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Uric acid detection using uv-vis spectrometer

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Abstract. The aim of this research is to detect uric acid (UA) concentration using Ultraviolet-Visible (UV-Vis) spectrometer in the Ultraviolet (UV) region. Absorption technique was proposed to detect different uric acid concentrations and its UV absorption wavelength. Current practices commonly take a lot of times or require complicated structures for the detection process. By this proposed spectroscopic technique, every concentration can be detected and interpreted into an absorbance value at a constant wavelength peak in the UV region. This is due to the chemical characteristics belong to the uric acid since it has a particular absorption cross-section, σ which can be calculated using Beer's Lambert law formula. The detection performance was displayed using Spectrasuite sofware. It showed fast time response about 3 seconds. The experiment proved that the concentrations of uric acid were successfully detected using UV-Vis spectrometer at a constant absorption UV wavelength, 294.46 nm in a low time response. Even by an artificial sample of uric acid, it successfully displayed a close value as the ones reported with the use of the medical sample. It is applicable in the medical field and can be implemented in the future for earlier detection of abnormal concentration of uric acid.

1. Introduction

Gout is a common disease affecting the human joints. Men over the age of 40 years is the common population which suffers from this disease [8]. It is influenced by an excessive amount of uric acid in the human body due to the daily intake issue containing more purines than the optimal daily amount. In other words, high purine food may cause Gout disease to occur [3]. Uric acid is caused by the abnormal amount of purine which is broken down into uric acid and poorly dissolved in the human blood. Therefore, it gets concentrated and crystallized forming numerous crystallites which normally inhibit in the human joints [5]. Additionally, it also may riskily cause other diseases such as chronic renal failure due to the existence of uric acid stone. It is also known as a risk factor of Hyperuricemia (high level of uric acid) [9]. Sudden intense pain and swelling in the joints are classified as the common symptoms due to the condition [4]. Particular research studies has been carried out by researchers till today for the technology and diagnosis advancement in term of reducing the risk of this disease [3]. Gout diagnosis is necessary for the inspection of this disease even from the earlier stage. The uric acid level or concentration can be assumed as a 'fingerprinting' of the risk of this disease to happen [6,7]. There are various techniques used to detect uric acid and an abnormal concentration of uric acid in human blood or serum sample. However, the techniques take a long time for the analysis phase and even require complicated structures. An effective sensor has to be proposed by considering the simplicity, cost effectiveness and fast response time

[1].Optical fiber sensing system or UV-Vis spectrometer is well known as a steadfast technology in the medical, biomedical and industrial sector due to its high elasticity and effective characteristics [10].The characteristics afford high sensitivity, high selectivity, high accuracy, fast time response, low detection limit and it even comes in a small size [11,12]. In this research study, a UV-Vis spectrometer is used to detect the uric acid by examining its current concentration and wavelength absorption in the UV region. Absorption cross-section of each uric acid concentration is defined by the derivation of Beer's Lambert formula. It used to describe the ability of the sample area to absorb photons of UV light wavelength. That is the reason why uric acid only absorbs at a constant UV wavelength. Basically, different concentrations have a constant absorption cross-section which is due to the chemical characteristics of uric acid to absorb the UV light radiation.

The relationship of absorbance, A and absorption cross-section, σ can be interpreted by the following formula of Beer's Lambert law or equations.

$$A = -ln\ (I/I_0) = ln\ (I_0/I) = \sigma Nb = \varepsilon cb \qquad (1)$$

$$A = log_{10}\ (I_0/I) = \frac{\sigma Nb}{2.303} = \varepsilon cb \qquad (2)$$

$$\sigma = \frac{2.303\ log10\ (I_0/I)}{Nb} = \varepsilon cb \qquad (3)$$

2. Material and methods

2.1 Instrumentations

2.1.1 Instrumentation. A UV-VIS Ocean Optics Maya 2000 Pro spectrometer is used with 10mm cuvette (cell) for the detection and measurement of uric acid.

2.1.2 Reagent. Uric acid stock standard (1 mg/ml) is prepared by dissolving 100mg of uric acid powder in 15ml of distilled water. Heat this mixture up to 60˚C on a hot plate. It should be stirred using a magnetic stirrer on the hot plate. Cool it down to a comfort room temperature, 20-26˚C. Add it up with 85 of distilled water to make it 100ml of uric acid stock. This uric acid stock can be refrigerated for one week and can be repetitiously used for scientific research.

Uric acid working samples (0.8 mg/ml, 0.6 mg/ml, 0.4 mg/ml). Molarity formula, $M_1V_1 = M_2V_2$ is used to make calculations for the dilutions. Start it by preparing the first uric acid working sample, 0.8 mg/ml. Dilute 16ml of the uric acid stock standard with 4ml of distilled water to make it to 20ml. For the second uric acid working sample (0.6 mg/ml), dilute 15 ml of the first working sample with 5ml of distilled water. Afterward, dilute 13.33 ml of the second working sample with 6.67ml of distilled water to produce the third working sample, 0.4mg/ml. Thus, three diluted working samples are resulted with three different uric acid concentrations by the serial dilution.
*Note → Dr., I didn't use any term 'downsampled' here . "The temperature readings were then downsampled to 30 minutes per sample per channel. Check the term downsampled and please provide sampling rate instead e.g. 1 S/sec per channel etc. (standard representation)."

