CHAPTER 3

RESULTS AND DISCUSSION

3.1 Optimization of flow based – ASV system using bismuth film electrode as a working electrode

Bismuth is suitable to be used as a working electrode in voltammetry because it provides a wide negative potential window similar to mercury electrode. Moreover, it is negligibly toxic comparing to mercury. However, like other solid electrodes, carry over usually occurs on the use of this electrode in batch system. In this work, flow based ASV using BiFE as a working electrode was investigated. Some parameters were studied as follow.

3.1.1 Concentration of cleaning solution

Concentrations of nitric acid solution has a crucial role on the efficient cleaning of the working electrode surface. In cleaning step, potential of 0.60 V was applied to the working electrode while nitric acid was flowed through the flow cell. This helped cleaning the working electrode better than by batch method and lead to achieve better precision in repeatedly use of BiFE. Solution containing 5 mg/L Bi(III) in acetate buffer and standard solution of Cd(II) and Pb(II) at 100 µg/L were used in this experiment. Then, effect of nitric acid concentration was studied in the concentration range of 0.2-1.0 M. By considering the percentage of relative standard deviation of peak current of the analyte metals as presented in Table 3.1, it was found that 1.0 M nitric acid provided better precision, due to effective cleaning of the electrode by this solution.

Table 3.1 The effect of concentration nitric acid for cleaning GCE (n=7)

Nitric acid (M)	The % relative standard deviation of peak current (n=7)	
	Cd(II)	Pb(II)
0.2	2.90	3.60
0.4	1.39	2.08
0.6	1.70	1.87
0.8	1.09	1.19
1.0	0.29	0.83
>1.0	poor reproducibility due to the evolution of air bubble at electrode surface	

3.1.2 Effect of times for polishing and storage of GCE

The storage and polishing of glassy carbon electrode affected on the determination of Cd(II) and Pb(II) on solid working electrodes in anodic striping voltammetry. This procedure could keep the surface electrode to be fresh and decrease the blocking of electrode surface from organic compound and others. The method of storage and times for polishing of glassy carbon electrode was carried out with 50 µg/L each of Cd(II) and Pb(II) at deposition time of 60 s. The peak current about 30 µA of Cd(II) and 25 µA of Pb(II) were obtained.

It was found that the special storage (0.1M NaOH) [51] of glassy carbon electrode help to decrease time for polishing, when compare with the normal storage. As shown in Table 3.2 and Table 3.3, the fresh surface of electrode was obtained by storage the electrode in 0.1 M NaOH and polishing for about 2 min. Then, electrode was immersed three times into water and into ethanol alternatively to remove the remaining Al_2O_3 particles. This storage of electrode in 0.1 M NaOH was chosen for further experiments.

Table 3.2 The times of electrode polishing with normal storage of electrode within 1 day.

Times of polish	Peak current (μA)	
	Cd(II)	Pb(II)
1	4.80	8.68
2	15.00	12.30
3	13.20	10.60
4	13.70	9.70
5	18.50	14.60
6	12.40	8.37
7	17.40	12.40
8	20.20	15.40
9	30.00	27.60

Table 3.3 The times of electrode polishing and special storage of electrode in 0.1 M NaOH within 2 days.

Times of polish	Peak current (µA)			
	Cd(II)		Pb(II)	
	Day 1	Day 2	Day 1	Day 2
1	18.50	28.80	15.40	20.50
2	36.30	34.80	25.10	25.30

3.1.3 Concentration of acetate buffer pH 4.5

Electrolyte concentration affects the ionic strength of the solution and hence the peak current of metal ions. Moreover, it could affect the bismuth film formation on electrode surface. For the study on effect of this parameter, standard solutions of Cd(II) and Pb(II) in the range of 10 to 30 µg/L were used for constructing calibration graphs. Effect of concentration of acetate buffer pH 4.5 was studied by considering slope of calibration graphs when varying concentration of acetate buffer in the range of 0.01 to 0.40 M as shown in Figure 3.1. It was observed that the sensitivity for Cd(II) and Pb(II) increased rapidly with the increasing ionic strength up to about 0.1 M and the calibration graph with higher slope than other concentrations was achieved. Concentration of acetate buffer at 0.1 M was chosen to be the optimum because it gave highest sensitivity.

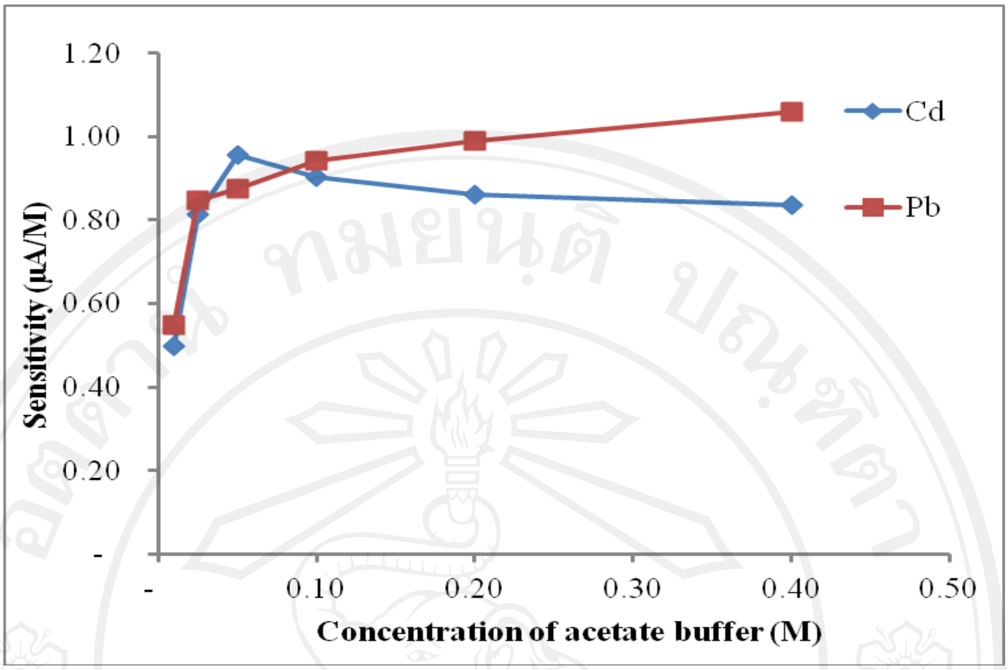


Figure 3.1 Effect of concentration of acetate buffer on sensitivity (slope) of Cd and Pb determination. Experimental conditions: acetate buffer pH 4.5 containing 0.50 mg/L Bi(III), deposition potential -1.5 V, deposition time of 90 s, cleaning potential of 0.6 V, cleaning time of 60 s, equilibration time of 30 s, voltage step of 6 mV, amplitude of 50 mV frequency 50 Hz and flow rate of the sample or standard solution at 0.5 ml/min.

