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ไอออนโลหะหนัก



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DEVELOPMENT OF PORTABLE SPECTROPHOTOMETRIC DEVICE WITH FLOW-
BASED ANALYSIS SYSTEM FOR DETECTION OF HEAVY METAL IONS

Miss Metida Srikullaphat



A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Chemistry

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ตะกั่วและแคดเมียมถูกตรวจวัดปริมาณโดยใช้การวิเคราะห์แบบไหลร่วมกับการตรวจวัดเชิงสเปกโทรโฟโตเมตรี ซึ่งควอซิทินและซิทิลไตรเมทิลแอมโมเนียมโบรไมด์ถูกใช้เป็นคอมเพลกซิงก์เอเจนต์และสารลดแรงตึงผิวตามลำดับ การดูดกลืนแสงของสารประกอบเชิงซ้อนสีเหลืองถูกตรวจวัดในทริสไฮดรอกซีเมทิลอะมิโนมีเทนบัฟเฟอร์ในช่วงความยาวคลื่น 420-470 นาโนเมตร ซึ่งสภาวะที่เหมาะสมที่สุดได้มาโดยใช้ควอซิทินรีเอเจนต์เข้มข้น 800 มิลลิกรัมต่อลิตร ปริมาตร 20 ไมโครลิตร ในสารละลายที่มีซิทิลไตรเมทิลแอมโมเนียมโบรไมด์เข้มข้น 3.0 มิลลิโมลต่อลิตร ปริมาตร 150 ไมโครลิตร สารละลายตัวอย่างถูกตรวจวัดโดยซีควอนเซียลอินเจกชันสเปกโทรโฟโตเมตรีโดยใช้น้ำที่ถูกกำจัดไอออนเป็นตัวนำส่ง ซึ่งความเป็นเส้นตรงของความสัมพันธ์ระหว่างการดูดกลืนแสงและความเข้มข้นของโลหะอยู่ในช่วงความเข้มข้นจาก 20-100 ไมโครกรัมต่อลิตร ทั้งแคดเมียมและตะกั่ว ซึ่งค่าต่ำสุดที่วิเคราะห์ได้ของแคดเมียมคือ 2 ไมโครกรัมต่อลิตร และปริมาณต่ำสุดที่สามารถวัดเชิงปริมาณได้คือ 8 ไมโครกรัมต่อลิตร ค่าต่ำสุดที่วิเคราะห์ได้ของตะกั่วคือ 2 ไมโครกรัมต่อลิตร และปริมาณต่ำสุดที่สามารถวัดเชิงปริมาณได้คือ 7 ไมโครกรัมต่อลิตร ค่าส่วนเบี่ยงเบนมาตรฐานสัมพัทธ์ เท่ากับ 3.6% และ 3.4% สำหรับแคดเมียมและตะกั่วที่มีความเข้มข้น 20 ไมโครกรัมต่อลิตรตามลำดับ สภาวะที่เหมาะสมที่สุดถูกนำมาใช้ร่วมกับอุปกรณ์ที่ถูกพัฒนาสำหรับการตรวจวัดปริมาณของแคดเมียมและตะกั่วในน้ำ แหล่งกำเนิดแสงที่เป็นตัวเลือกคือไดโอดเปล่งแสงซึ่งถูกใช้เพื่อวัตถุประสงค์การทำขนาดให้เล็กลง

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METIDA SRIKULLAPHAT: DEVELOPMENT OF PORTABLE SPECTROPHOTOMETRIC DEVICE WITH FLOW-BASED ANALYSIS SYSTEM FOR DETECTION OF HEAVY METAL IONS. ADVISOR: PASSAPOL NGAMUKOT, Ph.D., 55 pp.

Lead (II) and cadmium (II) were determined by a flow-based analysis with spectrophotometric detection. Quercetin (Querc) and cetyltrimethylammonium bromide (CTAB) were used as a complexing agent and a surfactant, respectively. The absorbance of the yellow-colored complexes (Pb-Querc and Cd-Querc) were measured in Tris(hydroxymethyl)aminomethane buffer (pH 8.9) within the wavelength of 420-470 nm. The optimal condition was obtained by using 800 mgL^{-1} quercetin reagent (20 μL) in solution with 3.0 mmol L^{-1} CTAB (150 μL). The sample solutions were determined by a sequential injection spectrophotometer using deionized water as a carrier. The linear relationship between absorbance and concentration of metals were obtained over the concentration range from 20-100 $\mu\text{g L}^{-1}$ for both Cd (II) and Pb (II). The limit of detection (LOD) of Cd (II) was $2 \mu\text{g L}^{-1}$ and the limit of quantification (LOQ) was $8 \mu\text{g L}^{-1}$. The limit of detection (LOD) of Pb (II) was $2 \mu\text{g L}^{-1}$ and the limit of quantification (LOQ) was $7 \mu\text{g L}^{-1}$. The relative standard deviations were 3.6% (n=7) and 3.4% (n=7) for $20 \mu\text{g L}^{-1}$ Cd (II) and Pb (II), respectively. The optimal condition was applied with the developed device for the determination of Cd (II) and Pb (II) in water. The alternative light source, LED (light-emitting diode), was also used for a miniaturization purpose.

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LIST OF ABBREVIATIONS

g	gram
L	liter
μL	microliter
mol	mole
LOD	limit of detection
LOQ	limit of quantification
SD	standard deviation
v/v	volume/volume
M	molarity
mL	milliliter
mg	milligram
nm	nanometer
cm	centimeter
min	minute
Querc	Quercetin
CTAB	cetyltrimethylammonium bromide
LED	light-emitting diode
SIA	sequential injection analysis
R^2	coefficient of determination

CHARTER I

INTRODUCTION

1.1 Introduction

Heavy metals are used in many industries such as paint factory, sheet metal, plastic, batteries etc. They can contaminate both surface water and ground water due to an improper release of waste to environments. These heavy metals are hazardous toxic because they tend to bioaccumulate in biological organisms. There is a risk of health through the consumption of food and drinking water. They are contaminated due to unsuitable treatment. Heavy metal is the high degree of toxicity such as lead, arsenic, cadmium, mercury, chromium and manganese. Specially, contamination of lead and cadmium are the serious danger to the kidney, liver, heart, blood system and immune system of the body [1]. They are found in the food chain of the organism, which can be analyzed from samples of water and food [2-4].

Quercetin (3, 3', 4', 5, 7-pentahydroxyflavone) is the most common flavonol compound in plant and food such as herbs, oils, nuts, fruits, vegetables and beverages. Quercetin is able to interact with metals because of the chelating metal ions and scavenging free radicals [5]. The structure of quercetin is shown in (Figure 1).

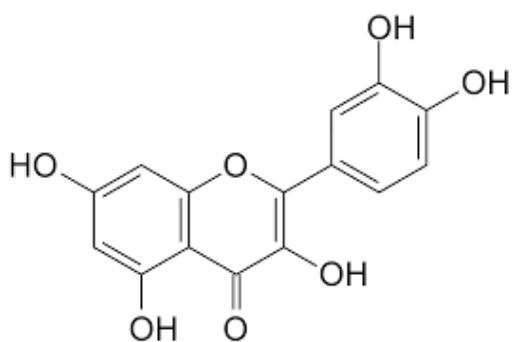


Figure 1.1 Structure of quercetin

Heavy metal can be determined by various techniques such as flame atomic absorption spectrometry (FAAS), graphite furnace atomic absorption spectrometry (GFAAS) [6, 7], inductively coupled plasma optical emission spectroscopy (ICP-OES) [8-10] and inductively coupled plasma mass spectrometry (ICP-MS) [11]. These techniques provide high sensitivity and reliability, but they involve expensive instrument and time-consuming sample preparation steps. On the other hand, spectrophotometric techniques are fast and simple [12]. It can be also coupled with a flow analysis system technique (flow - based analysis) [13], which is an automatic analytical technique with continuously flowing solution within a small inner diameter tube. Spectrophotometric technique combined with flow analysis technique is a high efficient and portable detection device, rapid, easy to use and cost-effective.

Generally, the spectrometer consists of the light source, wavelength selector and detector. It is modified to the portable device. For example, the light source can improve to a small size and inexpensive than the commercial device by using LED tube (light-emitting diode) as the alternative light source.

The aim of the study is to develop a portable spectrophotometric device with flow-based analysis system by measurement of the signal of complex caused by metal ion binding to the complexing agent for the determination of lead and cadmium in wastewater.

1.2 Objective of the research

- To develop a portable spectrophotometric device with flow-based analysis system for the determination of lead and cadmium in wastewater.

CHAPTER II

THEORY AND LITERATURE SURVEY

This chapter explains the theory that associated with the determination of cadmium and lead by the characteristics of metal, toxicity and effects on the health were illustrated. It explains the principles and theory of quercetin, CTAB, the reaction between the metal and the complexing agent, spectrophotometry, sequential injection spectrophotometer and LED (light-emitting diode). In addition, it shows the development of research involved the analysis of lead and cadmium by using spectrophotometric technique combined with flow analysis technique.

2.1 Cadmium

Cadmium is a silver-white, soft, lustrous heavy metal. The atomic number and atomic mass of cadmium are 48 and 112.4 g mol^{-1} , respectively (Figure 2.1). In nature, it is found as a fine-grained gray in the form sulfide compounds which are combined with zinc. Most cadmium is used in the electroplating industry because it has the corrosion and resistance characteristics. In addition, it is also used as a component of paint, the stable of plastic, the battery, and the welding metal process [14]. This process is the cause of contamination in the water sources due to the wastewater release into the environment over the limit by the standard wastewater, not more than to 0.03 mg L^{-1} [15].



Figure 2.1 characteristic of cadmium

Cadmium can enter the body through ingestion and inhalation, which is a severe gastrointestinal and pulmonary irritant. The acute ingestion affects the health such as dizziness, nausea, abdominal pain, diarrhea, shock, convulsion and loss of consciousness. The human may die if it is accumulated in the body for a long time. It causes Itai-itai due to accumulated in the bones. In addition, it causes lung cancer, kidney cancer, and prostate cancer [1, 14].

2.2 Lead

Lead is a naturally occurring bluish-gray heavy metal, soft, ductile, highly malleable, and the relatively poor conductor. The atomic number and atomic mass of lead are 82 and 207.2 g mol^{-1} , respectively (Figure 2.2). It is used for the industrial application such as the lead-acid battery, ammunition, sheet lead, pigment, and chemical [16]. The standard of lead has not more than to 0.2 mg L^{-1} in the wastewater [15].



Figure 2.2 characteristic of lead

Lead can enter the body through ingestion of the contaminated food, water, and paint, and through inhalation of the contaminated dust or air. It is the most toxic for the organs in the body and the central nervous system. It can effect on the health, which associates between decreased intelligence and blood level toxin such as decreased IQ level, decreased hearing, growth retardation, delayed

neurobehavioral development, poor attention span, anemia, especially, in the children [16, 17].

2.3 Quercetin

Quercetin or 3, 3', 4', 5, 7-pentahydroxyflavone is a common dietary flavonoid, which is found in plant and food such as herbs, oils, nuts, fruits, vegetables and beverages. Quercetin is not absorbed in the body in order to poor solubility. The quercetin-metal complexes present biological activities such as antioxidant, anti-tumor, anti-bacterial, and kinds of enzymatic activity [18-20]. It is able to chelate with many metal ion because it contains the hydroxyl group at 3, 3', 4', 5, 7 position and carbonyl group at fourth position. It is colored complexing agent, which absorb two major absorption maximum in range 300-400 nm (band I) and 240-285 nm (band II). Band I and band II are associated with the absorption of B ring (cinnamoyl system) and A ring (benzoyl system), respectively (Figure 2.3) [21]. The absorption of band I and band II relates with the π - π^* transition in the aromatic ring of quercetin. Both bands of the quercetin-metal complexes are a bathochromic shift by increased conjugated system of complexation. In the complex formation, the binding of 3, 4 positions are stronger than 3', 4' position due to the 3 and 4 positions are the first binding position in order to the 3-OH group has the acid proton more than 3'-OH group and 4-oxo group has charges more than the other oxygen. Moreover, the 5-OH group is intercepted form the first complex formation due to it has the steric hindrance and the few acid proton [22].

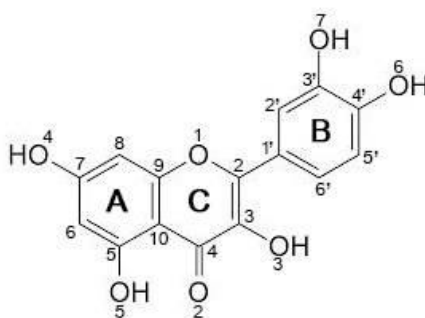


Figure 2.3 Structure of quercetin

2.4 CTAB

CTAB or $[(C_{16}H_{33})N(CH_3)_3]Br$ or cetyl trimethyl ammonium bromide is a cationic surfactant, which contains a quaternary amine as hydrophilic group, a long chain hydrocarbon as hydrophobic group, and bromide ion as counter ion (Figure 2.4). The property of cationic surfactants is that the positively charged surfactants can adsorb on the negatively charged substrates leading to the antistatic effect and hydrophobic effect [23, 24].

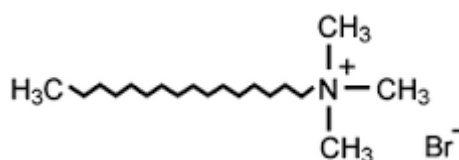


Figure 2.4 Structure of CTAB

The CTAB micelles interact with quercetin by the both of hydrophobic interaction and electrostatic attraction (Figure 2.5). The CTAB micelles will promote the deprotonation on 3, 7 position of quercetin, which is A and C ring of quercetin. The electrostatic attraction between the positive group of CTAB micelles and the negative group of quercetin increases, including the hydrophobic interaction of them. Thus, The CTAB micelles can incorporate with of quercetin in order to increase the solubility of quercetin. Lead and cadmium ions can chelate with quercetin at 3'-OH and 4'-OH position that cause the increased complexes of lead and cadmium [25]. The absorbance of complexes relate $\pi-\pi^*$ transition in the aromatic ring of quercetin which delocalized in molecule of quercetin. The electrons are excited to excited state easily in order to the gap of $\pi-\pi^*$ orbitals are small in width. The transition energy increases for the excited of electron. The absorbance of complexes absorbs at the increased wavelength that cause the spectrum of complexes is bathochromic shift.

