuterium and tungsten halogen lamp as the light source. Therefore, it is interesting to investigate whether the spectrophotometer system with an LED as the light source can affect the performance of the system. In this work, we make a comparison between the spectrophotometer system that utilizes a halogen lamp and an LED as the light source. Based on the experimental results, it is found that the spectrophotometer system with an LED has better sensor sensitivity than that with a halogen lamp as the light source.

The spectrophotometer configuration for uric acid detection is shown in Fig. 1. It consists of a light source, an input and output optical fiber, a sample compartment, and a detector. In this experiment, two types of light sources were utilized for comparison: Ocean Optics SL201 stabilized tungsten halogen lamp and 1 W high power LEDs with central wavelengths of 460, 525, and 630 nm. The input powers for both halogen and LED were controlled at 9 W and 16.5 mW, respectively, such that they can produce a constant 14,000 photons at the intended measured wavelength. In this work, the input light was coupled in and out of the sample compartment through a 25 cm length and 400 μm diameter Ocean Optics QP400-0025-SR optical fiber. The sample of uric acid was placed in the sample compartment that has a path length of 10 mm. The spectrum and power of the output light were measured using an Ocean Optics USB4000-UV-VIS spectrometer and an optical power meter, respectively.

The detection of uric acid in this work is based on energy absorbed by sodium urate, which is a chemical

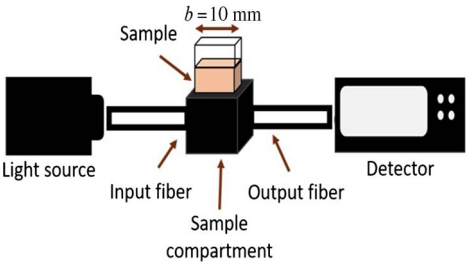


Fig. 1. Configuration of spectrophotometer.

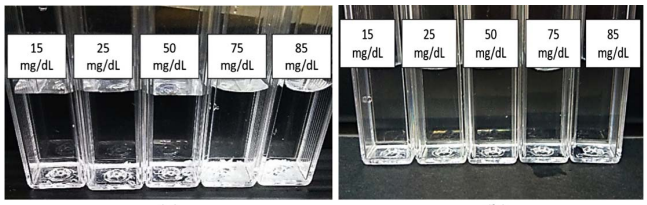
product of uric acid and sodium hydroxide (NaOH) solution[15]. The sample was prepared by mixing uric acid powder with 0.1 M NaOH solution. Different uric acid concentrations of 15, 25, 50, 75, and 85 mg/dL were then transferred into the sample compartment for measurement. The measurement was carried out at room temperature after a 3 min stirring process. Figures 2(a) and 2(b) show the different uric acid concentrations before and after the stirring process, respectively. Before the stirring process, as illustrated in Fig. 2(a), it can be seen that sodium urate crystals accumulate at the bottom of the sample compartment, and the amount of sodium urate crystals increases as the concentration of uric acid gets higher. On the contrary, after the stirring process, as shown in Fig. 2(b), the crystals do not appear at the bottom, showing that they are fully diluted in the NaOH solution.

Comparison of luminous efficacy L_e between the halogen and LED light source is firstly carried out. Luminous efficacy is basically a ratio of the interacted luminous flux $\varphi_{\mathrm{interacted}}$ to the optical beam power P_{beam}, as indicated in Eq. (1). The $\varphi_{\mathrm{interacted}}$ in turn is related to the optical beam energy E_{beam}, the surface area of fiber core A_{core}, and the surface area of the light beam A_{beam}. Their relationship is illustrated in Eq. (2). Both $E_{\mathrm{beam}} = P_{\mathrm{beam}}/A_{\mathrm{core}}$ and A_{beam} are measured at 10 mm distance from the input optical fiber:

$$L_e = \frac{\varphi_{\mathrm{interacted}}}{P_{\mathrm{beam}}}, \tag{1}$$

$$\varphi_{\mathrm{interacted}} = E_{\mathrm{beam}} \times \frac{{A_{\mathrm{core}}}^2}{A_{\mathrm{beam}}}. \tag{2}$$

The beam area A_{beam} is obtained from the recorded beam image at 10 mm distance from the input fiber tip.