3.1.4 Effect of pH of acetate buffer solution

The pH of acetate buffer solution is an important parameter for the deposition of bismuth film on glassy carbon by in situ plating [52]. The influence of the pH of the deposition upon the bismuth film electrode performance was examined by varying the pH range from 3.5 to 6.0. The effect of acetate buffer pH on metal stripping signals were studied with 20 µg/L each of Cd(II) and Pb(II). When the pH of acetate buffer was increased from 3.5 to 4.5, the stripping peak currents increased sharply, as displayed in Figure 3.2. Thereafter, a decline of signal was observed by further increasing the pH up to 6.0 because Bi(III) ion can undergo hydrolysis in non-complexing neutral and basic pH condition [38,52]. The acetate buffer of slightly acidic condition should be appropriate for metal accumulation. Thus, an acetate buffer pH 4.5 was selected for next experiment.

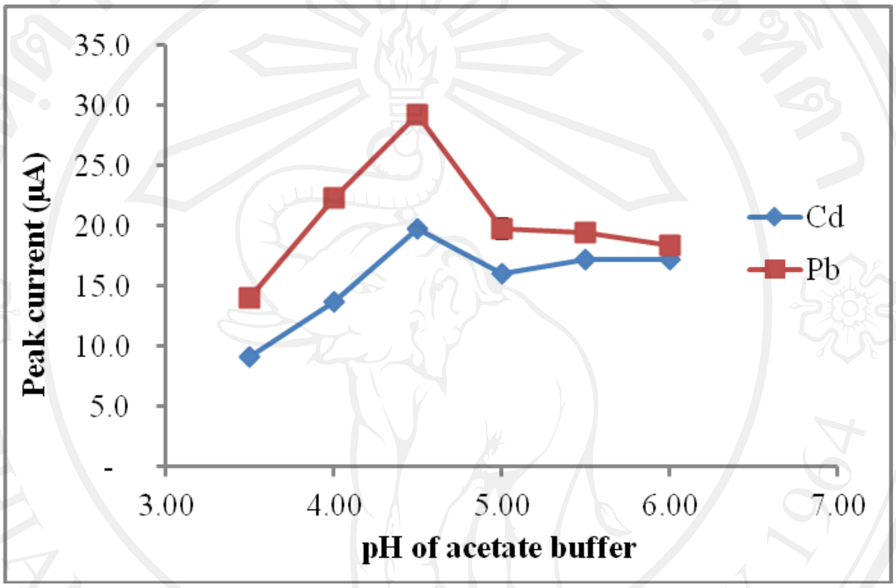


Figure 3.2 Effect of pH on metal decumulation at electrode (20 µg/L each of Cd(II) and Pb(II)). Experimental conditions: 0.10 M acetate buffer containing 0.50 mg/L Bi(III), deposition potential -1.5 V, deposition time of 90 s, cleaning potential of 0.6 V, cleaning time of 60 s, equilibration time of 30 s, voltage step of 6 mV, amplitude of 50 mV frequency 50 Hz and flow rate of the sample or standard solution at 0.5 ml/min.

3.1.5 Effect of concentration of Bi(III) plating solution

Concentration Bi(III) plating solution affected the thickness of bismuth film on the glassy carbon electrode [52]. Concentration of Bi(III) plating solution was studied in the range from 0 to 1.0 mg/L. As shown in Figure 3.3, metal stripping signals were studied with 10 and 30 µg/L each of Cd(II) and Pb(II). It was found that peak currents sharply increased with the increase of Bi(III) concentration and reached the maximum at about 0.6 mg/L. Beyond that, the peak current decreased due to the saturated Bi film block the conductive surface the bare electrode [53]. At the Bi(III) concentration higher than 0.6 mg/L, the decrease of the peak currents was resulted due to the saturation of Bi on the electrode surface. The concentration Bi(III) could attribute to the thick bismuth film coated on the electrode surface together with target metal ions. For further experiments a concentration Bi(III) plating solution at 0.60 mg/L was used.

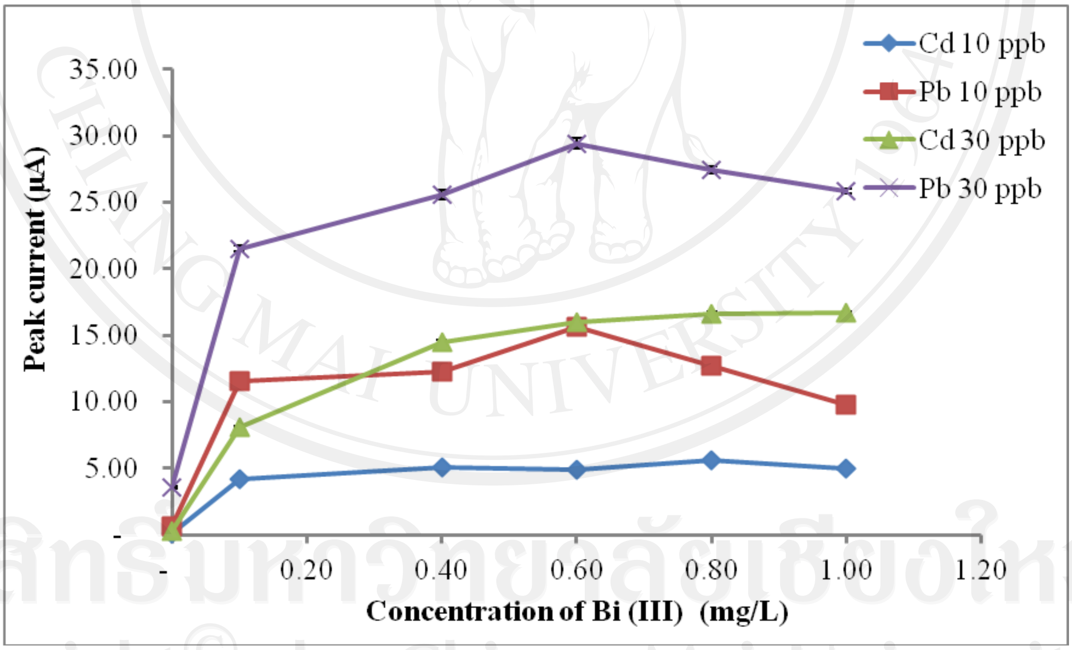


Figure 3.3 Effect of Bi(III) concentration (10 and 30 µg/L each of Cd(II) and Pb(II)). Experimental conditions: 0.10 M acetate buffer pH 4.5, deposition potential -1.5 V, deposition time of 90 s, cleaning potential of 0.6 V, cleaning time of 60 s, equilibration time of 30 s, voltage step of 6 mV, amplitude of 50 mV frequency 50 Hz and flow rate of the sample or standard solution at 0.5 ml/min.