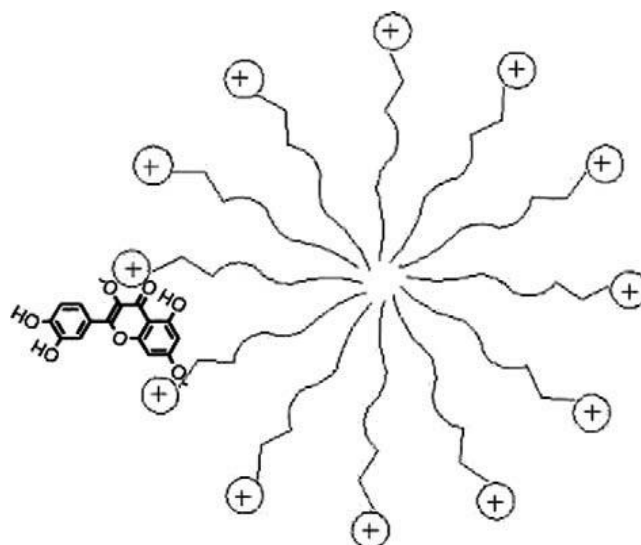


Figure 2.5 Interaction between CTAB micelles with quercetin.

2.5 Spectrophotometry

Spectrophotometry is the analytical technique used to measure the intensity of absorption or emission light as it passes a solution. The wavelength range of spectrophotometry is 200-2500 nm. This technique can assay the qualitative and quantitative analysis of atom or molecule. It involves with changes energy level, which is the characteristic of atom or molecule. The analytical instrument is divided according to the wavelength range such as IR spectrophotometry and UV-Vis spectrophotometry [26, 27].

2.5.1 UV-Vis spectrophotometry

The instrument uses to measure the absorption of the sample in the range of ultraviolet and visible light, which is the wavelength in the range of 200-800 nm. The wavelength ranges of UV and visible are 200-400 nm and 400-800 nm, respectively. It can assay the qualitative and quantitative analysis of the colored complex compound by the light passes through the sample solution. The absorbed light changes the electronic transition from ground state to excited state, which produces the energy in the form of the photons. The concentration of the sample is calculated according to Beer-Lambert Law (Figure 2.6) [26, 28].

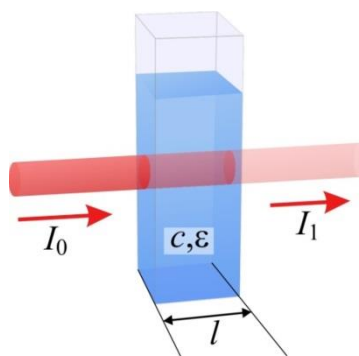


Figure 2.6 schematic of absorption.

Beer-Lambert Law:

$$A = \log_{10} I_0/I_1 = \epsilon l C$$

Where: A = measured absorbance

I_0 = Intensity of incident light

I_1 = Intensity of transmitted light

ϵ = Molar absorptivity ($\text{L mol}^{-1} \text{cm}^{-1}$)

l = Path length (cm)

C = Concentration (mol L^{-1})

2.6 Flow injection analysis system

Flow injection analysis (FIA) is the analytical technique with continuously flowing solution within a small inner diameter tube by the few sample solution is injected into the carrier solution. The sample solution interacts with reagent solution and forms the sample zone by dispersion. Then, the signal of the interested sample zone is measured by the carrier solution transfers the sample zone via flow through cell into the detector. FIA system is an automatic system, simplicity, miniaturized technique and green analytical chemistry, which is able to apply in versatile analysis such as the analysis of metals in the water. After that FIA

has developed the instrument and software to reduce the size of instrument and be friendly environment because the volume of chemical, sample, and waste are reduced [13, 29].

2.6.1 Sequential injection analysis system (SIA)

SIA is an automatic technique with continuously flowing solution by using the computer controls valve and pump, which is developed from FIA system (Figure 2.7). The carrier solution, reagent solution, and sample solution are aspirated into the system and kept in holding coil, which is controlled the volume and flow rate by the pump. In holding coil, the sample solutions disperse into the reagent solution lead to the sample zone. Then, the signal of the sample zone is measured by dispensing via flow through cell into the detector [29, 30].

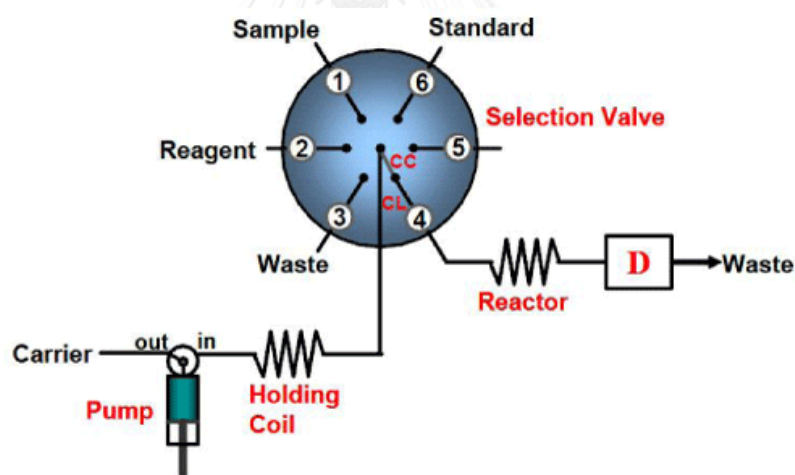


Figure 2.7 schematic of SIA.

SIA has the following components:

Pump:

Pump controls the flow rate of the carrier stream to various parts of the system with a constant rate of speed. The peristaltic pump is used in this research, which is reproducible, precise, and uses the small volume of reagent.

Selection valve:

SIA system uses the multi-port valve in place of injection valve by one port connects with the peristaltic pump. The other ports connect with the carrier solution, reagent solution, sample solution, holding coil, and detector by using Teflon tubing.

Reactor and holding coil:

The holding coil is placed between the selection valve and the pump, which is the area for storing the overall solution before it is dispensed into the detector.

The reaction coil is placed between the selection valve and the detector, which is the area for mixing of overall solutions and transfers to the detector.

Detection system:

SIA system uses detection system like FIA system by equipped with flow through cell. The diameter of flow through cell must suitable for the low pressure of the solution. Furthermore, the computer must connect with interface card and software, which controls valve, pump, and the overall analytical instrumentation

2.7 LED (light-emitted diode)

LED or light-emitted diode is essentially the pn junction diode, which is made from a thin layer of fairly heavily doped semiconductor material. When battery applies the voltage leading to the forward-biased junction, the electrons from the n-type inject into the p-type and combine with the hole. But the hole from the p-type inject into the n-type is very less due to the flow of electrons into the p-type. From this process emits energy in the form of the photons, which is a monochromatic light (Figure 2.8) [31, 32].

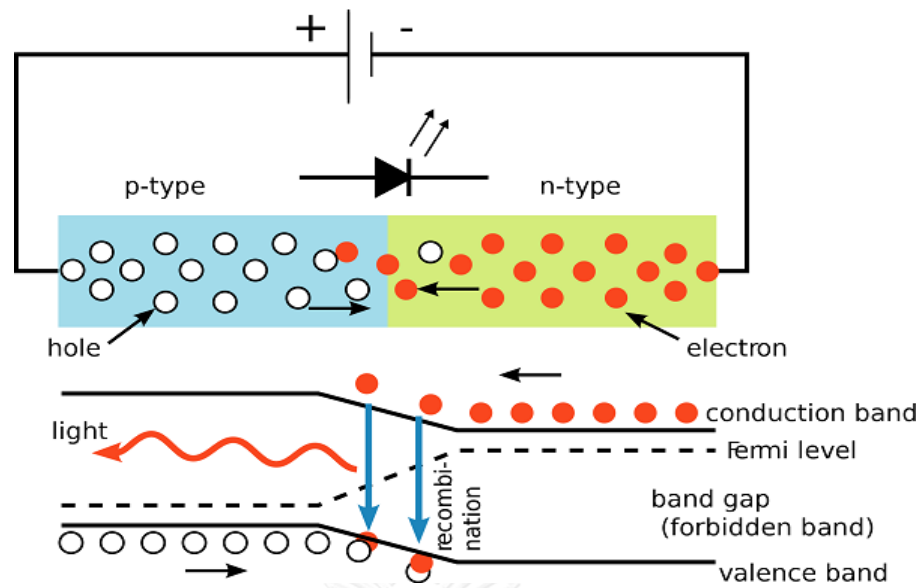


Figure 2.8 Mechanism of the pn junction diode.

Advantages of LEDs:

- LED is small size and hence light weight.
- The lifetime of LED has longer than the other lights.
- LED is low temperature because the mechanism of LED does not produce heat.
- The energy of LED has more efficient than the traditional lights.
- LED does not contain the toxic chemical.
- LED can emit the colored light without the use of color filter.
- LED operates in frequent on/off operation very quick, which it must not warm up.

2.8 Literature reviews

In 2002, Yuanqian et al [33] determined Zn, Cd, and Pb in water, beverage, powdered corn and powdered milk by flow injection-diode array detector-absorption spectrophotometry using partial least square (PLS) method. The colored complexes were measured, which was the reaction of metal ions with meso-tetra (4-

trimethylammoniumphenyl) porphyrin (TAPP). The overlapped spectra and multi-wavelength are collected by charge coupled device (CCD)-diode array detector and PLS algorithm, respectively. The study investigated that the recoveries of the metals were 89.6-103.2% with R. S. D in range of 2.8-7.1%.

In 2004, Udnan et al [34] determined iron (III) in pharmaceutical preparations by flow injection analysis system, which it used the green LED and the photodiode as light source and detector, respectively. The red complex of Fe (III) with salicylate reagent was measured using salicylate reagent obtained from aspirin. The study investigated that the linear range of Fe (III) was obtained in the range of 1-20 mg L⁻¹. The limit of detection (LOD) of Fe (III) was 0.5 mg L⁻¹. The relative standard deviations (R. S. D.s) were obtained in the range of 1.4-5.4% (n=3, for 1-20 mg L⁻¹).

In 2006, Liu et al [25] studied the interaction between quercetin and the surfactant with different charges by UV-Vis spectra, cyclic voltammetry method, and fluorescence spectra. In this study, the anionic and cationic surfactants were sodium dodecyl sulfate (SDS) and cetyl trimethyl ammonium bromide (CTAB), respectively. The study investigated that the main driving force of quercetin with the negative charged SDS micelles was the hydrophobic interaction because quercetin has aromatic rings and low solubility. For the positive charged CTAB micelles was the both of hydrophobic interaction and electrostatic attraction, which influences the π - π^* excitation of quercetin. In the cyclic voltammetry method, the result showed that CTAB micelle facilitates its oxidation but SDS makes hard oxidation due to the oxidant peak of quercetin in CTAB micelle moves to lower potential. The study investigated that the interaction site and interaction mode of quercetin with SDS and CTAB micelles were very different.

In 2010, Dai et al [35] determined the trace lead in drinking water by using flow injection spectrophotometer. The complexing agent is I⁻-EV⁺-PVA, Which is the reaction of reagent solution consist of potassium iodide-L-ascorbic acid, polyvinyl alcohol, and ethyl violet. The colored complex of Lead (II) with I⁻-EV⁺-PVA was measured. The study investigated that linearity of lead (II) was obtained over the

concentration range of 5-80 $\mu\text{g L}^{-1}$. The limit of detection (LOD) was 0.9 $\mu\text{g L}^{-1}$. The relative standard deviation (R. S. D.) was 1.10% (n=10) at 40 $\mu\text{g L}^{-1}$.

In 2010, Norfun et al [36] developed the determination of Al (III) in tap water by a simple reverse flow injection (rFI) spectrophotometric procedure. Al (III) was reacted with quercetin and cetyltrimethylammonium bromide (CTAB) in an acetate buffer medium (pH 5.5). The yellow colored complex of Al (III) was measured at 428 nm. The study investigated that linearity of Al (III) was obtained over the concentration range of 0.02–0.50 mgL^{-1} . The correlation coefficient was 0.9998. The limit of detection (LOD) was 0.007 mgL^{-1} . The limit of quantification (LOQ) was 0.024 mgL^{-1} . The repeatability was 1.10% (n = 11) for 0.2 mgL^{-1} Al (III).

In 2014, Ravichandran et al [37] determined the stability constant of the synthetic complex between quercetin and cadmium ion by UV–Vis spectrophotometry, infrared spectroscopy, thermogravimetry and differential thermal analysis techniques (UV–Vis, IR, TGA, and DTA). The study investigated that stability constant value of quercetin–cadmium complex was 2.27×10^6 and 7.80×10^6 at pH 4.4 and pH 7.4, respectively. The stoichiometric composition of quercetin and cadmium ion was 1:1 in both pH 4.4 and pH 7.4.

CHAPTER III

MATERIALS AND METHODS

3.1 Reagents

Quercetin (Querc) was purchased from Sigma. The standard solutions of 1000 mg L⁻¹ lead (II) and cadmium (II) were purchased from Sigma. Cetyltrimethylammonium bromide (CTAB) was purchased from Sigma. All chemicals were the analytical reagent grade that they were prepared with deionized water.

Quercetin stock solution (800 mg L⁻¹) was dissolved in ethanol/water mixture (60% v/v) as the solvent. Buffer solution, at pH 8.9, was prepared from 0.1 M Tris(hydroxymethyl)aminomethane (Sigma) and 0.1 M hydrochloric acid (Sigma) by dissolving 6.05 g. of Tris(hydroxymethyl)aminomethane in 500 mL of deionized water in 500 mL volumetric flask and adjusted pH to 8.9 by using hydrochloric acid and diluted with water in a 500 mL volumetric flask.