(a) (b)

Fig. 2. Samples with different uric acid concentrations (a) before and (b) after stirring.

In this stage, intensity analysis of the beam concentration is done using the ImageJ software based on the gray-scale percentage of the pixels. Figure 3(a) shows the beam image of the halogen light, and its corresponding beam diameter in Fig. 3(b) is 6.5 mm, yielding $A_{\text{beam}} = 33.18\ \text{mm}^2$ at 90% gray scale. On the contrary, the beam image of the LED is more focused, as indicated in Fig. 3(c), and this is validated by the lower beam diameter of 3.4 mm and beam area of $A_{\text{beam}} = 9.08\ \text{mm}^2$ in Fig. 3(d).

The luminous efficacy L_e is then calculated for both halogen and LED light sources. Following Eqs. (1) and (2), L_e is calculated based on the obtained values of P_{beam}, A_{beam}, and $\varphi_{\text{interacted}}$, as tabulated in Table 1. It is evident that the LED light offers better luminous efficacy at 1.7401 mlm/W compared to only 0.4762 mlm/W for the halogen light. The better efficacy for the LED is attributed to its emission of focused light rather than divergent light for the halogen light source.

Comparison of sensor sensitivity between the systems with halogen and LED light sources is then carried out. Figures 4(a) and 4(b) show the output intensity spectra for the halogen and LED (460 nm wavelength) light source, respectively. In both cases, it is evident from the spectra that the output light intensity reduces as the concentration of uric acid increases, and this is agreeable with the Beer–Lambert law[16]. Based on the input I_o and output light intensity I, the light absorbance $A = -\log\left(\frac{I}{I_o}\right)$ at the three wavelengths of 460, 525, and 630 nm is then calculated. With variations of uric acid concentrations, Figs. 5(a) and 5(b) present the light absorbance for both halogen and LED light sources, respectively. The absorbance is found to be linear with the uric acid concentrations, and the sensor sensitivity can be extracted from the slope of the linear fit equation. In general, the spectrophotometer system with LED light has better sensitivity compared to that with halogen light for all tested wavelengths, as presented in Table 2. For example, at a 460 nm wavelength, the sensitivity for the system with an LED is 0.0046 dL/mg, which is 73% higher than that with halogen light that records 0.0012 dL/mg. The enhanced sensitivity for the system with an LED is attributed to the higher luminous efficacy L_e for the LED light. As a result, a larger amount of flux interacts with the sample, and this leads to the improvement of the system sensitivity. Reproducibility of the proposed method is calculated to be 1.62% ($N = 3$) with limit of quantification (LOQ) of 14.52 mg/dL, whereas the limit of detection (LOD) is 4.79 mg/dL, which is comparable with the LOD that has been reported in Ref. [3] and records 0.58 mM or 9.75 mg/dL.

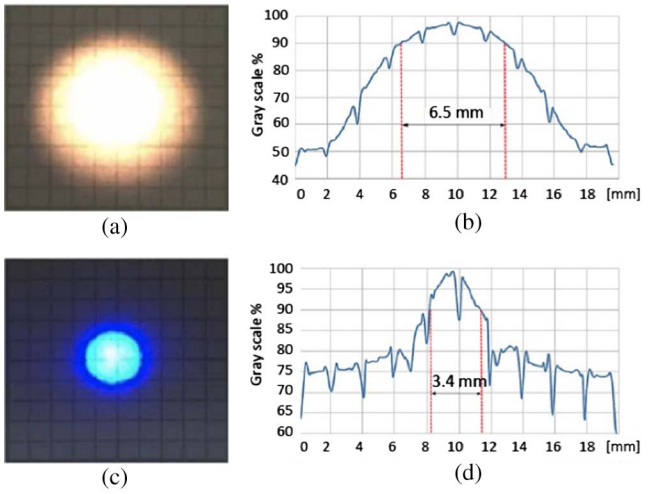


Fig. 3. Light beam at 10 mm distance from the input fiber. (a) Halogen beam. (b) Gray-scale percentage for halogen light. (c) LED beam (460 nm wavelength). (d) Gray-scale percentage for LED light.