2.2 Methods

2.2.1 Experimental Procedure. The uric acid stock and working samples are tested using a UV-Vis spectrometer in the UV wavelength region. Connect it to the CPU using a USB cable to integrate with

Spectrasuite software installed on the PC. Before running the experiment, warm the Deuterium light source up to 20 minutes. Place the sample of uric acid stock or uric acid working sample into 10-mm path-length cuvette before placing it into the cuvette holder. Every sample has to be tested separately. Set up the integration time till there is no saturation displayed in the graph of intensity versus intended wavelength, 200-400nm UV wavelength. Transmission rate is considered by choosing UV absorption peak below 1.0 abs since the light transmission rates 1% at 2.0 abs and 100% at 0 abs. Fig. 2 shows a complete experimental setup for the spectroscopic research.
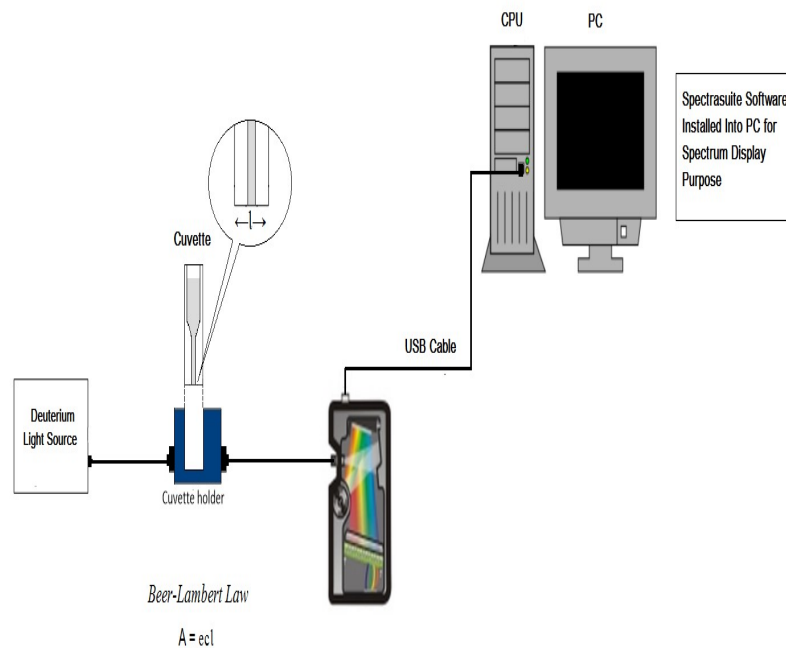

Figure 1. A complete experimental setup for spectroscopic study.

3. Results and discussion

The four different concentrations of uric acid showed four different absorbance values at a same UV wavelength peak in the UV region, 200nm to 400nm using Spectrasuite. The absorption cross-section value was calculated using Beer's Lambert law formula by substituting the absorbance and concentration value of uric acid. Each concentration of uric acid resulted a different absorbance value. However, uric acid constantly absorbs UV light radiation at a same wavelength peak. The UV-Vis spectrometer which was set to UV wavelength region successfully operated to observe the current concentration (each sample) and the UV absorption wavelength of uric acid. Fortunately, the samples were prepared with the artificial powder of uric acid which was mixed up with distilled water. The result shows that the UV absorption wavelength or absorption cross section was acquired at a very close value to the ones reported in the previous research studies. The previous research studies were carried out either by artificial sample (powder) or medical sample of uric acid which was extracted from the human serum.

Fig. 1 illustrates four different concentrations (absorbances) of uric acid acquired at maximum absorption wavelength peak, 294.46 nm using Spectrasuite software. There are some specific criteria had to be considered in the concentration measurement. The absorbance value should be below 1 absorbance (abs). This is because that it is related to the transmission rate which is one of the criteria used to indicate the light penetration and absorption. The light transmission rates 0% at 3.0 abs and 1% at 2.0 abs. 0 abs