Cetyltrimethylammonium bromide stock solution (3×10^{-3} mol L⁻¹) was prepared by dissolving 0.547 g. of cetyltrimethylammonium bromide in water and adjusted in a 500 mL volumetric flask.

3.2 Apparatus

The pH meter (SevenEasy pH, Mettler-Toledo, Switzerland) was used for adjusting the buffer solution. The flow injection spectrophotometer using standard 1.00 cm quartz cell and AvaSpec-2048-USA2-UA spectrophotometer (Avantes, The Netherlands) was used for measuring the absorbance of complexes. Complexes were measured by sequential injection spectrophotometer by sequential injection manifold consisted of a syringe pump (Nexus 3000, USA) and six port valve (DC/Dex Motor, USA). Tubing (Tub FEP Nat 1/16) was used as flow line for metal standard solutions, buffer, quercetin, and CTAB. All solutions were mixed at mixing coil and

through a flow cell of the spectrophotometer (AvaSpec-2048-USA2-UA spectrophotometer). Arduino Leonardo micro-controller board (Arduino, Italy), Ambient light illuminance level sensor module (GY-30, BH1750FVI, Arduino, Italy) and a single-wavelength light-emitting diode (450 nm) were purchased from the local electronics store in Bangkok, Thailand.

3.3 Cost-effective instrument

A home-made spectrometer has been developed in this work, (Figure 3.1). The spectrometer was constructed using Arduino Leonardo micro-controller board as a motherboard. In order to reduce cost and dimension, a single-wavelength LED (blue, 450 nm) and ambient light illuminance level sensor module were used as a light source and detector, respectively. A sample compartment was designed to accept not only a conventional 1 cm x 1 cm cuvette but also a cuvette-based configuration flow cell. All electronic parts were assembled and placed in a small plastic container. This portable device has been applied with the optimal condition for the determination of Cd (II) and Pb (II) in wastewater.

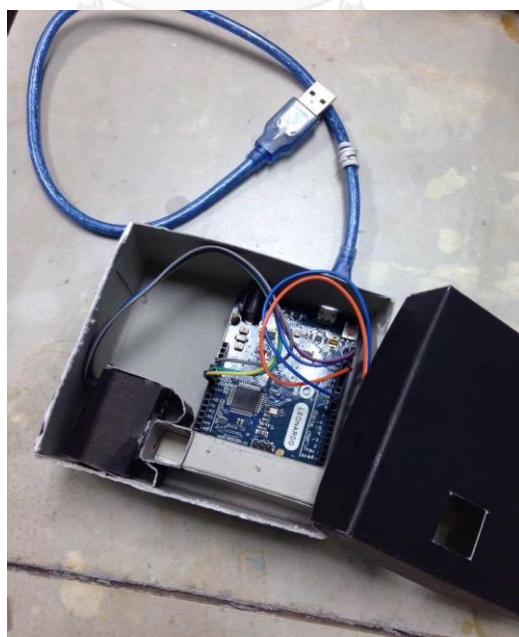


Figure 3.1 Portable device was modified light source using LED (Light-emitting diode)

3.4 Procedure

Using the SIA system setup as shown in (Figure 3.2), the operation sequences were summarized for the determination of lead and cadmium by SIA with spectrophotometry in (Table 3.1). The absorbance of Pb-Querc and Cd-Querc yellow-colored complexes was measured at 440 and 450 nm, respectively.

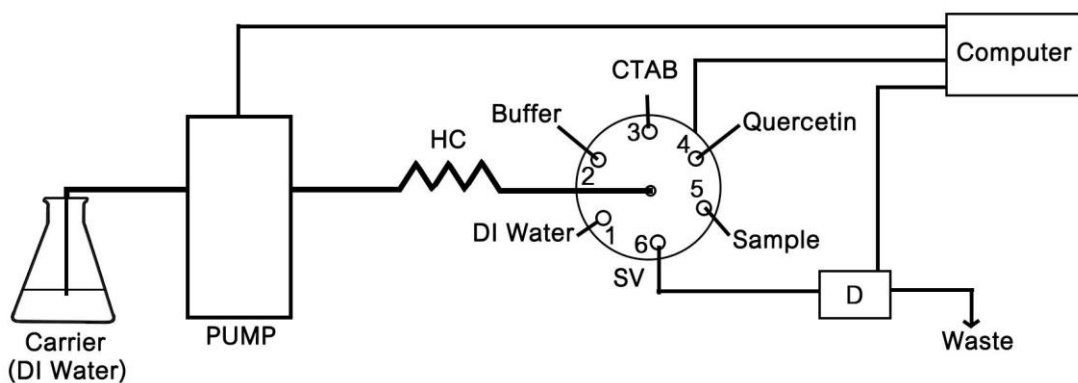


Figure 3.2 SIA system for the determination of Pb (II) and Cd (II). HC: holding coil, SV: selection valve and D: detector

Table 3.1 Operation sequences for the determination of lead and cadmium by SIA with spectrophotometry. SV: selection valve, SP: syringe pump.

Step	SV position	SP flow rate ($\mu\text{L min}^{-1}$)	SP operation	SP volume (μL)	commentary
1	1	1000	Aspirated	3000	DI water in the holding coil
2	6	5500	Dispensed	3000	Cleaning of tubing and eliminating of residue
3	1	1000	Aspirated	1200	DI water in the holding coil
4	2	1500	Aspirated	150	Buffer solution in the holding coil
5	3	3500	Aspirated	150	CTAB solution in the holding coil
6	4	2500	Aspirated	20	Quercetin reagent in the holding coil
7	5	3500	Aspirated	100	Metal standard solution in the holding coil
8	6	5500	Dispensed	1620	The mixed solution into the detector

CHAPTER IV

RESULTS & DISCUSSION

4.1 The choice of complexing agent

The complexing agents were studied for the determination of lead and cadmium such as dithizone, alizarin red s, and quercetin. The linear range of complexes between metal standards and complexing agents was obtained. The results showed that complexes of quercetin and metal standard obtained the best sensitivity. So, the quercetin reagent was chosen as the optimum complexing agent for the determination of lead and cadmium (Appendix: A1-A4).

4.2 Effect of pH

The formation of complex between the metal standard and ligand is pH dependence. Quercetin is a ligand capable of chelating in wide range of pH values, from acidic to slightly basic. Consequently, the complexation reaction of Pb (II) and Cd (II) standard solutions and quercetin reagent was studied in the pH range of 3.0-11 (Appendix: A5-A9) by using Tris(hydroxymethyl)aminomethane buffer solution in the wavelength range of 400-515 nm. The buffer solution was mixed with 2 mg L⁻¹ metal standard solution, 300 mg L⁻¹ quercetin reagent, and 3.5 × 10⁻³ mol L⁻¹ CTAB solution. The results indicated that pH 8.9 is the best one that obtained the best absorbance of Pb (II) and Cd (II) complexes. The property of quercetin oxidation occurred at high pH cause the loss of hydrogen atom from molecules, which obtained the negative charge of oxygen. CTAB solution was active at alkaline pH, which caused the positive charge of quaternary amine. The interaction of negative and positive charge between quercetin reagent, CTAB solution, and metal ion was complexes of metal at pH 8.9.

4.3 Effect of CTAB concentration

CTAB was commonly used as sensitizing and solubilizing agent to accelerated the reaction of complex. The effect of CTAB concentration for the determination of Pb (II) and Cd (II) was studied in the range of 0.5×10^{-3} to 5.0×10^{-3} mol L⁻¹ in the wavelength range of 400-515 nm. The results showed that the absorbance of Pb (II) increased over the absorbance of reagent blank when the concentration of CTAB was 1.5×10^{-3} to 3.0×10^{-3} mol L⁻¹. The absorbance of Cd (II) increased over the absorbance of reagent blank when the concentration of CTAB was 3.0×10^{-3} to 5.0×10^{-3} mol L⁻¹. Consequently, the concentration of 3.0×10^{-3} mol L⁻¹ CTAB was chosen as optimum for the determination of Pb (II) and Cd (II) due to it obtained the best absorbance of complexes (Appendix: A10-A11).

4.4 Absorption spectra of complexes

The absorption spectra of yellow-colored complexes were obtained from reaction between the metal standard and quercetin with CTAB in solution with buffer pH 8.9. The absorption spectra of complexes were measured at the wavelength range of 200-600 nm using flow injection spectrophotometer. The maximum absorbance of Pb (II)-Querc and Cd (II)-Querc was shown at 415 and 424 nm, respectively (Figure 4.1).

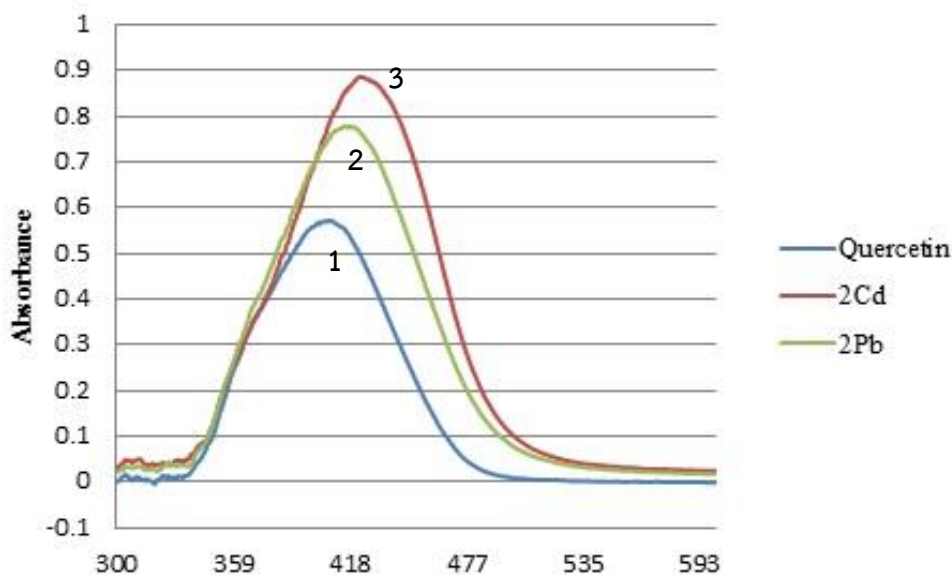


Figure 4.1 Absorption spectra of quercetin (1), Pb (II)-Querc. (2) and Cd (II)-Querc. (3) at pH 8.9. The standard concentration is 2 mg L^{-1} , Quercetin concentration is 25 mg L^{-1} and CTAB concentration is $3.0 \times 10^{-3} \text{ mol L}^{-1}$.

4.5 Effect of quercetin concentration

The effect of quercetin concentration for the determination of 0.3 mg L^{-1} Pb (II) and Cd (II) was studied by varying concentration of $100\text{-}1000 \text{ mg L}^{-1}$ quercetin solution at pH 8.9 in the wavelength range of $400\text{-}515 \text{ nm}$. The results showed that the quercetin concentration of 800 mg L^{-1} provided the best absorbance of Pb-Querc and Cd-Querc at wavelength 440 and 450 nm , respectively. The absorbance of Pb (II) and Cd (II) are similar at the same concentration. Consequently, the concentration of 800 mg L^{-1} quercetin was chosen as optimum (Figure 4.2). The concentration of quercetin involved the formation of complex between metal ions and quercetin. The concentration of quercetin was less than the optimum concentration, the complexes would less obtain thus the absorbance of complexes was low. But the concentration of quercetin was more than the optimum concentration that it affected the changed ratio between quercetin and metal ions. The complexes were obtained so the absorbance of complexes was low.

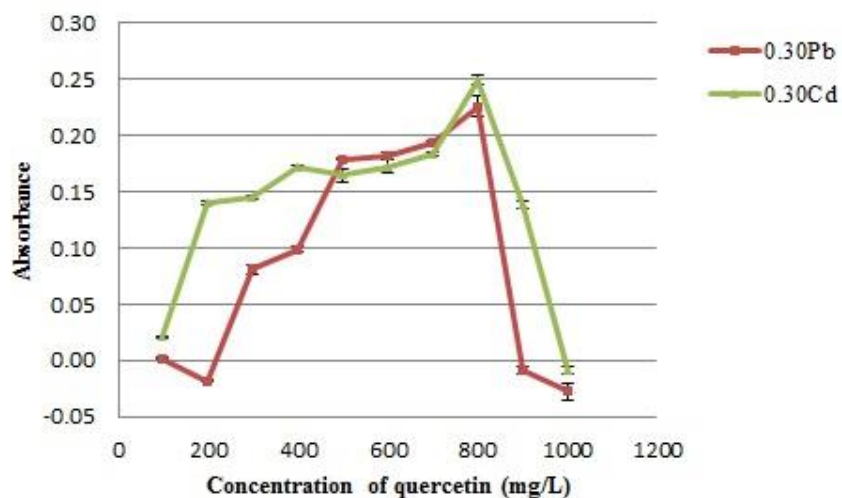


Figure 4.2 Determination of 0.3 mg L^{-1} standard and quercetin ($100\text{-}1000 \text{ mg L}^{-1}$) at wavelength 440 and 450 nm .

4.6 Effect of volume of standard solution

The effect of Pb (II) and Cd (II) standards volume were studied by varying volume of 0.3 mg L^{-1} standard solutions in range of $50\text{-}300 \mu\text{L}$. They were reacted with 800 mg L^{-1} quercetin at pH 8.9 in the wavelength range of $420\text{-}470 \text{ nm}$. The results showed that the volume of $100 \mu\text{L}$ standard solutions was chosen as optimum (Figure 4.3). The volume of standard affected the ratio of metal ion and quercetin that it involved the formation of complex. The quercetins were low chelated with metal ions. The complexes were low obtained so the absorbance of complexes was low. Moreover, the large volume affected the dilution effect in the solution so the complexes were low obtained so the absorbance of complexes was low.

