

Uric Acid Detection in UV Region

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Abstract— The aim of this research is to analyze uric acid (UA) concentration using Ultraviolet (UV) spectrometer. Absorption technique was proposed in the application to detect different uric acid concentrations. Current practice is commonly done by using enzymatic or colorimetric technique which may drag take a lot of times especially at the analysis phase. By this proposed spectroscopic technique, every concentration can be contemplated into UV absorption wavelength displaying on Spectrasuite. It shows fast time response i.e., 1s. Each value of diluted uric acid concentration indicates a different absorption potential. Absorption cross-section of uric acid can be calculated by Beer's Lambert law formula. The experiment proves that different concentrations of uric acid were successfully detected using UV spectrometer at wavelength, 294.46 nm, every concentration showing a different absorbance value. Meanwhile, the absorption cross-section of uric acid molecules shows the close values for the different concentrations.

Keywords— uric acid; ultraviolet (UV); UV absorption wavelength; concentration

1. INTRODUCTION

Gout is a typical inflammatory disease affecting the human joints. Men over the age of 40 years is the most typical population which suffers from painful joints caused by this disease (Schlesinger et.al, 2013). It is influenced by an excess amount of uric acid in the human body due to the daily intake issue containing more purines than the optimal daily amount (Dial-A-Dietitian Nutrition Information Society Of B.C., 2003). In other words, high purine food may cause Gout disease to happen (Jordan et.al, 2007). Based on the analysis, uric acid is resulted from the purine and it is poorly dissolved in blood. Thus, it will get saturated and crystallized forming numerous crystallites which inhibit in the human joints (Holmar et.al, 2012). Additionally, it also may riskily cause other diseases such as kidney stones and Hyperuricemia (high level of uric acid) (Grases et.al, 1957). As a common obvious symptom, this disease may cause sudden intense pain and swelling in the joints especially when the person is doing any intense exercise or movement (Dadig, B., & Wallace, A. E., 2011). A specific research has been medically undergoing by researchers for the technology and diagnosis advancement in term of reducing the risk of this disease (Choi et.al, 2005). Gout diagnosis is highly required in term of inspecting this disease from the earlier indicator or stage. The seriousness issue relies on the uric acid level in the human blood as a 'fingerprinting' or so-called a marker (Kutzing, M. K., & Firestein, B. L., 2008; Idborg et.al, 2005). There are some measurement of the interference among the blood molecules such as glucose, protein, hemoglobin (blood), albumin, bilirubin, cholesterol, HGB and triglyceride. These interferences have to be considered in the measurement to selectively excrete the uric acid concentration. There are some measurement of the interference among the blood molecules such as glucose, protein, hemoglobin (blood), albumin, bilirubin, cholesterol, HGB and triglyceride. These interferences have to be considered in the measurement to selectively excrete the uric acid concentration. This is also so-called spectroscopic study which is necessary to solve the cross-sensitivity issue (Dhinaa, A. N., & Palanisamy, P. K., 2010; Guan, W., Duan, X., & Reed, M. A., 2014; De Oliveira et. al, 2013). There are various techniques used to detect an early formation of abnormal concentration of uric acid in human blood. However, the techniques take hours for the diagnosis or analysis besides requiring a complicated implementation. Therefore, it is vital to propose an effective type of sensor fitted in the application by considering the simplicity, cost effectiveness and fast response time (Ali et.al, 2012). Optical fiber sensing has been well known

as a steadfast technology in the medical, biomedical and industrial sector due to its high elasticity and effective characteristics (Taffoni et.al, 2013). The characteristics afford high sensitivity, high selectivity, high accuracy, fast time response, low detection limit and it even comes in a small size (Lee et.al, 2012; Lou et.al, 2014)

2. MATERIAL AND METHODS

Materials

Instrumentation

A UV-VIS Ocean Optics Maya 2000 Pro spectrometer (Figure 1) was used with 10mm cuvette or cell for the detection and measurement of uric acid concentration.



