Table 1. Reduction efficiency of mixed standard of nitrate, nitrite and ammonium being detected as ammonium nitrogen (N-NH_4^+) by FIC method.

Mixed solution (mgL^{-1} N)			**Detected (mgL^{-1})***	**Reduction (%)**
NH_4^+	**NO_2^-**	**NO_3^-**		
-	-	15.00	16.34 ± 0.60	109
-	-	30.00	30.48 ± 0.07	102
-	-	45.00	38.60 ± 0.01	86
-	-	60.00	49.16 ± 0.72	82
10.00	-	20.00	29.01 ± 1.23	97
10.00	20.00	-	29.11 ± 0.37	97
10.00	10.00	10.00	27.61 ± 0.45	92

*mean of triplicate injections

Table 2

Table 2. Analytical characteristics of flow injection conductometric system for determination total inorganic nitrogen in soil.

Category	**Value**
Precision(RSD)	1.06 (30 mgL^{-1} n=11)
LOD	0.06 mgL^{-1}
LOQ	0.19 mgL^{-1}
Linear range	2.00-60.00 mgL^{-1}
NaOH consumption	0.20 $g.sample^{-1}$
Sample throughput	20 $samples.h^{-1}$
Tolerance limit (mgL^{-1})	› 200 mgL^{-1} positive peak (Ca^{2+}) › 500 mgL^{-1} negative peak (Mg^{2+}) and positive peak (SO_4^{2-}) › 1000 mgL^{-1} (Cl^-, CO_3^{2-}, Cu^{2+}, Fe^{3+}, Zn^{2+})

Table 3

Table 3. Content of TIN (determined by FIC method) and TKN (determined by modified Kjeldahl method) in the soil samples.

Sample	**TIN*** **(mgL^{-1})**	**TKN*** **(mgL^{-1})**
1	9.33 ± 0.26	24.81 ± 1.39
2	10.18 ± 0.20	13.07 ± 0.39
3	11.45 ± 0.61	14.92 ± 0.64
4	16.37 ± 0.46	36.30 ± 0.51
5	10.87 ± 0.15	38.38 ± 0.47
6	15.09 ± 0.14	32.13 ± 0.41
7	6.69 ± 0.05	23.83 ± 0.20
8	3.84 ± 1.02	3.10 ± 0.52
9	7.44 ± 0.12	27.67 ± 0.73
10	15.93 ± 0.33	56.33 ± 019

* mean of triplicate measurements

Figure 1
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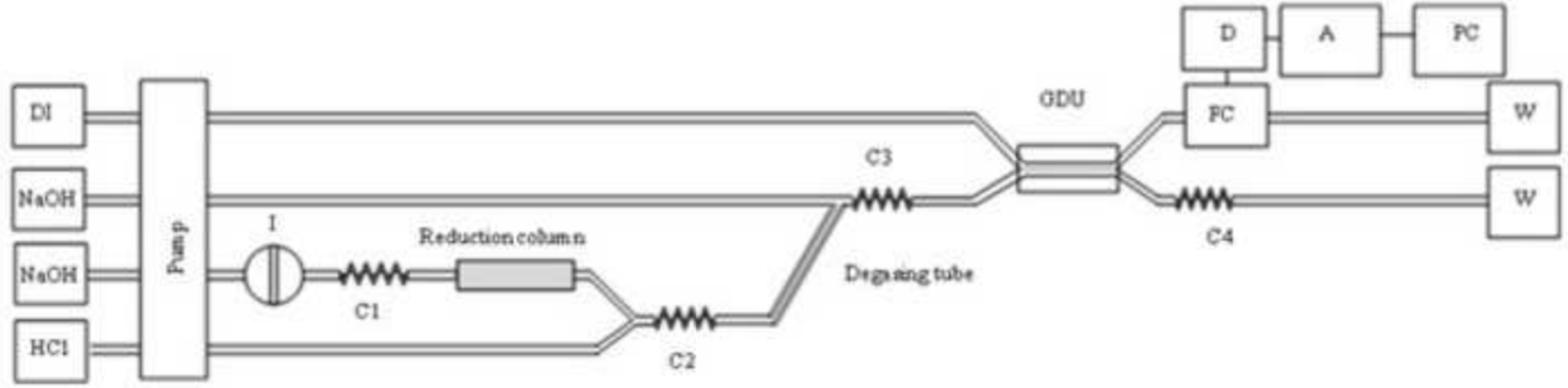

Figure 2

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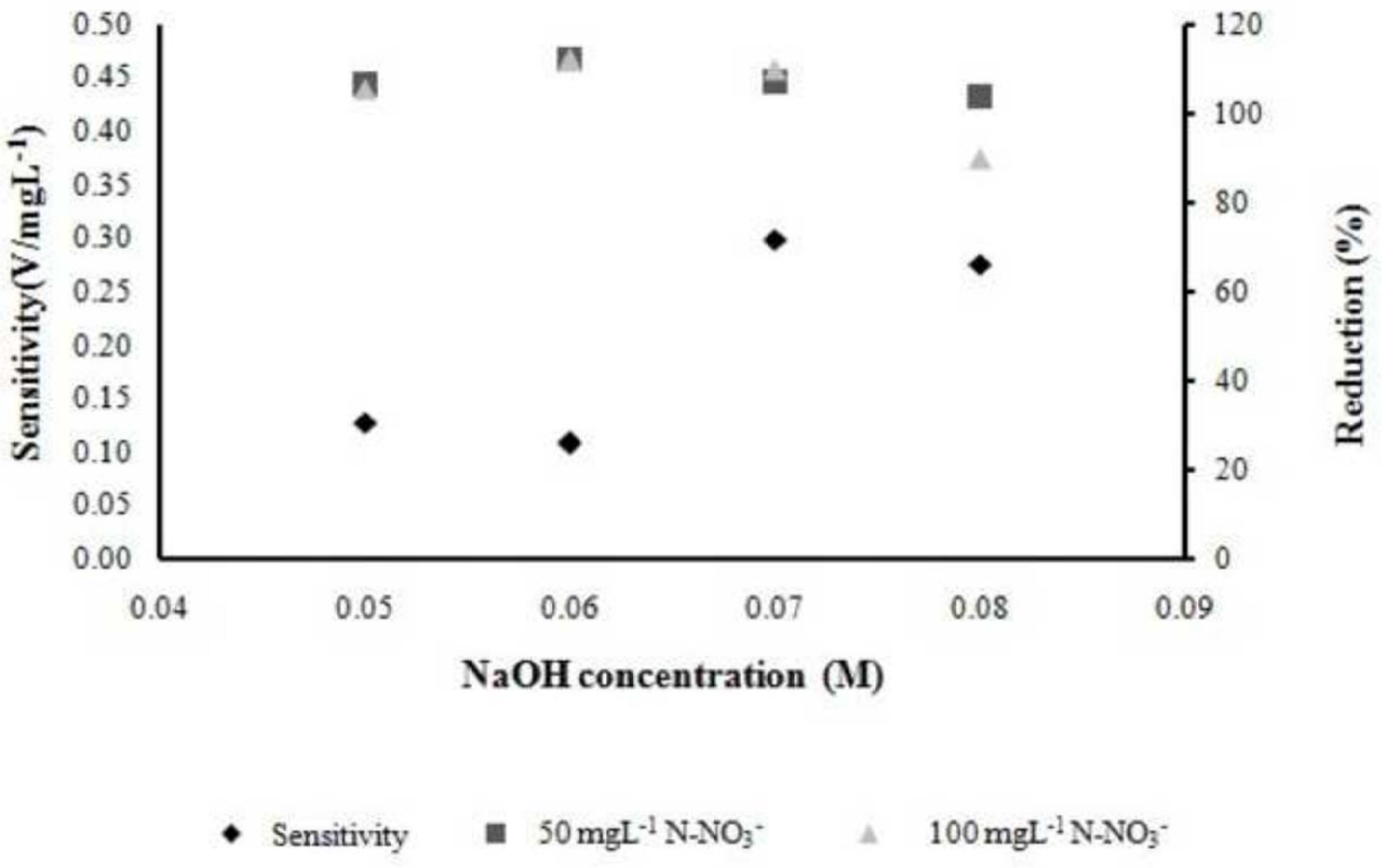
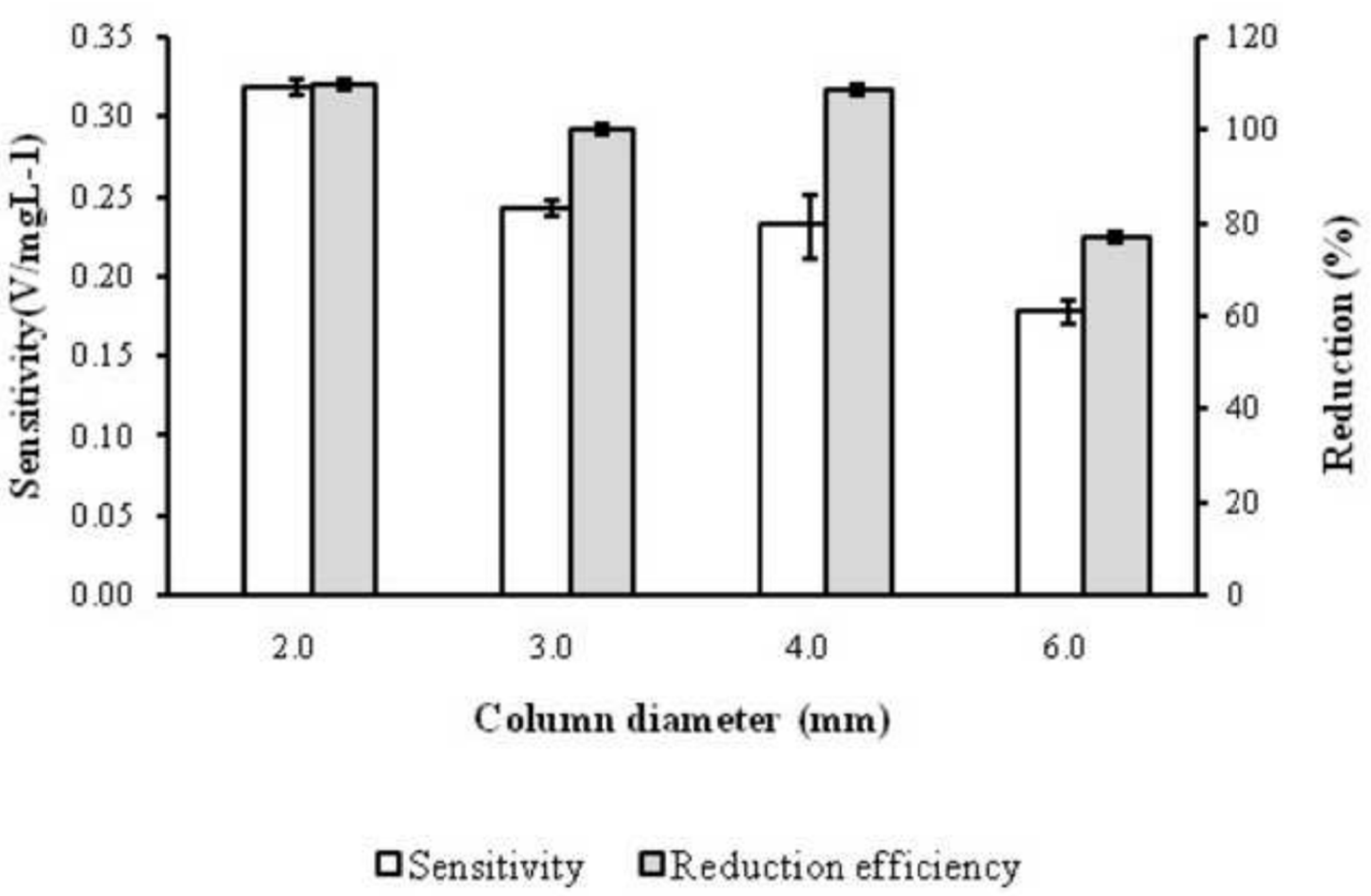


Figure 3


Sensitivity(V/mgL-1)
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0.25
0.20
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0.05
0.00
Reduction (%)
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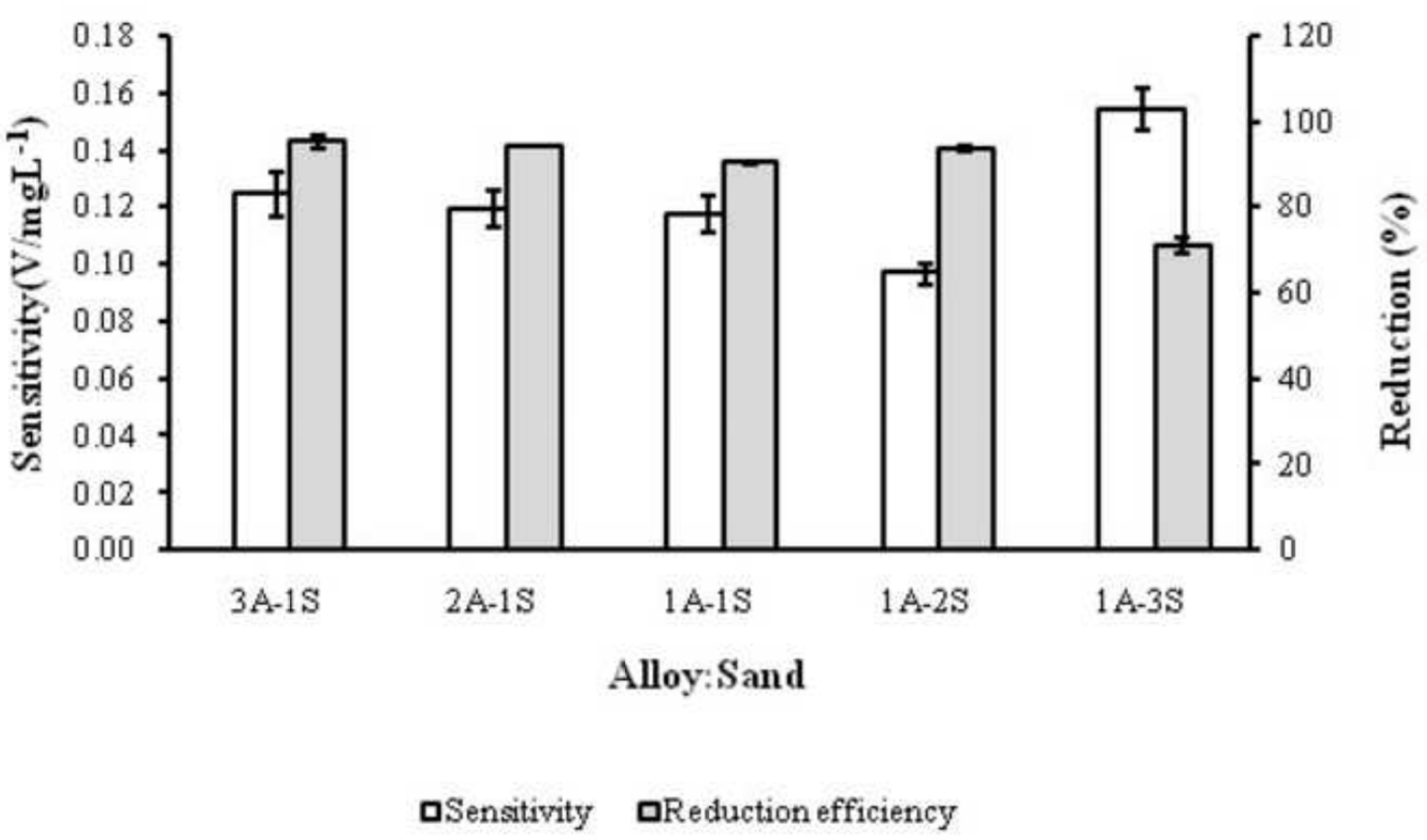
100
80
60
40
20
0
2.0
3.0
4.0
6.0
Column diameter (mm)
☐ Sensitivity
▨ Reduction efficiency

Figure 4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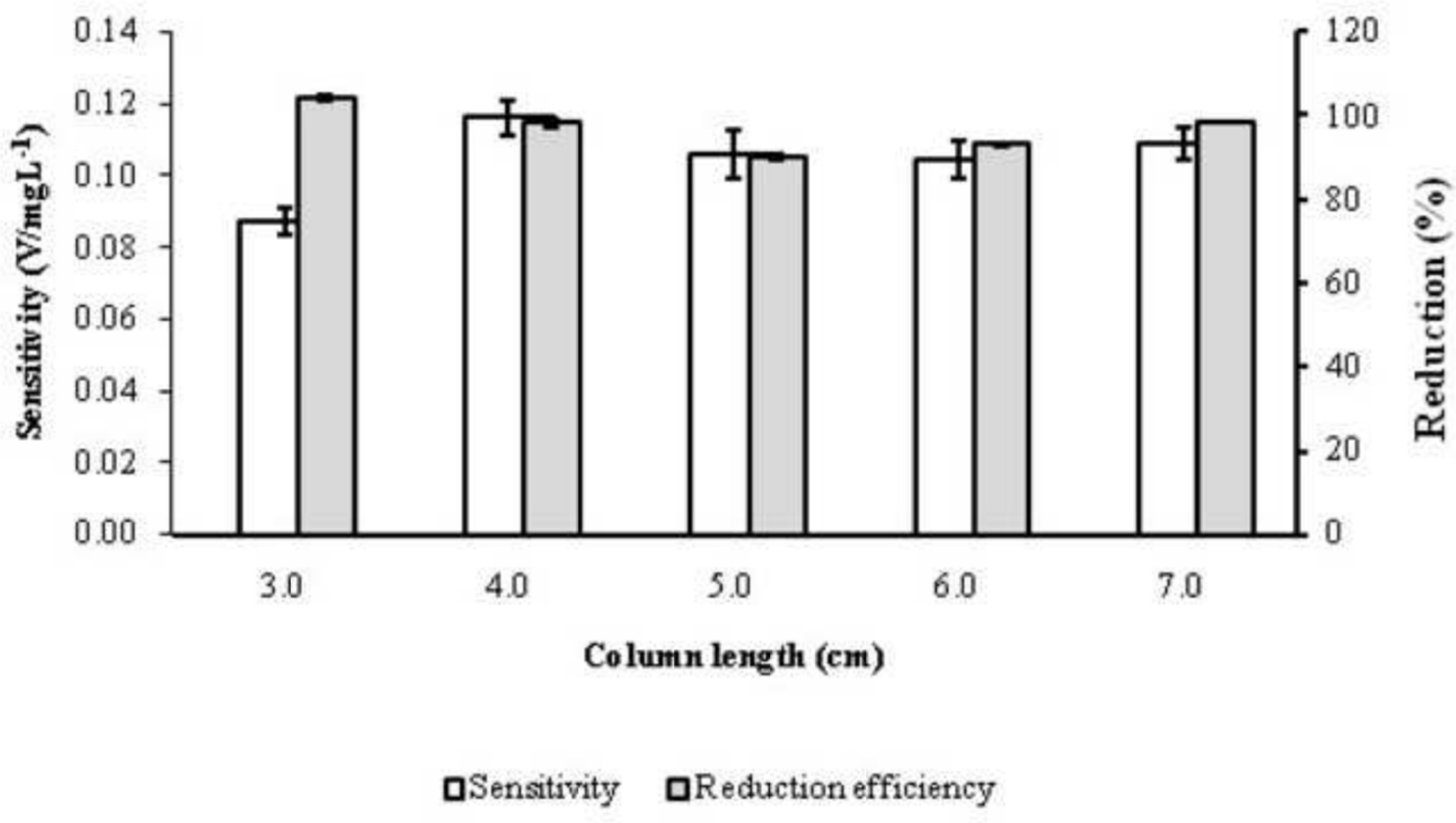
0.08
0.06
0.04
0.02
0.00
120
100
80
60
40
20
0
Reduction (%)
3A-1S
2A-1S
1A-1S
1A-2S
1A-3S
Alloy:Sand
□Sensitivity
▨Reduction efficiency

