

**Mahidol University Global Education Office  
International Relations Division  
Mahidol University**

**Report for MU Scholarships for Postgraduate  
Student Exchange Program**

**Mr. WALEED ALAHMAD**

## ❖ Overview about Okayama University

Okayama University (岡山大学 Okayama Daigaku) is a national university in Japan. The main campus is located in Tsushima-Naka, Okayama, Okayama Prefecture. The school was founded in 1870 and it was established as a university in 1949. The university's motto is "**Creating and fostering higher knowledge and wisdom**"



### • **Faculties (undergraduate schools)**

There are twelve faculties in Okayama University as following:

- Faculty of Letters
- Faculty of Education
- Faculty of Law
- Faculty of Economics
- Faculty of Science
- Medical School
- Dental School
- Faculty of Pharmaceutical Sciences
- Faculty of Engineering
- Faculty of Environmental Science and Technology
- Faculty of Agriculture
- Matching Program Course

### • **Graduate schools**

There are seven schools as following:

- Graduate School of Education (Master's courses/Professional Degree Course)
- Graduate School of Humanities and Social Sciences (Master's/Doctoral)
- Graduate School of Natural Science and Technology (Master's/Doctoral)
- Graduate School of Health Sciences (Master's/Doctoral)
- Graduate School of Environmental and Life Science (Master's/Doctoral)
- Graduate School of Medicine, Dentistry and Pharmaceutical Sciences (Master's/Doctoral)
- School of Law

My research lab belongs to Graduate School of Natural Science and Technology.

### • **Campus**

There three campuses in Okayama University are Tsushima, Shikata and Misasa campus. Each campus is in the central part of Okayama-city, except the Misasa Campus.

### ❖ Overview about my supervisor in Okayama University

Takashi Kaneta

Professor

Research field: Analytical Chemistry



### Education

B.Sc. (Hokkaido University of Education, Sapporo), M.Sc. (Hokkaido University), D.Sc. (Hokkaido University)

### Academic Career

- Assistant Professor, Department of Applied Chemistry, Kyushu University (1992.4-1994.12)
- Associate Professor, Department of Applied Chemistry, Kyushu University (1995.1-2011.3)
- Professor, Department of Chemistry, Okayama University (2011.4-)

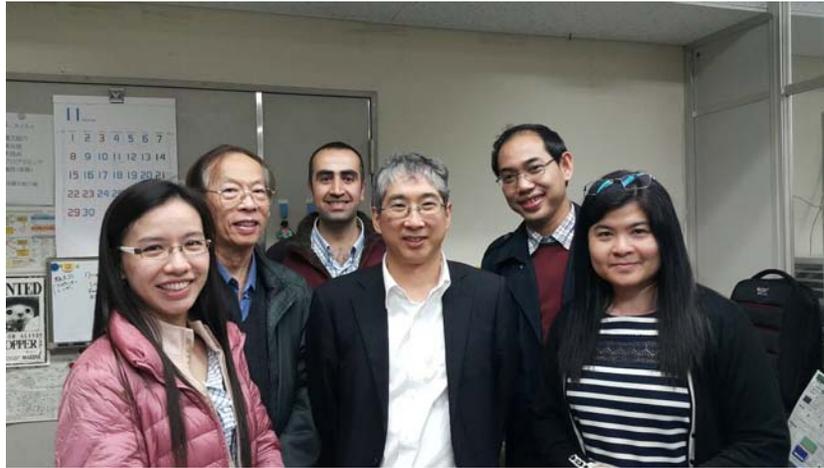
### Research Interests

- Laser-Induced Fluorometry
- Capillary Electrophoresis
- Single Molecule Detection
- Single Cell Analysis
- Microfluidic Paper-Based Analytical Device.

