CHAPTER 3

RESULTS AND DISSCUSION

3.1 Analytical characteristics of the method

3.1.1 Precision

The precision of the FAAS instrument was studied by repeating the measurement of 1 μg ml^{-1} standard solution of Cd and 10 μg ml^{-1} standard solution of Pb for ten times. The results obtained are shown in **Table 3.1**. The result of the precision was exhibited with replicate injection and expressed as the relative standard deviation (%RSD) which were 1.10% and 1.95% for Cd and Pb, respectively. These indicated that the FAAS instrument provided very good repeatability (less than 5 %RSD) for Cd and Pb under the optimum conditions. The calculation of relative standard deviation (%RSD) and detection limit value are shown in Appendix B. The calibration curves of Cd and Pb determined by FAAS were plotted between the absorbance and the concentrations of analyte. The linear range of calibration curves were in the range of 0.50-2.50 μg ml^{-1} and 1.00-20.00 μg ml^{-1} for Cd and Pb, respectively. The obtained calibration equation of Cd was $y = 0.168x + 0.0236$ and R^2 was 0.9988 as shown in **Table 3.2** and **Figure 3.1**. The sensitivity defined as the slope of regression line for Cd was 0.168 ppm^{-1}. The obtained calibration equation of Pb was $y = 0.0086x - 0.0009$ and R^2 was 0.9996 as shown in **Table 3.3** and **Figure 3.2**. The sensitivity obtained from slope of the regression line for Pb was 0.0086 ppm^{-1}.

Table 3.1 The precision of the FAAS instrument for Cd and Pb analysis

No.	Abs. of Cd (1 μg ml^{-1})	Abs. of Pb (10 μg ml^{-1})
1	0.180	0.112
2	0.183	0.118
3	0.183	0.117
4	0.184	0.117
5	0.182	0.114
6	0.180	0.112
7	0.178	0.117
8	0.181	0.112
9	0.185	0.114
10	0.181	0.114
Mean	0.1817	0.1147
SD	0.0020	0.0022
%RSD	1.10	1.95

Table 3.2 The absorbance of Cd concentration by FAAS

Concentration of Cd (μg ml^{-1})	Absorbance
1% HNO_3	0.000
0.50	0.108
1.00	0.187
1.50	0.279
2.00	0.365
2.50	0.439

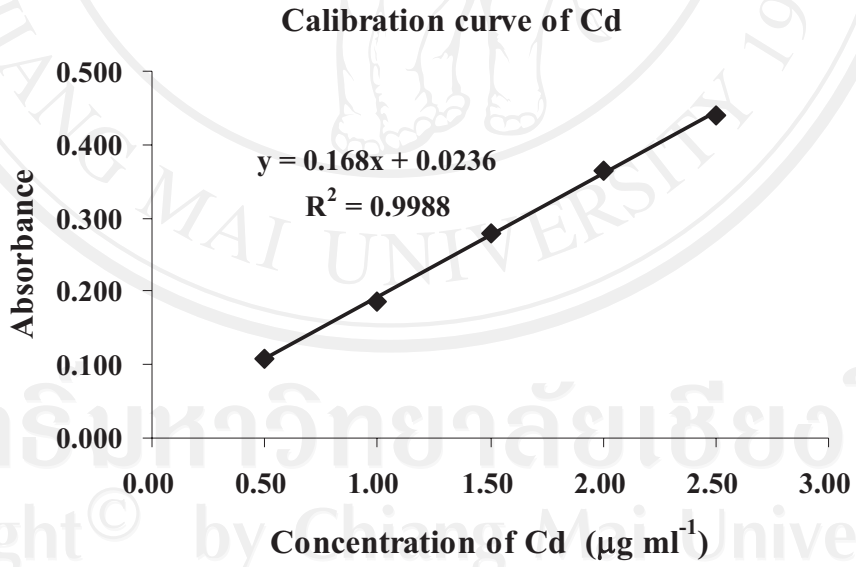


Figure 3.1 The calibration curve of Cd determination

Table 3.3 The absorbance of Pb concentration by FAAS

Concentration of Pb (μg ml^{-1})	Absorbance
1% HNO_3	0.000
1.00	0.007
5.00	0.043
10.00	0.086
15.00	0.126
20.00	0.172

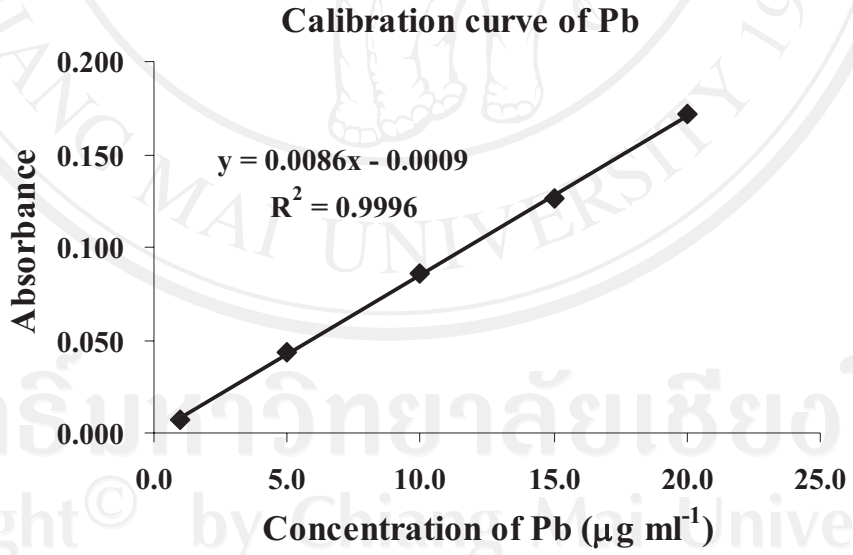


Figure 3.2 The calibration curve of Pb determination

3.1.2 Detection limit

The detection limits of the proposed method for determination of Cd and Pb were investigated by analyzing blank solution of 1% HNO_3 for ten times. The results are shown in **Table 3.4**. The detection limits of Cd and Pb were 0.03 and 0.61 μg ml^{-1}, respectively. The detection limits were sufficiently low and the calculations of detection limits are shown in Appendix B.