Figure 5


Sensitivity (V/mgL-1)
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Reduction (%)
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100
80
60
40
20
0
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5.0
6.0
7.0
Column length (cm)
☐ Sensitivity
▨ Reduction efficiency

Figure 6

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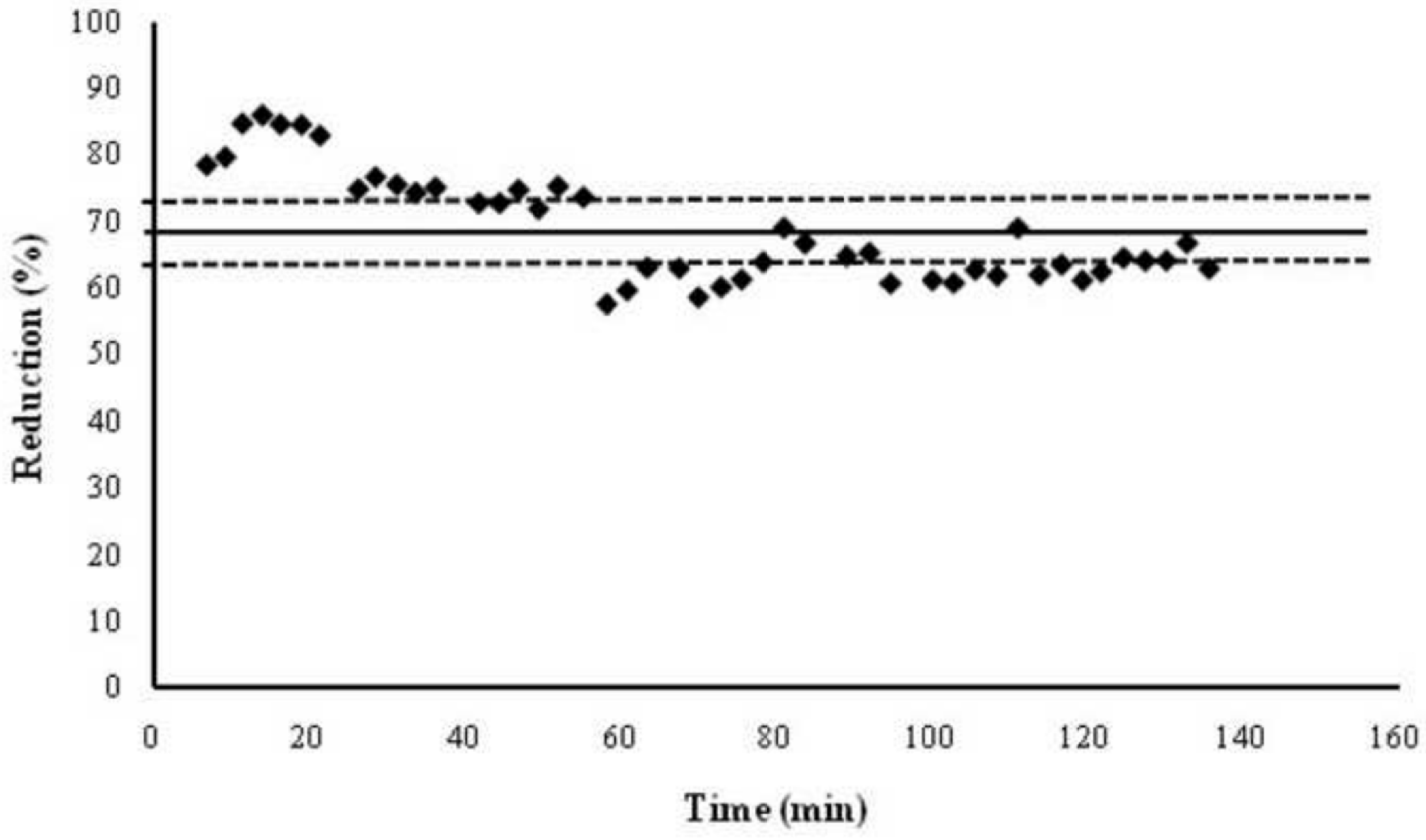
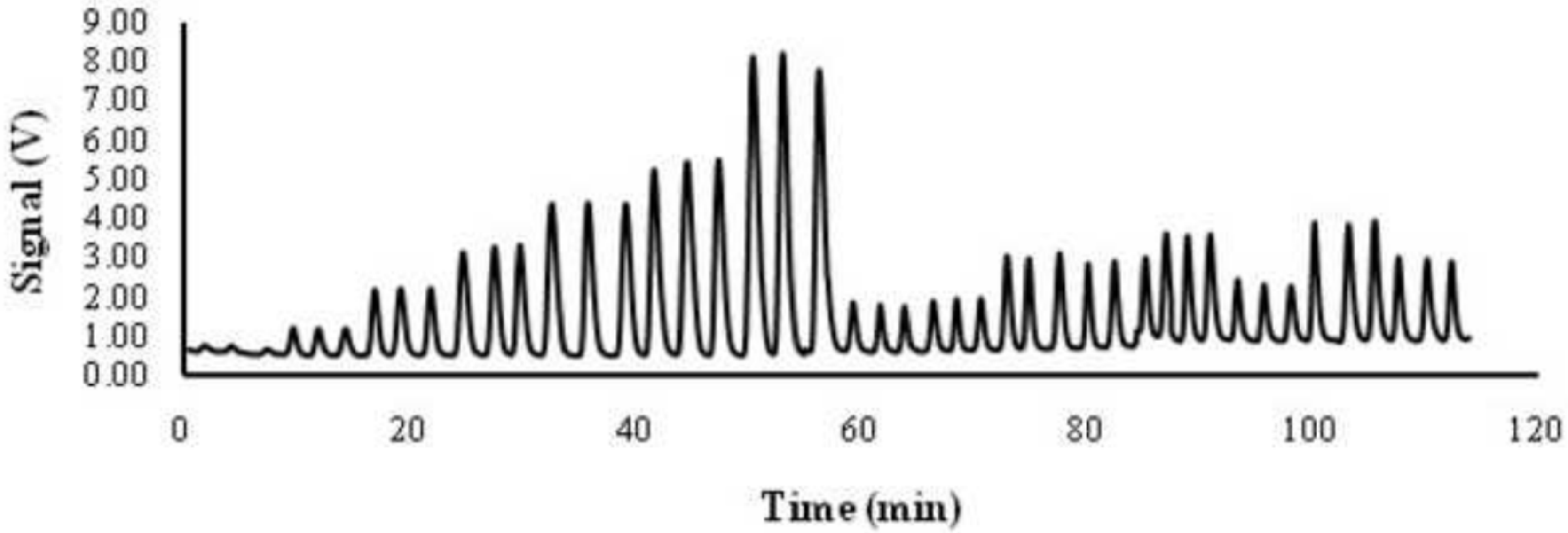


Figure 7


Signal (V)
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8.00
7.00
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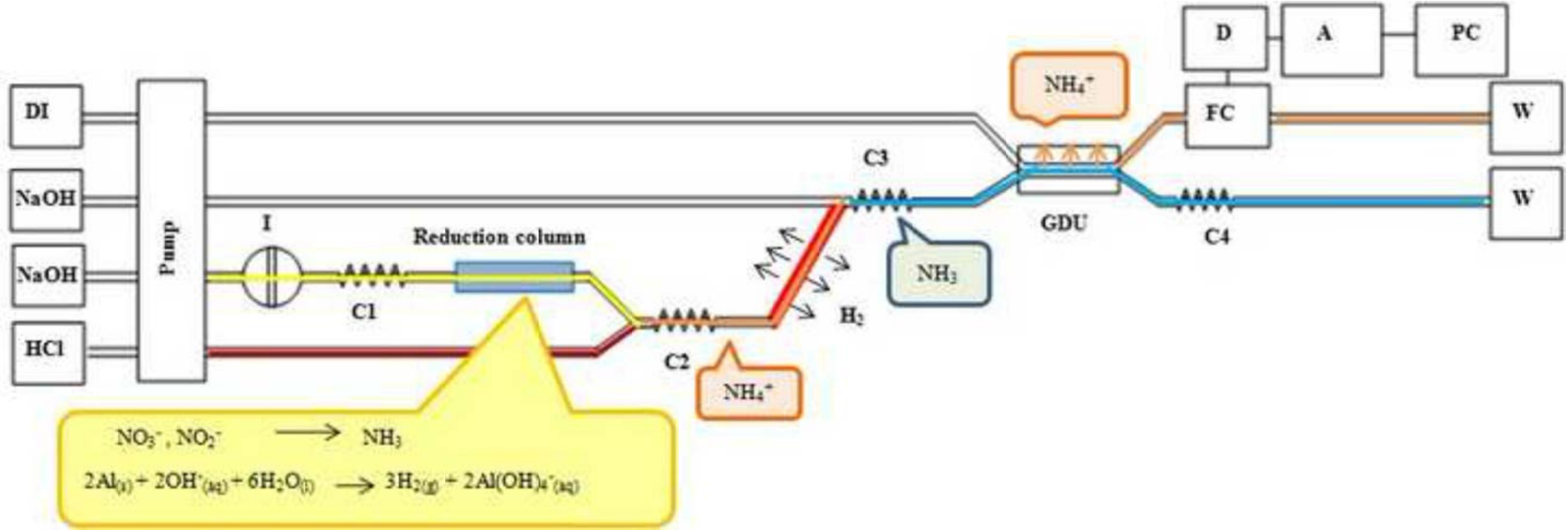
4.00
3.00
2.00
1.00
0.00
0
20
40
60
80
100
120
Time (min)


DI
NaOH
NaOH
HCl
Pump
I
C1
Reduction column
C2
NH_4^+
H_2
C3
NH_3
NH_4^+
GDU
FC
D
A
PC
C4
W
W
$NO_3^-, NO_2^- \longrightarrow NH_3$
$2Al_{(s)} + 2OH^-_{(aq)} + 6H_2O_{(l)} \longrightarrow 3H_{2(g)} + 2Al(OH)_4^-{}_{(aq)}$

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Greener Anodic Stripping Voltammetric Method Employing *In Situ* Plated Bismuth Film Electrode for Determination of Cadmium and Lead

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Abstract

In this research, an in-line preparation of in situ plated bismuth film on glassy carbon electrode was employed for anodic stripping voltammetric determination of cadmium and lead. This electrode is more environmentally friendly than the commonly used mercury working electrode. Bismuth film electrode could be prepared by three procedures, namely, in situ plating, ex situ plating and modifying the substrate with bismuth compounds. Although the first procedure is the simplest and cheapest, in batch wise analysis it usually gives irreproducible results. In the flow system, standard or sample solution was mixed on-line with Bi(III) plating solution before entering a voltammetric cell. Then, the deposition of the metals was carried out by applying a constant potential of -1.50 V versus Ag/AgCl to the working electrode. Next, a square wave waveform was scanned from -1.50 to 0.60 V and a voltammogram was recorded. The linear ranges were 2 – 40 μg/L for both cadmium and lead. Relative standard deviations were 0.76-1.57% for 11-replicate injections of 5, 20 and 40 μg/L of both Cd(II) and Pb(II). Detection limits of 1.4 and 0.9 μg/L for Cd and Pb respectively. The method has percentage recoveries for the determination of those metals in range of 92.8 – 102.8. A sample throughput was 13 h^{-1}. The proposed method provided high sensitivity and convenient operation for the determination of cadmium and lead at trace level. Application for analysis of real water samples was performed.

Keywords: Flow injection, Anodic stripping voltammetry, Bismuth film, Trace metals

1. Introduction

Heavy metals such as cadmium and lead are used mainly in metal processing industries. They are found naturally in small quantities in air, water and soil. Their toxicity are well known such as cadmium seriously affects on health, causing “Itai-Itai” disease and lead interrupts physical development and nervous system. Many techniques are employed for trace metals determination such as atomic absorption spectrometry (AAS), inductively coupled plasma-optical emission spectroscopy (ICP-OES), inductively coupled plasma-mass spectrometry (ICP-MS), atomic fluorescence spectrometry (AFS) and electroanalytical techniques such as anodic stripping voltammetry (ASV). ASV can be considered as the most powerful technique for in-field analysis, due to its excellent detection limit, high sensitivity, capacity to multi element determination, high speed, simplicity and relatively low cost. Mercury electrode provides a wide cathodic potential limit for reduction of several metals and allows the formation of amalgams for accumulative preconcentration of the metals leading to very high sensitivity and reproducibility for ASV determination. However, due to toxicity of the mercury [1-2], mercury electrode is recently limited use for voltammetric analysis and other alternative electrodes are being developed.

Many studies have demonstrated the applicability of bismuth film electrode (BiFE) as a possible alternative for electrochemical stripping analysis of trace heavy metals, with electroanalytical behavior similar to that of mercury-based electrodes [3-5]. However, using of BiFE in batch voltammetry is tedious and not effective. Low reproducibility and carry over are usually concerned. The method that combined stripping voltammetry with flow system has been proposed. It provides significant advantages over batch analysis, such as offering high level of automation, fast analysis, less risk of contamination and better reproducibility [6-9].

Aims: In this work, the flow based anodic stripping voltammetric system has been developed for determination of cadmium and lead with using of BiFE as a working electrode. The flow system improves the analytical performance of ASV for determination of cadmium and lead (such as high reproducibility and sensitivity and more convenient, rapid, inexpensive and environmental friendly operation). The real application of the proposed method to the analysis of water samples is demonstrated.

2. Materials and Methods

2.1 Chemicals

All chemical used are analytical reagent grade and all standard and reagent solution were prepared with ultrapure water (Milli Q water, resistivity of 18.2 MOhm cm^{-1}) obtained from a Millipore water purification system (Millipore, Sweden).

A stock solution of Bi(III) (1000 mg l^{-1}) was prepared by dissolving 0.2356 g of $Bi(NO_3)_3.5H_2O$ in 0.5 M HNO_3 solution and adjusting to the volume of 100 ml. A stock solution of Cd(II) (1000 mg l^{-1}) was prepared by dissolving 0.2032 g of $CdCl_2.2H_2O$ in 0.1 M HCl solution and adjusting to the volume of 100 ml. A stock solution of Pb(II) (1000 mg l^{-1}) was prepared by dissolving 0.3128 g of $Pb(NO_3)_2$ in 0.1 M HNO_3 solution and adjusting to the volume of 100 ml. Working standard solutions of Cd(II) and Pb(II) were daily prepared by appropriate dilution of the stock standard solution with a 0.01 M nitric acid solution pH 2. A 0.10 M acetate buffer pH 4.5 was prepared by dissolving 1.20 g of $CH_3COONa{\cdot}3H_2O$ in water and adding 1 ml of CH_3COOH, then adjusting to the volume of 250 ml with water.

2.2 Instrument Set Up

The proposed flow based voltammetric analysis system with bismuth film electrode (VA-BiFE) is shown in Fig. 1. It consisted of peristaltic pump (Ismatec, Switzerland) with pump tubing, two 3-way solenoid valves (Biochemvalve, USA) for injection in line, a mixing coil (PTFE, i.d. 0.05 mm), and a voltammograph (VA 757, Metrohm, Switzerland) with an electrochemical flow-cell. The electrochemical flow-cell (cross-flow cell, Model MF- 1093, BAS, USA) composes of a 6 mm diameter glassy carbon working electrode (GCE), a Ag/AgCl reference electrode (RE) and a stainless steel auxiliary electrode (AE). The system was controlled by a personal computer using a software program written in-house.

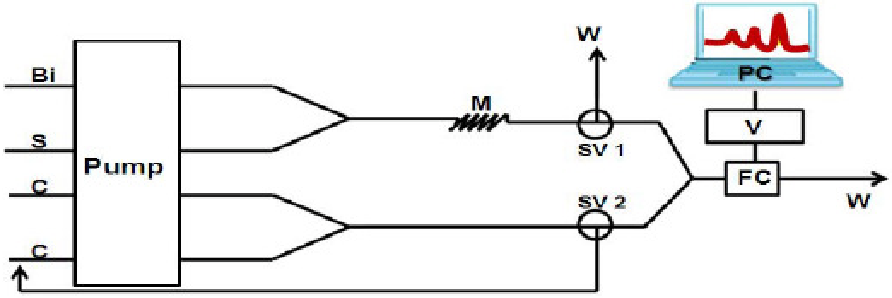

Figure 1. Manifold of flow based anodic stripping voltammetric system for determination of cadmium and lead; S = standard/sample, Bi = Bi(III) plating solution, C= cleaning solution (1 M HNO3), Pump = peristaltic pump, M = mixing coil, SV 1-2 = solenoid valves, FC= electrochemical flow cell, V= voltammetric analyzer, PC= personal computer, W = waste

2.3 Analytical Procedure:

In the flow system, standard or sample solution was mixed on-line with Bi(III) plating solution before entering a voltammetric cell. When the analyte metals reached the flow cell was applying potential of -1.50 V versus Ag/AgCl to the glassy carbon electrode and the flow was briefly stop to the stripping. Next, the square wave anodic stripping voltammograms were recorded between -1.50 and 0.60 V. The electrode was cleaning by applying a potential of 0.6 V to the working electrode while nitric acid 1M was flowed through the flow cell before to the next analysis cycle. A voltammogram was obtained, which peak potential and peak current of each metal were determined. A linear calibration graph of each metal was obtained by plotting between concentration of metal and peak current.