### His lab members



❖ **My Thai and Japanese supervisors**



**My Japanese supervisor Prof. Dr. Kaneta in the middle, my Thai supervisor Assoc. Prof. Dr. Duangjai Nacaprichaon his left-hand side and my Thai co-supervisor Assoc. Prof. Dr. Prapin Wilairat on his right-hand side.**

❖ Overview about culture activity

**Visiting Korakuen Garden**



**Visiting Okayama castle**



**Hiking stairs at Kagawa prefecture**



## ❖ Overview about my research in Japan

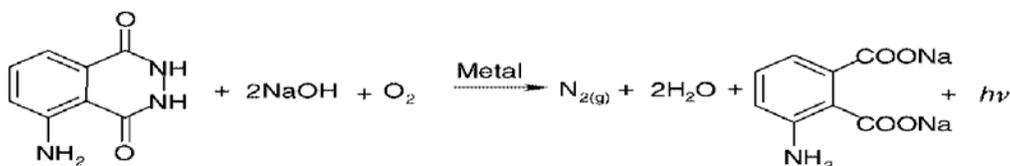
### Determination of Chromium (III) in Water Samples by using $\mu$ PAD and Chemiluminescence Detector

#### Aim of this work

To determine chromium (III) in water samples by using  $\mu$ PAD consists 6 detection zones, the first five for calibration curve and the last one for sample.

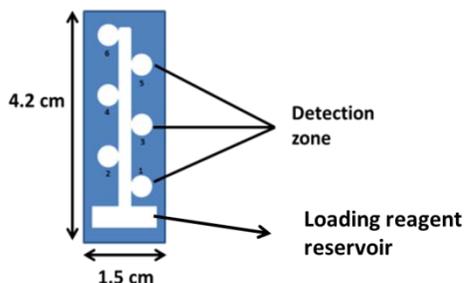
#### Principle

The CL reaction is based on luminol oxidation by hydrogen peroxide in basic aqueous solution catalyzed by chromium(III). In this case Cr(III) can be determined selectively in the presence of Cr(VI) and other metal ions in the aqueous environment.



#### Fabrication of the paper device

The  $\mu$ PAD design drew on PowerPoint and printed by wax printer. This  $\mu$ PAD consists 6 circular detection zones (each zone consists 0.20  $\mu$ L standard chromium (III) ) and one rectangle reservoir for loading the chemiluminescence (CL) reagent (25  $\mu$ L in this work). The CL reagent will flow sequentially to the detection zones and the signal can record. (The design is able to change according to unexpected problems).

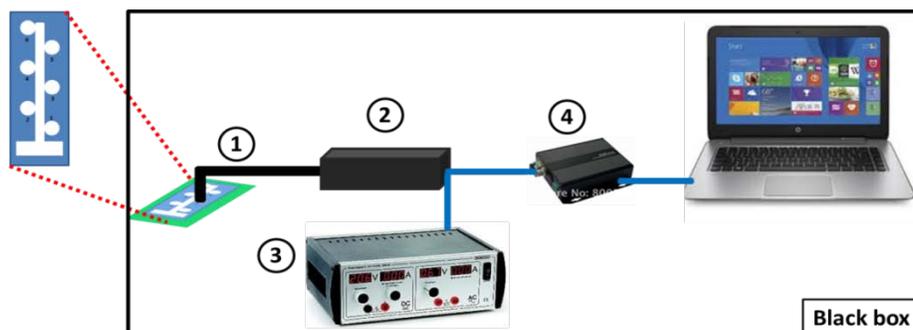


**The CL reagent consists:** luminol, hydrogen peroxide, NaBr and EDTA in  $\text{NaHCO}_3$ – $\text{Na}_2\text{CO}_3$  buffer. The sodium bromide and carbonate buffer used to enhance the CL signal while EDTA was added to remove the interference of other metal ions. The working standard chromium solutions were prepared in  $1.0 \times 10^{-2}$  mol/L EDTA prior to use.