Table 1. Comparison of Light Characteristics Between Halogen and LED Light Sources at 460 nm Wavelength

Parameter	Halogen	LED
P_{beam} (μW)	15.4	1.7
A_{beam} (mm^2)	33.18	9.08
$\varphi_{\text{interacted}}$ (nlm)	7.3337	2.9582
L_e (mlm/W)	0.4762	1.7401

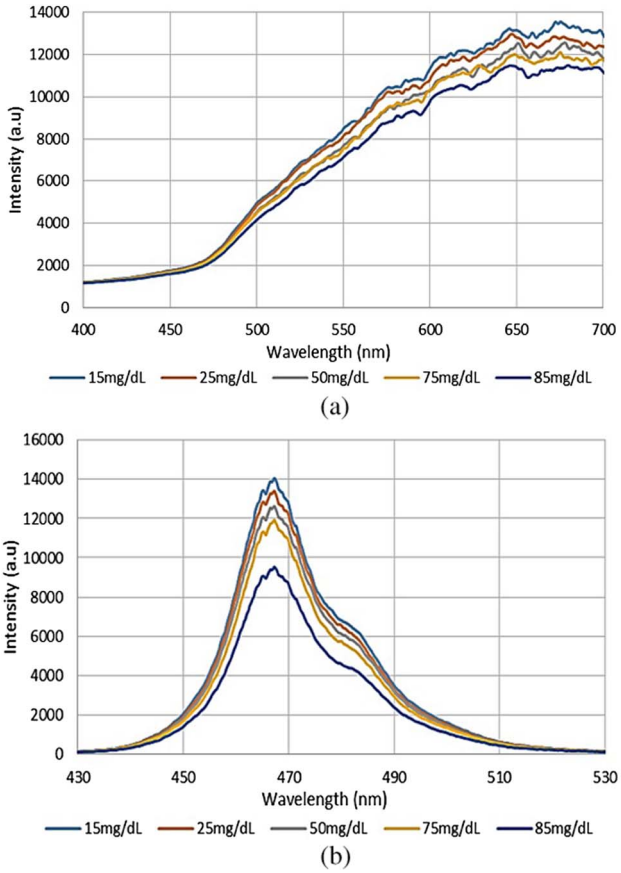


Fig. 4. Spectrometer output spectra. (a) Halogen. (b) LED (460 nm wavelength).

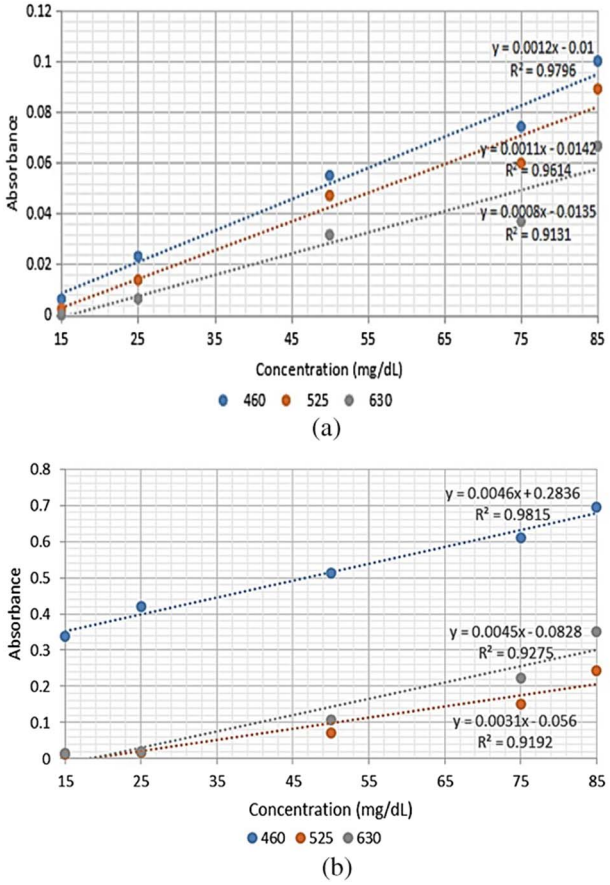


Fig. 5. Light absorbance with variations of uric acid concentration at wavelengths of 460, 525, and 630 nm. (a) Halogen. (b) LED.

