CHAPTER 3

RESULTS AND DISCUSSION

The determination of Cd and Pb in seafood samples were carried out by GFAAS after preconcentration using octadecyl silica membrane disks modified by 8-hydroxyquinoline.

3.1 Optimization of graphite furnace atomic absorption spectrophotometer (GFAAS)

3.1.1 Pyrolysis temperature

The purpose of the pyrolysis step is used to remove as much of matrix as possible prior to atomization. This decreases the possibility of chemical interference and reduces the magnitude of the background signal (AAnalyst 800, Perkin-Elmer). The atomization temperatures for Cd and Pb in this experiment were 1,400 and 1,500 ^{o}C respectively. The results are shown in Table 3-1 and Figure 3-1.

Table 3-1 The absorbance of 2.0 µg L^{-1} Cd and 50.0 µg L^{-1} Pb at the different pyrolysis temperatures.

Pyrolysis Temperture (^{o}C)	Absorbance (A.s)	
	Cd	Pb
400	0.0778	0.0878
500	0.0772	0.0904
600	0.0774	0.0921
700	0.0778	0.0908
800	0.0767	0.0913
900	0.0762	0.0913
1,000	0.0761	0.0900
1,100	0.0494	0.0891
1,200	0.0114	0.0671
1,300	-	0.0236

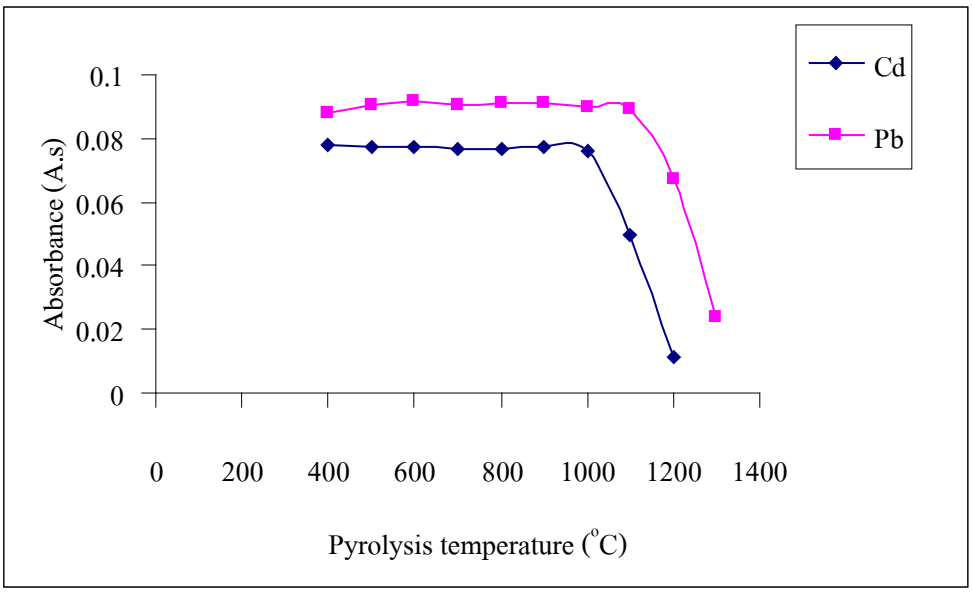


Figure 3-1 The absorbance of 2.0 µg L^{-1} Cd and 50.0 µg L^{-1} Pb at the different pyrolysis temperatures.

From Table 3-1 and Figure 3-1 the optimum pyrolysis temperature for Cd and Pb were 700 and 800 °C, respectively. Therefore, this temperature was selected for the next experiment because they give the high absorbance and good peak shape.

3.1.2 Atomization temperature

The purpose of the atomization step is used to atomize the analyte to be a free atom with high temperature which should be high enough to guarantee complete volatilization of the analyte (AAnalyst 800, Perkin-Elmer). The results are shown in Table 3-2 and Figure 3-2.