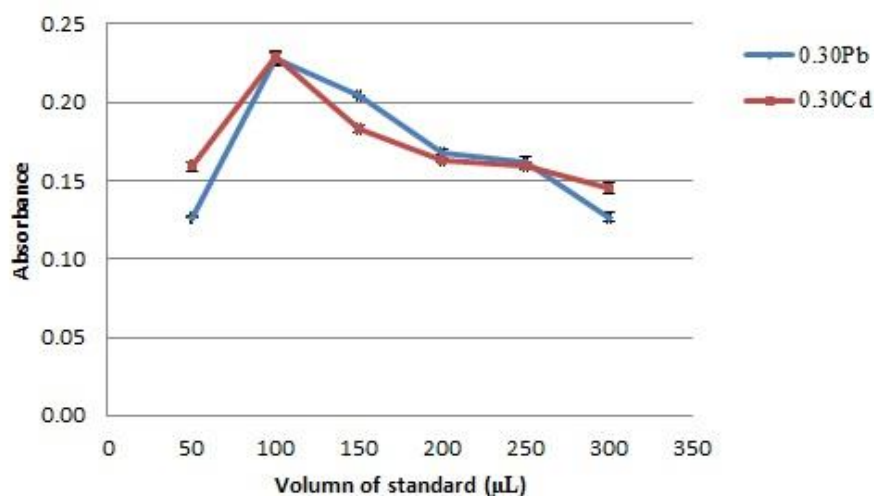


Figure 4.3 Determination volume of standard (50-300 μL) and 800 mg L^{-1} quercetin at wavelength 440 and 450 nm.

4.7 Effect of quercetin volume

The effect of quercetin volume was studied by varying volume of 800 mg L^{-1} quercetin reagent in the range of 10-70 μL . It was reacted with 0.3 mg L^{-1} metal standard solutions at pH 8.9 in the wavelength range of 420-470 nm. The results showed that the volume of 20 μL quercetin reagent was chosen as optimum because 20 μL quercetin reagent provided the maximum absorbance of complexes (Figure 4.4). The volume of quercetin reagent affected the ratio of CTAB and quercetin. In the method, the concentration of CTAB solution was fixed that it involved the dissolution of quercetin reagent in solution. The quercetin reagent was low dissolved so the quercetin reagents were low chelated with metal ions. The complexes were low obtained so the absorbance of complexes was low.

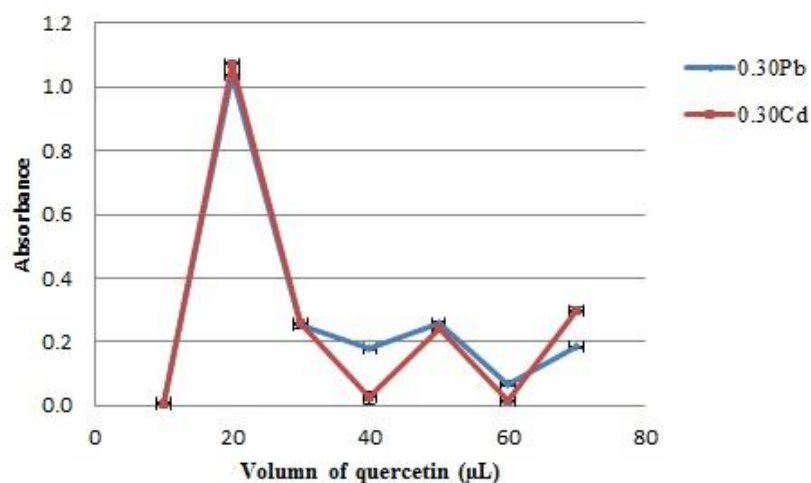


Figure 4.4 Determination volume of 800 mg L^{-1} quercetin (10-70 μL) and 0.3 mg L^{-1} metal standard solutions at wavelength 440 and 450 nm.

4.8 Effect of CTAB volume

The effect of CTAB volume was studied by varying volume of $3.0 \times 10^{-3} \text{ mol L}^{-1}$ CTAB in range of 50-300 μL . It was used as a surfactant of the reaction between 0.3 mg L^{-1} metal standard solutions and 800 mg L^{-1} quercetin reagent at pH 8.9 in the wavelength range of 420-470 nm. The results showed that the volume of 150 μL CTAB solution was chosen as optimum because 150 μL CTAB solution provided the maximum absorbance of complexes (Figure 4.5). The volume of CTAB affected the ratio of CTAB and quercetin. In the method, the concentration of quercetin reagent was fixed that it involved the dissolution of quercetin reagent in solution. The quercetin reagent was low dissolved so the quercetin reagents were low chelated with metal ions. The complexes were low obtained so the absorbance of complexes was low.

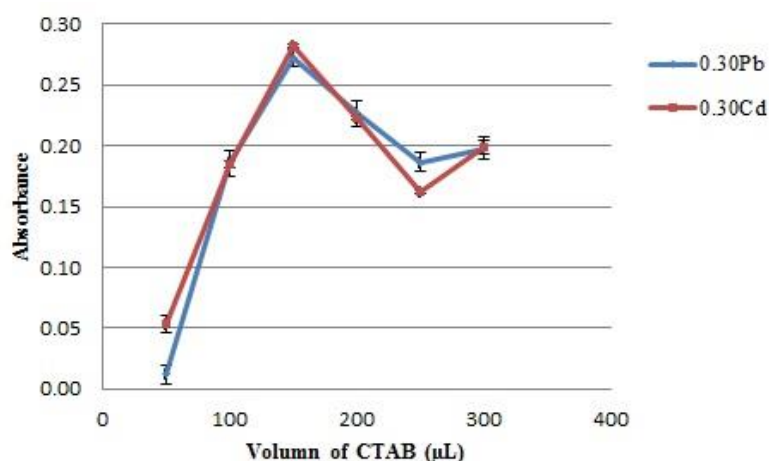


Figure 4.5 Determination volume of $3.0 \times 10^{-3} \text{ mol L}^{-1}$ CTAB (50-300 μL) and 0.3 mg L^{-1} metal standard solutions at wavelength 440 and 450 nm.

4.9 Effect of buffer volume

The effect of buffer volume was studied by varying volume of buffer pH 8.9 in range of 50-300 μL in the reaction between 0.3 mg L^{-1} metal standard solutions and 800 mg L^{-1} quercetin reagent at the wavelength range of 420-470 nm. The results showed that the volume of 150 μL buffer was chosen as optimum because 150 μL buffer solution provided the maximum absorbance of complexes (Figure 4.6).

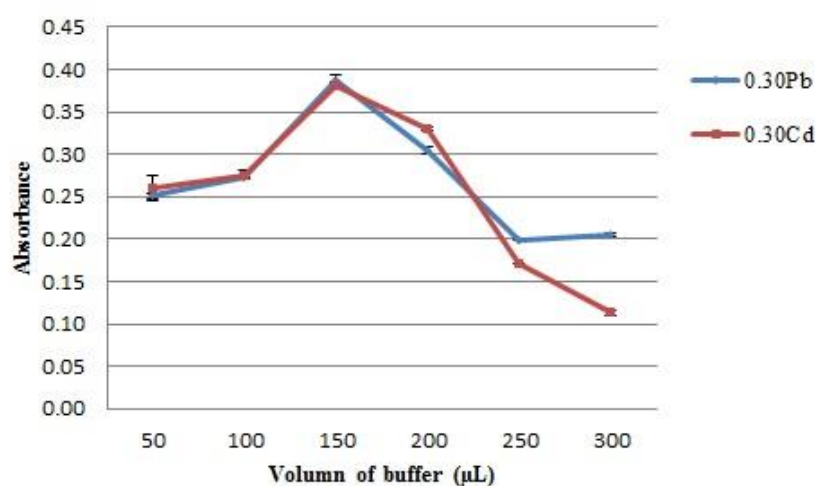


Figure 4.6 Determination volume of buffer (50-300 μL) at wavelength 440 and 450 nm.

4.10 Effect of deionized water volume

The effect of deionized water volume was studied by varying volume of deionized water volume in range of 100-1500 μL in the reaction between 0.3 mg L^{-1} metal standard solutions and 800 mg L^{-1} quercetin reagent at the wavelength range of 420-470 nm. The results showed that the volume of 900-1100 μL could not provide a good mixing ability between sample and other reagents. It offered a relatively low signal-response due to an incomplete chemical reaction. At the volume over 1200 μL , the chemical reaction might reach nearly 100% but the reaction product (complexes specie) could be dispersed. In this condition, the dispersion of the system was too high and the signal-response decreased due to a dilution effect.

The volume of 1200 μL deionized water was chosen as an optimum condition because it provided the maximum signal-response of the complexes (Figure 4.7).

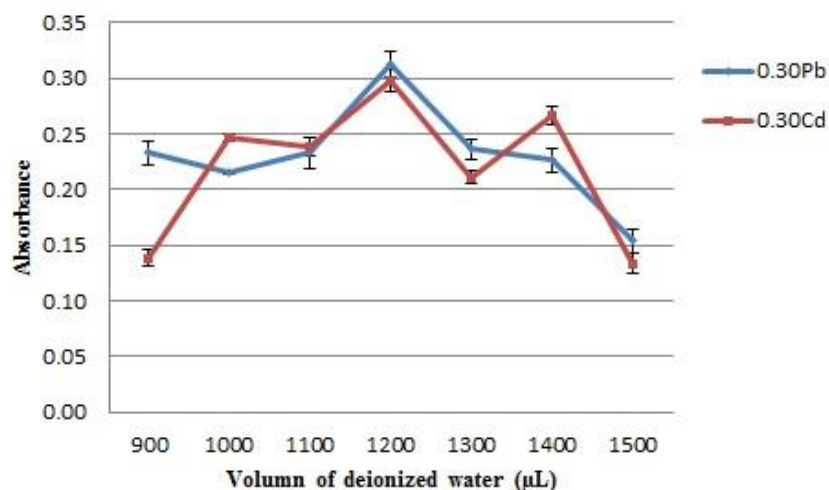


Figure 4.7 Determination volume of deionized water (100-1500 μl) at wavelength 440 and 450 nm.

4.11 Effect of flow rate

The effect of flow rate of deionized water, buffer, CTAB, quercetin, standard solutions and dispended flow rate were studied over the range 500-5000 $\mu\text{L min}^{-1}$. The results showed that the optimum flow rate of them were 1000, 1500, 3500, 2500, 3500 and 5500 $\mu\text{L min}^{-1}$, respectively (Table 4.1). However, the flow rate increased, the stream of reagents moved too fast. Therefore, the partial reagent might be chelate with the metals leading to smaller amounts of complexes. The absorbance of complexes decreased because of the shorter reaction time.

Table 4.1 Summarize the optimum conditions for the complexation of Pb (II) and Cd (II) using sequential injection spectrophotometry.

Variable	Range studied	Optimum value
concentration of quercetin (mg L^{-1})	100-1000	800
volumn of quercetin (μL)	10-100	20
volumn of metal standard (μL)	50-300	100
concentration of CTAB (mg L^{-1})	0.0005-0.005	0.003
volumn of CTAB (μL)	50-300	150
pH	3.0-11.0	8.9
volumn of buffer (μL)	50-300	150
volumn of deionized water (μL)	100-1500	1200
Flow rate of metal standard ($\mu\text{L min}^{-1}$)	500-5000	3500
Flow rate of quercetin ($\mu\text{L min}^{-1}$)	500-5000	2500
Flow rate of CTAB ($\mu\text{L min}^{-1}$)	500-5000	3500
Flow rate of buffer ($\mu\text{L min}^{-1}$)	500-5000	1500
Flow rate of deionized water ($\mu\text{L min}^{-1}$)	500-5000	1000
Flow rate of dispended ($\mu\text{L min}^{-1}$)	500-6500	5500

4.12 Linear range

Under the optimum conditions (Table 4.1), the determination of Pb (II) and Cd (II) with quercetin was studied over the concentration range from 0.02-0.1 mg L⁻¹ at 440 and 450 nm, respectively. The calibration equation of Pb (II) is $y = 3.8086x - 0.002$, $R^2 = 0.9991$. The limit of detection (LOD, defined as 3SD) of Pb (II) was 0.002 mg L⁻¹ and the limit of quantification (LOQ, defined as 10SD) was 0.007 mg L⁻¹ (Figure 4.8). The calibration equation of Cd (II) is $y = 3.3795x + 0.0078$, $R^2 = 0.996$. The limit of detection (LOD, defined as 3SD) of Cd (II) was 0.002 mg L⁻¹ and the limit of quantification (LOQ, defined as 10SD) was 0.008 mg L⁻¹ (Figure 4.9). The relative standard deviations were 3.6% (n=7) and 3.4% (n=7) for 0.02 mg L⁻¹ Cd (II) and Pb (II), respectively.

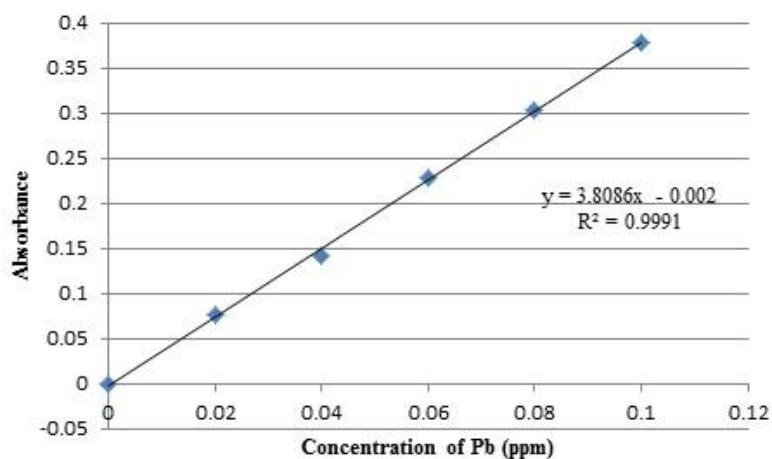


Figure 4.8 Calibration curve of Pb (II) standard solution in concentration of standard range 0.02-0.1 mg L⁻¹ at 440 nm.