Figure 1 UV-VIS Ocean Optics Maya 2000 Pro Spectrometer

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 by using a magnetic stirred which is integrated in the hot plate. Cool it down to a comfort room temperature, 20-26°. Dilute it to 100ml with distilled water. This uric acid stock can be refrigerated for one week and can be repetitiously used for scientific research work.

Uric acid working samples (0.8 mg/ml, 0.6 mg/ml, 0.4 mg/ml). 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.

Experimental Procedure

The uric acid stock and working samples are tested using a UV spectrometer. Connect it to the CPU by 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 in 10-mm path-length cuvette before placing the cuvette in its 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.

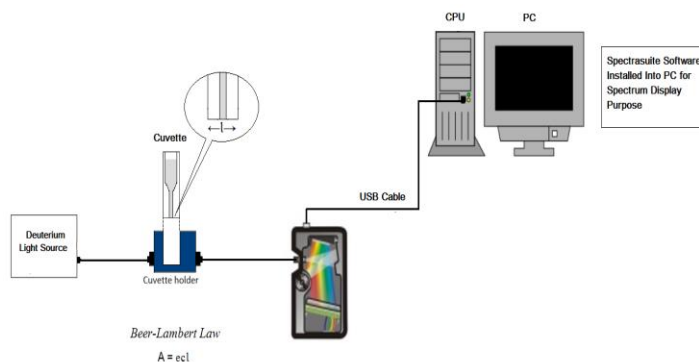


Figure 2 A Complete Experimental Setup for Spectroscopic Study

3. RESULT AND DISCUSSION

An analysis of uric acid concentration was carried out by examining the concentrations resulted by the serial dilution. The concentration of uric acid stock was also included in the analysis. Thus, four absorbance values were displayed on the Spectrasuite since four samples were spectroscopically experimented. UV light absorption also relies on the characteristics of uric acid compound such as molar absorptivity or coefficient and the space among the molecules. Based on colorimetric perspective, the whitish scale created by each uric acid dilution also gave an effect upon the absorption measurement. In addition, every concentration of uric acid obtained different absorbance value or spectrum within a same optimal wavelength region. The maximum absorption was obtained at 294.46 nm. It indicates that uric acid molecules absorbed UV light well at this wavelength peak which belongs to a part of uric acid characteristics. In previous scientific researches, it was reported that uric acid concentration was successfully detected at 293nm, 292nm or another close value in the intended UV wavelength region.

Fig. 1 illustrates four different concentrations of uric acid acquired at maximum absorption peak, 294.46 nm by using Spectrasuite software. There are some specific criteria had to be considered in the concentration measurement. Transmission rate is one of the criteria used to indicate the light penetration and absorption. Therefore, the most optimal graph or curve should be taken below 1.0 absorbance since the light transmission only value 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 an intended UV wavelength, 200nm-400nm. The highest absorbance spectrum was obtained by the uric acid stock, 1 mg/ml. The other three absorbance spectrums were obtained by the rest concentrations; 0.8 mg/ml, 0.6 mg/ml and 0.4 mg/ml resulted by the serial dilution. The differences of the absorbance spectrum describe the current uric acid concentration proportionally. It used to mean that there is a good potential of the spectrometer to acquire different uric acid concentrations in a real-time monitoring process.

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. Basically, different concentrations have a constant absorption cross-section which is due to the typical characteristics of uric acid to absorb the UV light. The relationship of absorbance, A and absorption cross-section, σ can be interpreted by the following formula of Beer's Lambert law and equations.