occurs when the light transmission rates 100%. It means that the absorption value below 1.0 abs rates multiple series of transmission light percentage. The curves of absorbance indicate the values of four uric acid concentrations against the intended UV wavelength region, 200nm-400nm. The highest absorbance spectrum was obtained by the uric acid stock, 1 mg/ml following by the other three absorbance spectrums obtained by the concentrations; 0.8 mg/ml, 0.6 mg/ml and 0.4 mg/ml. These three concentrations were resulted by the serial dilution which was based on the calculation with the molarity formula. The differences of the absorbance spectrum describe the current concentration of uric acid proportionally. Since every experimental sample is uric acid, it constantly absorbs the UV light radiation at a same wavelength peak, 294.46nm. This proved that there is a good potential of the spectrometer to acquire different uric acid concentrations at a constant UV wavelength peak in a real-time monitoring process.
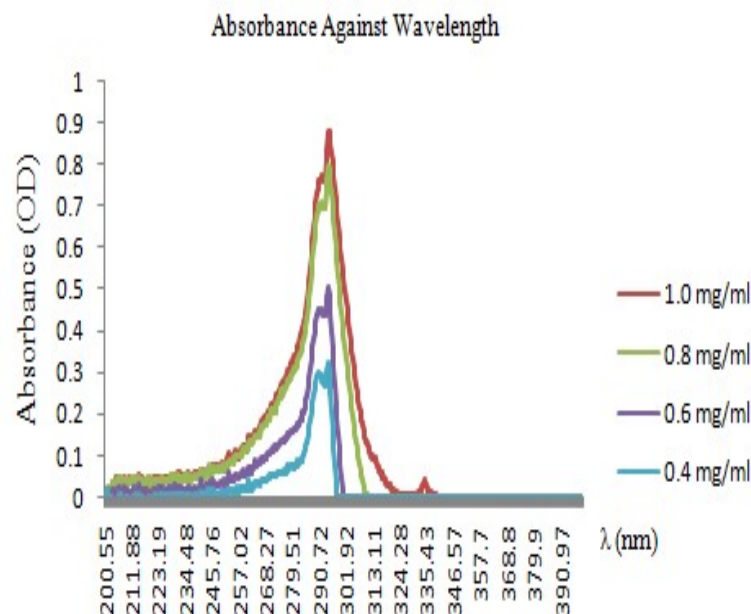
Figure 2. Absorption spectrums of four concentrations of uric acid.

Fig.3 illustrates absorption cross-section of uric acid concentration with four different samples or concentrations against the intended UV wavelength region. The absorption cross-section, $\sigma = 8.0 \times 10^{-19}$ molecules/cm^2 is selected as a maximum absorption value and it is relative to the molar absorptivity of uric acid. It is a constant variable that different uric acid concentrations should indicate the same or very close values of absorption cross-section.
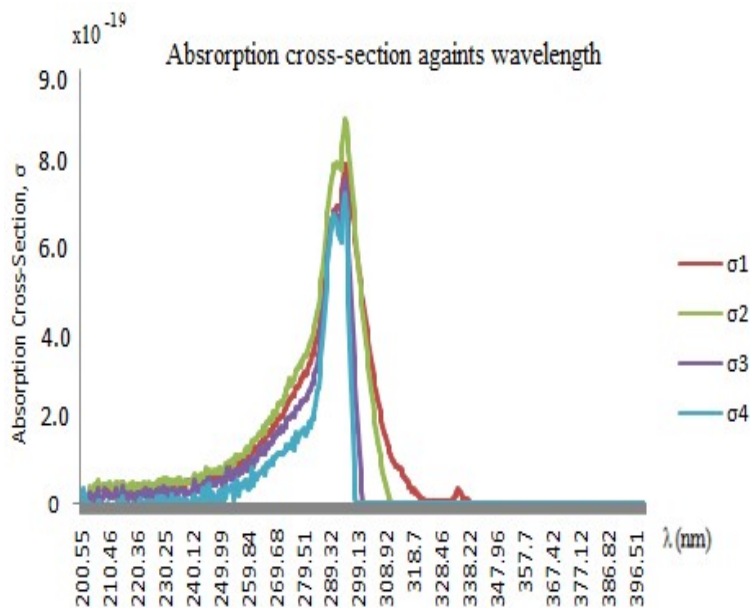
Figure 3. Absorption cross-section of uric acid.

Table 1 shows the values of absorption cross-section of each uric acid concentration.

Table 1. Uric Acid Concentration and Absorption Cross-Section.

ple Concentration	ross-Section, σ
₃/ml	?06e-19
ng/ml	?00e-19
ng/ml	?27e-19
ng/ml	?21e-19

　　　These four concentrations including the uric acid stock indicate a very close or discrete value of absorption cross-section. The variable differences are considerable since they are discrete values. This proves that uric acid has a particular molar absorptivity which is relative to its special chemical characteristics towards UV light radiation. The spectrometer was successfully functioned to measure the current concentration and observe the UV absorption wavelength of uric acid.

4. Conclusions
The research study was carried out to analyze the different concentrations of uric acid by respecting to the absorption technique. The maximum absorption wavelength peak was obtained at 294.46 nm. It is relative to the absorption cross-section of uric acid that it specifically absorbs the UV light radiation at that UV wavelength peak. The absorption cross-section was calculated using Beer's Lambert law formula by considering the current concentration and absorbance value of uric acid. Through the analysis, the spectrometer was able to observe the current concentration of uric acid and its UV absorption wavelength. Even by four different concentrations of uric acid, it was still able to indicate that all of them absorbed the UV light at a constant wavelength peak. This is due to the chemical characteristics or attributes belong to uric acid itself. Optical fiber sensor or UV-Vis spectrometer can be assumed as an effective sensor which may give a fast time response. Additionally, the experimental setup is simple and it doesn't require complicated structures for generating the detection process. It is applicable technology in medical or

biomedical field in the future since it may give a solution for a fast detection system.

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