Table 3-2 The absorbance of 2.0 µg L^{-1} Cd and 50.0 µg L^{-1} Pb at the different atomization temperatures

Atomization Temperture (^{o}C)	Absorbance (A.s)	
	Cd	Pb
1,000	0.0207	0.0012
1,100	0.0681	0.0019
1,200	0.0890	0.0358
1,300	0.0799	0.0956
1,400	0.0780	0.0908
1,500	0.0743	0.0928
1,600	0.0712	0.0859
1,700	0.0674	0.0834
1,800	0.0682	0.0815
1,900	0.0695	0.0807
2,000	0.0690	0.0815
2,100	0.0693	0.0853
2,200	0.0726	-

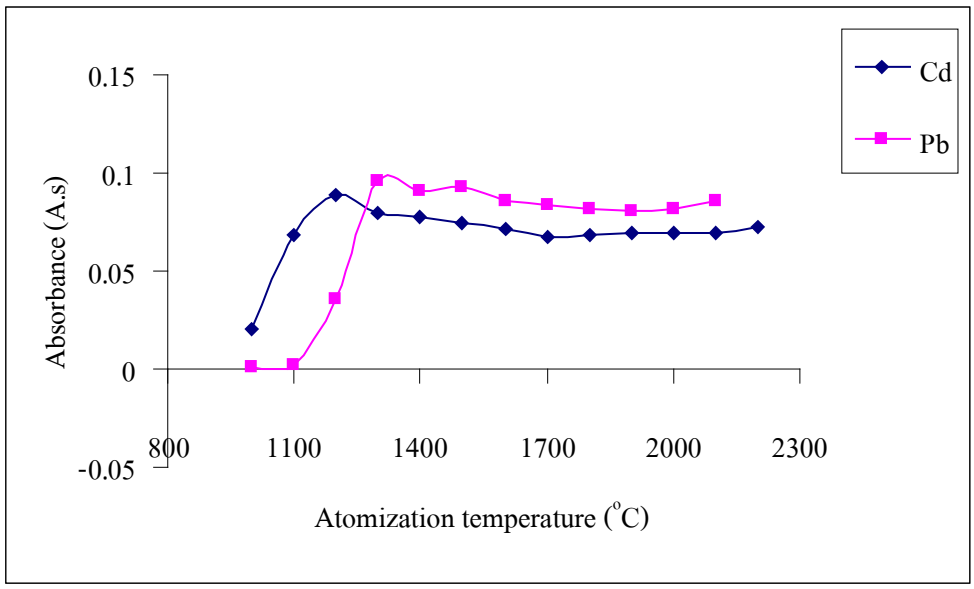
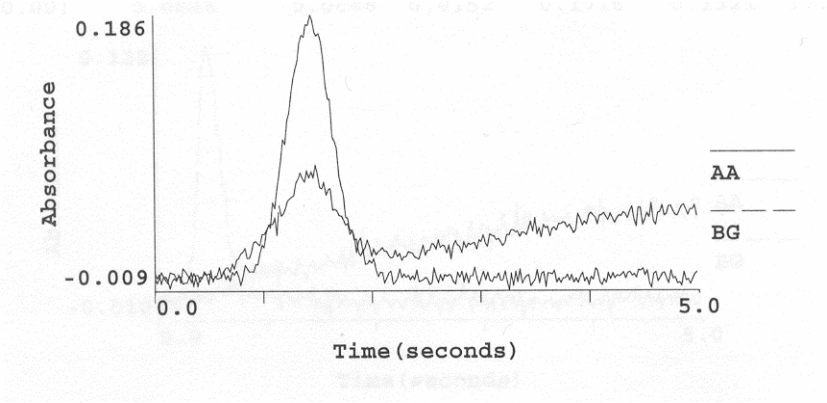
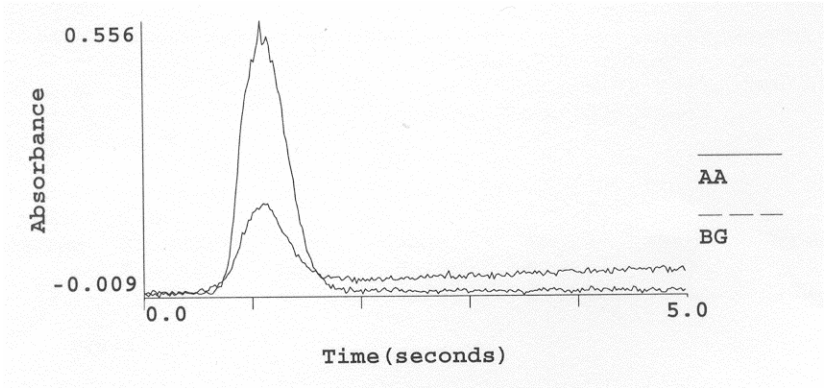