3. Results and Discussion

3.1 Optimization of the Parameters

The parameters which affected analytical performance and the optimum condition are presented in Table 1. Under the optimal condition, the striping peak currents at peak potential of about -850 and -550 mV versus Ag/AgCl were proportional to concentrations of Pb(II) and Cd(II), respectively. The voltammograms are depicted in Fig. 2(A). The calibration graphs of Cd(II) and Pb(II) in range of 2 to 40 µg/L of each metal were constructed by plotting peak current (µA) versus concentration of metal ions (µg l^{-1}). The calibration curves exhibit excellent linearity with a correlation of 0.9977 for Cd(II) and 0.9995 for Pb(II). The peak current increased linearity with metal ion concentration, with slopes of 1.6478 µA/µg for Cd(II) and 1.2219 µA/µg for Pb(II), respectively, as shown in Fig. 2(B). This system had very good reproducibility, the relative standard deviations were 0.76-1.57% for 11 replicate injections of 5, 20 and 40 µg/L of both Cd(II) and Pb(II).

Table 1. Optimization of Flow-ASV system using bismuth film electrode as a working electrode

Parameter	Optimum condition
Concentration of acetate buffer pH 4.5	0.10 M
Concentration of Bi(III) plating solution	0.60 mgL^{-1}
Deposition potential	-1.5 V
Deposition time	150 s
Flow rate	0.5 ml/L

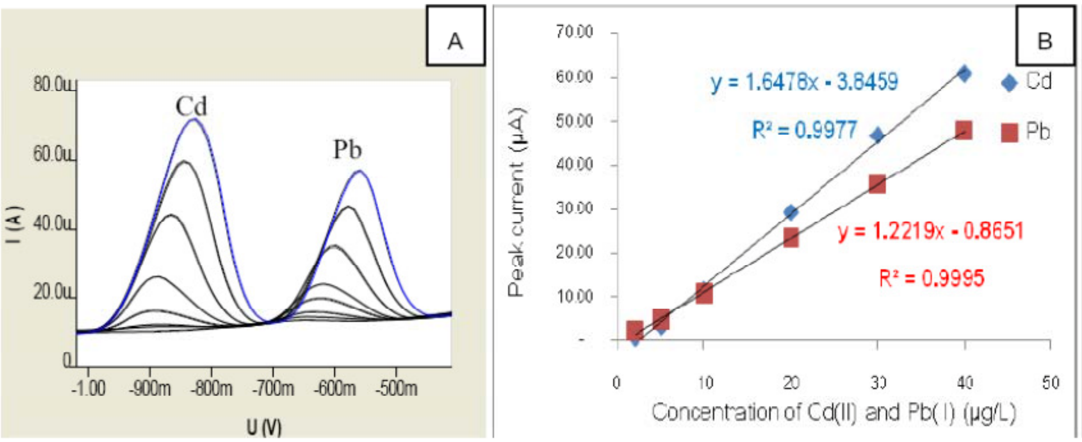

Figure 2. Square wave anodic stripping voltammograms obtained from VA-BiFE system of solution containing Cd(II) and Pb(II) of increasing concentration, from 2-40 µg/L (A) and calibration graphs of Cd(II) and Pb(II) (B).

3.2 Application to real samples

The developed method was applied to real water samples. The calibration graph was used for quantification of each metal. The results obtained for 9 water samples are summarized in Table 2. The correlation plot of the proposed method and ICP-OES method is shown in Fig. 3. It was found that the results from the proposed VA-BiFE method were in good correlation with those of ICP-OES method ($Cd(II)_{VA\text{-}BIFE} = 1.0606Cd(II)_{ICP\text{-}OES} + 0.1046$, $r^2 = 0.9779$) for Cd(II) and poor correlation with those of ICP-OES method ($Pb(II)_{VA\text{-}BIFE} = 0.7389Pb(II)_{ICP\text{-}OES} + 0.379$, $r^2 = 0.9801$) for Pb(II). Detection limits of 1.4 and 0.9 µg/L were achieved for Cd and Pb, respectively. The method has percentage recoveries for the determination of those metals in range of 92.8 – 102.8. A sample throughput was 13 h^{-1}. The method provided high sensitivity, low consumption and convenient operation for the determination of cadmium and lead at trace level. The method is simple, fast and can simultaneously determine two metals with high sensitivity. The developed method is suitable to be applied in the routine determination of cadmium and lead in water sample.

Table 2. Determination of cadmium and lead in real samples

Sample	Concentration of metals (mg/L)			
	Cd		Pb	
	ICP-OES	VA-BiFE	ICP-OES	VA-BiFE
1	0.50 ± 0.03	0.62 ± 0.00	2.06 ± 0.09	1.76 ± 0.01
2	1.08 ± 0.15	1.17 ± 0.01	4.16 ± 0.10	3.70 ± 0.01
3	2.02 ± 0.15	2.10 ± 0.01	12.06 ± 0.26	10.17 ± 0.03
4	0.71 ± 0.06	0.88 ± 0.00	8.26 ± 0.12	6.18 ± 0.00
5	1.52 ± 0.09	1.59 ± 0.01	15.53 ± 0.26	12.70 ± 0.00
6	1.82 ± 0.10	2.03 ± 0.00	5.36 ± 0.10	4.19 ± 0.04
7	1.24 ± 0.09	1.66 ± 0.00	2.35 ± 0.11	1.47 ± 0.01
8	2.51 ± 0.09	2.88 ± 0.01	13.52 ± 0.22	10.86 ± 0.03
9	0.20 ± 0.01	0.32 ± 0.00	21.26 ± 0.12	14.84 ± 0.07

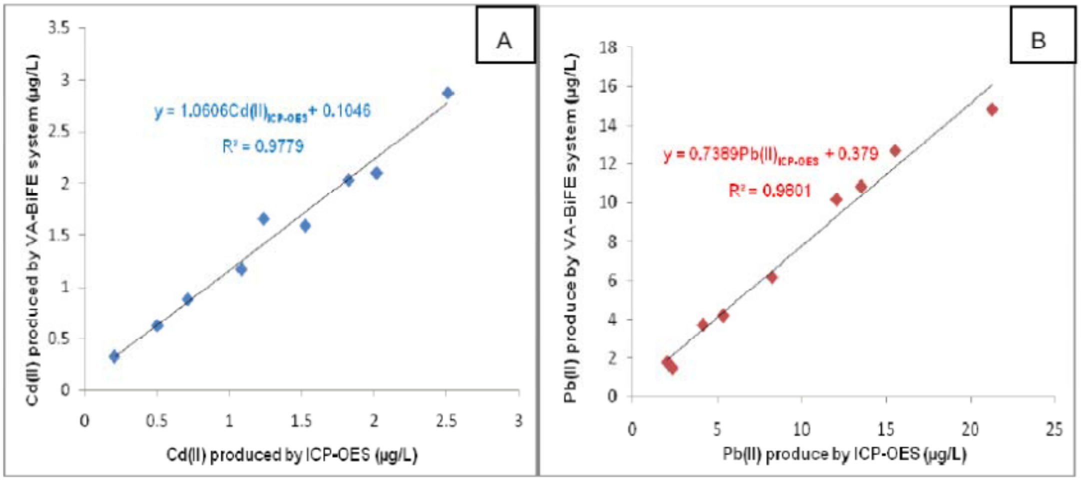

Figure 3. Correlation graphs of (A): Cd(II) and (B): Pb(II) contents determined by the proposed VABiFE method and ICP –OES method.

4. Conclusions

In this work, an in-line preparation of in situ plated bismuth film on glassy carbon electrode was employed for anodic stripping voltammetric determination of cadmium and lead. The proposed method provided high sensitivity and convenient operation for the determination of cadmium and lead at trace level. By employing flow system, the environmentally friendly BiFE can be used with a good reproducibility and no carry over. Application for analysis of real water was demonstrated.

5. Acknowledgements

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ภาคผนวก ค

รายการผลงานที่อยู่ในระหว่างการพิจารณาเพื่อตีพิมพ์ในวารสารวิชาการนานาชาติ (peer reviewed international journals)

1. Autchara Paukpol, Jaroon Jakmunee, Greener Anodic Stripping Voltammetric Method Employing Bismuth Coated Screen-printed Electrode for Determination of Cadmium and Lead, ***Chiang Mai J. Nat. Sci***., submitted.
2. Autchara Paukpol, Jaroon Jakmunee, Anodic Stripping Voltammetric Analysis of Cadmium and Lead in Solutions from Sequential Extraction of Soil and Sediment Samples, ***Anal. Chim. Acta***, MS in preparation.
3. Pimporn Insian, and Jaroon Jakmunee, Flow Injection Amperometric Detection and Off-line Sequential Extraction Column for Fractionation of Phosphorus in Sediment, ***Chiang Mai J. Sci.***, submitted.

Greener Anodic Stripping Voltammetric Method Employing Bismuth Coated Screen-printed Electrode for Determination of Cadmium and Lead

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ABSTRACT

A greener electrochemical platform was developed for determination of trace amounts of cadmium and lead. It is based on a disposable screen-printed carbon ink electrode modified with an in situ plated bismuth film employing as a more environmentally friendly working electrode alternative to the widely used mercury electrode. The bismuth coated screen-printed electrode (Bi-SPE) was used for the simultaneous determination of trace Cd(II) and Pb(II) by square-wave anodic stripping voltammetry (SWASV). Operational parameters such as Bi(III) concentration, deposition potential, deposition time and rotation speed during preconcentration of the metals were optimized. It was found that the Bi-SPE presented well-defined, reproducible and sharp stripping voltammogram. Peak current increased linearly with the metal concentration in a range of 5-40 µg L^{-1} for Cd(II) and 2-40 µg L^{-1} for Pb(II). Limit of detections were 1.7 µg L^{-1} for Cd(II) and 0.7 µg L^{-1} for Pb(II), which are better than those of flame atomic absorption method. Accuracy of the developed method was studied by examining the percent recovery of Cd(II) and Pb(II) that spiking into water samples.The proposed method could be applied for monitoring of trace cadmium and lead in river water sample.

Keywords: Bismuth coated screen-printed electrode, Square-wave anodic stripping voltammetry, Cd(II), Pb(II), Water samples

INTRODUCTION

The growing concerns about Cd(II) and Pb(II) poisoning have led to increasing demands for highly sensitive, reliable, reproducible, and low cost methods for quantitating them in water. Electrochemical stripping analysis historically has been recognized as a powerful technique for Cd(II) and Pb(II) detection. In stripping voltammetry, mercury film or hanging mercury drop electrode (MFE/HMDE) have been widely applied as a working electrode owing to its remarkable sensitivity. However the toxicity of mercury has stimulated the search for environmentally friendly material to produce greener mercury-free electrode. Bismuth film electrode (BFE) is considered as the best alternative material to MFE due to its ability to form fusing alloys with other metals (Arduini et al., 2010; Locatelli and Torsi, 2001 and Wang et al., 2000). Different materials have been used as substrates for BFEs, including glassy carbon (Wang et al., 2001a and Kefala et al., 2003), carbon paste (Krolicka et al., 2002 and Svancara et al., 2003), wax-impregnated graphite (Kefala et al., 2003), pencil-lead (Demetriadis et al., 2004) and screen-printed carbon ink (Wang et al., 2001b). The screen-printing technology is well established for the production of low cost, reproducible and sensitive electrochemical sensors. Screen-printed sensors have been widely used for environmental, biomedical and industrial monitoring (Goldberg et al., 1994 and Laschi et al., 2006).

In this work, bismuth film in situ coated on screen-printed carbon working electrodes have been used in square wave anodic stripping voltammetry (SWASV) for the determination of Cd(II) and Pb(II) in water samples. The optimization, characterization and attractive analytical performance of bismuth coated screen-printed electrode will be reported. The developed method provides high sensitivity and low detection limits which is better than the standard flame atomic absorption spectrometric (FAAS) method. Accuracy of the developed method was examined from percent recovery of Cd(II) and Pb(II) and found in the range of 86.4 to 110.6%. Moreover, the SWASV method involves inexpensive instrument and has lower operating cost.

MATERIALS AND METHODS

Chemicals and reagents

All the chemicals were of analytical reagent grade. Ultrapure water (Milli-Q water, resistivity of 18.2 MΩ cm^{-1}) obtained from a Millipore water purification system (Millipore, Sweden) was used throughout. The solution of Bi(III) (Carlo Erba, Italy), Cd(II) (Fluka, Switzerland) and Pb(II) (Merck, Germany) were prepared from 1000 mg L^{-1} standard solutions by appropriate diluting with Milli-Q water. An acetate buffer solution (0.1 M, pH 4.5), was prepared by mixing the acetic acid (Lab scan, Thailand) and sodium acetate (Carlo Erba, Italy) and served as a supporting electrolyte.

Apparatus

Voltammetric measurements were performed with a Metrohm model 757 VA computrace, GPES software for control of the voltammograph linked with a personal computer. A bismuth coated screen-printed carbon electrode was served as the working electrode, with the Ag/AgCl (3 M KCl) and platinum wire as the reference and counter electrodes, respectively.

Bismuth coated screen-printed carbon electrode (Bi-SPCE) preparation

SPCEs were produced with a homemade manually screen-printing apparatus. Carbon ink (Henkel, Germany) was used to print the working electrode. The substrate was a flexible polyester film. The diameter of the working electrode was 3 mm. SPCEs was modified with bismuth by in situ plating approach. Firstly, SPCEs were cleaned with air plasma and pretreated in 0.1 M acetate buffer solution, at pH 4.5 by applying a positive potential of +1.6 V vs Ag/AgCl for 120 s, followed by +1.8 V vs Ag/AgCl for 60 s and then were thoroughly rinsed with water.

Measurement procedure

The electrochemical deposition on SPCEs coated by bismuth was carried out according to the in situ procedure in the presence of dissolved oxygen. The SPCE was immersed in an electrochemical cell, containing the required concentration of

Bi(III) in an acetate buffer (0.1 M, pH 4.5) medium and simultaneously deposited Bi and the metals on the surface of the electrode under stirring. The analytical measurement was carried out in square wave anodic stripping voltammetric mode (SWASV). ASV experiments consisted of three conventional steps: time controlled electrochemical deposition with solution stirring, rest period and a positive going voltammetric stripping scan under the selected conditions. The individual conditions and parameters were optimized as described in the discussion section.

Analysis of real samples

Water samples were collected from the Ping River, Chiang Mai, Thailand as the sampling sites shown in Figure 1. Water samples were filtered through a 0.45 µm membrane. In the analysis, in situ plated Bi-SPCEs were prepared by spiking the sample with 1 mg L^{-1} Bi(III) in 0.1 M acetate buffer at pH 4.5. Calibration graph method was used for quantification of Cd(II) and Pb(II) in samples.

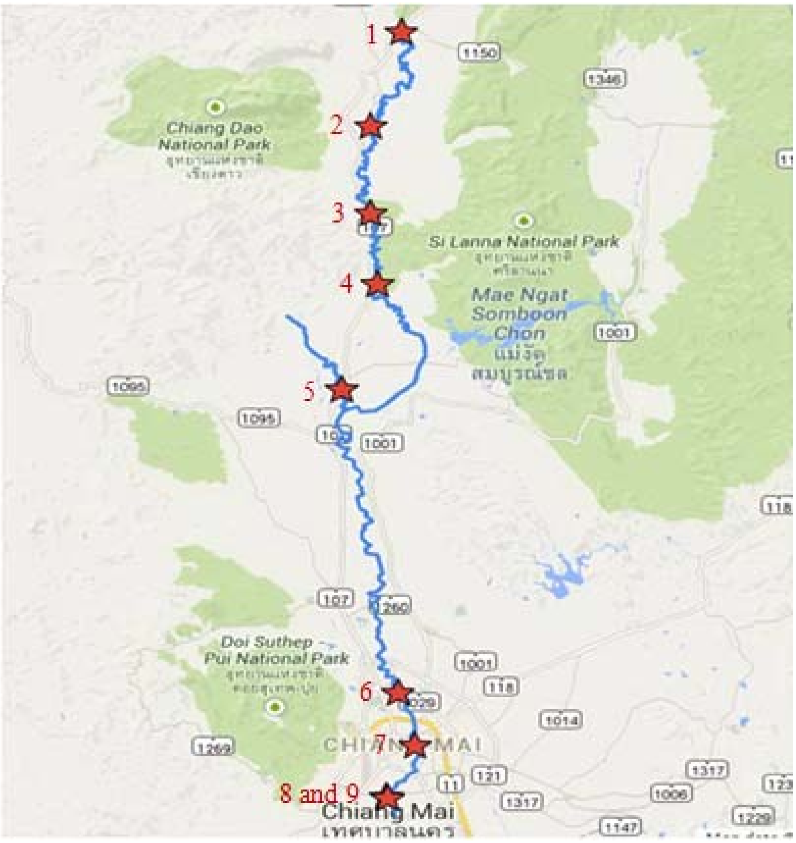


Figure 1. The sampling sites where water samples being collected from the Ping River, Chiang Mai, Thailand

RESULTS

Effect of experimental variables

1. Bi(III) concentration

Representative Cd(II) and Pb(II) peak currents for a solution containing 100 µg L^{-1} each of Cd(II) and Pb(II) in 0.1 M acetate buffer (pH 4.5) on in situ plated

Bi-SPCEs with different Bi(III) concentrations in the range 100-1400 µg L^{-1} are shown in Figure 2(a).

2. Deposition potential

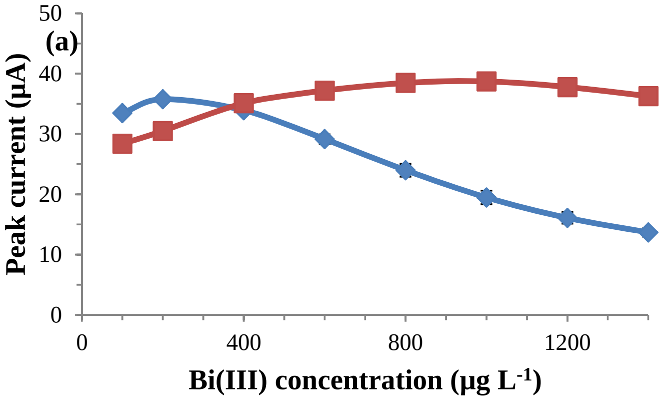
Effect of deposition potentials in the range of -0.75 to -1.60 V on the stripping currents of Cd(II) and Pb(II) were studied. As shown in Figure 2(b), Peak potential of Cd(II) and Pb(II) were -0.75 and -0.50 V, respectively.