## 1. Optimization Study

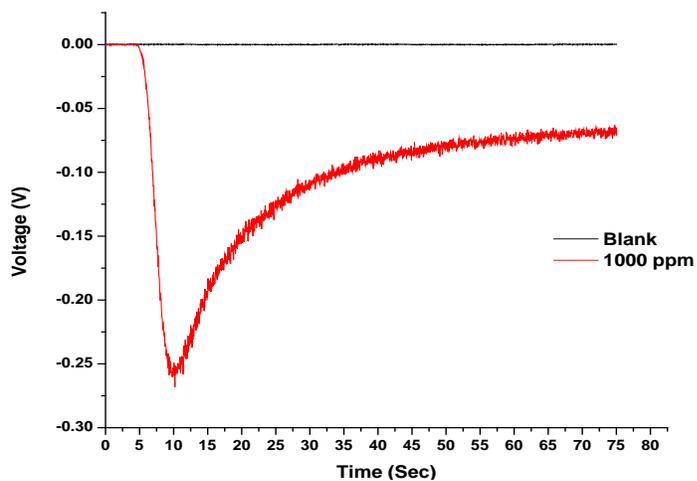
A series of experiments were carried out to establish the optimum conditions by using a Cr(III) concentration of 1000 ppm. In this optimization study, we used only the detection zone number 3.

### Manifold



1) Optical fiber. 2) Photomultiplier tube. 3) Voltage power supply. 4) Analog to Digital Voltage converter.

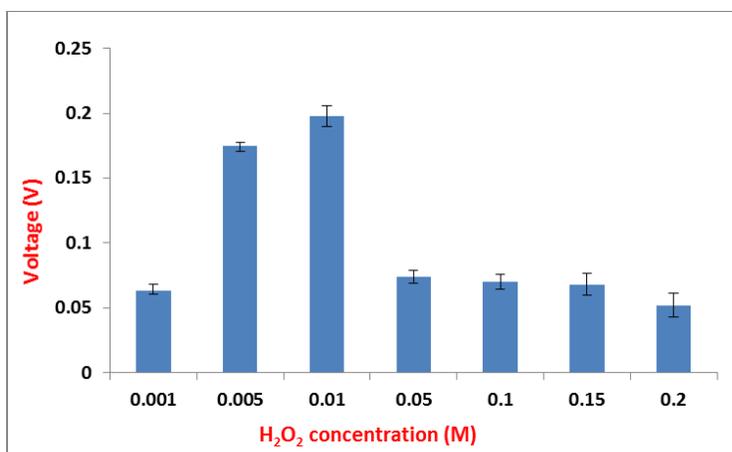
### Signal profile:



### 1.1. Effect of hydrogen peroxide

**Optimization range:**  $1.0 \times 10^{-3}$  -  $2.0 \times 10^{-1}$  mol/L.

**CL reaction solution:**  $1.0 \times 10^{-3}$  mol/L luminol,  $1.0 \times 10^{-1}$  mol/L NaBr and  $1.0 \times 10^{-2}$  mol/L EDTA in  $5.0 \times 10^{-2}$  mol/L  $\text{NaHCO}_3$ - $\text{Na}_2\text{CO}_3$  buffer, pH of CL reagent 12.1

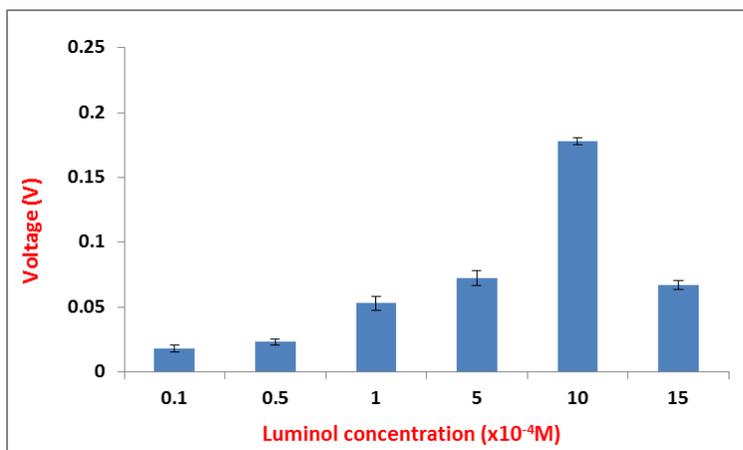


The concentration (0.01M) was chosen for next experiments.