Table 3.4 Absorbance obtained from blank solution

No.	Abs. of 1% HNO_3 for Cd	Abs. of 1% HNO_3 for Pb
1	0.001	0.001
2	-0.001	0.001
3	0.000	0.000
4	-0.002	0.001
5	0.002	0.005
6	0.000	0.003
7	0.001	0.003
8	0.001	0.000
9	0.001	0.002
10	0.001	0.004
Mean	0.0004	0.0020
SD	0.0011	0.0016
Slope	0.1169	0.0079
Detection limit (μg ml^{-1})	0.03	0.61

3.1.3 Accuracy

For Cd, the method was examined by determining the recoveries of the added 1 and 2 μg ml^{-1} of Cd into studied sample solutions. The results are presented in **Table 3.5**. For Pb, the method was examined by determining the recoveries of the added 10 and 20 μg ml^{-1} of Pb into studied sample solutions. The results are presented in **Table 3.6**. The recoveries were found in the range of 83.43 – 98.21% and 82.29 – 102.95% for Cd and Pb, respectively. The calculations of accuracy are shown in Appendix B.

Table 3.5 The recoveries of Cd analysis

Sample	Concentration of Cd (μg ml^{-1})		% Recovery
	Added	Found*	
1	-	ND	
	1.00	0.77 ± 0.00	87.50
	2.00	1.86 ± 0.01	98.21
2	-	ND	
	1.00	0.76 ± 0.01	89.68
	2.00	1.81 ± 0.01	97.02
3	-	ND	
	1.00	0.72 ± 0.01	86.51
	2.00	1.53 ± 0.01	83.43

*Mean ± SD (N=3)

Table 3.6 The recoveries of Pb analysis

Sample	Concentration of Pb (μg ml^{-1})		% Recovery
	Added	Found*	
1	-	ND	
	10	9.38 ± 0.15	102.78
	20	19.69 ± 0.39	102.95
2	-	ND	
	10	9.76 ± 0.18	100.69
	20	19.10 ± 0.34	97.05
3	-	ND	
	10	6.77 ± 0.00	82.29
	20	18.72 ± 0.10	100.87

*Mean ± SD (N=3)

3.2 Optimization of cloud point extraction (CPE)

In cloud point extraction (CPE), separation of the surfactant–rich phase and the aqueous supernatant phase requires appropriate experimental conditions. To determine the optimal condition for maximum extraction efficiencies of analytes, the effect of the experimental conditions on the extraction has to be thoroughly evaluated and optimized. The extracted solutions were subjected to analysis of Cd and Pb by FAAS. The optimum conditions in this study were obtained by the maximum value of analytical signal. The very high temperatures are not proper for CPE, because they

could reduce the stability of chelates and chelating agents. When working with the cloud point temperature of surfactants higher than 40°C, the main experimental difficulty to conquer is the loss of extraction efficiency with possible temperature decrease during the centrifugation or phase-separation step. Therefore, if high temperatures are not required by the experimental conditions they should be avoided, although they may give some improvement for the CPE. This research used Triton X-114 as a non-ionic surfactant. The cloud point temperature of Triton X-114 is 23-26ºC near room temperature which is preferred for cloud point temperature and analytical purposes. Because the use of high temperatures resulted in a decrease of recovery may be caused by the instability of the complex at high temperature. And it may lead to the decomposition of micelles and the reduction of analytical signal. Hence, the cloud point temperature at 40ºC was selected to perform in the experiment for avoiding the loss of extraction efficiency [49-51, 57]. In this work, the effect of the experimental variable parameters on the cloud point extraction procedure had been preliminary studied.

3.2.1 Effect of complexing time

Optimal complexing time is necessary to complete the reaction and to achieve easy phase separation as efficient as possible. Under certain conditions, the complexation reaction of metal and chelating agent has been completed. Associate data show that for complexing times of some minutes up to a few days, extraction efficiency increases very slightly and the phase-volume ratio decreases a little [52]. In this work, the dependence of extraction efficiency upon complexing time for 15 to 60

min at 40ºC was thoroughly optimized. The results of complexing time optimization are shown in **Table 3.7** and **Figure 3.3**.

Table 3.7 The effect of complexing time on the analytical signal for Cd (1 μg ml^{-1}) and Pb (10 μg ml^{-1})

Complexing time	**Abs.***	
(min)	**Cd**	**Pb**
15	0.114 ± 0.005	0.068 ± 0.003
20	0.117 ± 0.002	0.065 ± 0.003
25	0.109 ± 0.017	0.066 ± 0.008
30	0.118 ± 0.004	0.070 ± 0.002
35	0.107 ± 0.015	0.064 ± 0.008
40	0.109 ± 0.006	0.064 ± 0.002
45	0.098 ± 0.017	0.061 ± 0.007
50	0.082 ± 0.001	0.048 ± 0.002
55	0.078 ± 0.014	0.047 ± 0.008
60	0.077 ± 0.017	0.045 ± 0.005

*Mean ± SD (N=3)

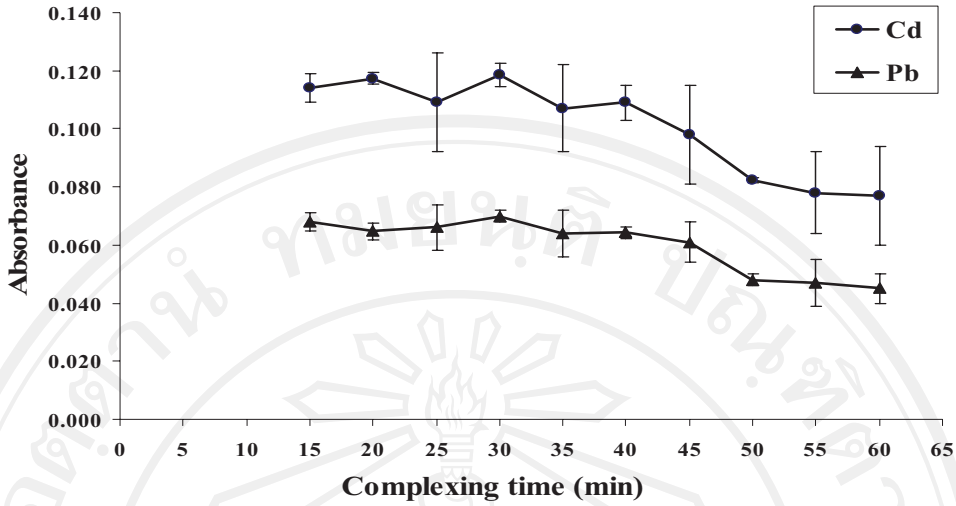


Figure 3.3 The effect of complexing time on the analytical signal for Cd and Pb

It was desirable to employ the shortest complexing time as a compromise between completion of extraction and phase separation efficiency. In both cases of Cd and Pb, the results have shown that the maximum analytical signal of complexing time obtained at 30 min. This certain complexing time was quite satisfactory to achieve small volumes of the surfactant-rich phase, quantitative extraction and experimental convenience. When increased complexing time more than 30 min, the analytical signals were decreased. Therefore, a time of 30 min was chosen as the optimum in order to achieve the highest possible extraction efficiency.