3.1.6 Flow rate

The flow rate of solution in the deposition step affected on the sensitivity of the analysis [11, 14]. Therefore, the influence of the flow rate on stripping peak currents was investigated in the range of 0.20 to 4.00 ml/min, with fixed sample volume of 1.60 ml of 20 µg/L each of Cd(II) and Pb(II), as shown in Figure 3.4. It was found that peak currents increased with the decrease of the flow rates, and at the flow rates in the range of 2.00 to 4.00 ml/min the peak currents were constant. The higher stripping current of both Cd(II) and Pb(II) was obtained at low flow rates due to more complete reductions of Cd(II) and Pb(II). But 0.5 ml/min gave enough sensitivity and short analysis time. Therefore, a flow rate of 0.50 ml/min of sample or standard solution was selected.

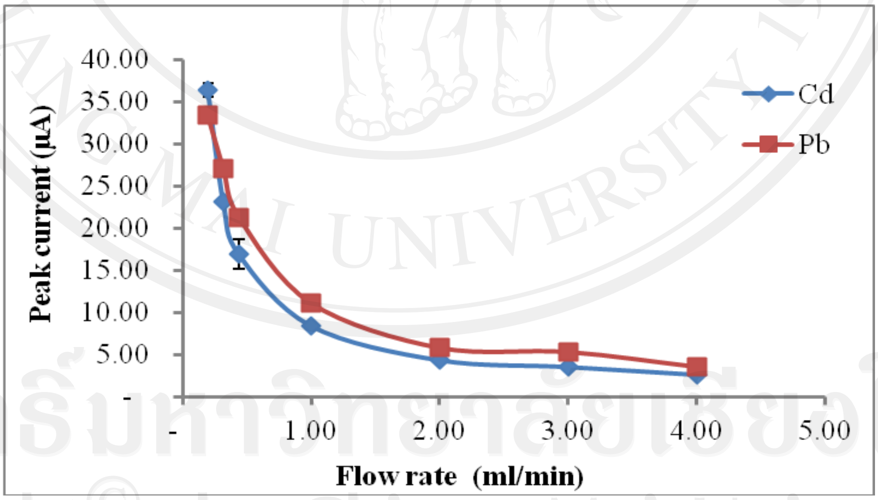
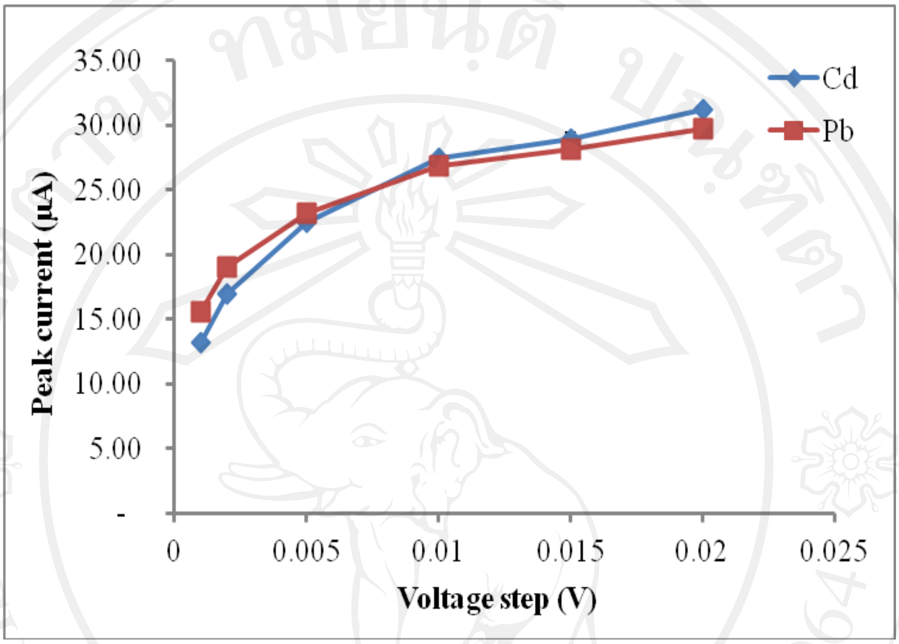


Figure 3.4 Effect of flow rate of sample or standard solution on metal stripping at 20 µg/L each of Cd(II) and Pb(II). Experimental conditions: 0.10 M acetate buffer pH 4.5 containing 0.60 mg/L Bi(III), deposition potential -1.5 V, cleaning potential of 0.4 V, cleaning time of 60 s, equilibration time of 30 s, voltage step of 6 mV, amplitude of 50 mV and frequency 50 Hz.

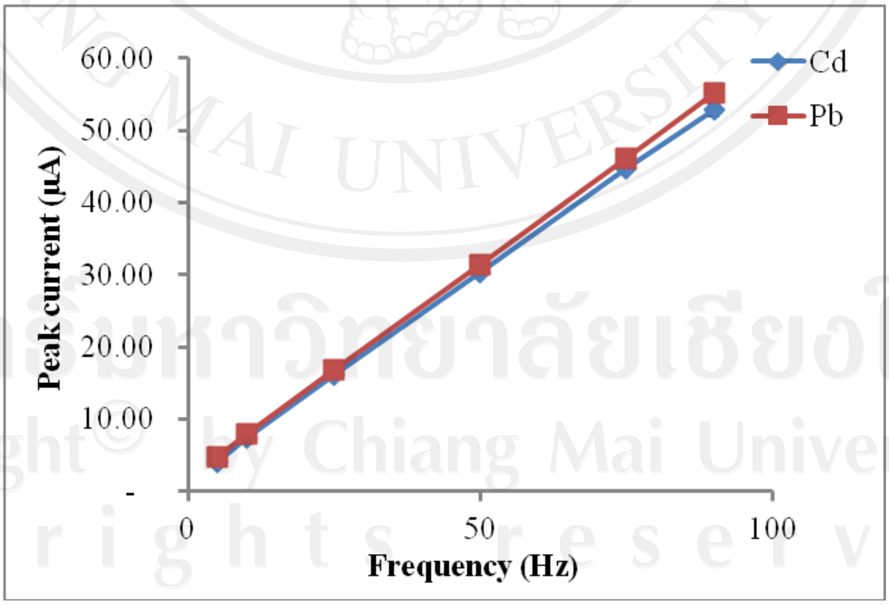
3.1.7 Sweep mode

The square wave mode provided in stripping step high sensitivity, high speed [54]. The bismuth film electrode is insensitive to dissolved oxygen which allowed the analysis of non-deoxygenated sample or standard solution. This electrode has low background current in the presence of dissolved oxygen [4, 55] At the same time in the AuNPs with dissolved oxygen can be reduction reaction on surface electrode [56]. So bismuth is suitable to be used as a working electrode in voltammetry for determination of Cd(II) and Pb(II) because it has low background current of dissolved oxygen, therefore it help to decrease the time-consuming in de-oxygenation step. Effect of square wave parameters including of frequency (Hz), amplitude (V) and voltage step (V) were investigated univariantly using a solution containing 20 µg/L each of Cd(II) and Pb(II) in 0.1 M acetate buffer pH 4.5. The effect of the frequency (Hz) was investigated in the range of 5 to 90 Hz and the voltage step was investigated in the range of 0.001 to 0.020 V. The peak potentials shifted to anodic direction with increasing frequency or voltage step (presumably as oxidation of metals became less reversible at higher frequencies) [45] while the peak heights for Cd(II) and Pb(II) increasing voltage step up to 0.010 V and frequency up to 50 Hz as shown in Figure 3.5 (a) and (b). At the higher values of voltage step or frequency, the peak potentials shifted and the peak heights become wider.