### 1.2. Effect of luminol concentration

**Optimization range:** (0.1, 0.5, 1.0, 5.0, 10, 15)  $\times 10^{-4}$  mol/L.

**CL reaction solution:**  $1.0 \times 10^{-2}$  mol/L hydrogen peroxide,  $1.0 \times 10^{-1}$  mol/L NaBr and  $1.0 \times 10^{-2}$  mol/L EDTA in  $5.0 \times 10^{-2}$  mol/L NaHCO<sub>3</sub>–Na<sub>2</sub>CO<sub>3</sub> buffer, pH 12.1



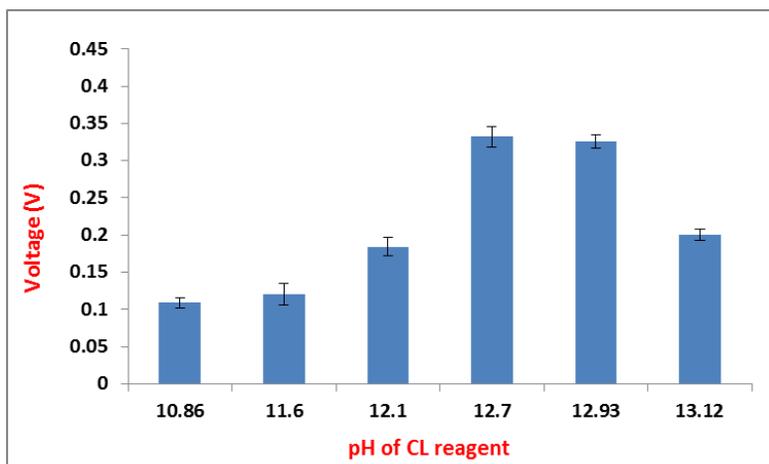
The concentration ( $10 \times 10^{-4}$  mol/L) was chosen for next experiments.

### 1.3. Effect of pH CL reagent.

The carbonate buffer which reported to enhance the CL signal in luminol–H<sub>2</sub>O<sub>2</sub> system was chosen and used in the preparation of CL solution. Furthermore, luminol–H<sub>2</sub>O<sub>2</sub> system can produce strong CL signal in the alkaline condition, so the effect of CL reagent pH was needed to investigate by adding NaOH to the reagent solution.

**Optimization range:** 10.86 – 13.12.

**CL reaction solution:**  $1.0 \times 10^{-2}$  mol/L hydrogen peroxide,  $1.0 \times 10^{-3}$  mol/L luminol,  $1.0 \times 10^{-1}$  mol/L NaBr and  $1.0 \times 10^{-2}$  mol/L EDTA in  $5.0 \times 10^{-2}$  mol/L  $\text{NaHCO}_3\text{-Na}_2\text{CO}_3$  buffer,



The pH 12.7 was chosen for next experiments. The CL signal started to drop after pH 12.7 because the decomposition of hydrogen peroxide.

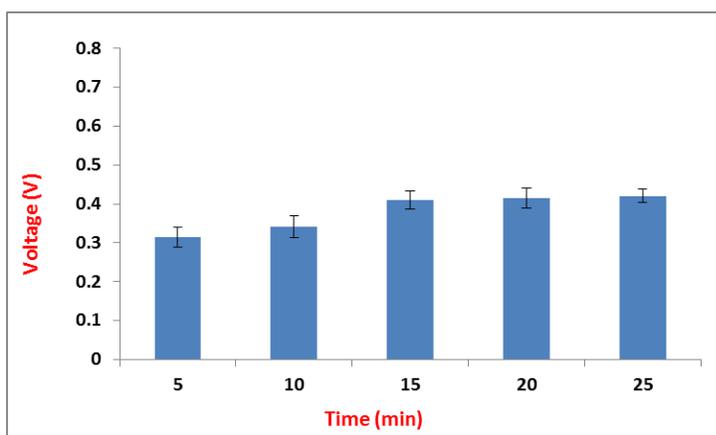
#### 1.4. Effect of needed time for stabilizing CL before use.