3.2.2 Effect of PAN concentration

The preconcentration of metal ions by the cloud point extraction involves the prior formation of a complex with sufficient hydrophobicity to be extracted into the small volume of the surfactant rich-phase. When 1-(2-pyridylazo)-2-naphthol (PAN) is added to a slightly acidic or neutral solution of many metal ions, pink or red colored chelate compounds are produced. The complexes are usually insoluble in water but soluble in organic reagents. Usually, the alkali and alkali earth metals do not form colored with dye. The dye may be used as an indicator in the complexometric titration [53]. PAN is also used as colorimetric reagent for the quantitative and qualitative determination of variety of metal ions. The mole ratio of complexation between PAN and metal is 1:1. The formation of PAN with metal is shown in **Figure 3.4**.

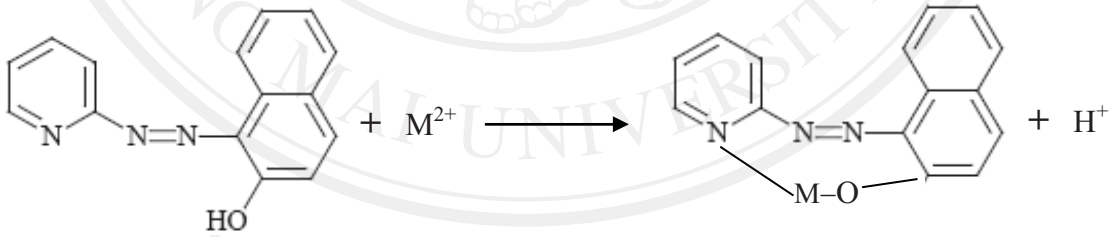


Figure 3.4 The formation of PAN with metal [53]

(This figure is modified from K.L. Cheng and R.H. Bray, 1995)

Experimental scheme of formation between metals and chelating agent in CPE is shown in **Figure 3.5**.

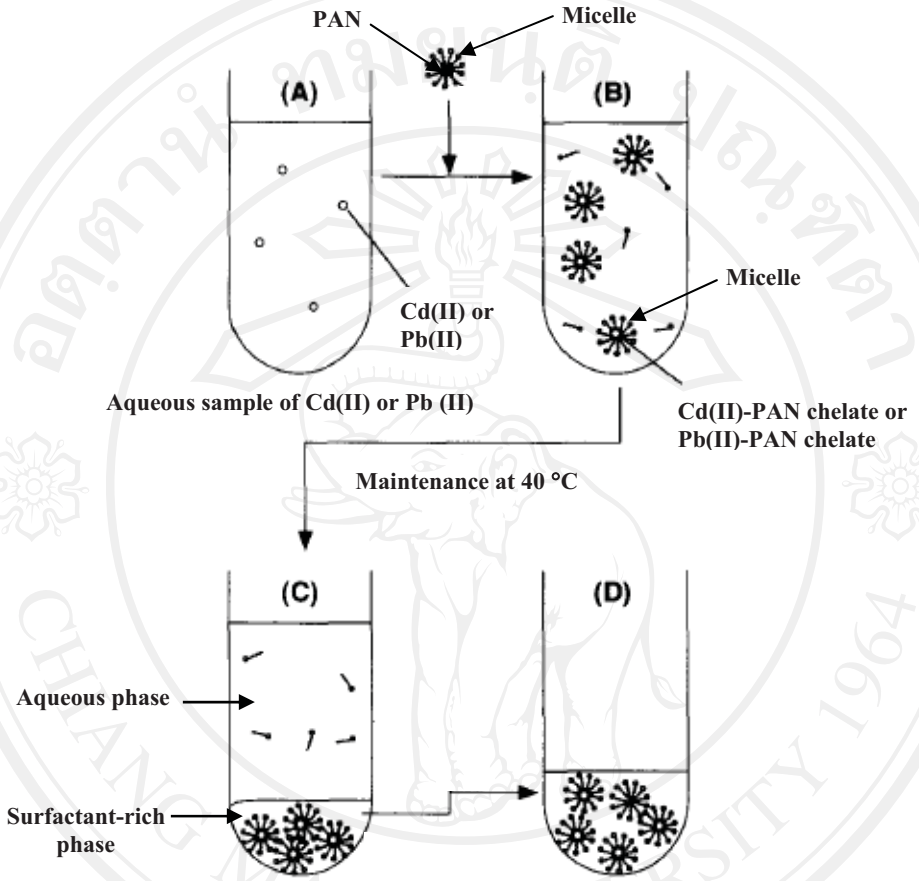


Figure 3.5 The prior formation of metal ions and chelating agent in CPE [55]

Figure 3.5(A) has illustrated the initial aqueous solution containing metal ions of Cd(II) or Pb(II). After added PAN into aqueous solution, the aqueous micelle solution in which water-insoluble metal chelates are solubilized as shown in **Figure 3.5(B)**. Then maintenance aqueous solution at 40ºC for 30 min, the phase is separated into two phases. The metal chelates are concentrated in a small volume of the surfactant-rich phase and separated from the aqueous supernatant phase as shown in

Figure 3.5(C). **Figure 3.5(D)** has shown the final solution of surfactant-rich phase, and can be introduced into FAAS after treated with organic solvent [55]. A sufficient chelating agent needed to form complex with trace metal ions under optimum conditions. The influence of the concentration of PAN is evaluated in the range of $2x10^{-5}$ to $1x10^{-3}$ mol l^{-1} and the results are shown in **Table 3.8** and **Figure 3.6**.