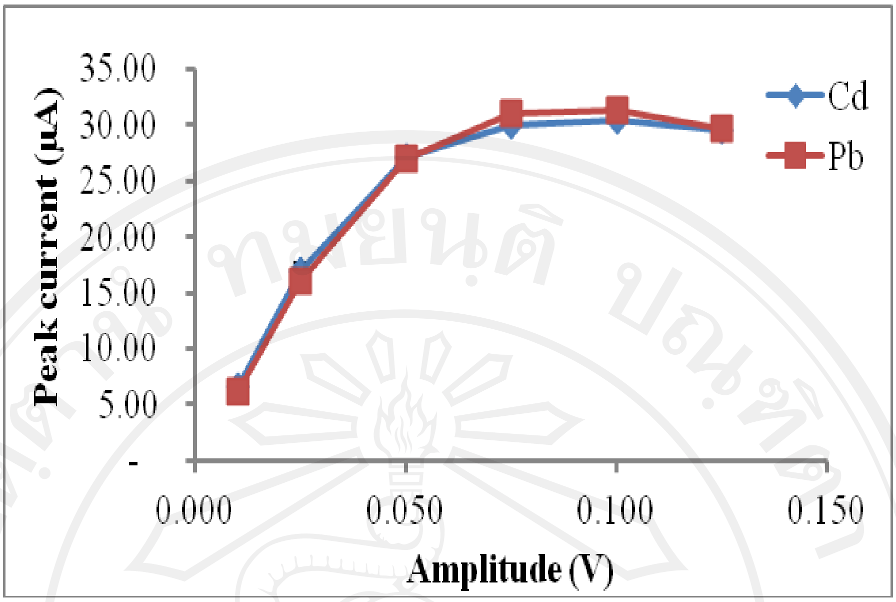
The effect of the amplitude was investigated in the range of 0.010 to 0.125 V. The peak potentials were shifted to cathodic direction with increasing amplitude up to 0.075 V as shown in Figure 3.5(c). At the higher values of amplitude the background deteriorate and the increase of peak widths occurred. The following square wave parameters, amplitude 0.075 V, voltage step 0.010 V and frequency 50 Hz provided a good sensitivity and higher resolution.



(a) Effect of voltage step was investigated in the range of 0.001 to 0.020V



(b) Effect of frequency was investigated in the range of 5 to 90 Hz



(c) Effect of amplitude was investigated in the range of 0.010 to 0.125V

Figure 3.5 Effect of of sweep mode on metal stripping at 20 µg/L each of Cd(II) and Pb(II). Experimental conditions: 0.10 M acetate buffer pH 4.5 containing 0.60 mg/L Bi(III), deposition potential -1.5 V, deposition time 90 s, cleaning potential of 0.6 V, cleaning time of 60 s and equilibration time of 30 s.

(a) voltage step was investigated in the range of 0.001 to 0.020V

(b) frequency was investigated in the range of 5 to 90 Hz

(c) amplitude was investigated in the range of 0.010 to 0.125V

3.1.8 Deposition potential

Deposition potential has significant influence on the selectivity of metals to be deposited on the electrode. Too high potential allowed other chemicals in the solution to co-deposite or react at the electrode. Effect of deposition potential on metal stripping signals was studied with 20 µg/L each of Cd(II) and Pb(II) and deposition potential in the range of -1.1 to -1.6 V. As shown in Figure 3.6, the deposition potentials from -1.1 to -1.5 V resulted in rapidly increase of the stripping peak currents of Pb(II) and Cd(II). In case of deposition potentials of higher than -1.5 V, the decrease and poor reproducibility in current signals were observed, due to the evolution of hydrogen bubble at the electrode surface. Thus, a deposition potentials of -1.5 V was selected for the next experiment.

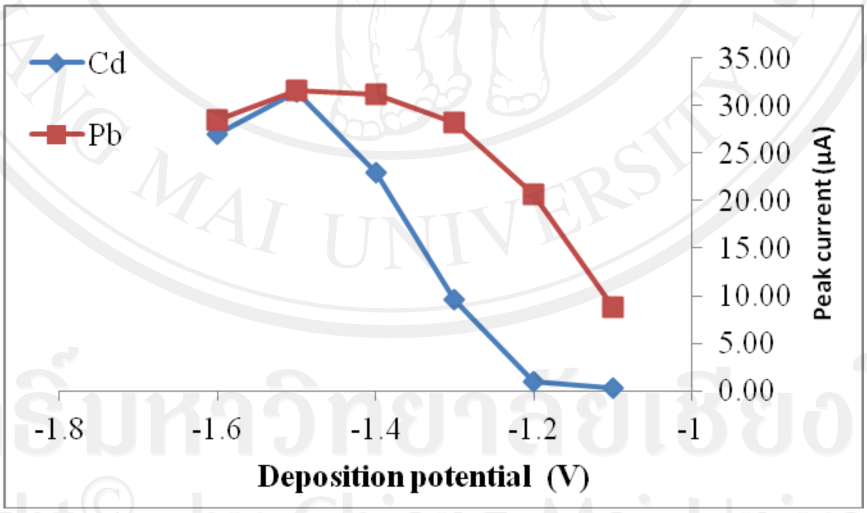


Figure 3.6 Effect of deposition potential on metal stripping at 10 and 20 µg/L each of Cd(II) and Pb(II). Experimental conditions: 0.10 M acetate buffer pH 4.5 containing 0.60 mg/L Bi(III), amplitude 0.075 V, voltage step 0.010 V, frequency 50 Hz, deposition time 90 s, cleaning potential of 0.6 V, cleaning time of 60 s and equilibration time of 30 s.

3.1.9 Deposition time

Deposition time or preconcentration time is the time to allow metal ions to be reduced on electrode surface, which the target metal ions form alloy with bismuth. This step the constant potential of -1.50 V versus Ag/AgCl was applied to the working electrode. And the standard or sample solution was carried to mix on-line with Bi(III) plating solution before entering a voltammetric cell. The deposition times in the range of 30 to 300 s was studied with 10 and 20 µg/L each of Cd(II) and Pb(II). As shown in Figure 3.7, the peak currents of Pb(II) and Cd(II) increased with the increase of deposition time. Deposition time of 150 s was chosen as the optimal accumulation time because this time provided enough sensitivity.