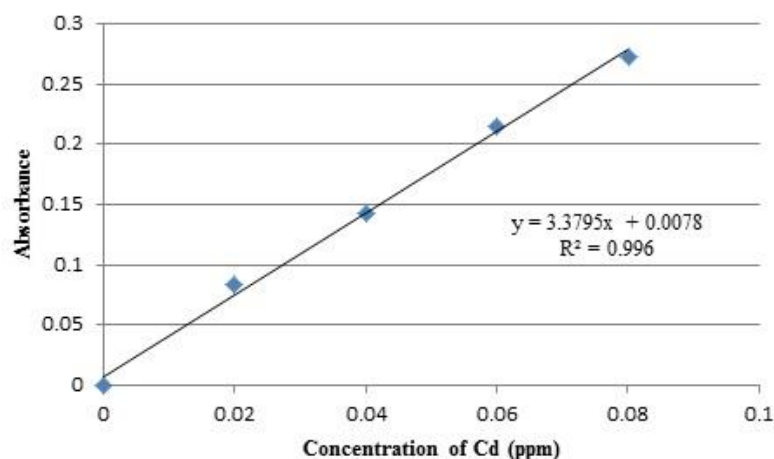


Figure 4.9 Calibration curve of Cd (II) standard solution in concentration of standard range 0.02-0.1 mg L⁻¹ at 450 nm.

4.13 Determination of lead and cadmium by cost-effective instrument

The determination of Pb (II) and Cd (II) reacted with quercetin was studied over the concentration range from 0.02-0.1 mg L⁻¹ under the optimum conditions (Table 4.1). The absorbance of complexes was measured by the sequential injection spectrophotometer with LED (Light-emitting diode) as the light source, wavelength 450 nm. The calibration equation of Pb (II) is $y = 1.58x + 0.0051$, $R^2 = 0.9953$. The limit of detection (LOD, defined as 3SD) of Pb (II) was 0.011 mg L⁻¹ and the limit of quantification (LOQ, defined as 10SD) was 0.037 mg L⁻¹ (Figure 4.10-4.11). The calibration equation of Cd (II) is $y = 2.1591x + 0.0033$, $R^2 = 0.9972$. The limit of detection (LOD, defined as 3SD) of Cd (II) was 0.011 mg L⁻¹ and the limit of quantification (LOQ, defined as 10SD) was 0.037 mg L⁻¹ (Figure 4.12-4.13).

The developed spectrometer has main limitation. Because of this spectrometer used a single wavelength LED light source, it can only be operated at 450 nm. Pb-Querc and Cd-Querc have maximum absorption wavelength at 440 and 450 nm, respectively, which their wavelengths have nearby the LED wavelength. The absorbance of the developed spectrometer has lower than the commercial instrument due to the photodiode sensor responses the best sensitivity in range of 800-900 nm which isn't include the absorption wavelength of complexes. Thus the sensitivity of developed spectrometer has sensitivity lower than the commercial

instrument. The instrumental and analytical characteristics of this home-made spectrometer are being carried out.

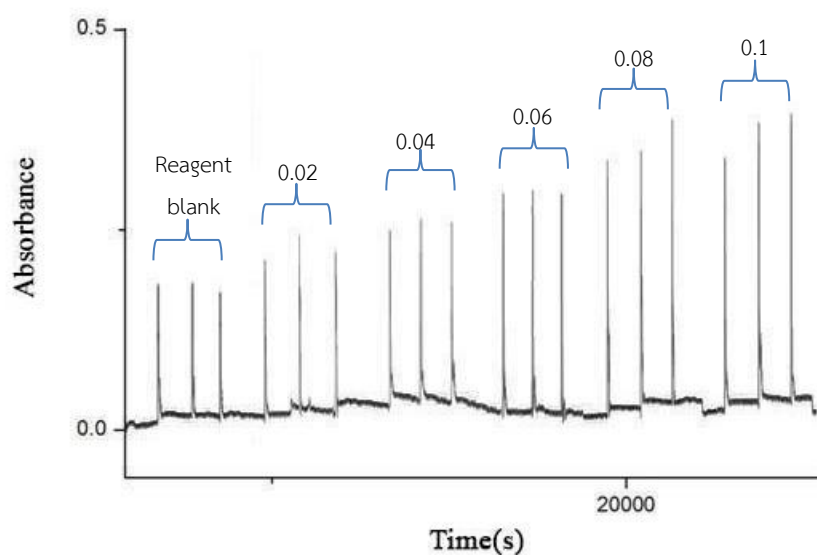


Figure 4.10 Absorption signals of 0.02-0.1 mg L^{-1} Pb (II) was measured by using sequential injection spectrophotometer with LED (Light-emitting diode) as the light source, wavelength 450 nm.

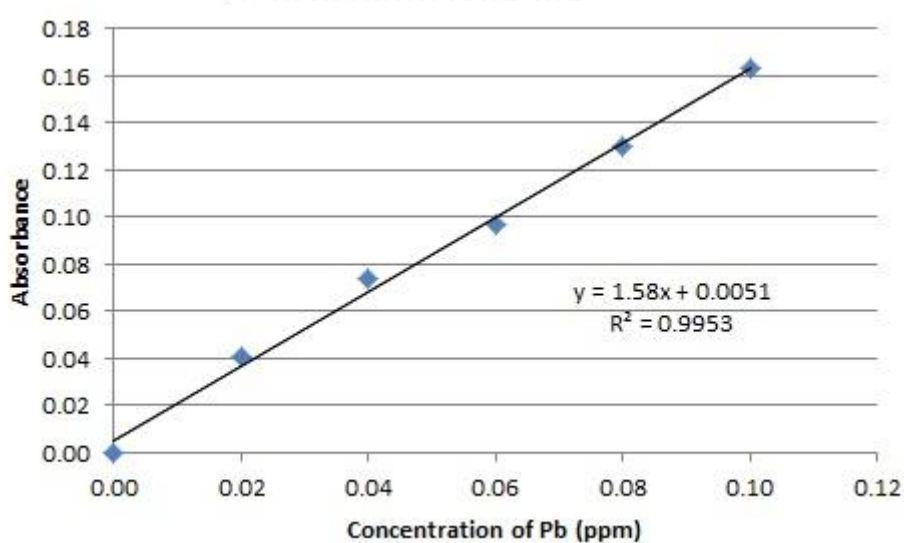


Figure 4.11 Calibration curve of Pb (II) standard solution in concentration of standard range 0.02-0.1 mg L^{-1} at 450 nm.

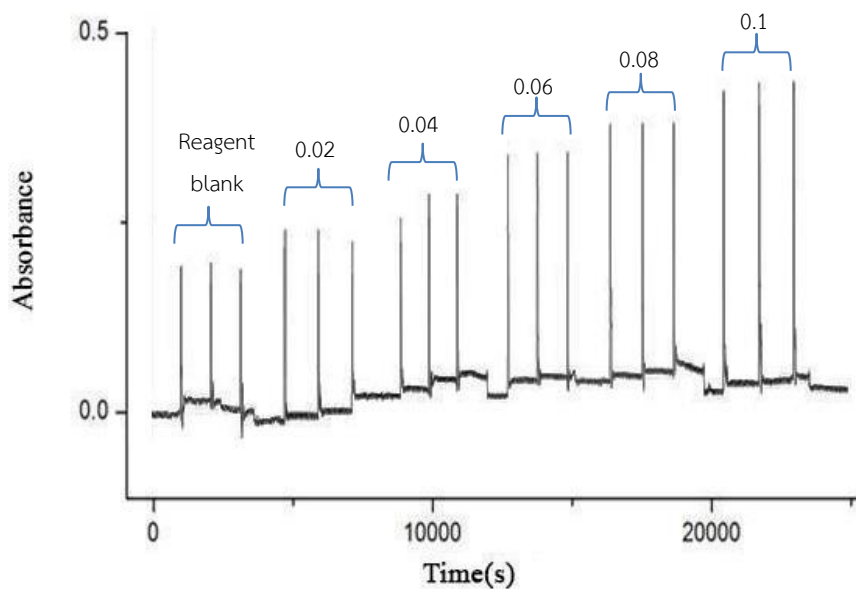


Figure 4.12 Absorption signals of 0.02-0.1 mg L⁻¹ Cd (II) was measured by using sequential injection spectrophotometer with LED (Light-emitting diode) as the light source, wavelength 450 nm.

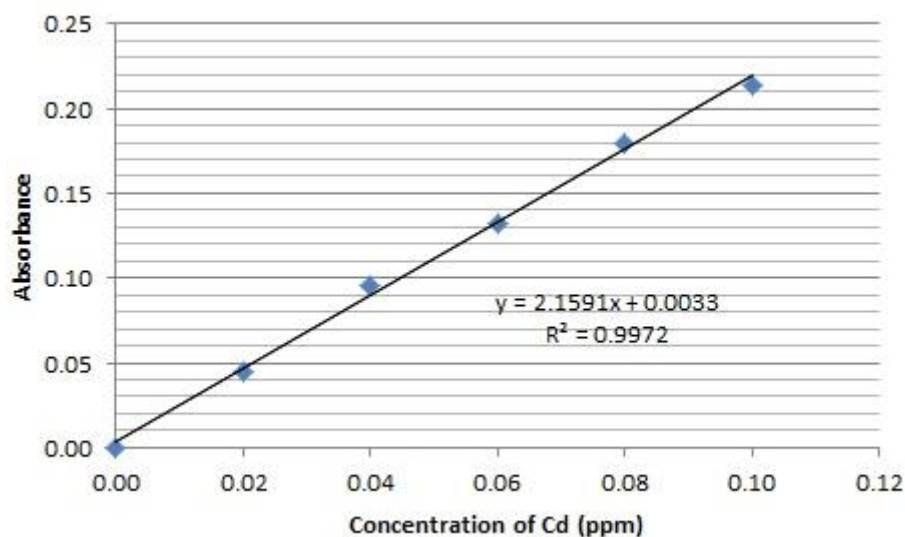


Figure 4.13 Calibration curve of Cd (II) standard solution in concentration of standard range 0.02-0.1 mg L⁻¹ at 450 nm.

4.14 Determination of lead and cadmium in wastewater samples

Determination of lead and cadmium in wastewater samples were studied at a concentration of 0.03, 0.05 and 0.07 mg L⁻¹ by added 1 mg L⁻¹ Pb (II) and Cd (II) 300, 500 and 700 µL in wastewater sample 10 mL (from the industrial area, Ratchaburi). The absorbance was measured using proposed conditions and modified device. The results showed that the percentage recoveries of 0.03, 0.05 and 0.07 mg L⁻¹ (n=10) of Cd (II) were 102.6, 108.5 and 101.3%, respectively (Table 4.2). The percentage recoveries of 0.03, 0.05 and 0.07 mg L⁻¹ (n=10) of Pb (II) were 94.4, 91.3 and 97.7%, respectively (Table 4.3). The experiments show that the proposed method provided acceptable the recoveries of Pb (II) and Cd (II) for the determination of Pb (II) and Cd (II) in wastewater.



Table 4.2 The percentage recovery of cadmium was studied at a concentration of 0.03, 0.05 and 0.07 mg L⁻¹ using the proposed method.

Time	Concentration of Cd (mg/L)						
	reagent blank	0.03		0.05		0.07	
		Concentration of Cd found (mg/L)	%recovery	Concentration of Cd found (mg/L)	%recovery	Concentration of Cd found (mg/L)	%recovery
1	0	0.0302	100.0	0.0530	106.0	0.0700	100.0
2	0	0.0310	103.0	0.0540	107.0	0.0700	100.0
3	0	0.0310	103.0	0.0540	108.0	0.0710	101.0
4	0	0.0301	100.0	0.0560	112.0	0.0710	102.0
5	0	0.0303	101.0	0.0500	100.0	0.0700	101.0
6	0	0.0307	102.0	0.0500	100.0	0.0710	101.0
7	0	0.0325	108.0	0.0570	113.0	0.0710	101.0
8	0	0.0310	104.0	0.0550	110.0	0.0700	101.0
9	0	0.0309	103.0	0.0560	111.0	0.0730	104.0
10	0	0.0306	102.0	0.0590	118.0	0.0720	102.0
Average	0	0.0308	102.6	0.0544	108.5	0.0709	101.3

Table 4.3 The percentage recovery of lead was studied at a concentration of 0.03, 0.05 and 0.07 mg L⁻¹ using the proposed method.

Time	Concentration of Pb (mg/L)						
	reagent blank	0.03		0.05		0.07	
		Concentration of Pb found (mg/L)	%recovery	Concentration of Pb found (mg/L)	%recovery	Concentration of Pb found (mg/L)	%recovery
1	0	0.0270	89.9	0.0482	96.3	0.0640	92.0
2	0	0.0300	101	0.0499	99.9	0.0760	108
3	0	0.0290	95.6	0.0404	80.8	0.0820	117
4	0	0.0270	91.1	0.0434	86.7	0.0690	97.9
5	0	0.0296	98.5	0.0506	101	0.0730	105
6	0	0.0292	97.3	0.0423	84.7	0.0630	89.3
7	0	0.0260	86.7	0.0434	86.8	0.0550	78.1
8	0	0.0287	95.6	0.0477	95.4	0.0590	84.9
9	0	0.0284	94.7	0.0426	85.2	0.0720	102
10	0	0.0280	93.5	0.0479	95.8	0.0720	103
Average	0	0.0283	94.4	0.0456	91.3	0.0685	97.7

4.15 Interference

Many heavy metals may contaminate in the wastewater from the industries. They may affect the determination of lead and cadmium. Therefore, the determination of the metal interferences was studied. The metal interferences are arsenic (As), mercury (Hg), chromium (Cr) and manganese (Mn) by studied the concentration of metal interferences interfere signal of Pb (II) and Cd (II), that it does not exceed 5% can acceptable. The determination of the metal interferences was studied over the concentration 0.01, 0.02, 0.1, 0.2, 1, 2, 10 and 20 mg L⁻¹ in the solution containing 0.05 mg L⁻¹ lead and cadmium using proposed conditions and modified device. The results found that the concentration of Pb (II) and Cd (II) remain the constant at 0.01-2 mg L⁻¹ As (III), but the concentration of Pb (II) and Cd (II) would

increase when the amount of As (III) increased 10 and 20 mg L⁻¹, respectively (Table 4.4). Hg (I) is similar to As (III), the concentration of Pb (II) and Cd (II) remain the constant in the concentration range 0.01-0.2 mg L⁻¹ Hg (I), but the concentration of Pb (II) and Cd (II) would increase when the amount of Hg (I) increased (Table 4.5). In the case of Cr (III), (Table 4.6), the result shown that the concentration of Pb (II) and Cd (II) is also the constant in the range 0.01-0.2 mg L⁻¹ Cr (III), but the concentration of Pb (II) and Cd (II) would decrease when the concentration of Cr (III) increased. In the case of Mn (II), (Table 4.7), the result shown that the concentration of Cd (II) would decrease when the concentration of Mn (II) increased, in part the concentration of Pb (II) would increase when the amount of Mn (II) increased. The experiment showed that all of metal interferences do not affect the determination of Pb (II) and Cd (II) in the range 0.02-0.1 mg L⁻¹, which this range was studied in this work.