It was reported in another work [1] that CL reagent was prepared and left to stand for 15 min before used.

From this point, we carry out experiment to investigate the effect of standing time on CL signal, by measure the signal at various times.

**Time range:** 5-25 minutes

**CL reaction solution:**  $1.0 \times 10^{-2}$  mol/L hydrogen peroxide,  $1.0 \times 10^{-3}$  mol/L luminol,  $1.0 \times 10^{-1}$  mol/L NaBr and  $1.0 \times 10^{-2}$  mol/L EDTA in  $5.0 \times 10^{-2}$  mol/L  $\text{NaHCO}_3\text{-Na}_2\text{CO}_3$  buffer, pH 12.7



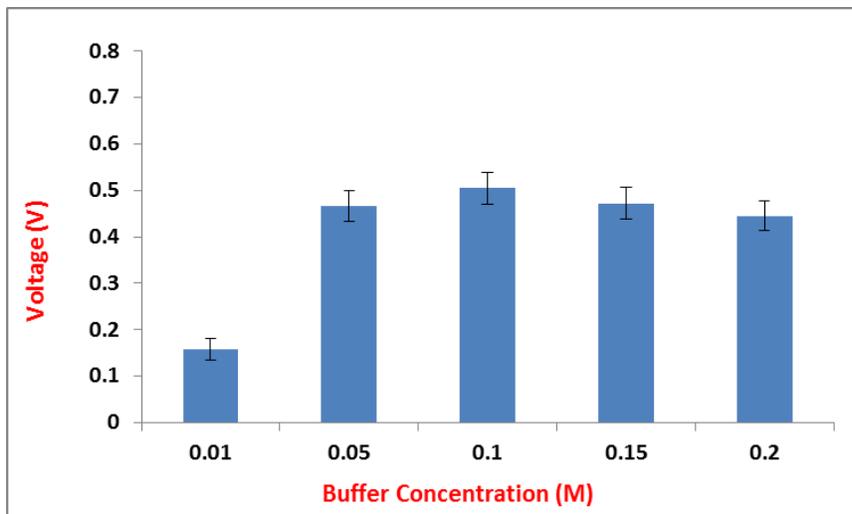
It was able to notice that there is a little difference between 5 to 15 min and the signal is stable after 15. we chose the time (15 min) for next experiments.

### 1.5. Effect of carbonate buffer concentration

The effect of carbonate buffer concentration was then examined by keeping the pH constant at 12.7.

**Optimization range:** 0.01 – 0.2 M.

**CL reaction solution:**  $1.0 \times 10^{-2}$  mol/L hydrogen peroxide,  $1.0 \times 10^{-3}$  mol/L luminol,  $1.0 \times 10^{-1}$  mol/L NaBr and  $1.0 \times 10^{-2}$  mol/L EDTA in  $???$  mol/L  $\text{NaHCO}_3\text{--Na}_2\text{CO}_3$  buffer, pH 12.7