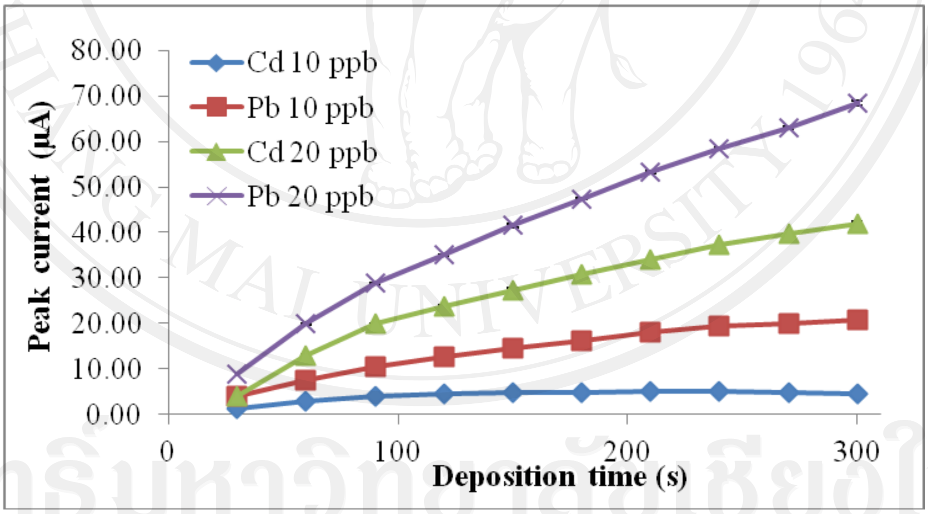


Figure 3.7 Effect of deposition potential on metal stripping at 10 and 20 µg/L each of Cd(II) and Pb(II). Experimental conditions: 0.10 M acetate buffer pH 4.5 containing 0.60 mg/L Bi(III), deposition potential -1.5 V amplitude 0.075 V, voltage step 0.010 V, frequency 50 Hz, cleaning potential of 0.6 V, cleaning time of 60 s and equilibration time of 30 s.

3.1.10 The optimum operational conditions

The parameters which affected analytical performance and the optimum condition for determination of Cd(II) and Pb(II) using Flow-ASV with bismuth film electrode as a working electrode are summarized in the Table 3.4.

Table 3.4 Optimization of Flow-ASV system using bismuth film electrode as a working electrode for determination of Cd(II) and Pb(II)

Parameter	Optimum value
Concentration of cleaning solution	1.0 M
Concentration of acetate buffer pH 4.5	0.10 M
The effect of pH of acetate buffer solution	4.5
Concentration of Bi(III) plating solution	0.60 mg/L
Flow rate	0.5 ml/L
Stripping sweep mode	amplitude 0.075 V
	voltage step 0.010 V
	frequency 50 Hz
Deposition potential	-1.5 V
Deposition time	150 s

3.1.11 The stability of sensitivity of the calibration graphs

The procedure for preparation of electrode surface have effect on sensitivity of the Cd(II) and Pb(II) analysis. Therefore, the stability of sensitivity (slope of calibration) of method determination was examined in the same day and different days. The calibration graphs were constructed in the concentration range of 1-30 μg/L of Cd(II) and Pb(II) at different times. It was found that the sensitivity of Cd(II) and Pb(II) within inter and intraday is not different, as shown in Table 3.5. Therefore, the developed method provide good stability and sensitivity.

Table 3.5 The comparative stability of sensitivity of Cd(II) and Pb(II) two times and 2 days for determination of Cd(II) and Pb(II).

Day	Times	Sensitivity (μAL/μg)	
		Cd(II)	Pb(II)
1	1	1.50	1.17
	2	1.48	1.18
2	1	1.70	1.07
	2	1.74	1.05

3.2 Analytical characteristics of the procedure

3.2.1 Calibration curves and limit of detection

Under the optimal condition as shown in Table 3.4, the stripping 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 Figure 3.8. The calibration graphs of Cd(II) and Pb(II) in the range of 2 to 40 μg/L of each metal were constructed by plotting peak current (µA) versus concentration of metal ions (µg/L). The calibration curves exhibited excellent linearity with a correlation coefficient of 0.9997 for Cd(II) and 0.9999 for Pb(II). The peak current increased linearity with metal ion concentration, with slopes of 1.6781 µA/µg for Cd(II) and 1.1678 µA/µg for Pb(II), respectively, as shown in Figure 3.9.

The detection limit was determined by the method reported by Miller and Miller [57], which was calculated from the linear regression of the calibration graph. The results are given in Appendix A. The detection limit of the proposed method was found to be 0.89 and 0.59 µg/L were achieved for Cd and Pb, respectively. The concentration at limit of detection (C_L) can be calculated from the equation 2.1-2.2.

$$\frac{Sy}{x} = \left(\frac{\Sigma(\mathrm{Yi} - \hat{\mathrm{Y}}\mathrm{i})^2}{\mathrm{n} - 2}\right)^{\frac{1}{2}} \qquad (2.1)$$

$$C_L = 3 \times \frac{Sy/x}{b} \qquad (2.2)$$

When: Yi = response vale from the instrument corresponding to the individual x- values

$\hat{\mathrm{Y}}\mathrm{i}$ = value of y on the calculated regression line corresponding to the individual x- values

n = number of points on the calibration line

b = slope of the straight line

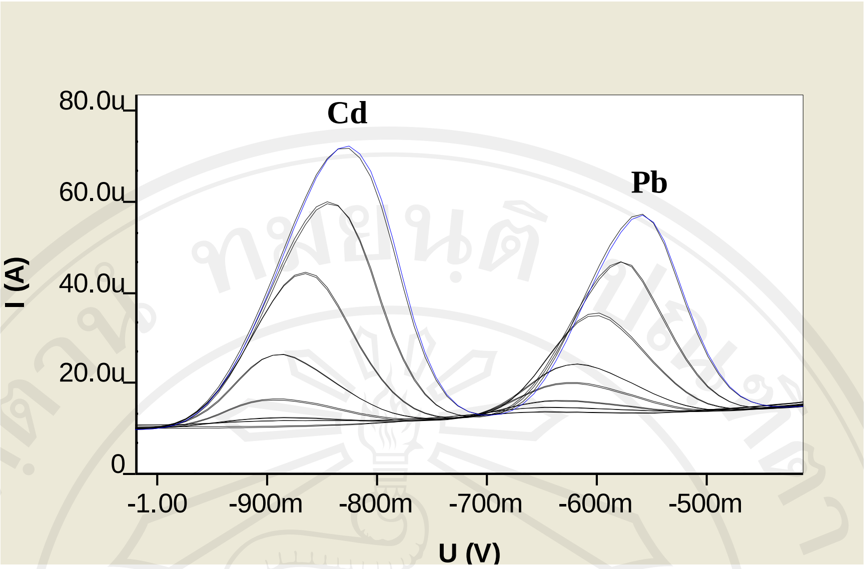


Figure 3.8 Square wave anodic stripping voltammograms obtained from VA-BiFE system of solution containing Cd(II) and Pb(II) of increasing concentration, from 2-40 µg/L