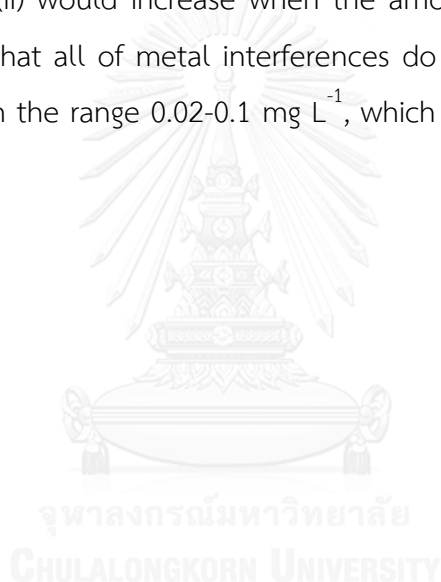


Table 4.4 The percentage interferences of arsenic were studied in the determination of 0.05 mg L^{-1} Pb (II) and Cd (II).

Heavy metal	mg/L	Cadmium (Cd) standard		Lead (Pb) standard	
		mg/L	Interference (%)	mg/L	Interference (%)
Cadmium (Cd) standard	-	0.060	0	-	-
Lead (Pb) standard	-	-	-	0.053	0
Arsenic (As)	0.010	0.060	0.164	0.053	0.983
	0.020	0.060	0.697	0.053	1.64
	0.10	0.060	1.27	0.054	1.97
	0.20	0.061	1.39	0.054	3.50
	1.0	0.061	2.46	0.056	5.46
	2.0	0.063	4.67	0.056	5.79
	10	0.066	9.68	0.061	15.9
	20	0.072	20.4	0.063	19.8

Table 4.5 The percentage interferences of mercury were studied in the determination of 0.05 mg L^{-1} Pb (II) and Cd (II).

Heavy metal	mg/L	Cadmium (Cd) standard		Lead (Pb) standard	
		mg/L	Interference (%)	mg/L	Interference (%)
Cadmium (Cd) standard	-	0.067	0	-	-
Lead (Pb) standard	-	-	-	0.053	0
Mercury (Hg)	0.010	0.069	1.84	0.053	0.544
	0.020	0.069	1.92	0.053	0.599
	0.10	0.070	3.40	0.055	3.81
	0.20	0.070	3.62	0.055	4.90
	1.0	0.078	16.1	0.056	5.44
	2.0	0.079	18.3	0.056	6.21
	10	0.086	29.4	0.061	15.4
	20	0.087	30.4	0.069	30.2

Table 4.6 The percentage interferences of chromium were studied in the determination of 0.05 mg L^{-1} Pb (II) and Cd (II).

Heavy metal	mg/L	Cadmium (Cd) standard		Lead (Pb) standard	
		mg/L	Interference (%)	mg/L	Interference (%)
Cadmium (Cd) standard	-	0.059	0	-	-
Lead (Pb) standard	-	-	-	0.054	0
Chromium (Cr)	0.010	0.059	-0.53	0.053	-2.3
	0.020	0.059	-0.80	0.053	-2.6
	0.10	0.059	-0.98	0.052	-3.2
	0.20	0.057	-3.8	0.052	-3.3
	1.0	0.056	-5.1	0.050	-7.8
	2.0	0.054	-7.9	0.047	-13
	10	0.037	-36	0.032	-40
	20	0.024	-58	0.018	-64

Table 4.7 The percentage interferences of manganese were studied in the determination of 0.05 mg L^{-1} Pb (II) and Cd (II).

Heavy metal	mg /L	Cadmium (Cd) standard		Lead (Pb) standard	
		mg/L	Interference (%)	mg/L	Interference (%)
Cadmium (Cd) standard	-	0.054	0	-	-
Lead (Pb) standard	-	-	-	0.050	0
Manganese (Mn)	0.010	0.054	-0.046	0.050	0
	0.020	0.053	-2.3	0.050	0
	0.10	0.053	-3.3	0.050	0
	0.20	0.052	-4.0	0.052	2.7
	1.0	0.052	-4.0	0.056	10
	2.0	0.051	-6.8	0.057	13
	10	0.046	-17	0.063	23
	20	0.036	-36	0.074	43

CHAPTER V

CONCLUSION AND FUTURE WORK

5.1 Conclusion

A flow-based analysis system with spectrophotometric detection of Pb (II) and Cd (II) was developed. Quercetin and CTAB were used as complexing agent and surfactant, respectively. The optimal condition of the complexes reactions was studied by a sequential injection analysis (SIA) system. The absorbance of the yellow-colored complexes (Pb-Querc and Cd-Querc) was measured in Tris(hydroxymethyl)aminomethane buffer medium (pH 8.9) at 440 and 450 nm, respectively. The results indicated that a concentration of 800 mg L^{-1} quercetin reagent in solution with $3.0 \times 10^{-3} \text{ mol L}^{-1}$ CTAB is an optimal condition. This optimal condition has also been applied with home-made spectrophotometer using a single-wavelength LED (Light-emitting diode) as the light source for the determination of Cd (II) and Pb (II) in wastewater. The home-made spectrometer is not only smaller and more cost-effective than the commercial instrument but also provides the acceptable recoveries. The LED light source used in the home-made spectrometer is a single wavelength LED. It can only be operated at 450 nm without using any monochromator. This approach offer a compact size of a whole spectrometer but an intensity of the LED source still be limited. That is a main reason why sensitivity of the home-made device is lower than a commercial instrument.

5.2 Suggestion for future work

Future works should focus on the home-made spectrometer. Circuit board modification including detector upgrade could provide a better sensitivity. Operational amplifiers (Op Amps) should be modified to improve a signal-to-noise (S/N) ratio. On-line sample preparation technique is another interesting approach. Selected solid-support or a commercial available solid phase micro extraction (SPME)

cartridge with a proper eluent could help increase a sample concentration before entering to the holding coil. A specific porous membrane or hollow fiber membrane could eliminate some interference and improve LOD, LOQ of the whole method.