Table 2. Comparison of Spectrophotometer Performance

Light Source	Wavelength (nm)	Sensitivity (dL/mg)	Accuracy (%)
Halogen	460	0.0012	86.51
	525	0.0011	91.17
	630	0.0008	84.27
LED	460	0.0046	96.62
	525	0.0031	77.57
	630	0.0045	79.43

Comparison between the system with halogen and LED light sources in terms of the sensor accuracy is also carried out. The accuracy is defined as[17]

$$\text{Accuracy} = 1 - \left| \frac{C_{\text{real}} - C_{\text{measured}}}{C_{\text{real}}} \right|, \tag{3}$$

where C_{real} is the practical uric acid concentration, and C_{measured} is the measured concentration obtained from the linear fit equation in Fig. 5.

Based on the accuracy data presented in Table 1, the highest accuracy obtained is 96.62% when the LED with a 460 nm wavelength is utilized as the light source. For other systems with different light sources, the sensor accuracy records the lower percentage value. This finding is consistent with what has been reported in Ref. [18], where the system with the absorbance value within the recommended range of 0.2 and 0.8 will have a lower margin of error. For the system with the 460 nm LED, the absorbance value falls within the range from 0.3 to 0.7, and, for this reason, it records the highest accuracy when compared to others.

This method is also applied for detection of uric acid in a real human urine sample. The concentration of uric acid in the sample is compared with the results obtained using the clinical method. The human urine samples are provided by a medical clinic. Out of seven urine samples, six samples are from male patients, and one sample is from a female patient. All of the patients have healthy kidney conditions. For detection process, these human urine samples are prepared by mixing 1 mL of urine with 0.5 mL of the NaOH solution. Then, these samples are transferred into a cuvette for testing, as shown in Figure 6. Absorbances for both synthetic uric acid and human urine are recorded and plotted in Figure 7. Both results have a similar pattern, where higher concentrations of uric acid exhibit higher values of absorbance. The sensitivity and accuracy of the developed method for the human urine sample are found to be 0.0016 dL/mg and 96.01%, respectively.

In conclusion, a comparison of spectrophotometer systems with halogen and LEDs as the light source is carried out in this work. The application of the spectrophotometer system is for the detection of uric acid in human urine. The mechanism of uric acid detection is based on energy absorbed by sodium urate, which is a chemical product of uric acid and NaOH solution. Measurement results indicate that the spectrophotometer with an LED light source has higher sensitivity than that with a halogen lamp. At the wavelength of 460 nm, the sensitivity for the spectrophotometer with an LED is 0.0046 dL/mg, as opposed to only 0.0012 dL/mg for the spectrophotometer with a halogen lamp. This represents 73% enhancement in the sense of sensor sensitivity. The reason for the

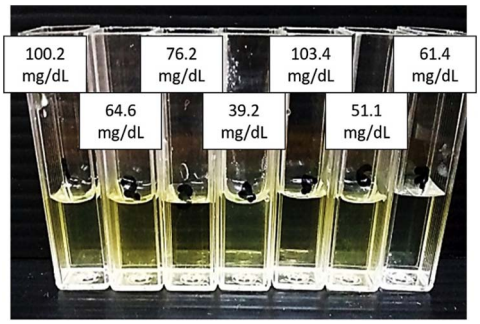


Fig. 6. Human urine sample in cuvette after mixing with NaOH solution.

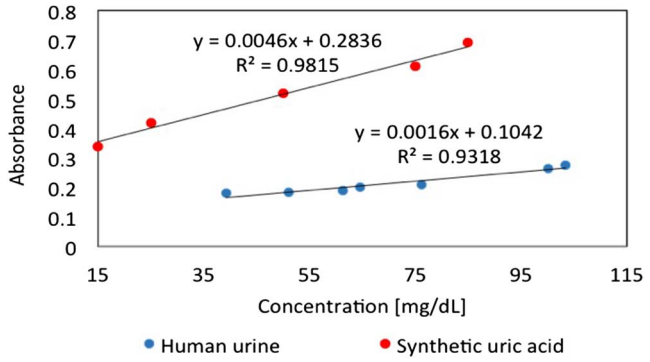


Fig. 7. Absorbance analysis on human urine sample at 460 nm wavelength.

enhancement is the higher luminous efficacy of the LED. Consequently, a larger amount of flux interacts with the sample, leading to the enhancement of the system sensitivity.

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