Table 3.8 The effect of PAN concentration on the analytical signal for Cd and Pb

PAN (mol l^{-1})	**Abs.***	
	Cd	**Pb**
$2x10^{-5}$	0.211 ± 0.004	0.155 ± 0.008
$4x10^{-5}$	0.211 ± 0.004	0.153 ± 0.006
$6x10^{-5}$	0.204 ± 0.004	0.156 ± 0.004
$8x10^{-5}$	0.196 ± 0.004	0.147 ± 0.010
$1x10^{-4}$	0.171 ± 0.007	0.128 ± 0.010
$2x10^{-4}$	0.225 ± 0.005	0.161 ± 0.001
$4x10^{-4}$	0.199 ± 0.008	0.153 ± 0.009
$6x10^{-4}$	0.189 ± 0.006	0.158 ± 0.005
$8x10^{-4}$	0.187 ± 0.007	0.146 ± 0.003
$1x10^{-3}$	0.166 ± 0.005	0.123 ± 0.004

*Mean ± SD (N=3)

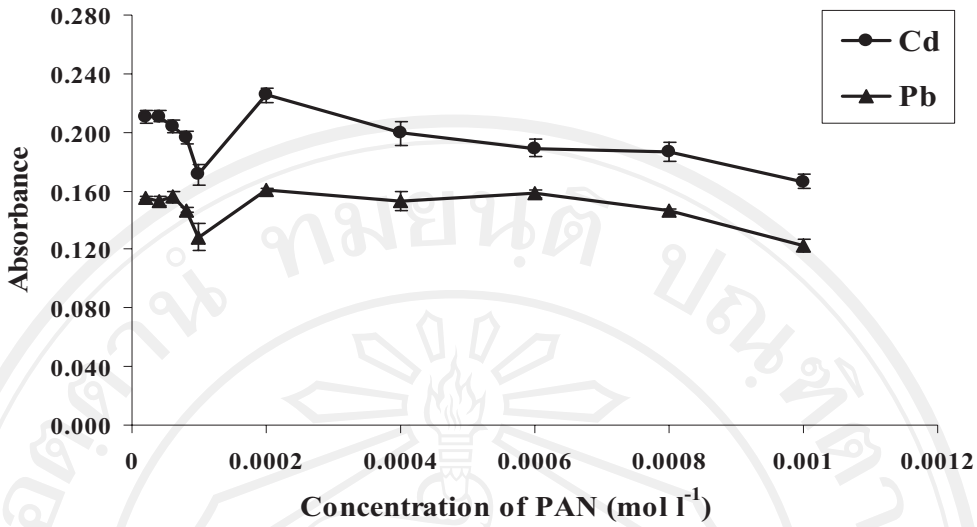
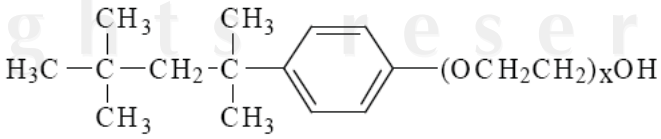


Figure 3.6 The effect of PAN concentration on the analytical signal for Cd and Pb

The extraction efficiency as a function of concentration of the chelating agent is presented in **Table 3.8** and **Figure 3.6**. In both elements, the analytical signals increased with the increasing of PAN concentration and leveled off at concentration greater than $2x10^{-4}$ mol l^{-1} PAN. The concentration of $2x10^{-4}$ mol l^{-1} PAN was sufficient for total complexation, and the obtained responses were improved. However, using an excessive amount of chelating agent, decrease in extraction performance was observed. Because the higher concentrations of chelating agent leading to the higher volume of organic solvent to enter the solution that can prevent the micelle formation and may reduce the extraction efficiency. In addition, at high concentrations of chelating agent instead of the formation of uncharged species, the charged species are formed and the extraction efficiency including method sensitivity will be reduced [56,58]. Therefore, the concentration of $2x10^{-4}$ mol l^{-1} PAN was selected as optimum concentration in order to achieve the highest possible extraction efficiency.

3.2.3 Effect of Triton X-114 concentration

The concentration of surfactant is a critical factor. A successful of cloud point extraction should maximize the extraction efficiency through minimizing the phase volume ratio, thus improving its concentrating ability. Triton X-114 is a nonionic surfactant that is often used in biochemical applications to solubilize proteins. In this research was selected Triton X-114 as a non-ionic surfactant, due to its commercial availability in a high purified homogeneous form, low toxicological properties and low cost. Moreover, it has low cloud point temperature (23-26ºC) and high density of the surfactant-rich phase, which simplifies phase separation by centrifugation. The "X" series of Triton X-114 is approximately 7-8, which produced from octylphenol polymerized with ethylene oxide. Triton X-114 permits its use in the extraction or preconcentration of a large number of molecules and chelates [57-58]. The phase separation mechanism can be ascribed to ethylene oxide segments in the micelle that repel each other at low temperature, when they are hydrated, and that attract each other as the temperature increases due to dehydration. This effect causes a decrease in the effective area occupied by the polar group on the micelle surface leading to increase the size of the micelle that can be considered to become infinite at the cloud point, resulting in the phase separation [43]. The structure of Triton X-114 is shown in **Figure 3.7**.



x = 7-8

Figure 3.7 The structure of Triton X-114

The variations in the analytical signal as a function of the concentration of Triton X-114 in the range of 0.02 – 0.20 %v/v were investigated. The influences of the concentration of Triton X-114 are shown in **Table 3.9** and **Figure 3.8**.