Figure 3-2 The absorbance of 2.0 µg L^{-1}Cd and 50.0 µg L^{-1} Pb at the different atomization temperatures.

From Table 3-2 and Figure 3-2 the optimum atomization temperature for Cd and Pb were 1,400 and 1,500 °C, respectively. At these temperatures give the high absorbance and good peak shape. In addition, Figure 3-3 to 3-4 are shown the peak shape of 2.0 and 8.0 µg L^{-1} Cd and 50.0 and 100.0 µg L^{-1} Pb standard working solution at optimum temperature.

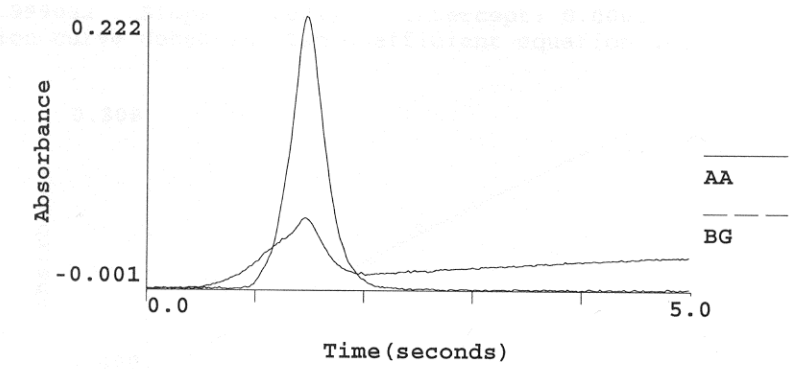


(a)

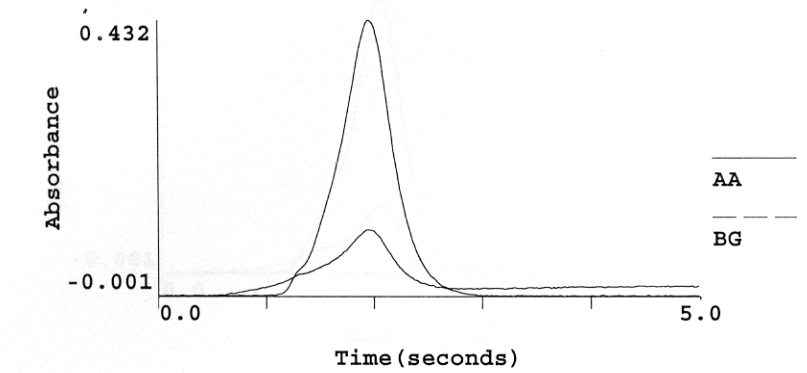


(b)

Figure 3-3 Peak shape of 2.0 µg L^{-1} Cd (a) and 8.0 µg L^{-1} Cd (b) with 0.06% (w/v) $Mg(NO_3)_2$ and 1% (w/v) $NH_4H_2PO_4$ matrix modifier at the optimum pyrolysis and atomization temperature.



(a)



(b)

Figure 3-4 Peak shape of 50.0 μg L^{-1} Pb (a) and 100.0 μg L^{-1} Pb (b) with 0.06% (w/v) $Mg(NO_3)_2$ and 1% (w/v) $NH_4H_2PO_4$ matrix modifier at the optimum pyrolysis and atomization temperature.

The optimum conditions for determination of Cd and Pb by GFAAS are summarized in Table 3-3.

