Construction of a laser diode fluorescence detection system for creatinine determination

Chien-Ming Chen[1], Ya-Li Tsai[1], Jen-Ai lee[2]
[1]*Department of Electro-Optical Engineering, National Taipei University of Technology*
[2]*School of Pharmacy, Taipei Medical University*
cmchen@ntut.edu.tw

Introduction

Serum creatinine is an important and the most commonly used indicator of renal function because creatinine is removed from the blood primarily by the kidney glomerular filtration. Any changes of creatinine levels in the blood are related to excretion and therefore reflect kidney function. There are two common determination methods of creatinine, one is enzymatic assay and the other one is spectrophotometric method based on the Jaffe reaction. The enzymatic assay exhibits good accuracy, but it is relatively expensive and highly complicated, and time consuming. The chemical picric acid used in the spectrophotometric method is inflammable. Creatinine will be reacting with 3,5-Dinitrobenzoic acid under the highly alkaline condition. The complexes have fluorescent characteristic that absorb excitation light at 400 nm and release fluorescence at 491 nm. In this study, we established a novel laser diode fluorescence detection system for the fast and low-cost determination of creatinine.

Materials and Methods

The creatinine fluorescent sensing system developed in this study used a 400-nm wavelength laser diode (LD) as the excitation light source with a wave width of ± 5 nm and an LD drive current of only 24 mA. With low energy consumption, small size and high coherence characteristics, then micro-spectrometer as a fluorescent receiver, the grating opening is 50 μm, and the spectrometer is connected with a two-dimensional translation stage, and it is adjusted to the optimal light receiving position, and select the capacity of 500 μL black double-sided light quartz cuvette containing the sample to be measured.

Creatinine solution with 0.3 mM of concentration was added with 0.625 M LiOH and 37.5 mM 3,5-dinitrobenzoate solution. After one minute, the excitation and emission spectra were measured by Fluorescence Spectrophotometer F-4500 (Hitachi, Ltd., Tokyo, Japan). Separately prepared 2.5 mM LiOH solution and 150 mM 3.5-dinitrobenzoate solution were mixed and 0.25 mL of the mixture was used as the test reagent. Each of the creatinine standard solution with the concentrations of 0, 5, 12.5, 25, 50, 75, 150, 300 μM was evenly shaked and placed in an optical fluorescence detection system set up in this laboratory for 20 minutes of fluorescence detection.

Results and Discussion

The results showed that the fluorescent bio-system had good linearity from 5 μM to 300 μM (R^2 = 0.9934) and the limit of detection (LOD) was 5 μM. The intra-day accuracy and precision ranged from 81.82 to 99.75% and 7.33 to 10.11%, respectively. The inter-day accuracy and precision ranged from 86.75 to 87.85% and 3.08 to 9.39%, respectively. Compared the results with those from National Laboratory Animal Center, the results showed good correlations with 0.80 of (R).

Conclusion

A new creatinine fluorescence sensing system was successfully developed using a micro-spectrometer combined with a laser diode and a three-dimensional translation stage. Compared with the existing measurement methods, it has high measurement sensitivity and is suitable for the determination of creatinine in the blood.

Bibliography

[1] Baojiao Gao, Yanbin Li, and Zhenguo Zhang, *Journal of Chromatography B*, **878(23)**, 2077-2086 (2010).
[2] Júlia M. C. S. Magalhães and Adélio A. S. C. Machado, *The Analyst*, **127(8)**, 1069-1075 (2002).
[3] Anthony J Killard and Malcolm R Smyth, *Trends in Biotechnology*, **18(10)**, 433-437 (2000).
[4] Anjal C. Sharma, Tushar Jana, Rasu Kesavamoorthy, Lianjun Shi, Mohamed A. Virji, David N. Finegold, and Sanford A. Asher, *Journal of the American Chemical Society*, **126(9)**, 2971-2977 (2003).
[5] Karl G. Blass, *Clinical Biochemistry*, **28(2)**, 107-111 (1995).