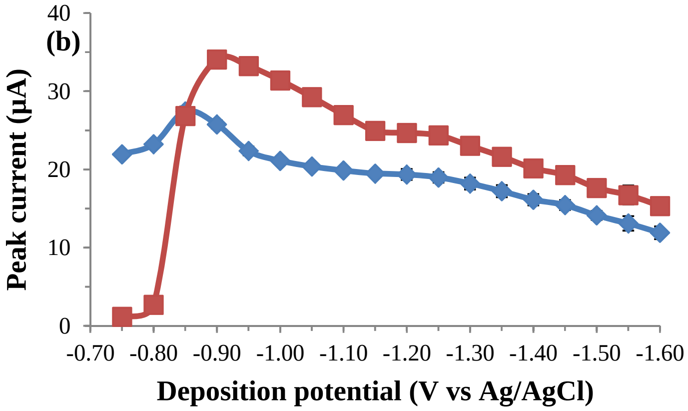
3. Deposition time

The metals deposition time was studied in the range of 60-420 s for the solution containing 100 µg L^{-1} each of Cd(II) and Pb(II) in 0.1 M acetate buffer (pH 4.5) as shown in Figure 2(c).

4. Rotation speed during preconcentration step

The rotation speed of the stirrer was investigated in the range of 0-3000 rpm as shown in Figure 2(d).





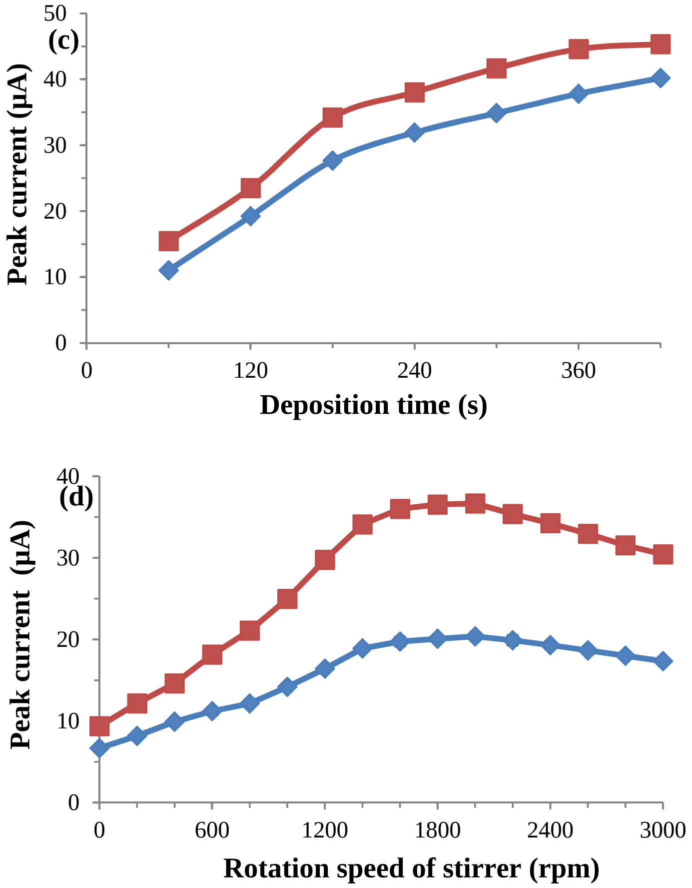


Figure 2. Effect of the Bi(III) concentration (a), deposition potential (b), deposition time (c) and rotation speed during preconcentration (d) on the stripping peak currents of Cd(II) (-■-) and Pb(II)(-◆-) in 100 µg L^{-1}Cd(II) and Pb(II) with 0.1 M acetate buffer pH 4.5 using in-situ prepared Bi-SPCE, n = 3. SWASV parameter; step increment 4 mV, frequency 35 Hz, pulse height 40 mV, cleaning potential 0.3 V and cleaning time 30 s.

Calibration data

Calibration was performed on Bi-SPCEs for the simultaneous determination of Cd(II) and Pb(II) by SWASV as shown in Figure 3. A small background current over a wide potential range was noticed. Calibration graphs had two different slopes in the range of 5-40 and 40-100 µg L^{-1} for Cd(II) and 2-40 and 40-100 µg L^{-1} for Pb(II). The analytical sensitivities were Cd(II): 1.21, 0.17 µA per µg L^{-1} (R^2 = 0.9995 , 0.9911) and Pb(II): 1.21, 0.15 µA per µg L^{-1} (R^2 = 0.9961, 0.9959). On the same electrode, the relative standard deviations were 7.0% for Cd(II) and 5.8 % for Pb(II) at 20 µg L^{-1} level (n=15). The same SPCE electrode could be used for at least 15-20 stripping cycles without loss of sensitivity. The limit of detections were calculated at a deposition time of 180 s, as 1.7 µg L^{-1} for Cd(II) and 0.7 µg L^{-1} for Pb(II).

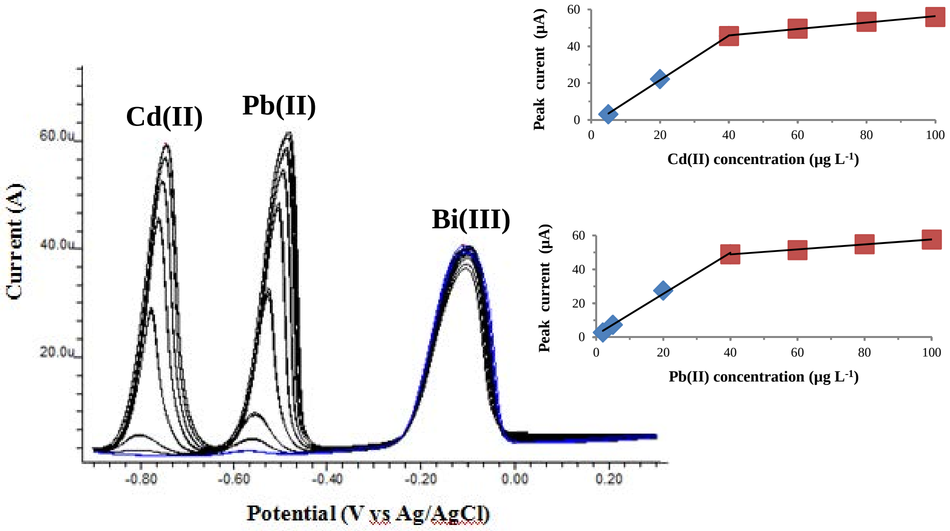


Figure 3. A series of voltammograms and calibration graphs for increasing concentrations of Cd(II) and Pb(II) in 0.1 M acetate buffer pH 4.5 using in-situ prepared Bi-SPCE (1 mg L^{-1} Bi(III)), n = 3. SWASV parameter; deposition potential -0.9V vs Ag/AgCl, deposition time 180 s, stirrer speed 2000 rpm, step increment 4 mV, frequency 35 Hz, pulse height 40 mV, cleaning potential 0.3 V and cleaning time 30 s.

Accuracy of the method

An accuracy of the system was studied by examining the percent recoveries of Cd(II) and Pb(II) at spiked concentration of Cd(II) and Pb(II) standard solution of 5 µg L^{-1} in water samples. The obtained results are given in the Table 1. Percent recoveries of Cd(II) and Pb(II) are close to 100%, in the range of 86.4 to 110.6%, under the optimal condition.

Application to river water

For the purpose of practical applicability, Bi-SPCEs were employed for the determination of Cd(II) and Pb(II) in river water samples. After that, the anodic stripping peak current was recorded for the determination of Cd(II) and Pb(II) with the calibration graph method under the optimized condition. The results obtained for nine water samples are summarized in Table 1.

Table 1. Determination of Cd(II) and Pb(II) in water samples by the proposed SWASV with Bi-SPCE

Water samples	Concentration of metals (µg L^{-1})		Recovery (%)	
	Cd(II)	Pb(II)	Cd(II)	Pb(II)
1	n.d.	n.d.	96.9	98.1
2	n.d.	n.d.	95.7	99.4
3	n.d.	n.d.	86.4	103.0
4	n.d.	n.d.	95.7	106.2
5	n.d.	n.d.	98.7	110.4
6	n.d.	n.d.	92.4	110.6
7	n.d.	2.10±0.01	91.4	106.7
8	n.d.	n.d.	103.1	104.3
9	n.d.	n.d.	103.7	105.6

* n.d. = not detected

DISCUSSION

The operational parameters are necessary due to indicating performance of analysis. The concentration of Bi(III) is a crucial parameter to be investigated. It controls the thickness of the Bi film. The thickness of the film does not affect the peak potential of any metals but rather affects the peak height. The results showed a decrease of peak current of the Cd(II) and Pb(II) with increasing thickness of the Bi film, especially the Pb(II) peak as shown in Figure 2(a). As Pb(II) has more positive reduction potential than Cd(II), Pb(II) can be determined at lower Bi(III) concentration. At high Bi(III) concentration, the peaks became wider and led to a substantial decrease in sensitivity over the Bi(III) concentration of 1000 µg L^{-1}, which was explained as a saturation effect of Bi film on the screen-printed carbon surface. This behavior was attributed to the increased number of nucleation sites and increased alloy formation. However, at Bi(III) concentrations greater than 1000 µg L^{-1}, a reduction in the peak intensity was observed, ascribed to the formation of a thick layer of Bi on the electrode surface that partially blocks the conductive surface of the electrode, reducing the number of electroactive site (Kachoosangi et al., 2007).Thus, Bi(III) concentration of 1000 µg L^{-1} was selected for further experiment.

The deposition potential affects the quality of bismuth film to form fusing alloys with metal ions. As shown in Figure 2(b), the stripping responses of Cd(II) and Pb(II) were found to occur at potential more negative than -0.75 V. As the deposition potential became more negative, the peak currents increased up to -0.90 V for Cd(II) and -0.85 V for Pb(II). The Pb(II) peak current did not increase significantly from -0.75 to -0.85 V due to its more positive reduction than Cd(II). The evolution of hydrogen gas from the SPCE surface started to occur at -1.50V that could damage the bismuth film and came about the reduction of interference when more negative potential was applied. A potential of -0.9 V was thus chosen as optimum deposition potential.

Deposition time is the time during which the Cd(II) and Pb(II) is reduced at the Bi-SPCE to form the Cd(Bi) and Pb(Bi) alloys. It is generally accepted that lower limits of detection (LODs) can obtained with longer deposition time (Castaneda et al., 2005). In Figure 2(c), Cd(II) and Pb(II) peak currents increased rapidly with the deposition time from 60-180 s. For the deposition time longer than 180 s, the current responses exhibited negligibly increase which were considered the longest practical time for a satisfactory compromise between high sensitivity and short analysis time. Metal ions were preconcentrated for 180 s in further experiments.

The mass transport behavior during preconcentration step was under the control of the rotation speed of the stirrer as shown in Figure 2(d). The stripping peak currents for Cd(II) and Pb(II) continuously increased with increment of rotation speed until 2000 rpm. The decrease of peak currents were observed at the rotation speed higher than 2000 rpm because of the bismuth film being mechanically damaged therefore, preconcentration was performed at 2000 rpm.

The SWASV with bismuth film working electrode can be applied to the simultaneous determination of Cd(II) and Pb(II) in real water samples by using calibration graph. This system was investigated for trace metals determination and it provides high sensitivity, low consumption and convenient operation. Concentrations of both the metals found in water samples are lower than the detection limit of FAAS method, therefore, this standard method could not be used for comparison with the developed method. The recovery study indicated that the proposed method can be used for analysis of real water sample with recoveries found in the range of 86.4 to 110.6%. The results obtained for nine water samples are summarized in Table 1. The concentration of both the metal ions in Ping river water is very low and should have no environmental impact. The permission levels of Cd(II) and Pb(II) in drinking water according to WHO guidelines are below 3 $\mu g\ L^{-1}$ and 10 $\mu g\ L^{-1}$, respectively. The developed method can be used for monitoring of these trace metals in water.

CONCLUSIONS

The developed anodic stripping voltammetric method with in situ coated Bi film on screen-printed carbon working electrode should be useful to be applied as a cost effective and a sensitive alternative analytical method for the simultaneous determination of trace Cd(II) and Pb(II) in water samples. SPCE, an inexpensive, widely available and disposable material requiring minimal pretreatment, has been shown to offer comparable performance to the more expensive glassy carbon electrodes for the simultaneous determination of Cd(II) and Pb(II). In addition with the low toxicity of bismuth, low background current and their small size, Bi-SPCEs offer great scope for applications in which compact instrumentation and low sample volumes are critical such as field measurement and on-site monitoring of heavy metals.

ACKNOWLEDGMENT

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Anodic Stripping Voltammetric Analysis of Cadmium and Lead in Solutions from Sequential Extraction of Soil and Sediment Samples

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Abstract

Sequential extraction is an important tool for the determination of different chemical forms of trace metals in soil and sediment. In this work, an environmental friendly anodic stripping voltammetric method (ASV) has been developed for determination of cadmium and lead in the extracted solutions of sequential extraction of soil and sediment samples. A bismuth coated screen printed carbon electrode (Bi-SPCE) was developed to be used instead of mercury based electrodes. The method was optimized for the determination in various extracting solutions of Tessier's extraction scheme. The developed method provided good sensitivity and precision. Linear ranges for cadmium and lead were 2-100 μgL^{-1} with detection limit of 1.7 μgL^{-1} and 0.7 μgL^{-1}, respectively. It was validated by using certified reference material of soil (CRM033), and applied to sequential extraction analysis of sediment sample. The method involves small instrument which is relatively low cost, portable and could be used as an alternative to the sophisticated instrument for study of sequential extraction of solid sample.

Keywords: Anodic stripping voltammetry; Bismuth coated screen-printed carbon electrode; Sequential extraction; Cadmium; Lead; Sediment; Soil

1. Introduction

The accumulation of heavy metals in sediment and soil samples causes a potential risk to human health due to the transfer of these elements to aquatic media, their uptake by plants, and their subsequent introduction into the food chain (Konradi et al., 2005). Metals present in solid sample such as soil can be related to several reactive components. Total metal analyses in solid sample provide limited information, they do not indicate the chemical nature or potential mobility and bioavailability of the metals (Silveira et al., 2006). Therefore, sequential extraction procedure is an important tool in environmental study to determine different chemical forms of trace metals in soil material (Tessier et al., 1979; Reinirkens, 1996). Sophisticated analytical techniques, e.g., electrothermal atomic absorption spectrometry (ET-AAS) (Gulmini et al., 1994), flame atomic absorption spectrometry (FAAS), inductively coupled plasma mass spectrometry (ICP-MS), and inductively coupled plasma optical emission spectrometry (ICP-OES) (Korn et al., 2006) are the standard techniques used for determination of cadmium (Cd) and lead (Pb) in the extracts of sequential extraction procedure. However, these instruments are expensive, complicated and inappropriate for field applications. In addition, the analysis must be performed in a specialized laboratory by skilled personnel.

Anodic stripping voltammetry (ASV) historically has been widely recognized as a powerful technique for Cd and Pb detection, due to its remarkable sensitivity. This is resulted from unique ability to cathodically preconcentrate target metals during the accumulation step onto the working electrode, and then stripped out from the electrode by applying an anodic potential scan (Wang, 2000; Locatelli et al., 2001). It can also be readily coupled with inexpensive and easy to use instrumentation (Arduini et al., 2010). Stripping voltammetry using screen-printed electrode (SPE) with mercury film has been widely used to determine Cd and Pb. However, the toxicity of mercury has stimulated the search for environmentally friendly material to introduce mercury-free electrode. Bismuth film electrode (BiFE) is considered as the best alternative to mercury film electrode (MFE), because it can form fused alloys with heavy metals, which is analogous to the amalgams that mercury forms (Economou, 2005). Moreover, it does not require the removal of dissolved oxygen during stripping analysis, and has wide negative potential window, which is a good analytical advantage (Locatelli et al., 2001).

The modification with carbon materials on the screen-printed carbon electrode (SPCE) is popular, which increases surface area of the working electrode, and improve sensitivity. Graphene and multiwall carbon nanotubes (MWCNTs) are two carbon materials, which have attracted attention. The graphene is mostly obtained from graphene oxide (GO) by electrochemically reduced method but the reduced graphene oxide (RGO) tends to aggregate due to π-π stacking interactions, thus modifications of RGO are necessary (Liu et al., 2010). The MWCNTs are rolled graphene sheets. They existed aggregation phenomenon during the period of use because of Van der Waals interactions (Zhang et al., 2010). Therefore, the combination of two dimensional (2D) graphene and 1D MWCNTs are attractive means to create 3D modified carbon materials to get rid of aggregation phenomenon and increase an electrode surface.

In this work, sequential extraction procedure and anodic stripping voltammetric method with employing bismuth film in situ coated on modified screen-printed carbon working electrode will be developed for the determination of the different chemical forms or ways of binding between metals (Cd and Pb) and soil components which are the novel concept for environmental studies. Some carbon materials, i.e., MWCNTs, RGO and RGO-MWCNTs were modified on SPCEs and investigated for simultaneous measurement of Cd and Pb by square wave anodic stripping voltammetry (SWASV). The sequential extraction procedure adapted by Tessier *et al.* was used for extracted soil and sediment samples (Tessier et al., 1979; Reinirkens, 1996). The conditions for operation of the system will be optimized and validated by using CRM033 certified reference material of soil. Addition, the development was applied to sediment sample.

2. Material and methods

2.1 Chemicals and Reagents

Multiwall carbon nanotubes (MWCNTs) were obtained from Nanomaterials Research Unit (Chiang Mai, Thailand). The graphene oxide (GO) was synthesized based on the modified Hummers method (Hummers et al., 1958). All chemicals were of analytical reagent grade. Ultrapure water (Milli-Q water, resistivity of 18.2 MΩ cm^{-1}) obtained from a Millipore water purification system (Millipore, Sweden) was used throughout. The solution of bismuth (Bi^{3+}) (Carlo Erba, Italy), cadmium (Cd^{2+}) (Fluka, Switzerland) and lead (Pb^{2+}) (Merck, Germany) were prepared from 1000 mgL^{-1} standard stock solutions. An acetate buffer solution (0.1 M, pH 4.5), was prepared by mixing the acetic acid (HOAc) (Lab scan, Thailand) and sodium acetate (NaOAc) (Carlo Erba, Italy) and served as a supporting electrolyte. Nitric acid (HNO_3) (Lab scan, Thailand), hydrogen peroxide (H_2O_2) (Carlo Erba, Italy), hydroxylamine hydrochloride ($NH_2OH*HCl$) (Fluka, Switzerland), ammonium acetate (NH_4OAc) (Loba Chemie, India) and hydrochloric acid (HCl) (Lab scan, Thailand) were used for preparation of extractants that used in sequential extraction. Certified reference material of soil (CRM033) (Fluka, Switzerland) was used to validate the developed method. All plastic and glassware were cleaned up by soaking in 10%(v/v) HNO_3 and rinsed with Milli-Q water prior to use.