$$A = -\ln(I/I_0) = \ln(I_0/I) = \sigma Nb = \epsilon cb \quad (1)$$

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

$$\sigma = \frac{2.303 \log_{10}(I_0/I)}{Nb} = \epsilon cb \quad (3)$$

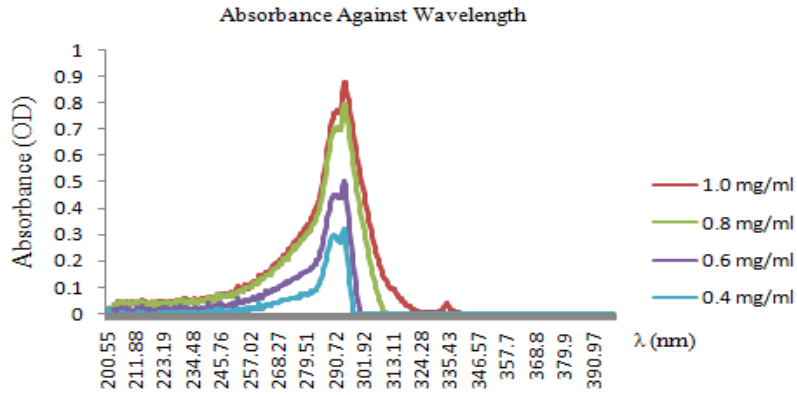


Figure 2: Absorption spectrums of four different uric acid concentrations

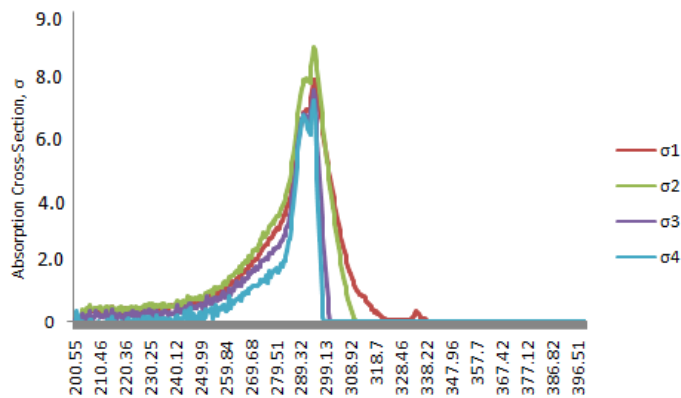


Figure 3: Absorption Cross-Section of Uric Acid

Table 1: Uric Acid Concentration and Absorption Cross-Section

Uric Acid Concentration	Absorption Cross-Section, σ
1mg/ml	7.8206e-19
0.8mg/ml	8.8800e-19
0.6mg/ml	7.4727e-19
0.4mg/ml	7.1221e-19

Fig. 3 illustrates absorption cross-section of each uric acid concentration against the intended UV wavelength. The absorption cross-section, $\sigma = 8.0 \times 10^{-19}$ molecules/cm² was selected at maximum absorption since 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.

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

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 too small. This proves that uric acid has a particular molar absorptivity which is relative to its special characteristics towards UV light absorption.

4. CONCLUSION

The research work was carried out to spectroscopically investigate different uric acid concentrations by respecting to absorption technique. All the samples were prepared by a serial dilution method to produce four different uric acid concentrations including the uric acid stock. The maximum peak was achieved at 294.46 nm. Previous research works also showed that the maximum peak was very close to this acceptable maximum peak value. Those four concentrations proportionally indicated different absorption value in the intended wavelength but each of them produced a similar value of absorption-cross section. The value of absorption-cross section was determined using Beer- Lambert Law formula. A common method such as enzymatic or colorimetric method normally requires a long time or complicated implementation for an analysis purpose. In fact, this spectroscopic absorption method is more effective and even may afford fast time response. Therefore, it is an effective implementation in medical or biomedical applications since it can reduce the duration of the analysis or diagnosis process. Further research works can be recommended by proposing a human serum as a medical sample besides solving cross-sensitivity issue caused by the common interference in the human blood.

ACKNOWLEDGMENT

I would like to thank my supervisor, Dr. Hadi bin Manap for his full support, guidance and the proof-reading. I would also like to thank all the laboratory staffs and science officers to provide me all the tools and instruments for the research use. I really appreciate all the knowledge and guidance as the directions for me complete this research.