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APPENDIX A

Optimization of spectrophotometric measurements

The choice of complexing agent

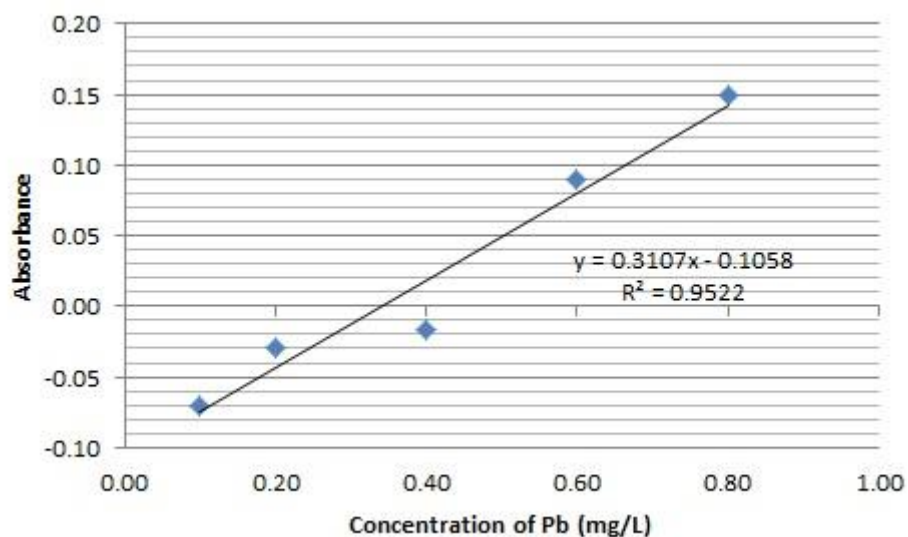


Figure A1 Linear range of Pb-dithizone at pH 3.2

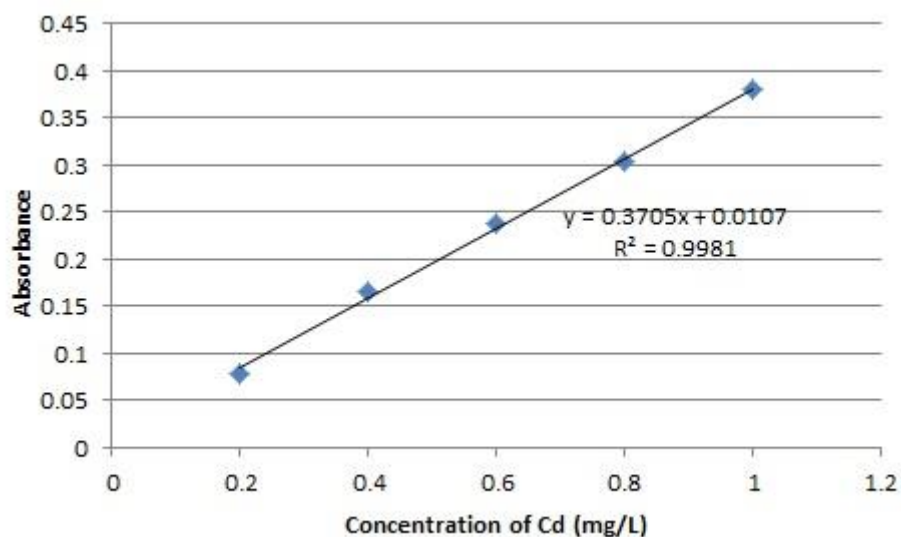


Figure A2 Linear range of Cd-dithizone at pH 3.2

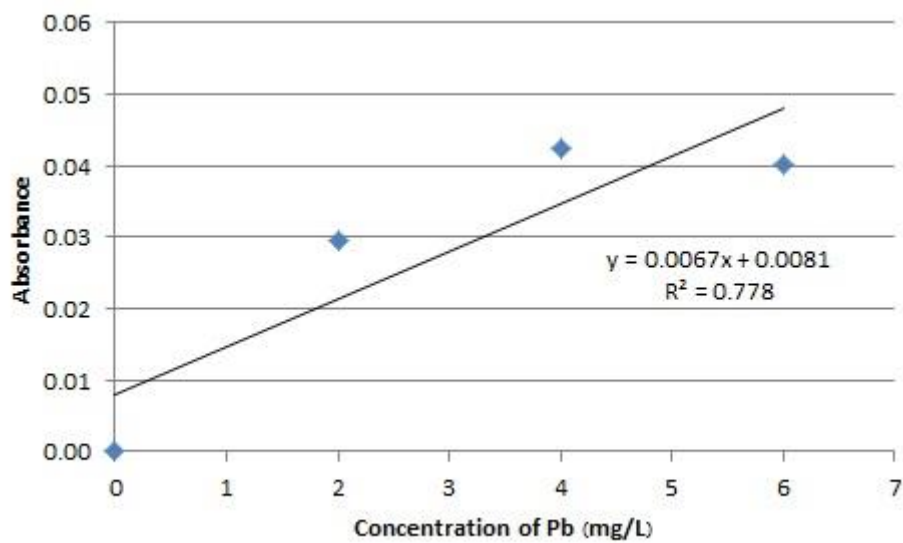


Figure A3 Linear range of Pb-alizarin red S at pH 5

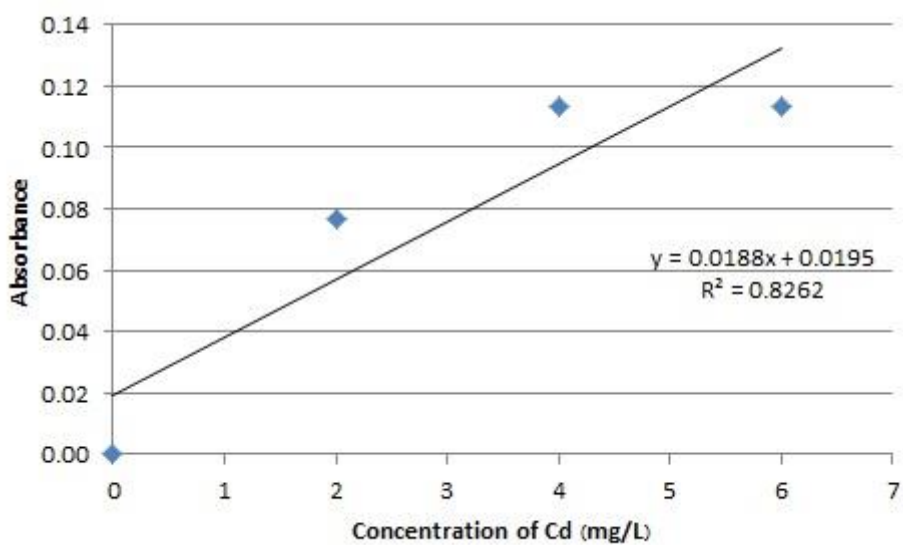


Figure A4 Linear range of Cd-alizarin red S at pH 5

The pH of Tris(hydroxymethyl)aminomethane buffer solution

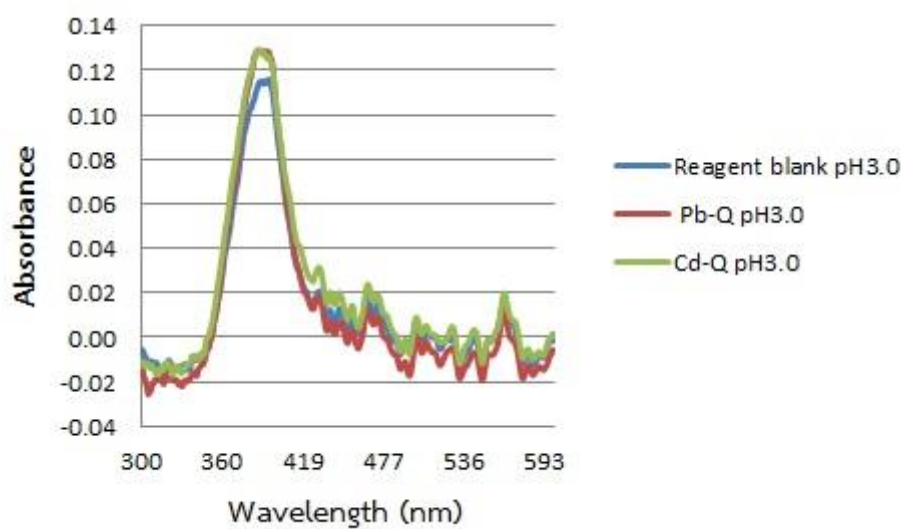


Figure A5 Spectrum of Cd-Querc and Pb-Querc at pH 3.0

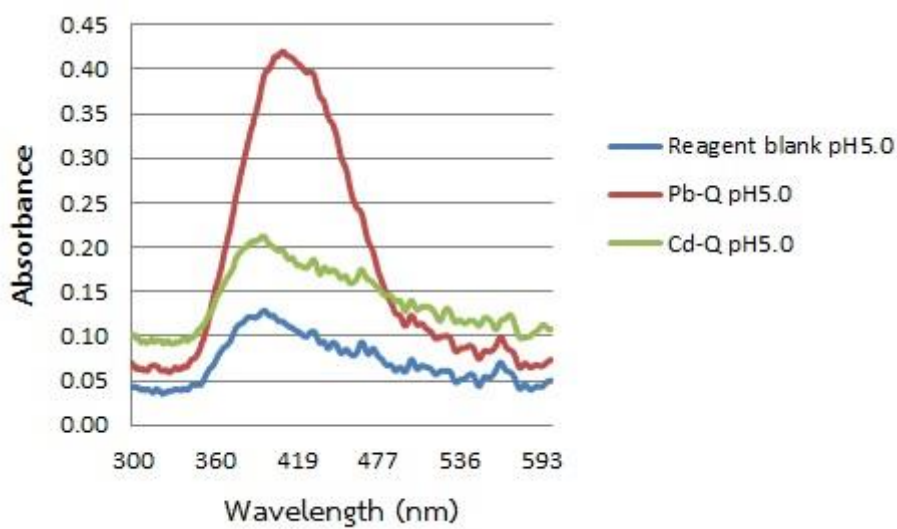


Figure A6 Spectrum of Cd-Querc and Pb-Querc at pH 5.0

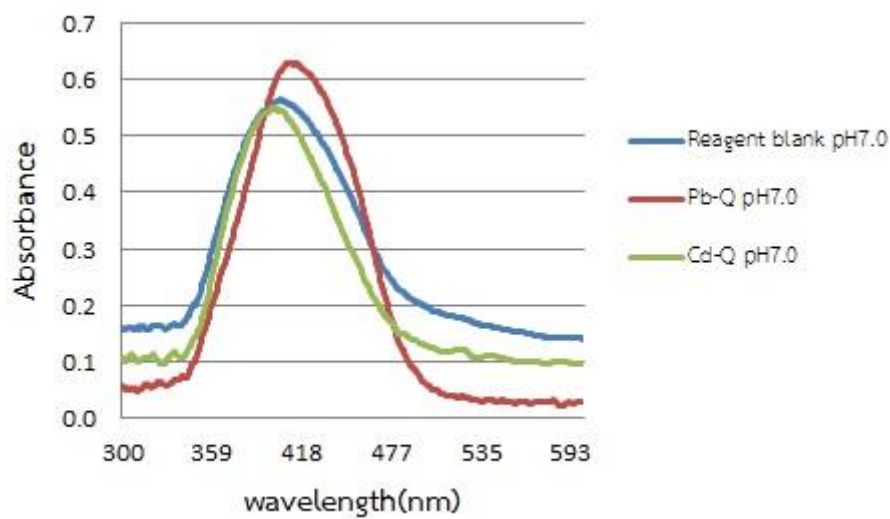


Figure A7 Spectrum of Cd-Querc and Pb-Querc at pH 7.0

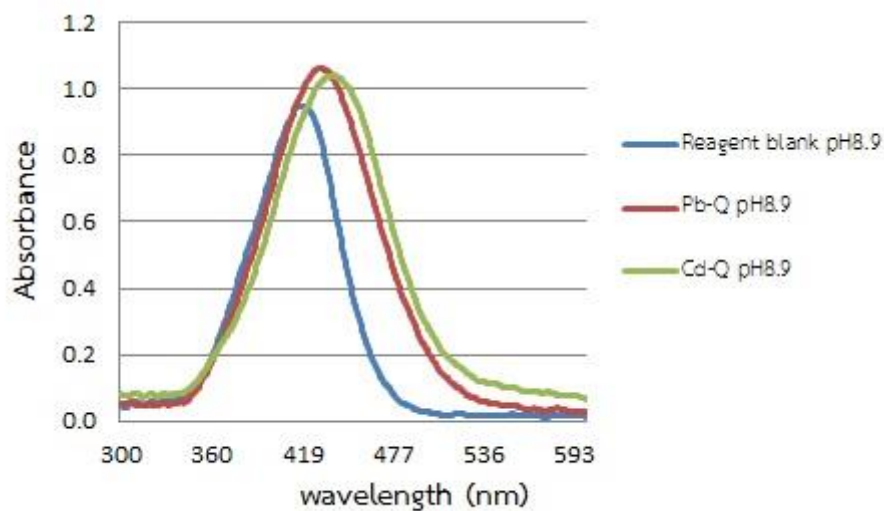


Figure A8 Spectrum of Cd-Querc and Pb-Querc at pH 8.9

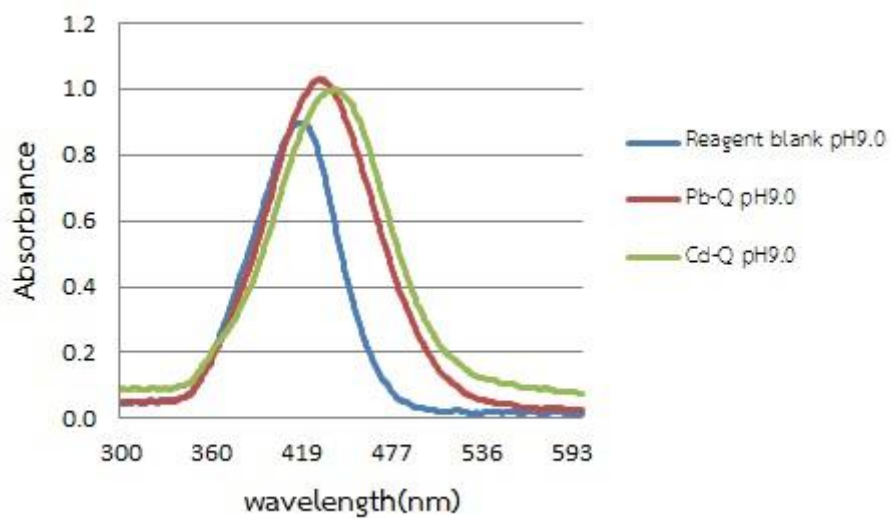


Figure A9 Spectrum of Cd-Querc and Pb-Querc at pH 9.0

The concentration of CTAB

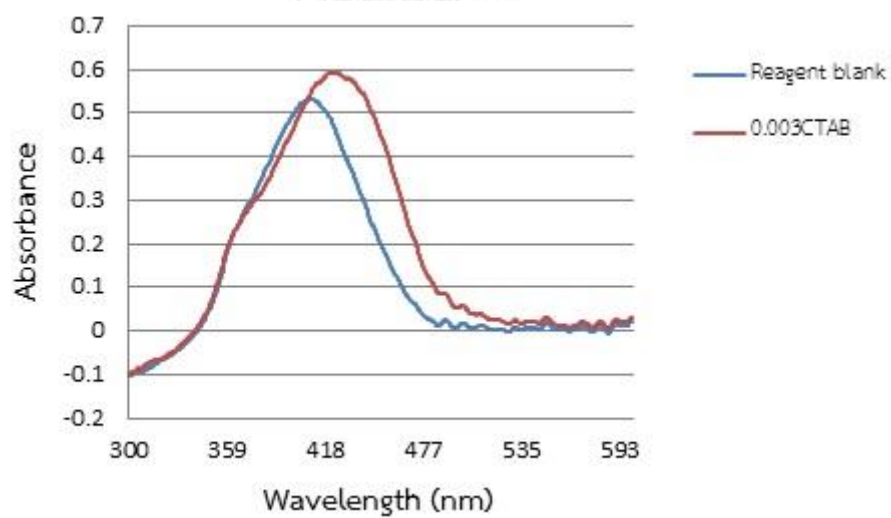


Figure A10 Spectrum of Cd-Querc using $3.0 \times 10^{-3} \text{ mol L}^{-1}$ CTAB at pH 8.9

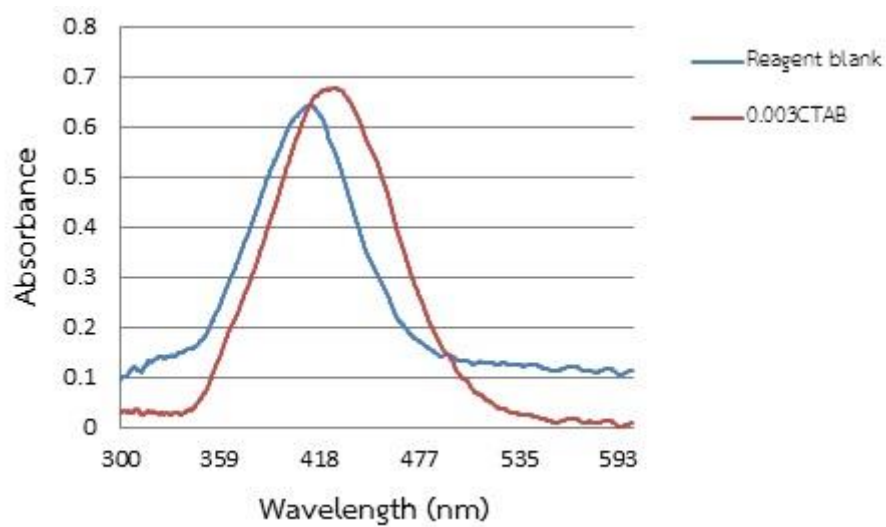


Figure A11 Spectrum of Pb-Querc using $3.0 \times 10^{-3} \text{ mol L}^{-1}$ CTAB at pH 8.9.



APPENDIX B
Analytical performance

Reproducibility by using SIA

Table B1 The reproducibility of lead standard solution with 800 mg L^{-1} quercetin at 440 nm by using SIA.

Replication of 0.02 mg/L Cd standard	Concentration
1	0.023
2	0.024
3	0.023
4	0.022
5	0.023
6	0.022
7	0.023
SD	0.00080
Average	0.023
RSD (%)	3.6
LOD (mg/L)	0.0020
LOQ (mg/L)	0.0080

Table B2 The reproducibility of cadmium standard solution with 800 mg L^{-1} quercetin at 450 nm by using SIA.

Replication of 0.02 mg/L Pb standard	Concentration
1	0.0213
2	0.0218
3	0.0211
4	0.0228
5	0.0207
6	0.0218
7	0.0219
SD	0.000700
Average	0.0220
RSD (%)	3.40
LOD (mg/L)	0.00200
LOQ (mg/L)	0.00700

Reproducibility by using cost-effective instrument

Table B3 The reproducibility of lead standard solution with 800 mg L^{-1} quercetin at 450 nm by using developed device.

Replication of 0.02 mg/L Pb standard	Concentration
1	0.038
2	0.028
3	0.037
4	0.038
5	0.032
6	0.029
7	0.031
SD	0.0037
Average	0.033
RSD (%)	11
LOD (mg/L)	0.011
LOQ (mg/L)	0.037

Table B4 The reproducibility of cadmium standard solution with 800 mg L^{-1} quercetin at 450 nm by using developed device.

Replication of 0.02 mg/L Cd standard	Concentration
1	0.021
2	0.030
3	0.023
4	0.023
5	0.028
6	0.029
7	0.024
SD	0.0037
Average	0.025
RSD (%)	14
LOD (mg/L)	0.011
LOQ (mg/L)	0.037

APPENDIX C

Interference

Linear range

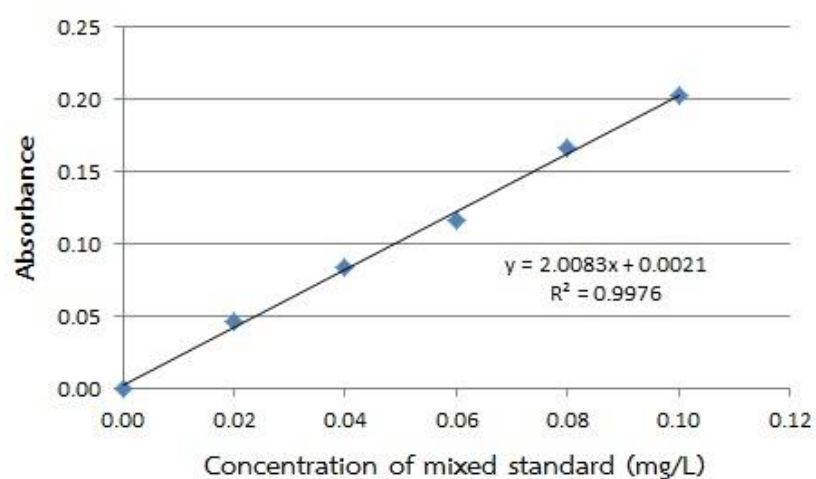


Figure C1 Calibration curve of cadmium standard solution with arsenic standard solution in concentration range 0.02-0.1 mg L⁻¹.