2.2 Apparatus

Voltammetric measurements were performed with a Metrohm model 757 VA computrace, with GPES software for control of the voltammograph linked with a personal computer. A bismuth coated screen-printed carbon electrode was served as the working electrode, with a Ag/AgCl (3 M KCl) and a platinum wire as reference and counter electrodes, respectively. Hotplate with magnetic stirrer (C-MAG HS7, IKA, Germany) was used for sample digestion. Centrifuge (Clay Adams Dynac, Becton Dickinson, US) operated at 12000x g was used for complete separation of the extracts.

2.3 Preparation of carbon materials

The MWCNTs were treated with 3 M HNO_3 solution for 24 h followed by filtering, rinsing with Mili-Q water until the filtrate pH became nearly neutral and drying in an oven at 80 °C for 24 h. This process is known as the carboxylation of MWCNTs to introduce –COOH groups at the ends or the sidewalls of the structure for dispersing in polar solvent and performed to ensure complete removal of transition metal ion catalyst as used in the production of MWCNTs (Mandil et al., 2012). 0.5 mg mL^{-1} MWCNTs dispersion was prepared by adding 1.0 mg of functionalized MWCNTs to 2.0 mL ethanol (EtOH) (Merck, Germany) and ultrasonication for 30 min. Even so, the obtained dispersion was not stable since most MWCNTs were hydrophobic.

The GO dispersion in Milli-Q water with concentration 0.5 mg mL^{-1} was obtained by 30 min ultrasonication. The homogeneous GO hydrosol was brown color. The preparation of reduced graphene oxide (RGO) was performed by electrochemically process as the cyclic scan between 0.0 V and −1.5 V for 10 cycles in 0.1M acetate buffer pH 4.5 to reduce the GO from the composite film modified on the working electrode (Lia et al., 2014).

To prepare the graphene oxide-multiwall carbonnanotubes (GO-MWCNTs) hybrid material, 1.0 mg functionalized MWCNTs and 2.0 mL 1.0 mg mL^{-1} GO hydrosol were mixed together with ultrasonication for 1 h. The GO–MWCNTs were electrochemically reduced as above by performing only 3 scan cycles because the incorporating of MWCNT significantly promotes the reduction of GO and acts as a conducting wire between the graphene sheets (Mani et al., 2013).

2.3 Modified of the screen-printed carbon electrodes (SPCEs)

SPCEs were produced with a homemade manual screen-printing. Carbon ink (Henkel, Germany) was used to print the working electrode, on a flexible polyvinyl chloride (PVC) substrate. The diameter of the working electrode was 3 mm. Firstly, SPCEs were cleaned with air plasma (PDC-32G, Harrick Plasma, USA) and modified with carbon materials (MWCNTs, RGO and RGO-MWCNTs hybrid) by dropping 5 µL of the solutions on SPCEs and drying at room temperature. After that, the electrodes were pretreated in 0.1 M acetate buffer solution, at pH 4.5 by applying a positive potential of +1.6 V for 120 s, followed by +1.8 V for 60 s and then were thoroughly rinsed with Mili-Q water. The electrochemical deposition of metals on Bi-SPCEs was carried out by in situ procedure in the presence of dissolved oxygen. The SPCE was immersed in an electrochemical cell, containing 1 mgL^{-1} Bi^{3+} in an acetate buffer (0.1 M, pH 4.5) medium and simultaneously deposited Bi and the metals on the surface of the electrode under stirring at 2000 rpm.

2.4 Sample preparation

Sediment sample was collected from the heavy metals contaminated area in Mae Sot district, Tak province, Thailand, by the Freshwater Biomonitor Research Laboratory (Chiang Mai, Thailand). Sediment sample was dried indoor at room temperature, crushed manually in a porcelain mortar and sieved through a 2 mm screen. Subsample of air-dried sediment was ground and sieved to obtain aggregates less than 0.1 mm to ensure uniformity of the material. The selected sediment sample was homogenized and stored in polyethylene vessel at room temperature in desiccators.

2.5 Digestion Procedures

2.5.1 Total metal digestion

Total metal content was determined by using EPA's acid digestion procedure 3050B as the conventional acid extraction method (USEPA, 1996). 1 g of sample was placed in 250 mL conical flask for digestion. The first step was to heat the sample to 95±5 °C with 10 mL of 50 %(v/v) HNO_3 without boiling. After cooling the sample, it was refluxed with repeated additions of 65 %(v/v) HNO_3 until no brown fumes observed. Then the solution was allowed to evaporate until the volume was reduced to 5 ml. After cooling, 5 mL of 18 %(v/v) H_2O_2 was added and warmed for peroxide reaction. Heat until effervescence subsides as complete reaction and cool the flask. Continue to add 1 mL 30 %(v/v) H_2O_2 with warming until the complete peroxide reaction. Continue heating the acid-peroxide digestion until the volume has been reduced to approximately 5 mL. After cooling, centrifuge for 30 min, dilute to 100 ml with Mill-Q water and store at 4°C for analyses.

2.5.2 Sequential extraction

The metals distribution in sediment and soil was evaluated by using the sequential extraction developed by Tessier *et al.* (Tessier et al., 1979). The steps in the sequence were operationally defined in terms of the targeted forms to be extracted as follows (Frentiu et al., 2007; Zimmerman et al., 2010):

Exchangeable (fraction1, F1): 1 g of sample extracted with 8 mL of 1 M NaOAc pH 8.2 for 1 h at room temperature with continuous agitation.

Bound to carbonates (F2): Residual from F1 extracted with 8 mL of 1 M NaOAc pH 5 for 1 h at room temperature with continuous agitation.

Bound to iron and manganese oxides (F3): Residual from F2 extracted with 20 mL of 0.04 M $NH_2OH*HCl$ in 25%(v/v) HOAc for 6 h at 96±3 °C with occasional agitation.

Bound to organic matter (F4): Residual from F3 extracted with 3 mL of 0.02 M HNO_3 and 5 mL 30 %(v/v) H_2O_2 pH 2. The mixture was heated to 85±2 °C for 5 h with occasional agitation, the second 3 mL 30 %(v/v) H_2O_2 pH 2 was then added and sample reheated to 85±2 °C for 6 h with intermittent agitation. After cooling, extracted with 5 mL of 3.2 M NH_4OAc in 20 %(v/v) HNO_3 was added and dilute to 20 mL with Milli-Q water for 1.5 h at 85±2 °C.

Residual (F5): Residual from F4 extracted with 10 mL aqua regia for 6 h at 96±3 °C. Finally, the sample was evaporated to near dryness and diluted to 25 mL volume.

Following each extraction or wash, mixtures were centrifuged for 30 min. Prior to the start of the next extraction step, residues were washed with water, centrifuged, the wash solutions were collected and store at 4°C for analyses.

2.6 Measurement procedure

The analytical measurement were carried out in square wave anodic stripping voltammetric mode (SWASV). ASV experiments consisted of three conventional steps: time controlled electrochemical deposition with solution stirring, rest period and a positive going voltammetric stripping scan under the selected conditions. For SWASV the frequency (f) was 35 Hz, step potential (E_{step}) 4 mV, and amplitude 40 mV; the deposition potential (E_{dep}) -0.9 V for 180 s and the potential was swept from -0.9 to -0.3 V after an equilibrium time (t_{eq}) of 15 s; the cleaning potential (E_{clean}) 0.3V for 30 s. The electrodeposition and precondition steps were stirred at 2000 rpm. In the analysis, in situ plated Bi-SPCEs were prepared by spiking the extracted sample solution as appropriate dilution with 1 mgL^{-1} Bi^{3+} in 0.1 M acetate buffer solution at pH 4.5. Standard addition method was used for quantification of Cd^{2+} and Pb^{2+} in samples.

3. Results and Discussion

3.1 Electrochemical characterization of modified SPCEs

Stripping voltammograms of different carbon electrodes (bare SPCE and, MWCNTs, RGO, and RGO-MWCNTs modified SPCEs) on which Bi was in situ plated are shown in Fig. 1. Stripping voltammograms were obtained with 1 mgL^{-1} Bi^{3+} in 0.1 M acetate buffer solution at pH 4.5 containing 100 μgL^{-1} each of Cd^{2+} and Pb^{2+}. For comparison of the resulting voltammograms of different electrodes, the baselines current increased in the order of bare, RGO, MWCNTs and RGO-MWCNTs modified SPCEs, respectively. This indicated the increase of electrode area after modification. However, the surface areas as increasing were not active sites for Cd^{2+} and Pb^{2+} detection. The data obtained from the voltammograms are summarized in Table 1. The Bi-SPCE exhibited the sharpest and the highest peak current. And the sensitivity of the electrode decreased in the order of bare, RGO, RGO-MWCNTs and MWCNTs modified SPCEs as shown in Table 2. The sensitivity of Bi-SPCE was 11 times higher than RGO-BI-SPCE for Cd^{2+} and 4 times for Pb^{2+}. Therefore, it was clear that the Bi-SPCE was more appropriate for determination of Cd^{2+} and Pb^{2+} using SWASV.

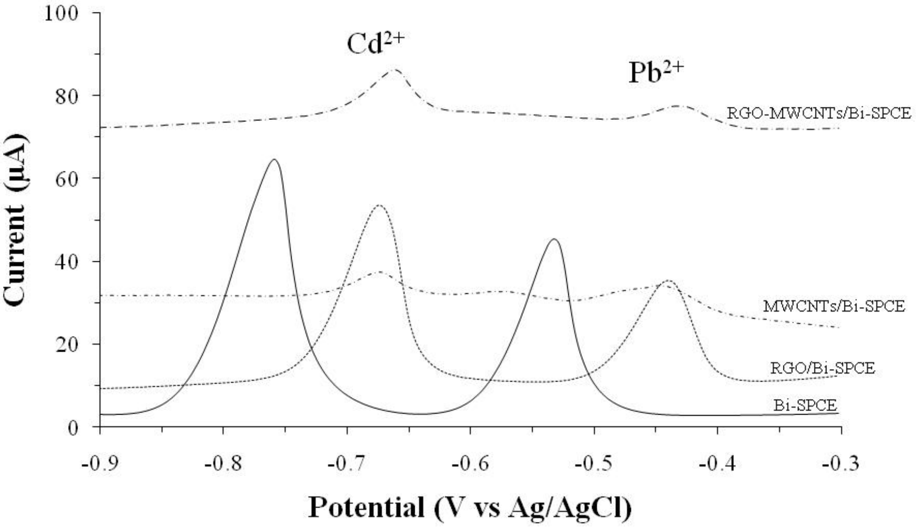

Fig. 1 Anodic stripping voltammograms of 100 μgL^{-1} Cd^{2+} and Pb^{2+} at in situ plated Bi film (1 mgL^{-1} Bi^{3+}) on different carbon materials modified SPCEs. Supporting electrolyte; 0.1 M acetate buffer solution (pH 4.5); E_{dep} -0.9 V; t_{dep} 180 s; t_{eq} 15 s; f 35 Hz; E_{step} 4 mV; amplitude 40 mV.

Table 1 Effect of the different carbon materials modified Bi-SPCEs on the SWASV peak current (i_p), peak potential (E_p) and peak widths ($W_{1/2}$) for a solution containing 100 μgL^{-1} each of Cd^{2+} and Pb^{2+} in 0.1 M acetate buffer solution (pH 4.5), the conditions as in Fig. 1

	Cd^{2+}			Pb^{2+}		
	i_p (μA)	E_p (V)	$W_{1/2}$ (V)	i_p (μA)	E_p (V)	$W_{1/2}$ (V)
Bi-SPCE	61.9	-0.76	0.59	34.2	-0.53	0.51
MWCNTs/Bi-SPCE	5.3	-0.68	044	1.3	-0.47	0.82
RGO/Bi-SPCE	42.9	-0.68	0.59	23.8	-0.44	0.59
RGO-MWCNTs/Bi-SPCE	13.4	-0.66	0.53	4.6	-0.43	0.57

Table 2 The sensitivity of different carbon materials modified Bi-SPCEs in range 0-40 μgL^{-1} Cd^{2+} and Pb^{2+}with 0.1 M acetate buffer solution (pH 4.5) using in-situ prepared Bi-SPCE, n = 3, other conditions as Fig. 1.

	Sensitivity (μA/μgL^{-1})			
	Bi-SPCE	MWCNTs/Bi-SPCE	RGO/Bi-SPCE	RGO-MWCNTs/Bi-SPCE
Cd^{2+}	0.8491	0.0200	0.0758	0.0580
Pb^{2+}	0.4401	0.0028	0.0980	0.0468

3.2 Analytical performances

Fig. 2 exhibits the evolution of square wave anodic stripping voltammograms were performed on Bi-SPCE for the simultaneous determination of Cd^{2+} and Pb^{2+} in range 0-100 μgL^{-1}. A small background current over a wide potential range was noticed. The resulting calibration plots, which considers as peak height, are linear over the entire range studied. They had two different slopes in the range of 2-40 and 40-100 μgL^{-1} for both Cd^{2+} and Pb^{2+}. The corresponding equations of calibration curves are y = 0.8491x-1.4767, y =

0.4975x+13.0450 (x: concentration/ µgL^{-1}; y: current/ µA) with the correlation coefficient of R^2 = 0.9982, 0.9930 for for Cd^{2+} and y = 0.4401x-0.2460 , y = 0.2731x+7.064 (x: concentration/ µgL^{-1}; y: current/ µA) with the correlation coefficient of R^2 = 0.9975, 0.9990 for for Pb^{2+}. The limit of detections (LODs) were calculated based on three times the standard deviation of blank at a deposition time of 180 s, were 1.7 µgL^{-1} for Cd^{2+} and 0.7 µgL^{-1} for Pb^{2+}.

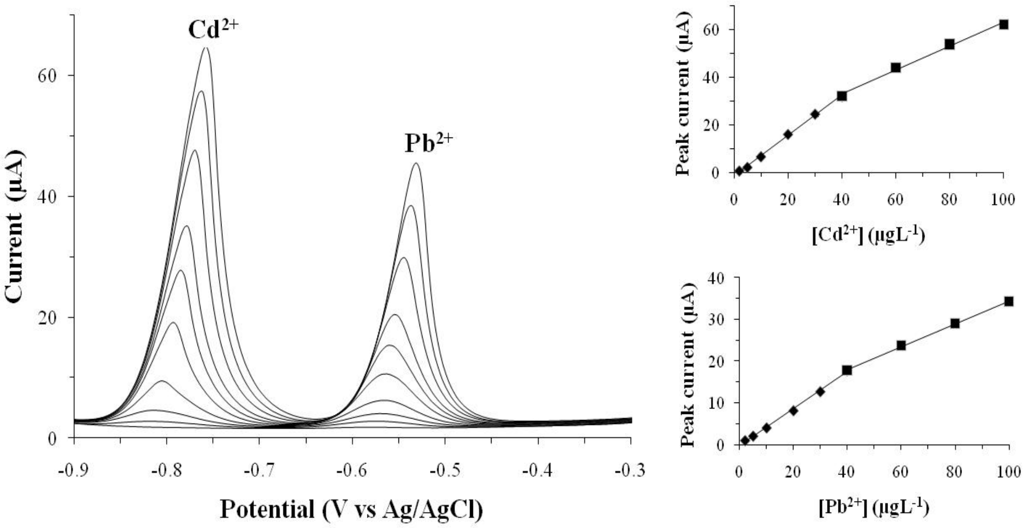

Fig. 2 A series of voltammograms and calibration graphs for increasing concentrations of Cd^{2+} and Pb^{2+} in 0.1 M acetate buffer pH 4.5 using in-situ prepared Bi-SPCE (1 mgL^{-1} Bi^{3+}), n = 3, SWASV parameter as Fig. 1.

The performances of Bi film deposition on SPCE have been tested and evaluated for Cd^{2+} and Pb^{2+} concentrations lower than 40 µgL^{-1} which are sufficient to analyze in the real samples. On the same electrode, the relative standard deviations (RSDs) were 7.6 % for Cd^{2+} and 9.4 % for Pb^{2+} at 20 µg L^{-1} level (n=30). The same SPCE could be used for at least 30 stripping cycles with slightly loss of sensitivity (RSD < 10 %) as shown in Fig.3. This electrode also permits to attain a repeatability of RSDs; 6.7 % for Cd^{2+} and 5.6 % for Pb^{2+} (n=3) considering seven successive measurements of the same solution.

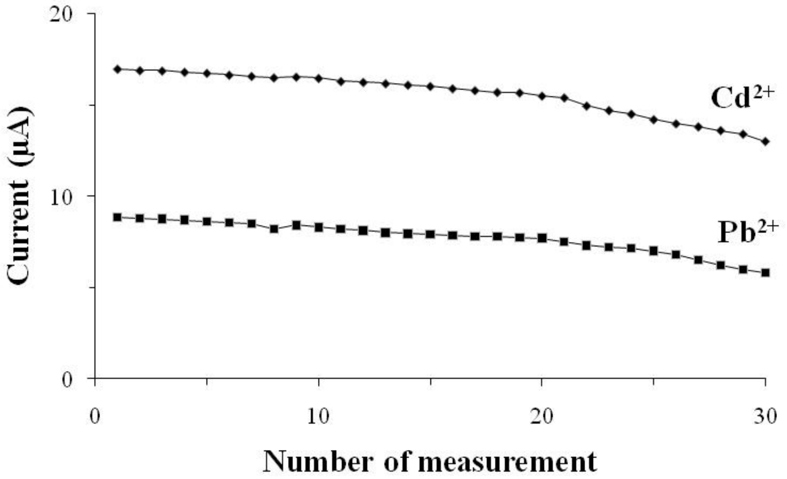

Fig. 3 Stability of 30 reproductive measurements for 20 µgL^{-1} Cd^{2+} and Pb^{2+} in 0.1 M acetate buffer pH 4.5 using in-situ prepared Bi-SPCE (1 mgL^{-1} Bi^{3+}), Other conditions as Fig. 1.