Table 3.9 The effect of Triton X-114 concentration on the analytical signal for Cd and Pb

Triton X-114	**Abs.***	
(%v/v)	**Cd**	**Pb**
0.02	0.110 ± 0.004	0.139 ± 0.005
0.04	0.106 ± 0.002	0.135 ± 0.002
0.06	0.106 ± 0.002	0.138 ± 0.004
0.08	0.104 ± 0.003	0.132 ± 0.004
0.10	0.096 ± 0.011	0.123 ± 0.008
0.12	0.102 ± 0.002	0.130 ± 0.006
0.14	0.095 ± 0.009	0.122 ± 0.006
0.16	0.102 ± 0.002	0.133 ± 0.006
0.18	0.106 ± 0.005	0.124± 0.011
0.20	0.093 ± 0.012	0.124 ± 0.015

*Mean ± SD (N=3)

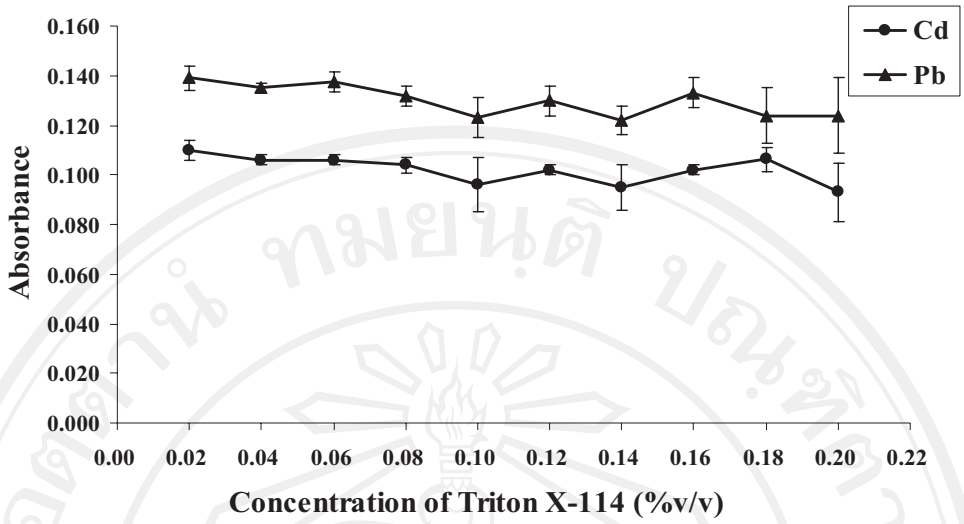


Figure 3.8 The effect of Triton X-114 concentration on analytical signal for Cd and Pb

From the results in **Table 3.9** and **Figure 3.8**, the analytical signals were decreased with increasing of Triton X-114 concentration due to an increase in the volume and the viscosity of the surfactant-rich phase. In both cases of Cd and Pb, the analytical signals in the range of 0.02-0.06 %v/v Triton X-114 were almost constant and higher than that in other concentrations. And 0.04 % Triton X-114 provided a very low standard variation. After the concentration of 0.08 %v/v Triton X-114, the analytical signals were decreased. These results might be related to the presence of the high amount of surfactant, resulting in an increase in the volume of the surfactant-rich phase and increase its viscosity. Because the increments in the volumes and the viscosity of surfactant-rich phase, leading to poor sensitivity by FAAS [58]. Therefore, an amount of 0.04% Triton X-114 was selected in order to achieve the greatest analytical signal and thereby the highest extraction efficiency.

3.2.4 Effect of pH

It is known that pH of the sample solution is one of the important factors affecting the formation of complexes. Therefore, the formation of metal complexes and its chemical stability is necessary to evaluate in CPE, because the pH plays a unique role on metal complexes and subsequent extraction. In this work, the optimization of pH was carried out in the range of pH 1–6, since the phase separation was not occurred and the mixture was precipitated at pH higher. The results are shown in **Table 3.10** and **Figure 3.9**.

Table 3.10 The effect of pH on the analytical signal for Cd and Pb

pH	Abs.*	
	Cd	Pb
1	0.085 ± 0.009	0.048 ± 0.000
2	0.085 ± 0.003	0.049 ± 0.000
3	0.092 ± 0.004	0.055 ± 0.002
4	0.090 ± 0.007	0.052 ± 0.003
5	0.087 ± 0.006	0.051 ± 0.002
6	0.084 ± 0.007	0.051 ± 0.002

*Mean ± SD (N=3)

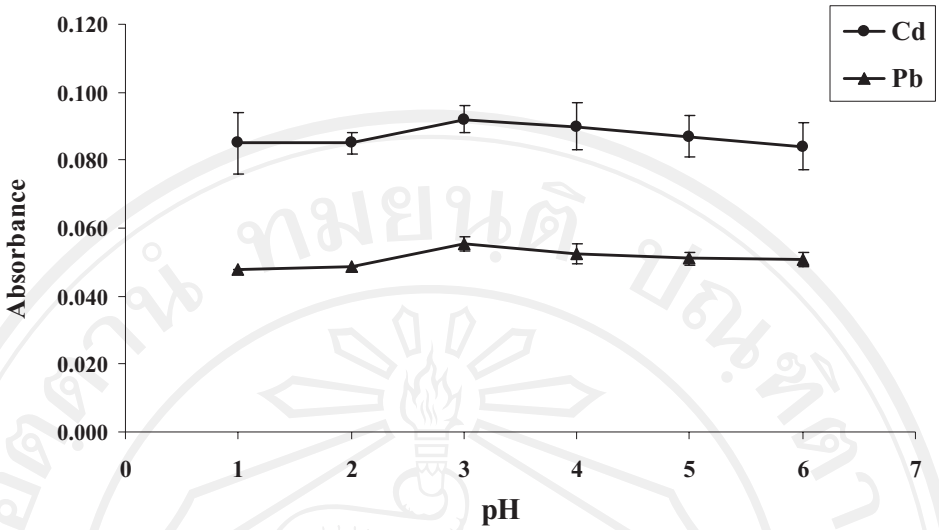


Figure 3.9 The effect of pH on the analytical signal for Cd and Pb

In both elements, the maximum analytical signals were found at pH 3. The low extraction efficiency at lower pH because the ligand is protonated, and its ionic characteristics increased leading to decreasing in its solubilization in the hydrophobic micelles. At higher pH, the ligand is de-protonated, it behaves like a hydrophilic molecule and easily gets solubilized in the micelles. In addition, at pH > 6 is attributed to the precipitation of $M(OH)_n$ or $M(OH)^+$ ions occurred in the form of hydroxides [56-57]. Therefore, a pH 3 was selected in order to achieve the optimum extraction efficiency and good selectivity for Cd and Pb.

3.2.5 Effect of the volume of acetate buffer solution pH 3

The acetate buffer solution pH 3 was used to control the pH of the analyte solution. In this work, the volume of acetate buffer solution pH 3 was optimized in the range of 0.20 – 3.00 ml. The results are shown in **Table 3.11** and **Figure 3.9**.