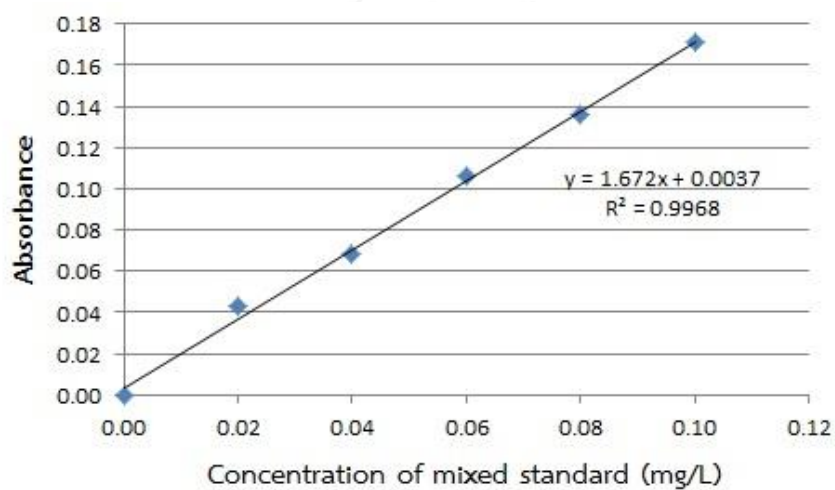


Figure C2 Calibration curve of lead standard solution with arsenic standard solution in concentration range 0.02-0.1 mg L⁻¹.

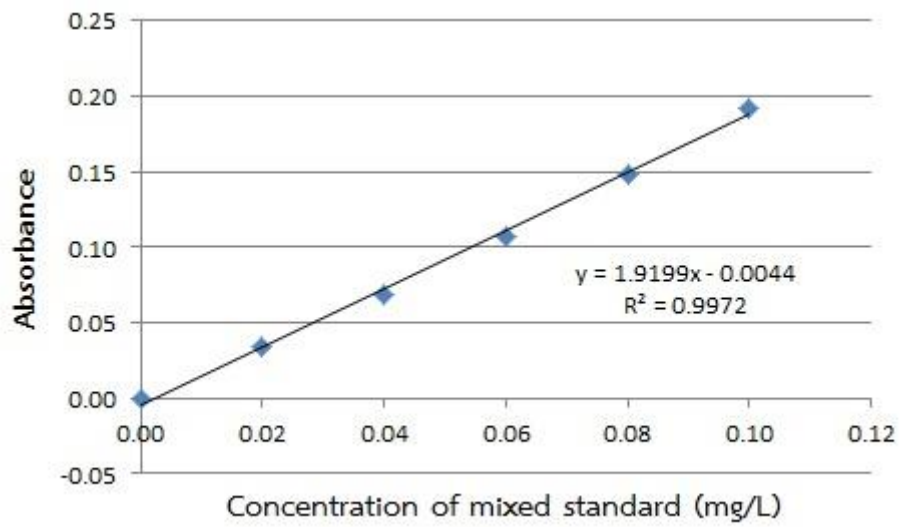


Figure C3 Calibration curve of cadmium standard solution with mercury standard solution in concentration range 0.02-0.1 mg L⁻¹.

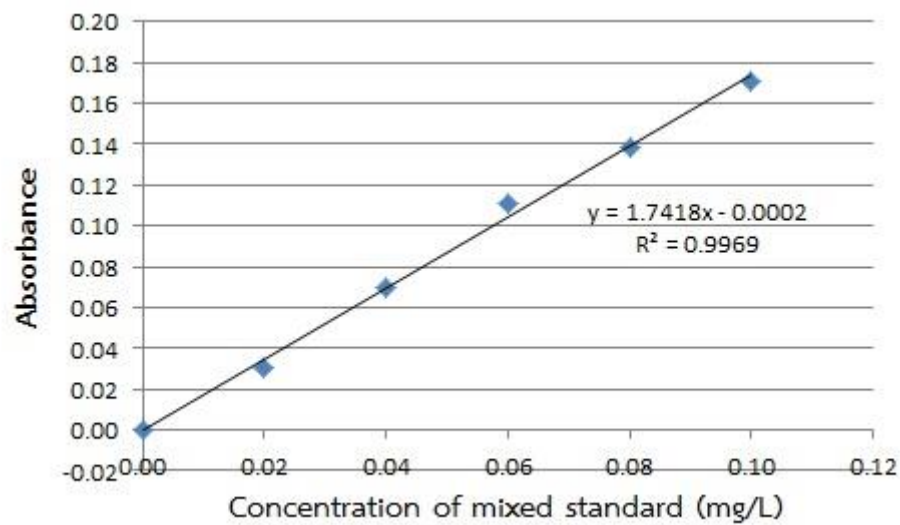


Figure C4 Calibration curve of lead standard solution with mercury standard solution in concentration range 0.02-0.1 mg L⁻¹.

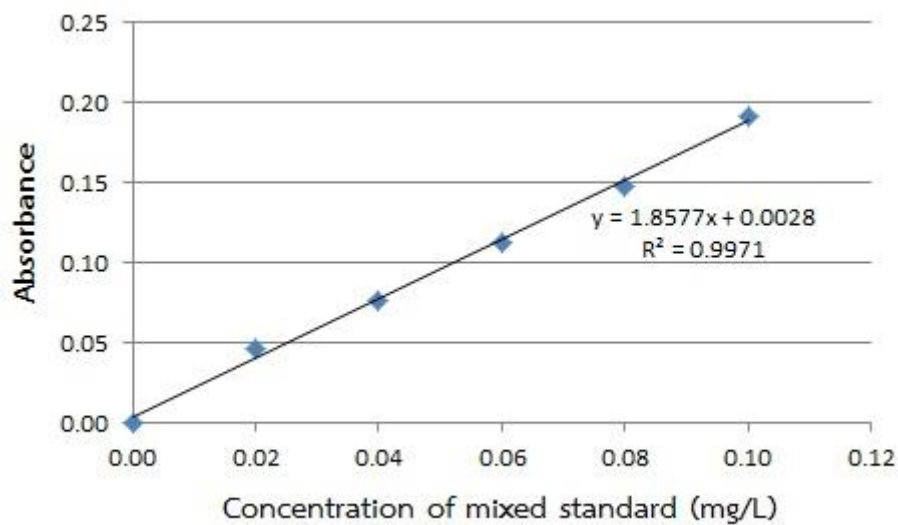


Figure C5 Calibration curve of cadmium standard solution with chromium standard solution in concentration range 0.02-0.1 mg L⁻¹.

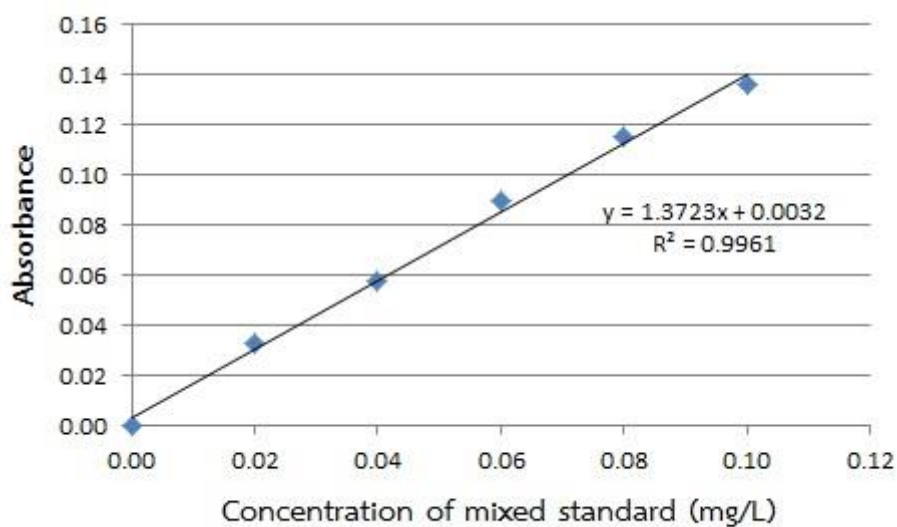


Figure C6 Calibration curve of lead standard solution with chromium standard solution in concentration range 0.02-0.1 mg L⁻¹.

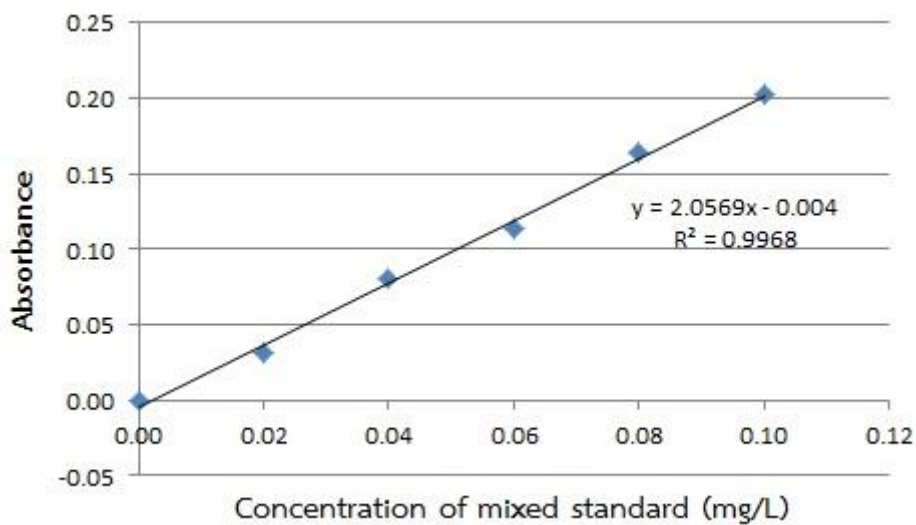


Figure C7 Calibration curve of cadmium standard solution with manganese standard solution in concentration range $0.02-0.1 \text{ mg L}^{-1}$.

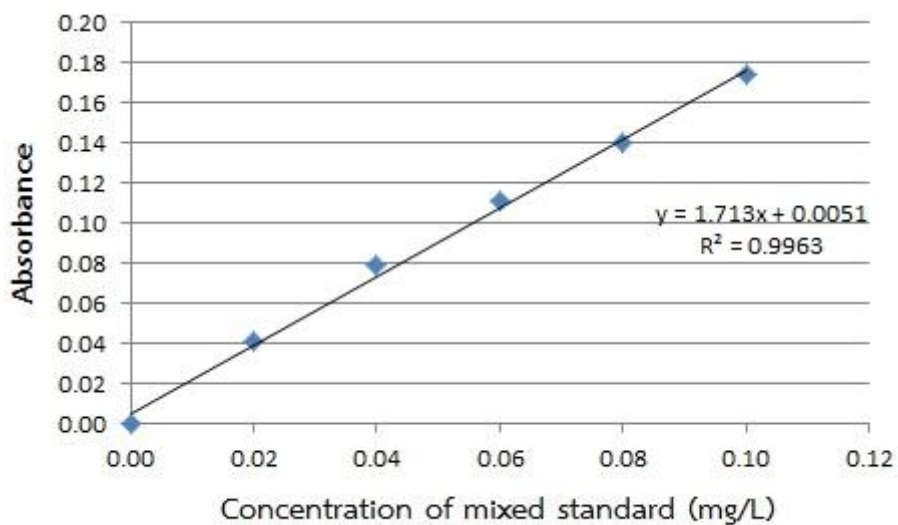


Figure C8 Calibration curve of lead standard solution with manganese standard solution in concentration range $0.02-0.1 \text{ mg L}^{-1}$.

APPENDIX D

Light-emitting diode (LED)

The spectrum range of blue LED

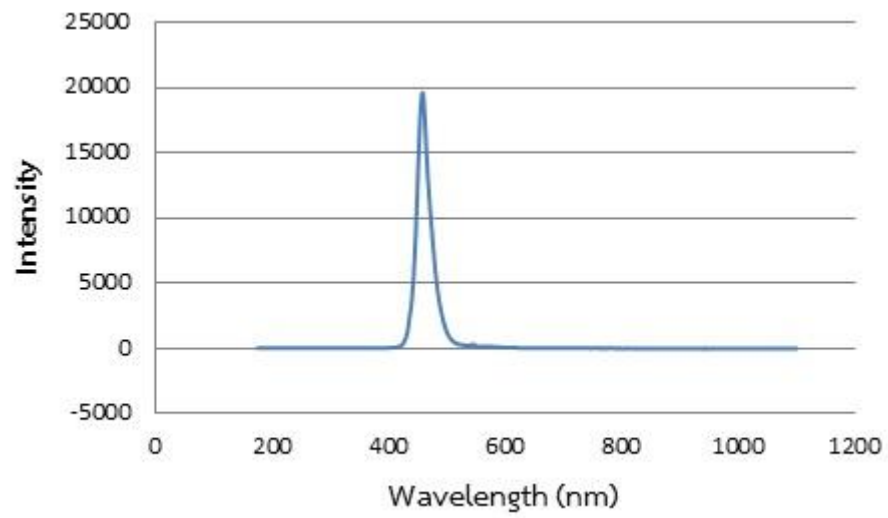


Figure D1 The spectrum of blue LED



VITA

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Proceeding:

Metida Srikullaphat, Passapol Ngamukot “Development of a portable spectrophotometric device with the flow-based analysis system for detection of heavy metal ions” Proceedings of Pure and Applied Chemistry International Conference 2016, Bangkok, BITEC, Bangkok, Thailand, February 9-11, 2016, pp 20-25.