3.3 Effect of sequential extractants in Tessier's procedure

The extractants for sequential extraction by Tessier's procedure are the solution of salt and acid so the extractants of each fraction were used as supporting mediums for the voltammetric analysis without sample preparation. The bound to organic matter (F4) and residual (F5) fractions were adjusted the conditions to be appropriate for electrochemical stripping analysis. Since the extractant for fraction 4 contained HNO_3, H_2O_2 and NH_4OAc, the excess H_2O_2 can be reduced to oxygen gas (O_2) on working electrode at negative potential in preconcentration step. Therefore, the solution was added with zinc (Zn) powder to reduce remaining H_2O_2 before the measurement. In addition, the residual fraction was strong acid solution, i.e., aqua regia. The reduction of remaining hydrogen ion (H^+) to hydrogen gas (H_2) in preconcentration step was occurred so the solution was adjusted pH (~4.5) with NaOAc before measurement. A small background current over a wide potential range was observed in each sequential extractant of five fractions. In each case, the calibration graphs of Cd^{2+} and Pb^{2+} in concentration range of 0-40 µgL^{-1} were constructed. The calibration data as sensitivity and R^2 of Cd^{2+} and Pb^{2+} are summarized in Table 3. The different extractants as supporting mediums provided different sensitivities, suggesting that a standard addition method should be used for quantification of the analytes. The linearity of the calibration graphs of both Cd^{2+} and Pb^{2+} are good as indicated by R^2 values closing to 1. Therefore, the SWASV was applicable to determine Cd^{2+} and Pb^{2+} that were extracted from soil and sediment by Tessier's procedure. All of the extractants can be used as supporting electrolyte, without any trouble, so it did not require sample preparation step before measurement. The ASV provides more convenient operation and cheaper than the commonly used spectrometry techniques.

Table 3 The calibration data of Cd^{2+} and Pb^{2+} at concentration range 0-40 µgL^{-1} in each Tessier's procedure sequential extractants by the proposed SWASV with Bi-SPCE

Fractions	Linear range (µgL^{-1})	Calibration data			
		Cd^{2+}		Pb^{2+}	
		Sensitivity (µA/µgL^{-1})	R^2	Sensitivity (µA/µgL^{-1})	R^2
Exchangeable	0-40	1.3663	0.9975	1.7975	0.9933
Bound to carbonates		1.4970	0.9951	1.5081	0.9945
Bound to Fe/Mn oxides		1.1488	0.9990	1.6296	0.9960
Bound to organic matter		2.1003	0.9960	2.6437	0.9993
Residual		0.5936	0.9891	1.4187	0.9944

3.4 Application to real samples

The validation of the developed method was studied by determining of Cd^{2+} and Pb^{2+} in the CRM033 certified reference material using standard addition method for quantification of the analytes in concentration range of 0-40 µgL^{-1}. The total concentrations as certified values were found to be very high, i.e., 126.7±1.3 mg Kg^{-1} for Cd and 107.5±0.3 mg Kg^{-1} for Pb. The relative distributions in each elemental fractions of Cd and Pb in soil by the Tessier's procedure were exchangeable (25.9; 1.5 %), bound to carbonates (9.0; 5.6 %), bound to iron and manganese oxides (1.0; 4.1 %), bound to organic matter (61.3; 41.4 %) and residual (2.8; 47.4 %), respectively. All the obtained results are given in the Table 4 and Fig. 4. The summation of Cd and Pb concentrations analyzed by sequential extraction were in good agreement with the results found in the total concentration using EPA's acid digestion procedure 3050B. Therefore, the SWASV with bismuth film working electrode can be applied to the simultaneous determination of Cd and Pb in real soil and sediment samples by using Tessier's sequential extraction procedure.

Table 4 The concentration of Cd and Pb in CRM033 analyzed using the proposed SWASV with Bi-SPCE by different extraction method[a].

Extraction method	Fractions	Concentration (mg Kg^{-1})	
		Cd	Pb
Sequential extraction	Exchangeable	33.7±0.3	1.7±0.0
	Bound to carbonates	11.7±0.0	6.2±0.1
	Bound to Fe/Mn oxides	1.3±0.0	4.5±0.1
	Bound to organic matter	79.7±0.2	45.9±0.9
	Residual	3.6±0.1	52.6±0.2
	Sum	130.0±0.6	110.9±1.3
Total concentration by digestion certified value		126.7±1.3	107.5±0.3

[a] Values represent the mean of three duplicates ± standard deviation.

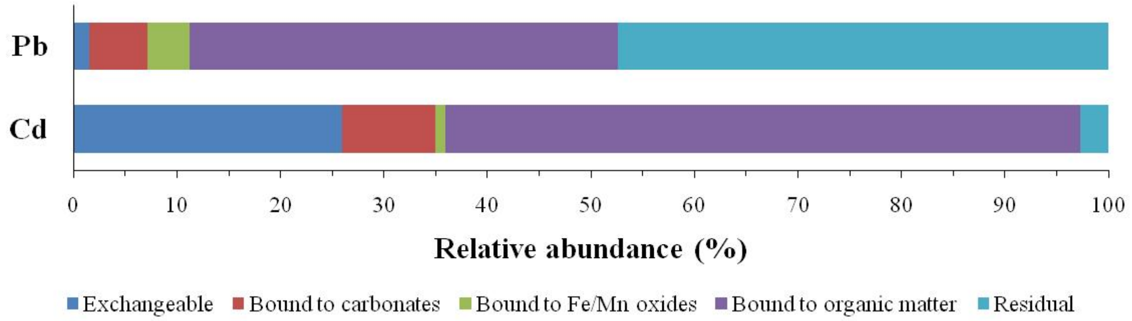

Fig. 4 The relative distributions of Cd and Pb among the various soil fractions extracted by the Tessier's procedure

For the purpose of practical applicability, the Tessier's procedure and SWASV method with employing bismuth film in situ coated on screen-printed carbon working electrode were used for the determination of the different chemical forms of Cd and Pb contaminants in sediment sample, which was collected from the Mae Sot district, Tak province, Thailand. The element contents determined in five fractions following the Tessier's procedure and the total concentrations of Cd and Pb are shown in Table 5, while the distribution among the fractions are presented in Fig. 5, consisting of exchangeable (2.0; 0.6 %), bound to carbonates (9.2; 2.7 %), bound to iron and manganese oxides (7.2; 11.1 %), bound to organic matter (24.8; 55.8 %) and residual (56.9; 29.7 %), respectively. The Tessier's partitioning shows the predominance of metals associated with the organic matter, residual fractions and smaller concentrations extracted from exchangeable, carbonates and oxides fractions. The exchangeable and bond to carbonates species, corresponding to the first and second fractions, are generally called "bio-available", as they exhibit a mobility relative to the environment and are potentially available for plants which introduce into the food chain. The results show that the amounts of Cd and Pb in bio-available form are very low in sediment so they do not risk to human health. For Cd and Pb increasing of the oxides, organic matter and residual fractions, excepting Cd bound to oxides, are non-bioavailable forms to general environment.

Table 5 The concentration of Cd and Pb in sediment sample analyzed using the proposed SWASV with Bi-SPCE by different extraction method[a].

Extraction methods	Fractions	Concentration (mg Kg^{-1})	
		Cd	Pb
Sequential extraction	Exchangeable	0.3±0.0	3.8±0.0
	Bound to carbonates	1.4±0.0	16.9±0.1
	Bound to Fe/Mn oxides	1.1±0.0	68.6±0.1
	Bound to organic matter	3.8±0.1	344.4±0.4
	Residual	8.7±0.2	183.6±0.2
	Sum	15.3±0.3	617.3±0.8
Total concentration		15.3±0.1	614.2±4.2

[a] Values represent the mean of triplicates ± standard deviation.

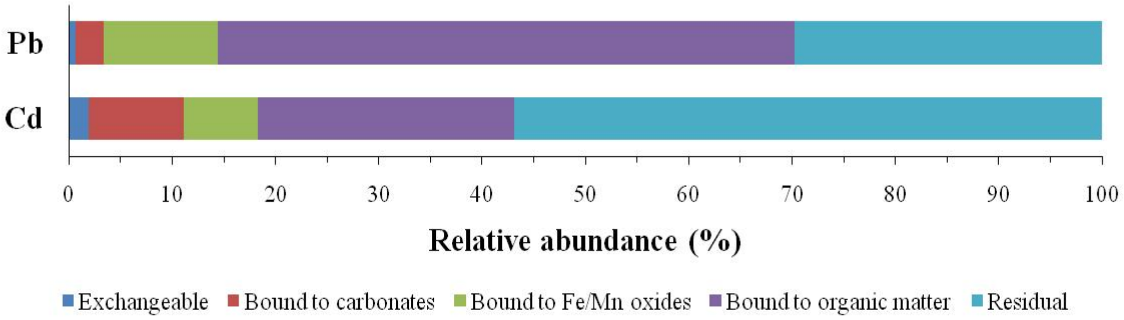

Fig. 5 The relative distributions of Cd and Pb among the various sediment fractions extracted by the Tessier's procedure

4. Conclusions

The experimental results indicate that the developed analytical method by using electrochemical stripping analysis for the determining different chemical forms of Cd^{2+} and Pb^{2+} which extract from soil and sediment by Tessier's sequential extraction procedure is successful. The method is applicable for direct simultaneous determination of Cd^{2+} and Pb^{2+} which are extracted from soil and sediment by sequential extraction using standard addition method for quantification of the analytes. Therefore, it is not require sample preparation before measurement. In addition Bi-SPCEs are sensitive alternative electrode for Cd^{2+} and Pb^{2+} with the high active surface area, low background current, low toxicity of Bi, disposable material requiring minimal pretreatment and their small size. Sequential extraction as Tessier's procedure coupled SWASV using Bi-SPCE offers great scope for applications in which it is simpler, less expensive, and low sample volume than the spectrometric methods for studying in environmental field.

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Flow Injection Amperometric Detection and Off-line Sequential Extraction Column for Fractionation of Phosphorus in Sediment

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ABSTRACT

Flow injection amperometric system has been developed for determination of phosphorus. It is based on in-line formation of 12-molybdophosphate complex, which is further electrochemically reduced on a glassy carbon working electrode at a constant applied potential of 0.20 V vs. Ag/AgCl. An electrical current is directly proportional to phosphate concentration. Calibration graphs plotting between peak height and phosphate concentration were obtained in range of 0.1-1.0 and 1.0-10.0 mg L-1 P-PO4, with a detection limit of 0.03 mg L^{-1} P-PO4. The precision of 2.1, 2.6, 1.5, 1.2 and 1.0% (n=11, 0.1, 0.5, 1.0, 5.0 and 10.0 mg L^{-1} P-PO4, respectively) and sample throughput of 89 h^{-1} were achieved. The method has lower interference (i.e., from silicate, colored and colloidal substances, and the Schlieren's effect) than the commonly used spectrophotometric method. Although it may be interfered by cations, e.g., Fe^{3+} and Al^{3+}, these ions could be eliminated by using Chelex 100 resin column. The proposed system was successfully applied to determine phosphorus that sequentially leached from sediments. An off-line leaching column was proposed for fractionation of phosphorus according to Hieltjes-Lijklema scheme (HLS). The results obtained from extraction by using the proposed column method and the batch shaking method were in good agreement (no significant difference at 95% confident level).

KEYWORDS: Flow injection amperometry, Phosphorus, Fractionation, Sequential extraction, Sediment

1. INTRODUCTION

Phosphorus (P) is an important element for the growth of plants and animals. It also plays a major role in eutrophication of aquatic system [1-3]. Determination of phosphorus in natural water and environmental solids is significant for assessing the sources, cycle and fate of phosphorus [4], whereas, the information on total phosphorus content is not enough to estimate the environmental risk [3,5,6]. In solid phases, various chemical forms of phosphorus have different behaviors, mobilities and bioavailabilities [3,7]. Consequently, it is necessary to study and determine phosphorus species in environmental solids using fractionation schemes [3,6,8].

Sequential extraction or fractionation procedures have been widely used to characterize the different forms of inorganic phosphorus exploiting acid and alkaline reagents [9,10]. Most modification or improvement of extraction schemes are originally suggested by Chang and Jackson [9]. The Hieltjes-Lijklema scheme [11] is one of the modified fractionation procedures that it is simple to operate and has been used by many researchers [9,12-19]. This method employs NH_4Cl to extract loosely bound P and partially remove carbonates, NaOH to dissolve Fe- and Al-bound P, and HCl to solubilize Ca-bound P.

Sequential extraction procedures have been performed in a batchwise manner, i.e., normally by shaking solid sample with extracting solution, which is much time consuming, large consumption of reagents and inconvenient. Flow based on-line fractionation methods were also proposed [9,18,19]. The detection of extracted phosphate could be done on-line or off-line, commonly by a spectrophotometric method based on a molybdenum blue reaction. However, the spectrophotometric detection using this reaction suffers from interferences such as silicate, colored and colloidal substances and the Schlieren's (refractive index) effect. Amperometric detection is one of electrochemical techniques that can be applied for phosphate determination and it does not suffer from the mentioned interferences [4,20].

In this work, the flow injection amperometric detection system was developed for determination of phosphate in different mediums of various extracting solutions according to the Hieltjes-Lijklema scheme. An on-line cation exchange column was incorporated into the proposed system to eliminate interference from metal ions. A batchwise off-line extraction column was also proposed for sequential extraction of sediment using the Hieltjes-Lijklema scheme. The method provides the results comparable to the standard shaking extraction method, but it is faster, lower cost, and more convenient operation.

2. MATERIALS AND METHODS

2.1 Chemicals

All chemicals used are analytical reagent grade. All standard and reagent solutions were prepared with deionized (DI) water, which obtained from Millipore water purification system (Millipore, Sweden). Potassium dihydrogenphosphate (Fisher Scientific, USA) 0.4394 g was dissolved with water and the solution was made up to 100.00 mL to obtain 1000 mg L^{-1} of phosphate stock standard solution. Working standard solution was prepared by appropriately diluting the stock solution with water. Solution of 0.1 M potassium chloride was prepared by dissolving 3.73 g potassium chloride (Carlo Erba, Italy) with water and the solution was made up to 500 mL. Ammonium molybdate (Carlo Erba, Italy) 2.50 g was dissolved with water. Then, sulfuric acid (QRëc, New Zealand) 12.5 mL was added into the solution. The solution was made up to 500 mL and the resulting solution contains 0.5%w/v molybdate and 2.5%v/v sulfuric acid solution.

Chelex 100 resin column was prepared by suspending 5.0 g of the Chelex 100 resin (50-100 mesh (sodium form), total capacity 0.4 meq mL^{-1}, density 0.65 g mL^{-1} (BIO-RAD, USA)) in water and pouring into 10 mL of plastic syringe (length 8.5 cm, I.D. 1.5 cm). After that, the resin column was washed with the large excess of 1 M HCl, H_2O, 1 M NaOH and H_2O, respectively, until pH of the effluent was less than 10.

Extracting solutions for sequential extraction of phosphorus were prepared as follow: Ammonium chloride (Scharlau, Spain) 26.75 g was dissolved with water. Then, the pH of solution was adjusted to 7.0 by adding ammonium hydroxide (Fluka, Switzerland). The solution was made up to 500 mL to obtain 1.0 M of ammonium chloride solution. Sodium hydroxide (Solvay, Belgium) 2.00 g was dissolved with water and the solution was made up to 500 mL to obtain 0.1 M of sodium hydroxide solution**.** Hydrochloric acid (Lab Scan, Poland) 20.8 mL was added into water and the solution was made up to 500 mL to obtain 0.5 M of hydrochloric acid solution.

2.2 Flow Injection Amperometric System

Flow injection amperometric system for phosphorus determination is shown in Figure 1. The system consisted of a peristaltic pump (Ismatec, Switzerland) with Tygon pump tubing, an injection device assembled from a set of three-way solenoid valves (Biochemvalve, USA), a flow through electrochemical cell (Cross-flow cell, Model MF – 1093, BAS, USA) composed of a 3 mm diameter glassy carbon working electrode (GCE), a Ag/AgCl reference electrode (RE) and a stainless steel auxiliary electrode (AE), a PalmSens handheld potentiostat/galvanostat (PalmSens, Netherland) and a personal computer. PTFE tube with inner diameter of 0.5 mm was used for assembling the flow system. The glassy carbon electrode was polished with water slurry of 0.3 and 0.05 μm alumina polish (Al_2O_3), respectively, on a polishing pad and polished about 100-300 times to obtain a fresh surface. Then, it was rinsed three times into water and into ethanol alternatively to eliminate the remaining Al_2O_3 particles from its surface.

The operation of FI system is as follow: A solution was flowed through the system at 3.2 mL min^{-1}. A standard/sample solution (75 μL) was injected into the stream of 0.1 M KCl and merged with the stream of 0.5%w/v acidic molybdate solution. Then, the mixture was flowed through a mixing coil (50 cm) to a flow through electrochemical cell. The 12-molybdophosphate complex produced from the reaction was then electrochemically reduced at a GCE working electrode, at a constant applied potential of 0.20 V *vs.* Ag/AgCl, producing an electrical current which is directly proportional to phosphate concentration. The calibration graph was plotted between peak height and phosphate concentration and used for quantification of phosphate in sample.

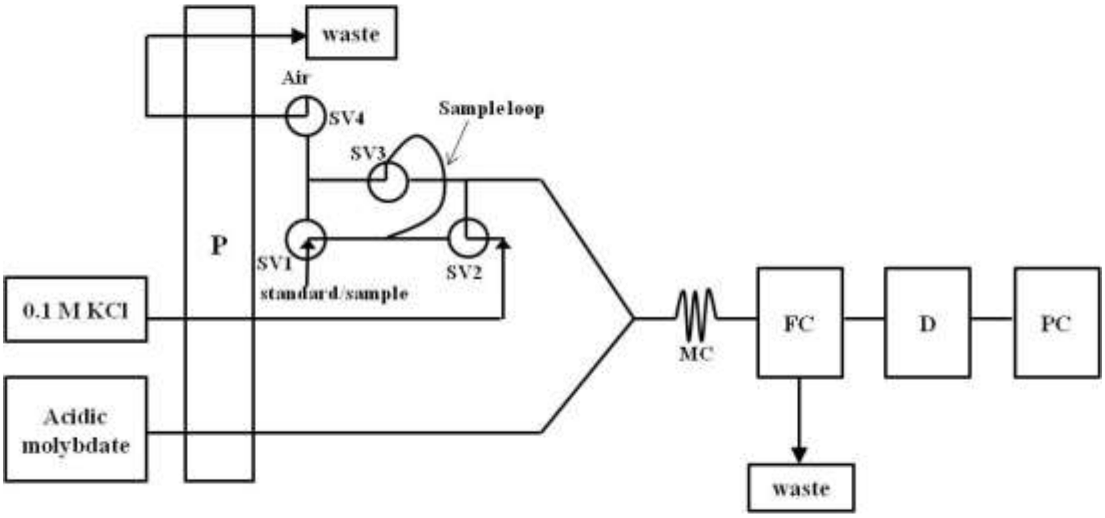

Figure 1. Flow injection amperometric system for phosphorus determination: P = peristaltic pump, SV1-4 = solenoid valves, MC = mixing coil, FC = flow through electrochemical cell (WE = glassy carbon I.D. 3 mm, RE = Ag/AgCl, AE = stainless steel), D = potentiostat/amperometric detector, and PC = computer.