Table 3.11 The effect of the volume of acetate buffer solution pH 3 on the analytical signal for Cd and Pb

Acetate buffer solution pH 3 (ml)	**Abs.***	
	Cd	**Pb**
0.20	0.130 ± 0.015	0.048 ± 0.002
0.40	0.134 ± 0.008	0.051 ± 0.005
0.60	0.135 ± 0.006	0.045 ± 0.008
0.80	0.125 ± 0.006	0.049 ± 0.003
1.00	0.125 ± 0.004	0.042 ± 0.005
1.20	0.123 ± 0.007	0.044 ± 0.005
1.40	0.120 ± 0.007	0.045 ± 0.005
1.60	0.123 ± 0.002	0.042 ± 0.004
1.80	0.123 ± 0.005	0.040 ± 0.004
2.00	0.123 ± 0.003	0.041 ± 0.000
2.20	0.118 ± 0.001	0.040 ± 0.004
2.40	0.120 ± 0.010	0.038 ± 0.006
2.60	0.128 ± 0.002	0.041 ± 0.004
2.80	0.132 ± 0.009	0.039 ± 0.003
3.00	0.119 ± 0.012	0.034 ± 0.001

*Mean ± SD (N=3)

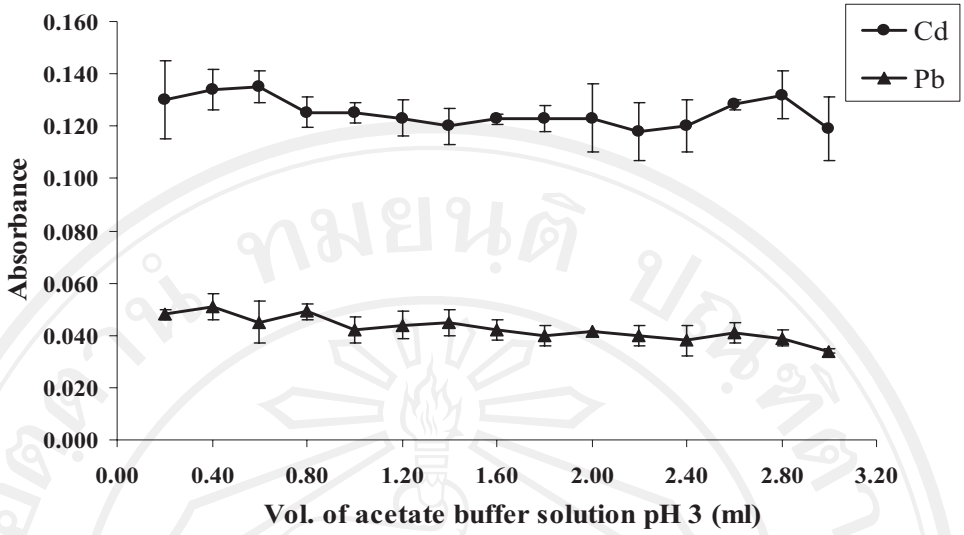


Figure 3.10 The effect of the volume of acetate buffer solution pH 3 on the analytical signal for Cd and Pb

In both elements, the results have shown that if 0.40 ml or larger volume of acetate buffer solution was added into 10 ml of sample solution, small variation took place in the analytical signals. For Cd analysis, the higher volume of acetate buffer solution pH 3 trended to the higher standard deviation (SD) of analytical signals. This effect may be caused by the buffer salts adhered on the burner head in desolvation step, resulting in the incomplete atomization of the analyte in the flame of FAAS. Therefore it was concluded that addition of 0.40 ml of acetate buffer solution pH 3 throughout the course of experiment would serve the purpose.

3.2.6 Effect of the volume of MeOH in 0.1 mol l^{-1} HNO_3

Since the surfactant-rich phase obtained after the cloud point extraction is very viscous and the analysis by FAAS requires a low viscosity of sample solution to be nebulized prior to measurement. Therefore, the solution of methanol in 0.1 mol l^{-1}

HNO_3 was added to the surfactant-rich phase in order to decrease the viscosity without excessive dilution of the surfactant-rich phase to facilitate the introduction of the sample into the atomizer of flame atomic absorption spectrophotometer. In this work, the variations in the analytical signal as a function of the volume of methanol in 0.1 mol l^{-1} HNO_3 in the range of 100-1000 μl were investigated. **Table 3.12** and **Figure 3.10** are shown the change of the normalized analytical signals as function of the volume of methanol in 0.1 mol l^{-1} HNO_3 added into the surfactant-rich phase.

Table 3.12 The effect of the volume of MeOH in 0.1 mol l^{-1} HNO_3 on the analytical signal for Cd and Pb

MeOH in 0.1 mol l^{-1} HNO_3 (μl)	**Abs.***	
	Cd	**Pb**
100	0.108 ± 0.003	0.129 ± 0.002
200	0.110 ± 0.002	0.130 ± 0.002
300	0.107 ± 0.003	0.131 ± 0.003
400	0.111 ± 0.000	0.127 ± 0.005
500	0.116 ± 0.003	0.136 ± 0.004
600	0.111 ± 0.004	0.127 ± 0.005
700	0.112 ± 0.002	0.129 ± 0.001
800	0.108 ± 0.005	0.129 ± 0.003
900	0.097 ± 0.007	0.116 ± 0.005
1000	0.085 ± 0.007	0.105 ± 0.000

*Mean ± SD (N=3)