Table 3-3 The optimum conditions of GFAAS for determination of Cd and Pb

Stage	Temperature (^{o}C)		Ramp	Hold	Internal Flow
	Cd	Pb	Time (s)	Time(s)	(mL min^{-1})
Drying 1	110	110	1	30	250
Drying2	130	130	15	30	250
Pyrolysis	700	800	10	20	250
Atomization	1,400	1,500	0	5	0
Clean- up	2,450	2,450	1	3	250

3.1.3 Type of matrix modifier

Graphite furnace atomic absorption spectrometry (GFAAS) is the suitable technique for the determination of trace cadmium and lead in food and biological samples because of its speed, good sensitivity and low detection limit. However, the determination by this technique is difficult because the influence of a complicated matrix greatly affects the analytical results. Therefore, difference chemical modifiers are used for the stabilization of the analyte. The matrix modifier generally depresses the background absorbance signal and increases the sensitivity of metal determinations. The absorbance of 4.0 µg L^{-1}Cd and 100.0 µg L^{-1} Pb standard working solution with various types of matrix modifiers are shown in Table 3-4 to3-5 and Figure 3-5 to 3-6.

Table 3-4 The effect of different matrix modifier on the absorbance of 4.0 µg L^{-1} Cd in aqueous solution.

Modifier type	Absorbance (A.s)
No modifier	0.1668
0.06% (w/v) $Mg(NO_3)_2$	0.1885
1% (w/v) $NH_4H_2PO_4$	0.1779
0.06% (w/v) $Mg(NO_3)_2$ + 1% (w/v) $NH_4H_2PO_4$	0.1868

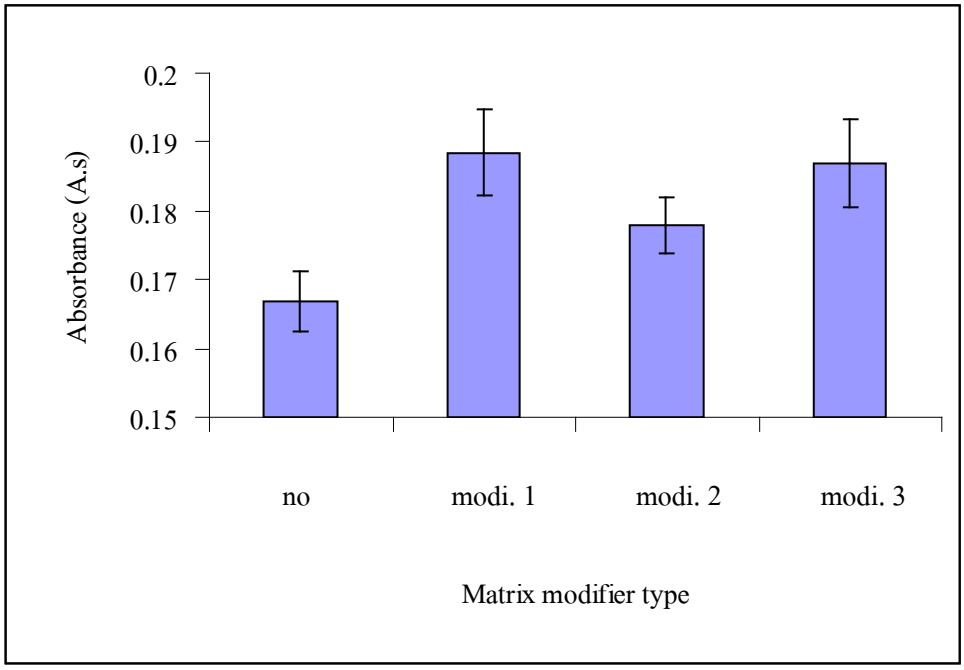


Figure 3-5 The effect of different matrix modifier on the absorbance of 4.0 µg L^{-1} Cd in aqueous solution.

no : No modifier

modi. 1 : 0.06% (w/v) $Mg(NO_3)_2$

modi. 2 : 1% (w/v) $NH_4H_2PO_4$

modi. 3 : 0.06% (w/v) $Mg(NO_3)_2$ + 1% (w/v) $NH_4H_2PO_4$

Table 3-5 The effect of different matrix modifier on the absorbance of 100.0 µg L^{-1} Pb in aqueous solution