2.3 Sample Collection and Preparation

Sediment samples were collected from 6 sites in Chiang Mai University, as illustrated in Figure 2. Sediment samples (depth 0-3 cm) were randomly collected in each sampling site and combined together. The samples were sieved through a 70 mesh sieve and kept at -20 °C before analysis. 1.0000 g of wet sediment sample was oven dried at 105 °C for 16-24 hours. After that, the dried sediment sample was put into desiccator, weighted and calculated percentages of solid and moisture.

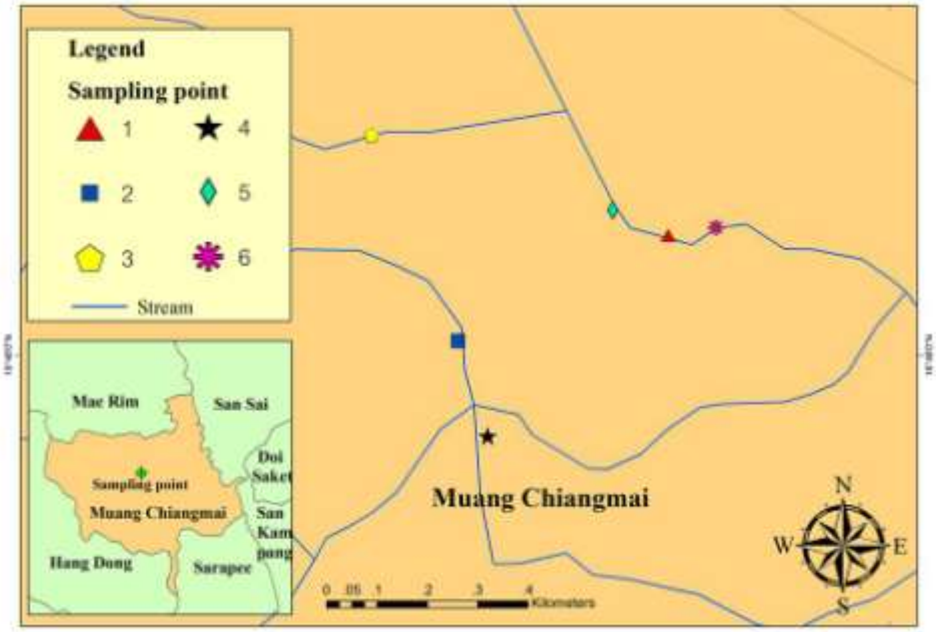

Figure 2. Map of sampling sites showing the location of 6 sampling points in Chiang Mai University.

2.4 Sequential Extraction Procedures

2.4.1 Sequential extraction of phosphorus from sediment by the off-line leaching column

The column leaching was carried out at room temperature (25±2°C) by the batch method employing a plastic syringe fitted at the bottom with cotton wool and a piece of filter paper to support the sample as shown in Figure 3. A portion (1.0000 g) of sediment was accurately weighed and put in the column. The first extractant (1.0 M NH_4Cl) 50.00 mL was carefully poured into the column and the leached solution was collected in a polyethylene bottle. Then, the next extractant (0.1 M NaOH) 50.00 mL was carefully poured into the column and the leached solution was collected in a polyethylene bottle. The last extractant (0.5 M HCl) 50.00 mL was carefully poured into the column and the leached solution was collected in a polyethylene bottle. Finally, the residue was digested with 2.00 mL of hydrochloric acid for an hour, then filtered through Whatman No.1 filter paper and made up to 50.00 mL with water.

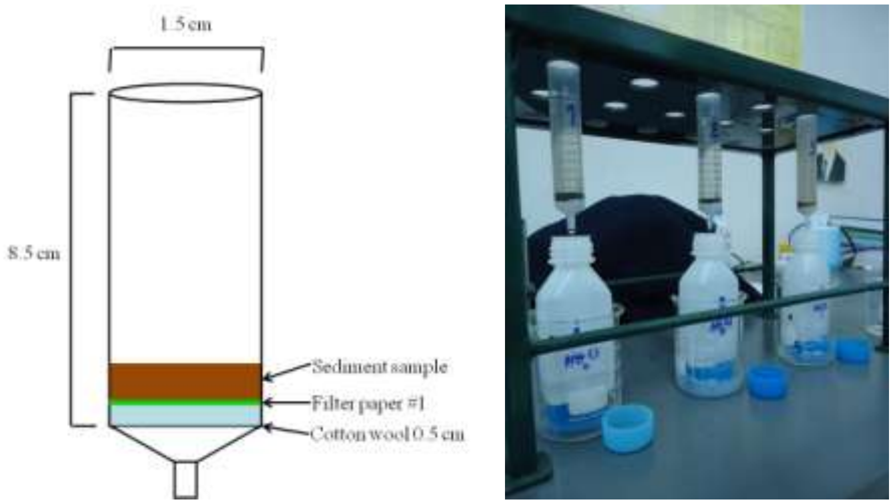

Figure 3. A leaching column for off-line sequential extraction of phosphorus from sediment samples.

2.4.2 Sequential extraction of phosphorus from sediment shaking method

A portion (1.0000 g) of sediment was extracted with 50.00 mL of 1.0 M NH_4Cl for 2 hours, then filtered through Whatman No.1 filter paper and collected in a polyethylene bottle. The residue was extracted with 1.0 M NH_4Cl again. After that, the residue was extracted with 0.1 M NaOH for 17 hours, then filtered through Whatman No.1 filter paper and collected in a polyethylene bottle. Next, the residue was extracted with 0.5 M HCl for 24 hours, then filtered through Whatman No.1 filter paper and collected in a polyethylene bottle. Finally, the residue was digested with 2.00 mL of hydrochloric acid for an hour, then filtered through Whatman No.1 filter paper and made up to 50.00 mL with water. Extraction was carried out at room temperature (25 ± 2 °C). Before changing to the next extractant, cleaning the sediment with water was done. The Schematic diagram of sequential extraction of phosphorus is summarized in Figure 4.

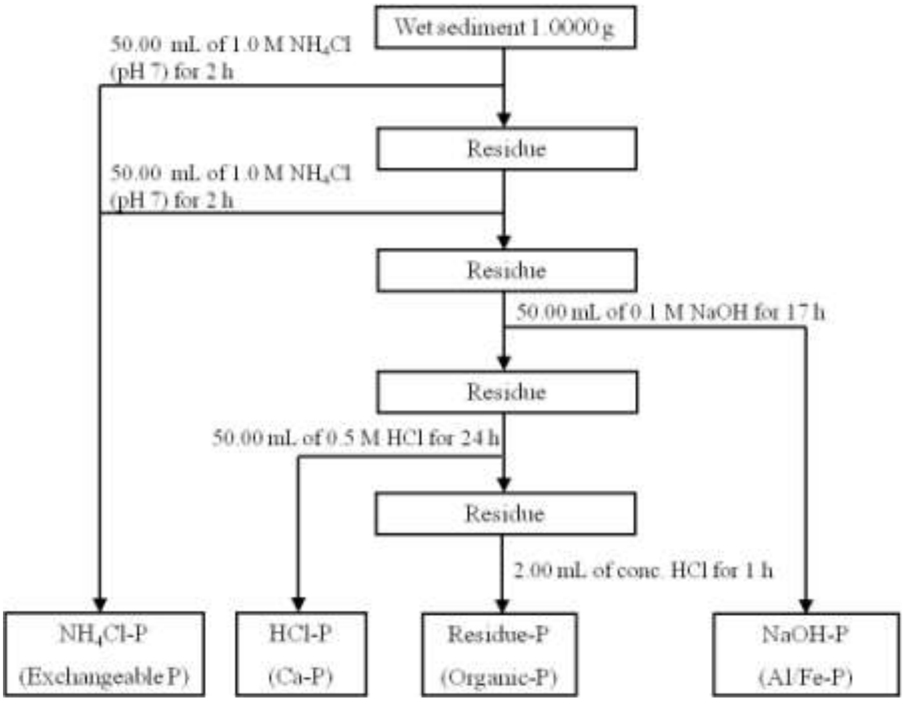

Figure 4. Schematic diagram of sequential extraction of phosphorus from sediment soil by the batch method.

3. RESULTS AND DISCUSSION

3.1 Flow Injection Amperometric Method for Phosphate Determination

FI-ampermetric method for phosphorus determination is based on the electrochemical reduction of the 12-molybdophosphate complex as shown in the equations below:

Phosphate (PO_4^{3-}) + Acidic molybdate → 12-molybdophosphate complex (1)

12-molybdophosphate complex (Mo^{VI})+ e^- → Molybdenum blue(Mo^{V}) (2)

The 12-molybdophosphate complex was reduced at a working electrode and produced an electrical current which is directly proportional to phosphate concentration.

The FI system as depicted in Figure 1 was employed and the previously reported conditions of the system [20] were preliminary used. It was found that applied potential of 0.20 V vs. Ag/AgCl gave a good sensitivity and stable baseline. Moreover, signal of silicate (1000 mg L^{-1}) and arsenate (5 mg L^{-1}) did not appear at this potential. The sensitivity increased when increasing ammonium molybdate concentration, however, at ammonium molybdate concentration higher than 0.5%w/v, the baseline drift was occurred due to the adsorption of the molybdenum blue at surface of GCE was occurred. Therefore, 0.5%w/v of ammonium molybdate was chosen. Sulfuric acid concentration of 2.5 %v/v was selected for the developed system since it furnished better sensitivity and stable baseline. At sulfuric acid concentration higher than 2.5%v/v, the sensitivity was higher but a dramatically noisy baseline was observed. The optimum conditions of the FI-amperometric system are summarized in Table 1.

Table 1. The optimum conditions of the FI-amperometric system for phosphate determination

Parameters	Optimum value
Flow rate	3.2 mL min^{-1}
Sample volume	75 μL
Ammonium molybdate concentration	0.5%w/v
Sulfuric acid concentration	2.5%v/v
Potassium chloride concentration	0.1 M
Mixing coil length	50 cm
Working electrode	Glassy carbon I.D. 3 mm
Reference electrode	Ag/AgCl
Auxiliary electrode	stainless steel
Applied potential	0.20 V

3.2 Analytical Characteristics of the Method

Under the optimum conditions in Table 1, the FIAgram of various concentrations of phosphate standard solution was obtained as shown in Figure 5. The calibration graphs were linear in the range of 0.1-1.0 mg L^{-1} P-PO_4 ($y = -0.5240x - 0.0053$, $r^2 = 0.9991$) and 1.0-10.0 mg L^{-1} P-PO_4 ($y = -0.3739x - 0.2250$, $r^2 = 0.9978$). The limit of detection (LOD) and the limit of quantitation (LOQ) were 0.03 and 0.11 mg L^{-1} P-PO_4, respectively.

Precision of the system was evaluated by eleven injections of standard phosphate solution concentrations of 0.1, 0.5, 1.0, 5.0 and 10.0 mg L^{-1} P-PO_4. The proposed method provided a good precision as indicated by percentages of relative standard deviation (%RSDs) of 2.1, 2.6, 1.5, 1.2, and 1.0 for 0.1, 0.5, 1.0, 5.0 and 10.0 mg L^{-1} P-PO_4, respectively. The proposed method consumed 75 μL of sample and 2.1 mL each of electrolyte and reagent solutions (corresponding to 16 mg of KCl, 11 mg of ammonium molybdate and 0.05 mL of sulfuric acid per analysis), which is much lower than the batch spectrophotometric method. Sample throughput of 89 h^{-1} was achieved.

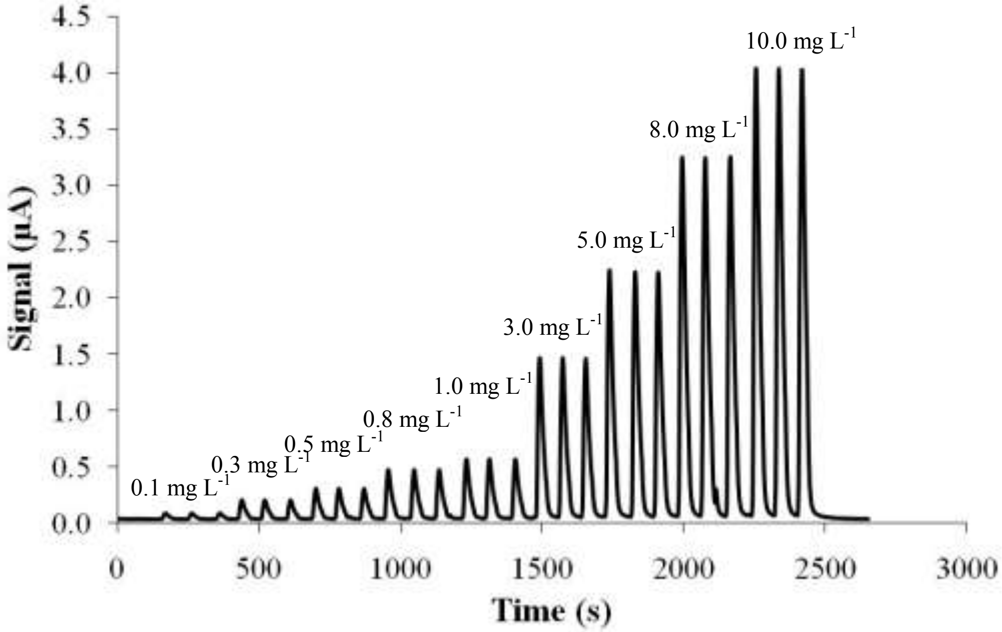

Figure 5. FIAgram of standard phosphate solutions by using the FI-amperometry.

3.3 Interference study

Effects of some potential interfering species have been investigated by adding different concentrations of the potential interfering substances into 0.5 mg L^{-1} standard phosphate solution. Then, the solutions were injected to the FI-ampermetric system and the peak height obtained was compared to that of 0.5 mg L^{-1} standard phosphate solution without interfering species. The tolerance limit of interfering substances was considered as the concentration that did not give %different in peak height more than ±5%. Effects of some anions were investigated, such as arsenate and silicate which can react with the acidic molybdate and interfere in spectrophotometric molybdenum blue method. It was found that the FI amperometric method can tolerate to arsenate and silicate up to 0.25 mg L^{-1} and 1000 mg L^{-1}, respectively, which is higher than the spectrophotometric method. Tolerance limits for carbonate, nitrate and chloride were 500 mg L^{-1}, 1000 mg L^{-1}, and 40 g L^{-1}, respectively. Unlike spectrophotometric method, amperometric detection did not suffer from high concentration of salt (Schlieren's effect) and colored substances in sample solution.

The FI-amperometric system can tolerate to some cations, such as Na^+ up to 26 g L^{-1}, Ca^{2+} and NH_4^+ up to 1000 mg L^{-1}, and Mg^{2+} up to 500 mg L^{-1}. In case of metal ions which can bind with phosphate and precipitate in the insoluble form (e.g., Cu^{2+}, Zn^{2+}, Pb^{2+}, Cd^{2+}, Mn^{2+}, Fe^{2+}, Fe^{3+} and Al^{3+}), the system can tolerate up to 10 mg L^{-1} of Cu^{2+}, Zn^{2+}, Pb^{2+}, Cd^{2+} and Fe^{2+}, and 5 mg L^{-1} of Mn^{2+}. Fe^{3+} and Al^{3+} had low tolerance limit at 0.1 and 0.05 mg L^{-1}, respectively. For Fe^{3+}, it provides the negative interference at 0.1-1.0 mg L^{-1}, but it gives the positive interference at the higher concentration, i.e., ≥ 5 mg L^{-1}.

In order to reduce interference from cations, an ion exchange resin was used to remove cations from the solution before being injected into the system. Chelex 100 is a chelate resin which is widely used to bind metal ions. It is styrene divinylbenzene copolymers with iminodiacetate exchange groups. The resin can strongly bind metal ions at high pH and poorly bind to metal ions at low pH [21]. The resin column was prepared as described in section 2.1 and tested for removal of Fe^{3+}. It was found that after passing through the Chelex 100 resin column the peak heights of phosphate solutions with and without Fe^{3+} were identical, indicating that Chelex 100 resin could eliminate Fe^{3+}.

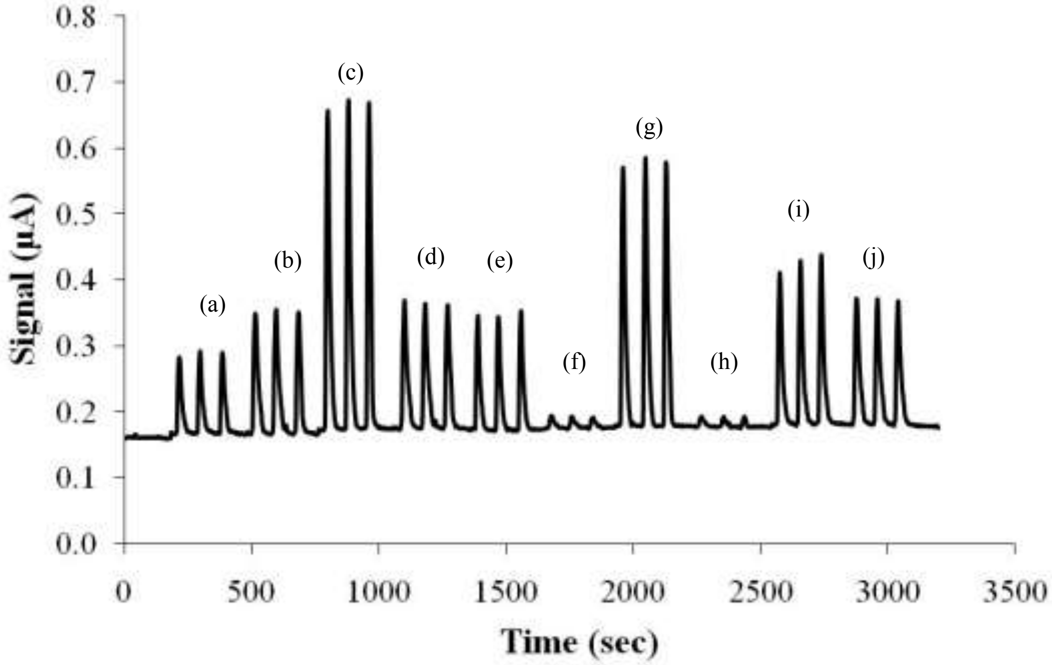

Figure 6. FIAgram from the FI-amperometric system for the injection of various solutions: (a) 5 mg L^{-1} Fe^{3+} + 0.5 mg L^{-1} P-PO_4, (b) 5 mg L^{-1} Fe^{3+} + 0.5 mg L^{-1} P-PO_4 (passed resin column), (c) 10 mg L^{-1} Fe^{3+} + 0.5 mg L^{-1} P-PO_4, (d) 10 mg L^{-1} Fe^{3+} + 0.5 mg L^{-1} P-PO_4 (passed resin column), (e) 5 mg L^{-1} Fe^{3+}, (f) 5 mg L^{-1} Fe^{3+} (passed resin column), (g) 10 mg L^{-1} Fe^{3+}, (h) 10 mg L^{-1} Fe^{3+} (passed resin column), (i) 0.5 mg L^{-1} P-PO_4 and (j) 0.5 mg L^{-1} P-PO_4 (passed resin column).