REFERENCES

- [1] Schlesinger, N. (2005). Diagnosis of gout: clinical, laboratory, and radiologic findings. *Am J Manag Care*, 11(15 Suppl)
- [2] Hamzah, H. H., Zain, Z. M., Musa, N. L. W., Lin, Y. C., & Trimbee, E. (2013). Spectrophotometric Determination of Uric Acid in Urine Based-Enzymatic Method Uricase with 4-Aminodiphenylamine Diazonium Sulfate (Variamine Blue RT Salt). *J Anal Bioanal Tech S*, 7, 2.
- [3] Dial-A-Dietitian Nutrition Information Society Of B.C.(2003). Diet For Gout.
- [4] Jordan, K. M., Cameron, J. S., Snaith, M., Zhang, W., Doherty, M., Seckl, J., Hingorani, A., Jacques, R. & Nuki, G. (2007). British Society for Rheumatology and British Health Professionals in Rheumatology guideline for the management of gout. *Rheumatology*, 46(8), 1372-1374.
- [5] Holmar, J., Fridolin, I., Uhlin, F., Lauri, K., & Luman, M. (2012). Optical method for cardiovascular risk marker uric acid removal assessment during dialysis. *The Scientific World Journal*, 2012.
- [6] Grases, F., Villacampa, A. I., Costa-Bauzá, A., & Söhnel, O. (1997). Uric acid calculi. *Scanning Microsc.*
- [7] Wyngaarden, J. B. (1957). Overproduction of uric acid as the cause of hyperuricemia in primary gout. *Journal of Clinical Investigation*, 36(10), 1508.
- [8] Dadig, B., & Wallace, A. E. (2011). CE/CME. *Clinician Reviews*, 21(7), 29.
- [9] Choi, H. K., Mount, D. B., & Reginato, A. M. (2005). Pathogenesis of gout. *Annals of internal medicine*, 143(7), 499-516
- [10] Kutzing, M. K., & Firestein, B. L. (2008). Altered uric acid levels and disease states. *Journal of Pharmacology and Experimental Therapeutics*, 324(1), 1-7.
- [11] Idborg, H., Zamani, L., Edlund, P. O., Schuppe-Koistinen, I., & Jacobsson, S. P.(2005). Metabolic fingerprinting of rat urine by LC/MS: Part 1. Analysis by hydrophilic interaction liquid chromatography–electrospray ionization mass spectrometry. *Journal of Chromatography B*, 828(1), 9-13.
- [12] Dhinaa, A. N., & Palanisamy, P. K. (2010). Optical nonlinearity in measurement of urea and uric acid in blood. *Natural science*, 2(02), 106.
- [13] Guan, W., Duan, X., & Reed, M. A. (2014). Highly specific and sensitive non-enzymatic determination of uric acid in serum and urine by extended gate field effect transistor sensors. *Biosensors and Bioelectronics*, 51, 225-231.
- [14] De Oliveira, E. P., Moreto, F., Silveira, L. V., & Burini, R. C. (2013). Dietary, anthropometric, and biochemical determinants of uric acid in free-living adults. *Nutr J*, 12(11).

- [15] Ali, S. M. U., Alvi, N. H., Ibupoto, Z., Nur, O., Willander, M., & Danielsson, B. (2011). Selective potentiometric determination of uric acid with uricase immobilized on ZnO nanowires. *Sensors and Actuators B: Chemical*, 152(2), 241-247.
- [16] Iveković, D., Japac, M., Solar, M., & Živković, N. (2012). Amperometric uric acid biosensor with improved analytical performances based on alkaline-stable H₂O₂ transducer. *Int. J. Electrochem. Sci*, 7, 3252-3264.
- [17] Taffoni, F., Formica, D., Saccomandi, P., Pino, G. D., & Schena, E. (2013). Optical fiber-based MR-compatible sensors for medical applications: An overview. *Sensors*, 13(10), 14105-14120.
- [18] Lee, B. H., Kim, Y. H., Park, K. S., Eom, J. B., Kim, M. J., Rho, B. S., & Choi, H. Y. (2012). Interferometric fiber optic sensors. *Sensors*, 12(3), 2467-2486.
- [19] Lou, J., Wang, Y., & Tong, L. (2014). Microfiber optical sensors: A review. *Sensors*, 14(4), 5823-5844.