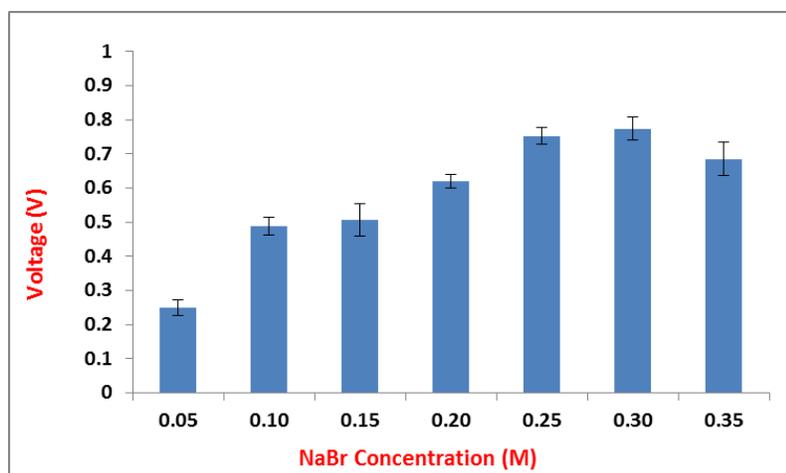


### 1.6. Effect of NaBr concentration

NaBr was added to the luminol solution in carbonate medium in order to enhance the CL signal. In the presence of bromide, the hydroxyl radical is converted into bromide radical which selectively reacts with luminol yielding the luminol radical. Bromide enhances the CL intensity by selectively increasing the steady-state concentration of luminol radical and facilitates its decomposition to give more excited luminol ions.

**Optimization range:** 0.05 – 0.35 M

**CL reaction solution:**  $1.0 \times 10^{-2}$  mol/L hydrogen peroxide,  $1.0 \times 10^{-3}$  mol/L luminol,  $?????$  mol/L NaBr and  $1.0 \times 10^{-2}$  mol/L EDTA in 0.1 mol/L  $\text{NaHCO}_3\text{--Na}_2\text{CO}_3$  buffer, pH 12.7



The concentration (0.3M) was chosen for next experiments.

**The optimal conditions:**

- Hydrogen peroxide (0.01M).
- Luminol concentration ( $10 \times 10^{-4}$ M).
- Carbonate buffer concentration (0.1M).
- NaBr concentration (0.3M).
- pH CL reagent (12.7).
- required time for stabilizing CL before use (15 min).

**2. Analytical features**

| <i>Analytical parameters</i> |                   |
|------------------------------|-------------------|
| Limit of detection (ppm)     | 0.020             |
| Limit of quantitation (ppm)  | 0.066             |
| Slope of calibration curve   | $0.350 \pm 0.016$ |
| Regression coefficient       | $0.992 \pm 0.014$ |
| Linear dynamic range (ppm)   | 0.05 - 1          |
| RSD (%), (0.5ppm) (n=10)     | < 6.5             |

\* Three injections per sample.

### 3. Analysis of real samples.

|            | This Work   |             |              | Validation method <sup>a</sup> |             |              |
|------------|-------------|-------------|--------------|--------------------------------|-------------|--------------|
|            | Added (ppb) | Found (ppb) | Recovery (%) | Added (ppb)                    | Found (ppb) | Recovery (%) |
| Hot Spring | 0           | ND*         |              | 0                              | 4.69±0.03   |              |
|            | 200         | 213±0.05    | 106.38       | 200                            | 199±0.01    | 96.96        |
|            | 600         | 593±0.10    | 98.82        | 600                            | 576±0.03    | 95.15        |
| Zasu River | 0           | ND*         |              | 0                              | 3.74±0.05   |              |
|            | 200         | 210±0.11    | 104.96       | 200                            | 206±0.01    | 101.14       |
|            | 600         | 607±0.11    | 101.18       | 600                            | 594±0.03    | 98.34        |
| Tap Water  | 0           | ND*         |              | 0                              | 4.16±0.03   |              |
|            | 200         | 203±0.20    | 101.65       | 200                            | 201±0.02    | 98.33        |
|            | 600         | 598±0.22    | 99.61        | 600                            | 604±0.03    | 99.90        |

\*Not Detected

<sup>a</sup> ICP-AES

## ❖ **Conclusion**

I would like to thank the Mahidol University Global Education office, International Relations Division for giving me this great chance to develop my academic skills in research and its related fields. Also, I would like to thank my supervisor Assoc. Prof. Dr. DuangjaiNacapricha for her help and guidance to find the suitable university for my research, and all the thanks for all whose helped me to achieve this research in Japan.