3.4 Application of FI amperometric Method to Study Fractionation of Phosphorus in Sediment

3.4.1 Effect of extractants to the FI-amperometric method

Characterization of various phosphorus species in soil is normally performed by sequential extraction employing three different extractant solutions such as 1.0 M NH_4Cl, 0.1 M NaOH and 0.5 M HCl (Hieltjes-Lijklema Scheme). The determinations of leached phosphorus in different extractants are usually done by using different conditions/systems [4,18,19]. In this work, the developed FI amperometric system was proposed to analyze all 3 extracted solutions. Effect of the extractants was examined before applying the proposed system to such analysis. Under the optimum conditions in Table 1, standard phosphate solutions (0.1-10.0 mg L^{-1}) in each medium (DI water, 1.0 M NH_4Cl, 0.1 M NaOH and 0.5 M HCl) were injected into the system. The FIAgrams obtained are shown in Figure 7. Comparison to standard solution prepared in DI water, 0.1 M NaOH did not affect on the phosphate determination, while 1.0 M NH_4Cl caused slightly reduction of sensitivity, and 0.5 M HCl caused dramatically reduction of sensitivity of the analysis. It might cause by the high molar ratio of [H^+] to [MoO_4^{2-}] (more than 80) resulted in slow and incomplete reaction to form phosphomolybdate complex [22]. Furthermore, it might be resulted from chloride ion which competed to phosphate ion to bind molybdate ion. Therefore, the standard solution of phosphate must be prepared in each extractant for use in analysis of each fraction.

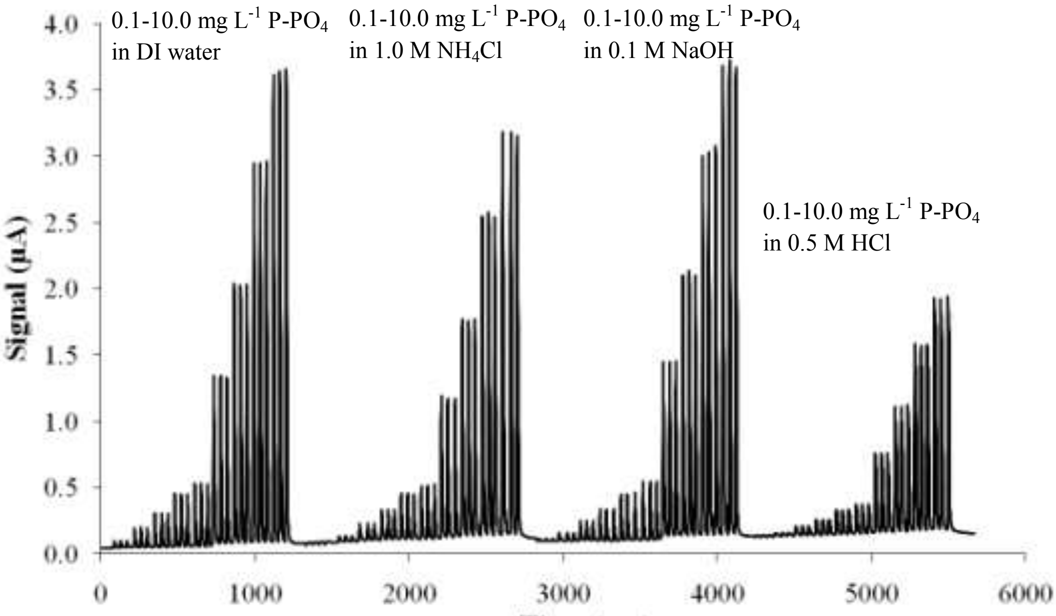

Figure 7. FIAgrams of standard phosphate solutions in the different mediums (DI water, 1.0 M NH_4Cl, 0.1 M NaOH and 0.5 M HCl).

3.4.2 Determination of phosphorus leached from sediment in various fractions

Firstly, percentage of solid and moisture in sediment samples were analyzed. It was found that the percentages of solid were between 45-73 and moistures were in the range of 28-55%. Content of phosphorus leached in each fraction was reported as mg kg^{-1} dry weight.

The sediment samples were sequentially extracted by using off-line column leaching, and standard shaking-filtering methods as described in section 2.4. Several samples can be carried out in parallel. Sample solutions in each fraction were injected into the FI-amperometric system and phosphorus contents were calculated by using corresponded calibration graphs. Phosphorus contents in various fractions obtained from the off-line leaching column and the batch shaking method are presented in Table 2. The total phosphorus (Total-P) content is the summation of phosphorus contents in the fractions of NH_4Cl, NaOH, HCl and the residue. The values of total phosphorus ranged from 307 to 831 mg kg^{-1} for the off-line leaching column and 315 to 856 mg kg^{-1} for the shaking method. The first fraction (NH_4Cl-P) is exchangeable phosphorus which is the most mobile one, being extracted by NH_4Cl. It was found at negligible levels in only sample No. 5 (13 mg kg^{-1} by off-line leaching column, and 11 mg kg^{-1} by shaking method). Phosphorus contents in NaOH-P, HCl-P and Residue-P ranged from 26 to 128, 57 to 434 and 179 to 500 mg kg^{-1}, respectively, for the off-line leaching column, and 18 to 103, 47 to 409 and 106 to 465 mg kg^{-1}, respectively, for the shaking method. Results from both the extraction methods were in good agreement. Both the results were compared with paired *t*-test at 95% confidence level, indicated that there was no significant difference between both methods ($t_{critical}$ = ± 2.571, t_{stat} of total-P = -0.562, t_{stat} of NaOH-P = 1.678, t_{stat} of HCl-P = -0.752 and t_{stat} of residue-P = 0.280). In addition, the off-line column leaching method is faster, simpler and more convenient operation as summarized in Table 3.

Figure 8 illutrated fractions of phosphorus being extracted from sediment samples by both methods. It was found that the trends of phosphorus contents were Residue-P > HCl-P > NaOH-P > NH_4Cl for sites 2, 3 and 4, and HCl-P > Residue-P > NaOH-P > NH_4Cl-P for sites 1, 5 and 6. For all the sediment samples from both extraction methods, the rank order of inorganic phosphorus fractions was HCl-P > NaOH-P > NH_4Cl-P, indicating that phosphorus in the sediment is in stable forms which do not easily leach into water under natural condition.

Table 2. Contents of phosphorus in sediment in different fractions leaching by off-line leaching column and the batch shaking method

Sample	**Method**	**Phosphorus (mg kg^{-1}) contents (mean ± S.D.)**				
		NH_4Cl-P	**NaOH-P**	**HCl-P**	**Residue-P**	**Total-P**
1	column	N.D.	128 ± 20	434 ± 202	269 ± 188	831 ± 199
	batch	N.D.	103 ± 8	320 ± 116	314 ± 179	736 ± 144
2	column	N.D.	49 ± 13	57 ± 7	201 ± 65	307 ± 67
	batch	N.D.	37 ± 11	98 ± 13	180 ± 26	315 ± 28
3	column	N.D.	26 ± 5	60 ± 11	227 ± 23	314 ± 17
	batch	N.D.	18 ± 2	47 ± 6	347 ± 12	412 ± 12
4	column	N.D.	32 ± 4	178 ± 60	500 ± 53	709 ± 97
	batch	N.D.	39 ± 12	352 ± 31	465 ± 163	856 ± 185
5	column	13 ± 3	91 ± 17	326 ± 48	228 ± 91	658 ± 122
	batch	11 ± 2	33 ± 13	409 ± 40	239 ± 40	692 ± 58
6	column	N.D.	54 ± 9	208 ± 25	179 ± 28	441 ± 37
	batch	N.D.	53 ± 12	216 ± 38	106 ± 5	376 ± 42

*N.D. = Not detected

Table 3. Comparison of the batch shaking/filtering and the off-line leaching column methods

Parameters	Shaking and filtering	Off-line leaching column
Weight of soil (g)	1	1
Volume of extractant (mL):		
- NH_4Cl fraction	50×2 times	50
- NaOH fraction	50	50
- HCl fraction	50	50
Operation time per sample (h)	45	6-14
Temperature	Room temperature	Room temperature
Instrument	Shaker, erlenmeyer flask, glass funnel, Whatman filter paper No. 1	Plastic syringe 10 mL, cotton wool, Whatman filter paper No. 1
Procedure	Shaking and filtering	Pouring the extractant solution into the column

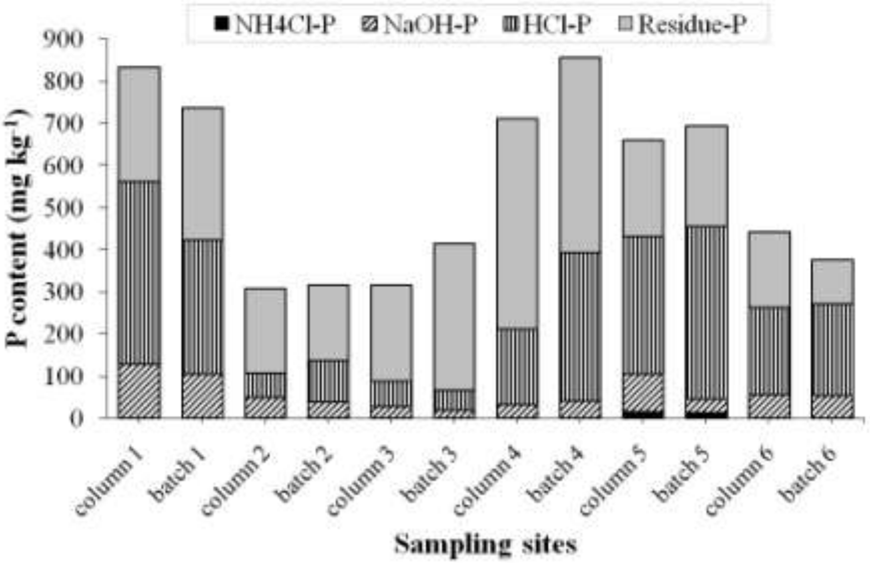

Figure 8. Contents of phosphorus in different sediment samples, being extracted into various fractions by using the off-line leaching column and the batch shaking methods. (sediment samples were taken from different sampling sites as shown in Figure 2).

4. CONCLUSIONS

Flow injection amperometric method was developed for determination of phosphate. The method has high sensitivity and selectivity but yet simple and more convenient operation. It has many advantages over the commonly used spectrophotometric method, such as did not affect by high concentration of salts and colored substances, and can tolerate to high concentration of silicate. The developed method was successfully applied for analysis of various extracting solutions obtained from sequential extraction of sediment. The off-line column extraction was also proposed for sequential leaching of phosphorus from solid material, which gave the results in good agreement with those obtained by the standard shaking extraction procedure.

ACKNOWLEDGEMENTS

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ภาคผนวก ง

ตัวอย่างเอกสารการฝึกอบรมและประยุกต์งานวิจัยในการวิเคราะห์ดินสำหรับเกษตรกร

เอกสารประกอบการฝึกอบรม

โครงการการถ่ายทอดเทคโนโลยี
เรื่อง เทคโนโลยีใหม่ในการหาปริมาณธาตุอาหารพืชเพื่อการจัดการดินและปุ๋ยอย่างเหมาะสม

วันศุกร์ที่ 20 พฤษภาคม 2554

ณ ศูนย์ความเป็นเลิศนวัตกรรมวิทยาศาสตร์และเทคโนโลยีการวิเคราะห์
มหาวิทยาลัยเชียงใหม่

อาคารคณะวิทยาศาสตร์ 40 ปี ห้องประชุมสัมมนา ชั้น 2
คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่

<u>โดย</u> คณะวิทยาศาสตร์ (ศูนย์วิจัยวิทยาศาสตร์พหุวิทยาการ) ร่วมกับ
ศูนย์ความเป็นเลิศนวัตกรรมวิทยาศาสตร์และเทคโนโลยีการวิเคราะห์
มหาวิทยาลัยเชียงใหม่

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I ANALY S T
Center of Excellence
Chiang Mai University

โครงการการถ่ายทอดเทคโนโลยี
เรื่อง เทคโนโลยีใหม่ในการหาปริมาณธาตุอาหารพืชเพื่อการจัดการดินและปุ๋ยอย่างเหมาะสม

วันศุกร์ที่ 20 พฤษภาคม 2554 เวลา 8.30 – 16.30 น.
ณ ศูนย์ความเป็นเลิศนวัตกรรมวิทยาศาสตร์และเทคโนโลยีการวิเคราะห์ มหาวิทยาลัยเชียงใหม่
อาคารคณะวิทยาศาสตร์ 40 ปี ห้องประชุมสัมมนา ชั้น 2 คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่

ภาคเช้า

เวลา	กิจกรรม
8.30 – 9.00 น.	ลงทะเบียนผู้เข้าร่วมประชุมเชิงปฏิบัติการ
9.00 – 9.15 น.	พิธีเปิดการอบรมเชิงปฏิบัติการ โดย ศ.ดร. เกตุ กรุดพันธ์ หัวหน้าศูนย์ความเป็นเลิศนวัตกรรมวิทยาศาสตร์และเทคโนโลยีการวิเคราะห์ มหาวิทยาลัยเชียงใหม่ และหัวหน้าศูนย์วิจัยวิทยาศาสตร์พหุวิทยาการ คณะวิทยาศาสตร์
9.15 – 9.45 น.	การบรรยาย: เรื่อง เทคโนโลยีใหม่ในการวิเคราะห์หาปริมาณธาตุอาหารพืช โดย: รศ.ดร. จรูญ จักร์มุณี
9.45 – 10.15 น.	พักรับประทานอาหารว่างและเครื่องดื่ม
10.15 – 10.45 น.	การบรรยาย: เรื่อง ปุ๋ยอินทรีย์น้ำ/น้ำหมักชีวภาพ กระบวนการผลิตแบบง่ายและการเก็บตัวอย่างเพื่อการวิเคราะห์ธาตุอาหารพืช โดย: นาย ชินกฤต สุวรรณคีรี
10.45 –11.15 น.	การบรรยาย: เรื่อง การเก็บตัวอย่างดินและพืชเพื่อการวิเคราะห์ธาตุอาหาร โดย: ดร. สิทธิชัย ลอดแก้ว
11.15 –11.45 น.	การบรรยาย: เรื่อง ระบบโฟลอินเจคชันสำหรับการวิเคราะห์หาปริมาณฟอสฟอรัสและไนโตรเจน โดย: รศ.ดร. จรูญ จักร์มุณี
11.45 – 12.00 น.	ตอบข้อซักถาม
12.00 – 13.30 น.	พักรับประทานอาหารกลางวัน

ภาคบ่าย

เวลา	กิจกรรม
13.30 – 15.30น.	ทำปฏิบัติการและชมการสาธิต (4 ปฏิบัติการ) โดย อ.ดร. กฤษณะ จิตมณี, ดร. จรูญ จันทรสมบูรณ์, นางสาวพิมพ์พร อินเสน และนางสาวปัทมา ยานุ
15.30 – 15.45 น.	พักรับประทานอาหารว่างและเครื่องดื่ม
15.45 – 16.15 น.	-การสรุปผลการปฏิบัติการ -ตอบข้อซักถามและแนวทางในการนำความรู้/เทคโนโลยีที่ฝึกอบรมไปใช้ โดย: รศ.ดร. จรูญ จักร์มุณี
16.15 – 16.30 น.	แจกประกาศนียบัตรแก่ผู้เข้าประชุมและกล่าวปิดการประชุม

ห้องปฏิบัติการวิจัยเพื่อนวัตกรรมเครื่องมือวิเคราะห์และไฟฟ้าเคมี
Research Laboratory for Analytical Instrument and Electrochemistry Innovation
ภาควิชาเคมีและศูนย์ความเป็นเลิศด้านนวัตกรรมทางเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่

หมายเลขอ้างอิง AIE-08-05 วันที่รายงาน 28 สิงหาคม 2556

รายงานผลการวิเคราะห์ตัวอย่าง
(Sample Analysis Report)

1. ข้อมูลตัวอย่าง

ชื่อผู้ส่งตัวอย่าง	นายวินัย สายไฮคำ
นามผู้ขอใช้บริการ	-
สถานที่ติดต่อ	154 หมู่ที่ 6 ต.ป่าป้อง อ.ดอยสะเก็ด จ. เชียงใหม่ 50220
โทรศัพท์	081-9503417
E-mail	-

2. ข้อมูลตัวอย่าง

รายละเอียดตัวอย่างดิน	
จำนวนตัวอย่าง	3 ตัวอย่าง

ผลการวิเคราะห์

เลขที่ (Lab NO)	ตัวอย่าง (Sample Designation)	พีเอช (pH)	อินทรีย์วัตถุ (OM) g/100 g	ไนโตรเจนทั้งหมด (Total N) g/100 g	ฟอสฟอรัส (P) mg/kg	โพแทสเซียม (K) mg/kg
S-12	แปลงที่ 1	6.23	2.3	10.0	24.4	14.7
S-13	แปลงที่ 2	4.73	2.3	20.0	20.9	22.4
S-14	แปลงที่ 3	4.76	2.5	30.0	38.2	32.0

รับรองผลการวิเคราะห์ถูกต้อง

(รองศาสตราจารย์ ดร. จรูญ จักร์มุณี)
หัวหน้าห้องปฏิบัติการฯ