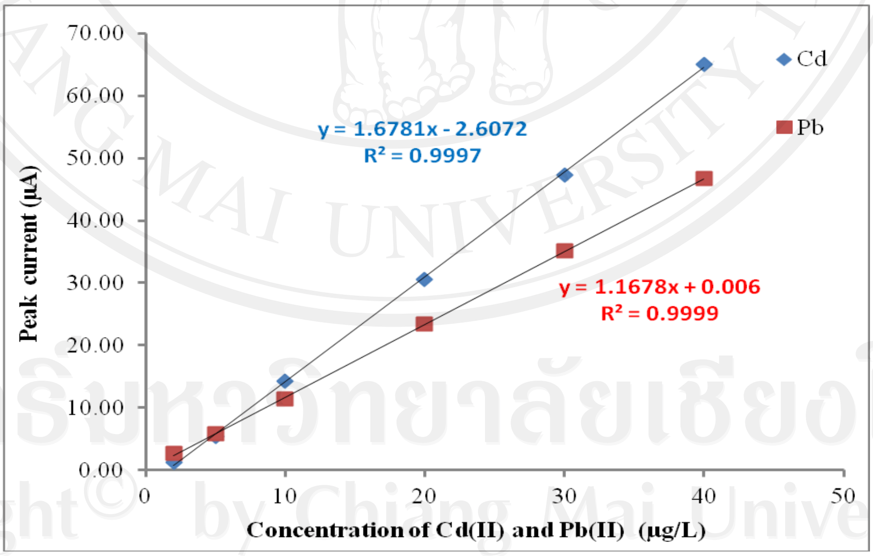


Figure 3.9 Calibration graphs of Cd(II) and Pb(II) from VA-BiFE system of solution containing Cd(II) and Pb(II) of increasing concentration, from 2-40 µg/L.

3.2.2 Precision study

The precision of the proposed method was verified by 11 replicated determinations of standard solutions of Cd(II) and Pb(II) at low, medium and high concentration levels ca. 5.0, 20.0 and 40.0 µg/L. The percentage of relative standard deviation (%RSD) values was used for evaluating the precision and can be calculated from the equation (2.3). The results are given in Table 3.6. 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 3.6 The precision study at the concentration of standard Cd(II) and Pb(II). (n=11)

Number of injection	Peak current (µA) obtained from standard Cd(II) and Pb(II) (µg/L)					
	5		20		40	
	Cd	Pb	Cd	Pb	Cd	Pb
1	3.43	4.81	29.60	23.90	61.40	47.90
2	3.42	4.84	29.30	23.70	61.20	48.30
3	3.41	4.85	29.60	23.70	60.90	48.10
4	3.35	4.81	29.50	23.70	60.80	48.20
5	3.34	4.80	29.40	23.60	60.90	48.40
6	3.35	4.80	29.40	23.70	61.00	48.40
7	3.29	4.82	29.30	23.70	59.90	47.60
8	3.33	4.79	29.60	23.70	59.80	47.80
9	3.33	4.82	29.70	23.70	59.50	47.70
10	3.34	4.75	29.50	23.70	59.90	47.80
11	3.26	4.83	30.10	24.30	59.30	47.30
Mean	3.35	4.81	29.55	23.76	60.42	47.95
SD	0.05	0.03	0.23	0.19	0.74	0.36
%RSD	1.57	0.56	0.76	0.80	1.23	0.74

3.2.3 Accuracy of system

The 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, 10, 15, 20 and 25 µg/L in water samples. The recovery percentage for each sample was calculated from the calibration equation to obtain concentration of Cd(II) and Pb(II) and calculated from the equation (2.4). The obtained results are given in the Table 3.7. Percent recoveries of Cd(II) and Pb(II) at different spiked standard solution were different. It was found that, when the spiked standard solution Cd(II) and Pb(II) increased from 5 to 25 µg/L in sample, the percent recoveries of Cd(II) and Pb(II) got close to 100%. Because, the added standard solution of Cd(II) and Pb(II) at 25 µg/L resulted in the total concentrations of Cd(II) and Pb(II) in sample at the higher end of the calibration graph. The concentration of Cd(II) and Pb(II) standard solution at 25 µg/L was selected to check accuracy of the system.

Under the optimal conditions, the flow based ASV with bismuth film working electrode can be applied to the simultaneous determination of Cd(II) and Pb(II) in real water sample by using calibration curve. The method has percentage recoveries for the determination of those metals in range of 92.8 – 102.8 for added standard solution of both Cd(II) and Pb(II) in the water sample at 25 µg/L. This system was investigated for trace metals determination and it provides high sensitivity, low consumption and convenient operation.

Table 3.7 The recovery percentages of sample on spiked of Cd(II) and Pb(II) at 5-25 µg/L in samples.

Added (µg/L)	Recovery %									
	Sample nunber									
	3		4		5		6		7	
	Cd	Pb	Cd	Pb	Cd	Pb	Cd	Pb	Cd	Pb
5	89.6	124.0	76.4	117.1	97.6	124.5	113.0	145.8	104.0	111.5
10	99.6	115.6	75.6	102.7	99.7	116.5	107.9	124.3	105.6	105.1
15	100.5	116.1	96.5	111.0	103.8	106.4	102.2	113.3	101.5	96.8
20	94.6	103.2	92.3	104.9	104.0	105.5	99.8	110.3	101.5	96.3
25	93.6	101.8	93.4	102.7	102.6	101.5	92.8	96.6	98.5	96.0

3.2.4 Interferences

Interference can be attributed to adsorption of surface active compounds, formation of intermetallic compounds, poor resolution between adjacent peaks and mechanical degradation of bismuth film. Possible interferences with the stripping voltammetric measurements of heavy metal ions at BiFE can be classified as: [52]

1. Fouling of the electrode surface by proteins or surfactants.

This is reason for erratic behavior of the electrode especially in the case of natural samples. In order to overcome this problem, pre- treatment of the samples should be done in order to reduce the amount of compounds able to foul the electrode surface.

2. Formation of intermetallic compounds. Regarding intermetallic compounds,

the well known Cu-Zn intermetallic compound that interferes with the analysis of Zn(II) in the presence of an excess of Cu has been observed on BiFE, this interference can be alleviated with the addition of Ga(III) ions. Also, a large excess of Cu was found to have a suppressing effect on the responses of Cd and Pb on BiFE [11]. Or ferrocyanide due to their action as masking agents.

3. Contamination from the environment. Before turning to the application to

natural samples, it is crucial to confront the problem of environmental contamination of the sample during preparation and analysis, providing some practical imformation about how to avoid it. For example in the practical experiments suggests that one should avoid laboratory glassware and should be washed several time with ultrapure 65% nitric acid diluted 1:10. In addition, it could be very useful to confirm daily, the purity of the reagents by measuring the solvent blank, and cleaning the electrochemical cell and electrode is necessary.

However, the interferences were negligible in the real environmental samples analysis, because their concentrations were low. This implies the possible direct application of the method to real sample which contain low concentration of interferences.