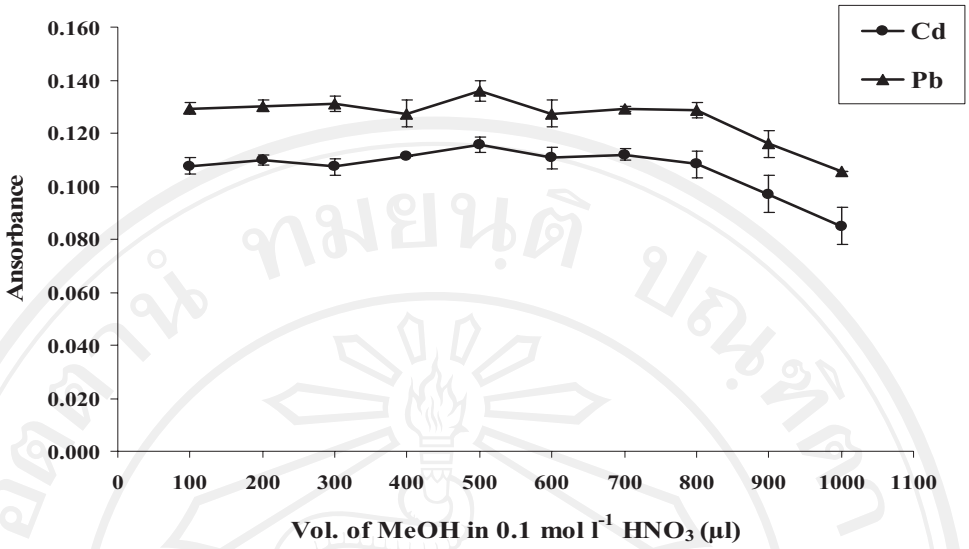


Figure 3.11 The effect of the volume of MeOH in 0.1 mol l^{-1} HNO_3 on analytical signal for Cd and Pb

From the results, the maximum analytical signal obtained was 500 µl of methanol in 0.1 mol l^{-1} HNO_3 with respect to both Cd and Pb analysis. For smaller volumes, the analytical signals were poor because the viscosity of the sample solutions remained high and not suitable for introducing into the atomizer of flame atomic absorption spectrophotometer. Whereas for larger volumes of acidified methanol, dilution was clearly predominated resulting in a gradual absorbance reduction, as also observed by other workers [43-44,58]. Therefore, the amount of methanol in 0.1 mol l^{-1} HNO_3 500 µl was selected in order to achieve the suitable and effective analysis of human hair samples for introducing into a flame atomic absorption spectrophotometer.

3.3 Effect of interfering elements on the recovery of Cd and Pb

In order to assess the possible analytical application of the recommended preconcentration by cloud point extraction procedure, the effects of possible metal ions in the human hair samples on the determination of Cd and Pb were investigated by adding the known concentrations of Fe, Cu, Zn and Mn into a model solution. The concentration of each interfering element was investigated in the range of 100-2000 μg ml^{-1}. The tolerance limits of the interfering elements are shown in **Table 3.13** and **Table 3.14**. In this work, the concentration of 1 μg ml^{-1} Cd and 10 μg ml^{-1} Pb were used for studying of percent recovery. From the results, the recoveries of Cd and interfering elements were in the range of 88.10-111.29% and 79.94-95.75%, respectively. The acceptable recovery of the 1 μg ml^{-1} analyte was in the range of 75-120%. The recoveries of Pb and interfering elements were in the range of 100.98-119.39% and 77.48-106.49%, respectively. The acceptable recovery of the 10 μg ml^{-1} analyte was in the range of 80-115% [59]. Thus, it could conclude that the determination of Cd was not interfered by interfering elements in the range of 100-2000 μg ml^{-1} with the acceptable recovery value. But the interfering elements might interfere the determination of Pb above 1500 μg ml^{-1} with the acceptable recovery value.

Table 3.13 The recoveries of interfering elements

Interfering elements	Concentration (μg ml^{-1})	%Recovery of analyte	
		Cd	Pb
Fe	2000	95.75	82.72
Cu	2000	79.94	87.51
Zn	2000	88.53	106.49
Mn	2000	88.44	77.48

Table 3.14 The concentration of interfering elements on the recovery of Cd and Pb

Concentration of interfering elements (μg ml^{-1})	% Recovery of analyte	
	Cd	Pb
100	111.29	100.98
500	97.73	106.74
1000	102.65	101.28
1500	88.10	107.60
2000	90.53	119.39

3.4 Optimum conditions of cloud point extraction

Summary of the optimum conditions of cloud point extraction for the determination of Cd and Pb in human hair samples are shown in **Table 3.15**.

Table 3.15 Optimum conditions of cloud point extraction

Parameters	Studied range	Optimum value
Complexing time (min)	15 - 55	30
PAN concentration (mol l^{-1})	$2x10^{-5}$ - $1x10^{-3}$	$2x10^{-4}$
Triton X-114 concentration (%v/v)	0.02 - 0.20	0.04
pH	1 - 6	3
Buffer solution volume (ml)	0.20 - 3.00	0.40
MeOH in 0.1 mol l^{-1} HNO_3 volume (μl)	100 - 1000	500

From the results in **Table 3.15**, they indicated that optimum conditions of the proposed method was improved the efficiency of cloud point extraction for Cd and Pb in human hair samples. This research used 1-(2-pyridylazo)-2-naphthol (PAN) as a complexing agent to form hydrophobic chelates with Cd and Pb and used Triton X-114 as surfactant. The literature reviews of the use of PAN and Triton X-114 in cloud point extraction have been rarely reported. In 2003, Manzoori *et al* [63] proposed the application of cloud point extraction using PAN and Triton X-114 to the determination of cobalt in urine samples. However, the use of PAN and Triton X-114 is rarely reported concerning the investigation of Cd and Pb in CPE, especially the application for human hair samples. This work has shown that the optimum concentration of PAN was $2x10^{-4}$ mol l^{-1}. The very low concentration of $2x10^{-4}$ mol l^{-1}

PAN was sufficient for total complexation and effective to preconcentration of Cd and Pb in human hair samples, as well as it improved to reduce the use of reagent in the laboratory. There are many literature reviews which reported the application of cloud point extraction using Triton X-114 as surfactant with others chelating agent such as bis ((1H-benzo [d] imidazol-2 yl) ethyl) sulfane (BIES) [56], *O*,*O*-diethyldithiophosphate (DDTP) [43,64-66], 1-(2-thiazolylazo)-2-naphthol (TAN) [44,67], ammonium pyrrolidinedithiocarbamate (APDC) [68] and dithizone [15,69]. For cloud point extraction using Triton X-114 as surfactant with various chelating agent, the concentration of Triton X-114 has been reported in the range of 0.05-5 %v/v [15, 43-44,56, 64-69]. In this work, the optimum concentration of Triton X-114 was 0.04 %v/v. Therefore, an amount of 0.04% Triton X-114 had improved the efficiency of cloud point extraction for Cd and Pb in human hair samples. Another improvement of cloud point extraction is the pH of sample solution because at low pH value can be avoided the effect of interfering ions.