3.3 Real sample analysis

3.3.1 Application of real samples water I

The developed method was applied to real water samples to simultaneous determination of Cd(II) and Pb(II) in real water samples which were collected from a draining pond of zinc mining in northern Thailand. The calibration graph was used for quantification of each metal. The results obtained for 9 water samples are summarized in Table 3.8. The method has percentage recoveries for the determination of those metals in range of 92.8 – 102.8.

Table 3.8 Determination of cadmium and lead in water sample by proposed Flow-VA- BiFE method

Sample	Dilution	Flow-VA-BIFE (mg/L)		% recovery	
		Cd(II)	Pb(II)	Cd(II)	Pb(II)
1	50	0.62 ± 0.00	1.76 ± 0.01	-	-
2	100	1.17 ± 0.01	3.70 ± 0.01	-	-
3	500	2.10 ± 0.01	10.17 ± 0.03	93.6	101.8
4	500	0.88 ± 0.00	6.18 ± 0.00	93.4	102.7
5	500	1.59 ± 0.01	12.70 ± 0.00	102.6	101.5

Table 3.8 (Continued)

Sample	Dilution	Flow-VA-BIFE (mg/L)		% recovery	
		Cd(II)	Pb(II)	Cd(II)	Pb(II)
6	500	2.03 ± 0.00	4.19 ± 0.04	92.8	96.6
7	500	1.66 ± 0.00	1.47 ± 0.01	98.5	96.0
8	500	2.88 ± 0.01	10.86 ± 0.03	-	-
9	(Cd(II) 100, Pb(II) 1000)	0.32 ± 0.00	14.84 ± 0.07	-	-

Samples were also analyzed by ICP-OES at the Office of Primary Industry and Mine Region 3, Chiang Mai for comparison. The results are given in Table 3.9.

Table 3.9 Comparative determination of cadmium and lead in water sample by proposed Flow -VA- BiFE system method and ICP-OES.

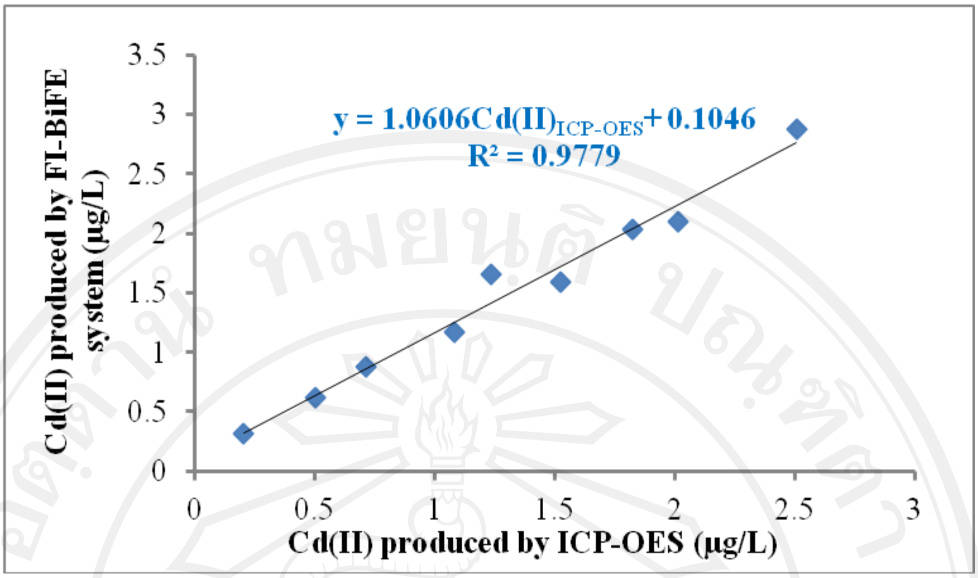
Sample	Concentration of metals (mg/L)			
	Cd(II)		Pb(II)	
	ICP-OES	Flow-VA-BiFE	ICP-OES	Flow-VA-BiFE
1	0.50 ± 0.03	0.62 ± 0.00	2.06 ± 0.09	1.76 ± 0.01
2	1.08 ± 0.15	1.17 ± 0.01	4.16 ± 0.10	3.70 ± 0.01
3	2.02 ± 0.15	2.10 ± 0.01	12.06 ± 0.26	10.17 ± 0.03
4	0.71 ± 0.06	0.88 ± 0.00	8.26 ± 0.12	6.18 ± 0.00
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

The correlation plot of the proposed method and ICP-OES method is shown in Figure 3.10. It was found that the results from the proposed Flow-VA-BiFE method were in good correlation with those of ICP-OES method ($Cd(II)_{Flow\text{-}VA\text{-}BIFE}$= 1.0606$Cd(II)_{ICP\text{-}OES}$ + 0.1046, r^2= 0.9779) for Cd(II) and poor correlation with those of ICP-OES method ($Pb(II)_{Flow\text{-}VA\text{-}BIFE}$= 0.7389$Pb(II)_{ICP\text{-}OES}$ +0.379, r^2= 0.9801) for Pb(II).

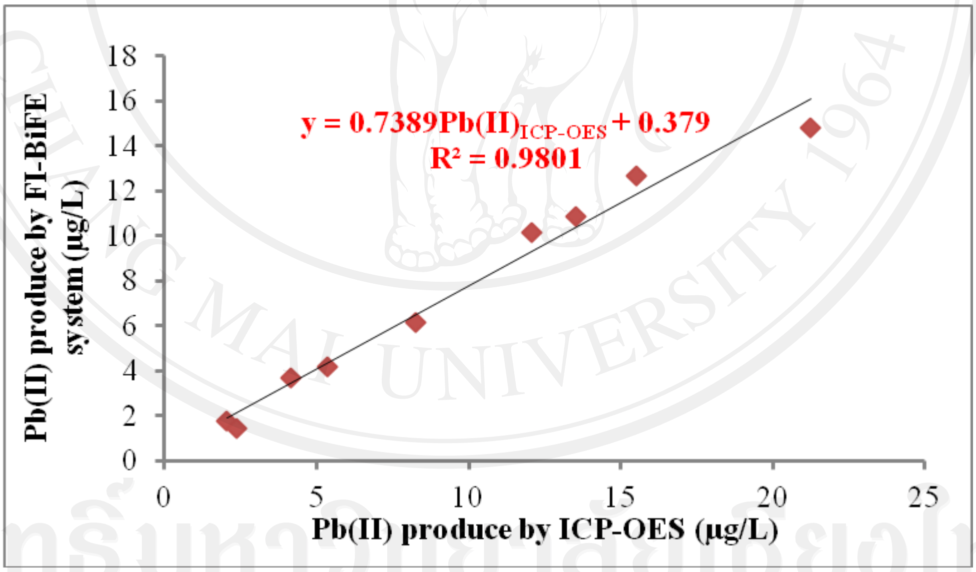
Both methods were also compared with t test at 95% confidence level (Appendix B), the result obtained from both method were in poor agreement for Cd(II) and good agreement for Pb(II) as shown in Table 3.10. This may be because of samples were kept too long before analysis by the proposed method.