The determination of trace elements in biological samples is particularly difficult because of the complex matrix and the low concentration of elements in such samples. In this research, the method of sample digestion was improved using microwave-assisted digestion instead of classical digestion technique. Microwave-assisted digestion is an extremely useful technique to increase the rate of chemical processes for rapid chemical decomposition. It provides higher digestion temperature, reduce digestion time and reduce the loss of volatile species. Cloud point extraction is widely used to preconcentration of trace elements in many biological samples such as blood, urine or serum, but it is rarely applied for human hair samples. Therefore, the

preconcentration technique based on cloud point extraction is reasonable to apply in human hair samples.

3.5 Determination of cadmium and lead in human hair samples

The optimized method was applied to the determination of Cd and Pb in human hair samples. The human hair samples including untreated hair sample (A-1), hair sample of a person worked in heavy metals laboratory (B-1), hair samples of workers who worked in electronic industry (C-1 to C-3) and dyed hair samples (D-1 to D-8) were studied. A 5.00 ml of each human hair sample was preconcentrated following the proposed method and the procedure explained in **Section 2.4** and **Section 2.5**. Then, the concentrations of Cd and Pb were detected by flame atomic absorption spectrophotometry (FAAS). The results are shown in **Table 3.16**.

Table 3.16 The amount of Cd and Pb in human hair samples

Samples	Cd content (μg/g) *	Pb content (μg/g) *
A-1	ND	ND
B-1	ND	10.50 ± 0.000
C-1	ND	6.60 ± 0.055
C-2	2.30 ± 0.008	ND
C-3	4.80 ± 0.003	ND
D-1	9.30 ± 0.011	ND
D-2	12.30 ± 0.003	ND
D-3	11.60 ± 0.008	6.60 ± 0.055
D-4	13.10 ± 0.006	ND
D-5	10.00 ± 0.011	ND
D-6	12.10 ± 0.005	2.70 ± 0.055
D-7	17.50 ± 0.032	ND
D-8	16.60 ± 0.003	ND

*Mean ± SD (N=3)

ND = Not detectable

The determination of Cd in human hair samples can be ordered as following: D-7 > D-8 > D-4 > D-2 > D-6 > D-3 > D-5 > D-1 > C-3 > C-2, respectively. Whereas sample A-1, B-1 and C-1 could not be detected the concentration of Cd by FAAS. The determination of Pb in human hair samples can be ordered as following:

B-1> C-1, D-3 > D-6, respectively. Whereas sample A-1, C-2, C-3, D-1, D-2, D-4, D-5, D-7 and D-8 could not be detected the concentration of Pb by FAAS. Example of calculation is shown in Appendix B.

The concentrations of Cd in dyed hair samples were higher than that in other samples. The results indicated that the increasing in the use of hair dye periods resulted in the increasing of Cd concentration. Hair dyes vary in toxicity, especially heavy metals. Some permanent hair dyes contain toxic metallic preparations based on cobalt (Co), copper (Cu), cadmium (Cd), iron (Fe), lead (Pb), nikel (Ni), silver (Ag) bismuth (Bi), arsenic (Ar) or tin (Sn) [60-62]. This can be harmful to human health. Whereas the lower concentrations of Cd were found in hair samples from the workers who worked in electronic industry for 15 and 8 years, respectively. The highest concentration of Pb was found in hair sample from the person worked in heavy metals laboratory because the laboratory was related with Pb throughout 4 years. The lower concentrations of Pb were found in hair sample of a worker who worked in electronic industry for 5 years which used Pb for manufacturing process and dyed hair sample for 2 years. And the minimum concentration of Pb was found in dyed hair sample for 8 years, due to one of the ingredients in hair dye consist of Pb.

The results indicated that Cd concentrations were higher than Pb concentrations in human hair samples. Normally, the formation constant of Cd with various chelating agents is higher than Pb. Higher value of formation constant resulting in the increasing of metal-complex stability. In addition, the dimensionless covalence parameter β of Cd (0.34) is lower than Pb (0.45). The lower β leading to the increasing of bond covalence, which can be increased the stability of metal-complex formation. Therefore, the formation of Cd(II)-PAN chelate is stronger than

Pb(II)-PAN chelate, resulting in the higher concentration of Cd in surfactant-rich phase.

Hair grows in cycles during the periods of growth. The follicle that is actively producing hair is called the anagen phase and hair is produced during 4-8 years for head hair with a rate of approximately 0.6-1.42 cm/month. After this period, the follicle enters a relatively short transition period of about 2 weeks, known as a catagen phase, which cell division stops and the follicle begins to degenerate. Following the transition period, the hair follicle enters the resting period, known as telogen phase, which the hair stops growing completely and hair growth begins to shut down. For scalp hair of an adult, the hair is approximately 85% in the growing period and the remaining 15% is in a resting period.

The follicle is located within the complex microenvironment of the skin, which has multiple layers of tissue and glands whose secretions bathe hair. Hair follicle is exposed to blood, lymph, and extracellular fluids. Multiple vascular systems transfer xenobiotics or toxic substances to hair. Since the amino acid cysteine is a key component of the keratin proteins in hair fiber, hair contains around 5% of sulfur. The sulfur in cysteine molecules is linked by disulfide chemical bonds, which exert high affinity for some heavy metals. On the other hand, heavy metals, xenobiotics and poisonous substances in human body are excreted pass through vascular systems to accumulate in hair. Moreover, many contaminants have been proven to reach hair via external environment (toxic substances reach the hair through hair treatment, smoke and dust). Therefore, metals can be transferred and accumulated within hair throughly exposure time. And the concentration of metals in hair increases with the increasing of exposure time.

In suggestion, the determination of metals in human hair should be used the scalp hair as a sample. Since the scalp hair is in the growing period of hair growth, which is a good representation of metal accumulation in human hair. Moreover, scalp hair is the metabolic end product that has a recognized ability to reflect the body metal burden. In addition, the elemental composition of human hair depends on gender, age and hair color. When comparing the effect of gender, similar trends of Cd concentration in male and female. Mostly, the concentration of Pb is found in male higher than that in female. For the effect of age, the higher level of Pb is found in children hair, but the level of Cd is not the function of age. And the concentrations of Cd and Pb are usually found in dark hair higher than fair hair.