Table 3.10 The results to t – test at 95% confident limit of Cd(II) and Pb(II).

T-test	Metal ions	
	Cd	Pb
t calculate	[4.41]	1.23
t Critical two-tail	2.26	2.26



(a) correlation graphs of Cd**(II)** contents determined by the two method



(b) correlation graphs of Pb(II) contents determined by the two method

Figure 3.10 Correlation graphs of (a): Cd(II) and (b): Pb(II) contents determined by the proposed Flow-VA-BiFE method and ICP –OES method.

3.3.2 Application of real samples water II

The developed method was applied to real water samples to the simultaneous determination of Cd(II) and Pb(II) in real water samples which were collected from a draining pond of zinc mining in northern Thailand. The results obtained for 8 water samples are summarized in Table 3.11. The method has percentage recoveries for the determination of those metals in range of 104.9 –115.3 for Cd(II) and 106.0 –119.5 for Pb(II).

Table 3.11 Determination of cadmium and lead in water sample by proposed Flow-VA- BiFE method

Sample	Dilution	Flow-VA-BiFE (mg/L)		% Recovery	
		Cd(II)	Pb(II)	Cd(II)	Pb(II)
1	100	0.43 ± 0.02	0.81 ± 0.01	108.9	118.3
2	100	0.64 ± 0.01	0.61 ± 0.01	104.9	106.0
3	100	0.88 ± 0.01	0.76 ± 0.02	115.3	112.8
4	200	0.97 ± 0.01	1.84 ± 0.05	109.2	114.1
5	100	0.58 ± 0.01	1.39 ± 0.01	112.9	114.2
6	100	0.43 ± 0.02	2.64 ± 0.05	113.3	115.7
7	100	0.76 ± 0.00	0.76 ± 0.02	111.5	119.3
8	100	0.96 ± 0.01	0.96 ± 0.02	108.6	119.5

Sample were also analyzed by ICP-OES at the Office of Primary Industry and Mine Region 3, Chiang Mai for comparison. It was found that the results was not significantly different. The results are given in Table 3.12.

Table 3.12 Comparative determination of cadmium and lead in water sample by proposed Flow-VA- BiFE system method and ICP-OES

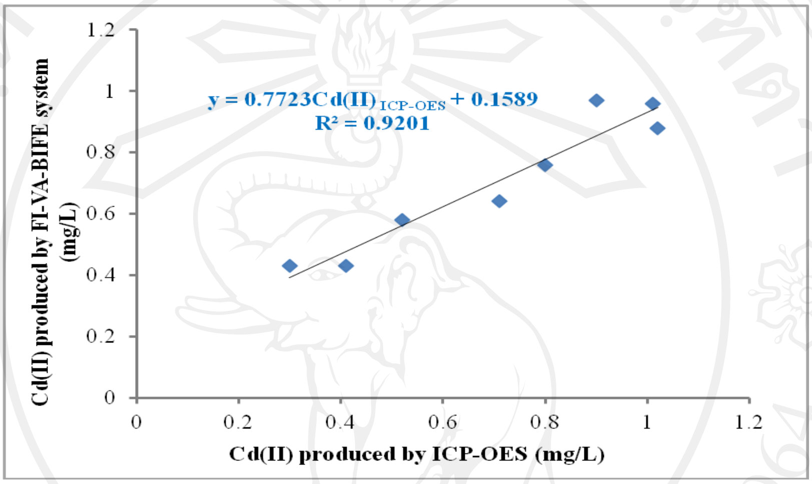
Sample	Concentration of metals (mg/L)			
	Cd(II)		Pb(II)	
	ICP-OES	Flow-VA-BIFE	ICP-OES	Flow-VA-BIFE
1	0.41	0.43 ± 0.02	1.01	0.81 ± 0.01
2	0.71	0.64 ± 0.01	0.52	0.61 ± 0.01
3	1.02	0.88 ± 0.01	0.80	0.76 ± 0.02
4	0.90	0.97 ± 0.01	2.12	1.84 ± 0.05
5	0.52	0.58 ± 0.01	1.52	1.39 ± 0.01
6	0.30	0.43 ± 0.02	3.01	2.64 ± 0.05
7	0.80	0.76 ± 0.00	0.80	0.76 ± 0.02
8	1.01	0.96 ± 0.01	0.51	0.96 ± 0.02

The correlation plot of the proposed method and ICP-OES method is shown in Figure 3.11. It was found that the results from the proposed VA-BiFE method were in poor correlation with those of ICP-OES method of Cd(II) and Pb(II) ($Cd(II)_{Flow-VA-BIFE}= 0.7759Cd(II)_{ICP-OES} + 0.1556$, $r^2= 0.9235$ and $Pb(II)_{Flow-VA-BIFE}= 0.7764Pb(II)_{ICP-OES} +0.2208$, $r^2= 0.9492$**.**

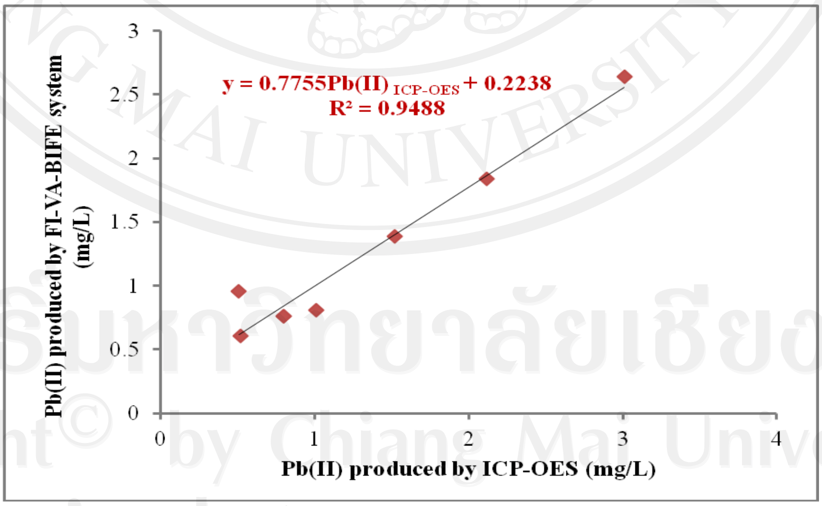
Both methods were also compared with t test at 95% confidence level, the results obtained from both method were in good agreement for Cd(II) and Pb(II) ($t_{critical} = 2.36$, $t_{calculate} = 0.11$ and 0.74 for Cd(II) and Pb(II), respectively).

Table 3.13 The results t – test at 95% confident limit of Cd(II) and Pb(II).

T-test	Metal ions	
	Cd(II)	Pb(II)
t calculate	0.02	0.17
t critical two-tail	2.36	2.36



(a) correlation graphs of Cd(II) contents determined by the two method



(b) correlation graphs of Pb(II) contents determined by the two method

Figure 3.11 Correlation graphs of (a): Cd(II) and (b): Pb(II) contents determined by the proposed Flow-VA-BiFE method and ICP –OES method.