Modifier type	Absorbance(A.s)
No modifier	0.2356
0.06% (w/v) $Mg(NO_3)_2$	0.2289
1% (w/v) $NH_4H_2PO_4$	0.2241
0.06% (w/v) $Mg(NO_3)_2$ + 1% (w/v) $NH_4H_2PO_4$	0.2362

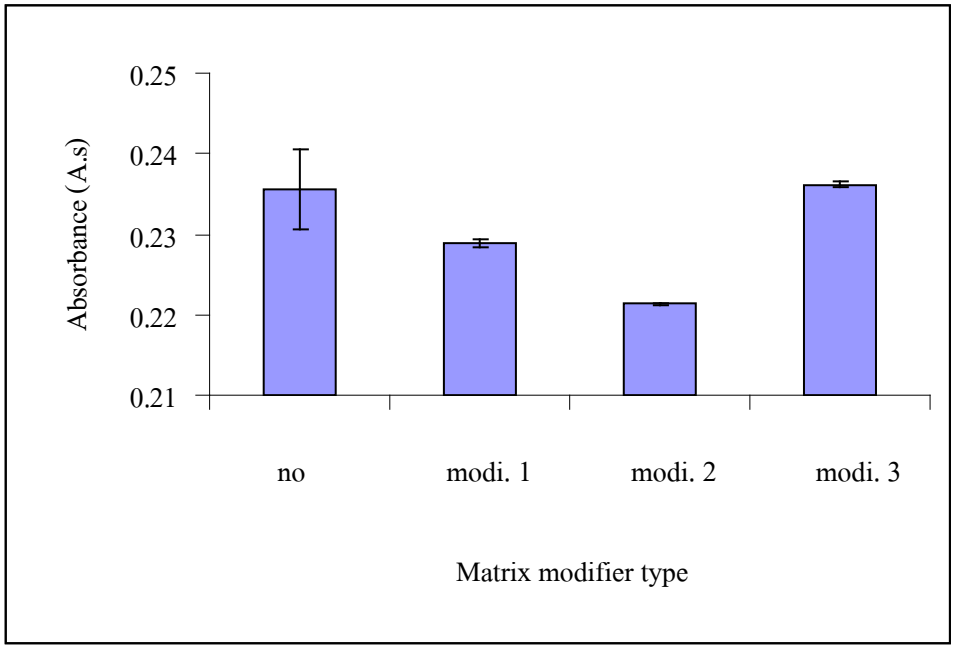


Figure 3-6 The effect of different matrix modifier on the absorbance of 100.0 µg L^{-1} Pb in aqueous solution

no : No modifier

modi 1 : 0.06% (w/v) $Mg(NO_3)_2$

modi 2 : 1% (w/v) $NH_4H_2PO_4$

modi 3 : 0.06% (w/v) $Mg(NO_3)_2$ + 1% (w/v) $NH_4H_2PO_4$

From the results in Table 3-4 and Figure 3-5 the absorbance of 4.0 μg L^{-1} Cd was low when without the matrix modifier. The absorbances were increased after the addition of various matrix modifiers. However, the optimum matrix modifiers for cadmium was the 0.06% (w/v) $Mg(NO_3)_2$ combined 1% (w/v) $NH_4H_2PO_4$ because this matrix modifier provided much higher signal enhancement.

The effects of matrix modifier on absorbance signal for 100.0 μg L^{-1} Pb were also investigated. From the results in Table 3-5 and Figure 3-6, the combined matrix modifier between 0.06% (w/v) $Mg(NO_3)_2$ and 1% (w/v) $NH_4H_2PO_4$ provided higher absorbance than another type of matrix modifiers and the absorbance of 100.0 μg L^{-1} Pb without modifier was nearly to the absorbance of Pb with combined matrix modifier due to the standard solution has not much matrix. Seafood samples have a lot of matrix which can interfere the analysis so that matrix modifier can reduce the matrix interference. Therefore, 0.06% (w/v) $Mg(NO_3)_2$ and 1% (w/v) $NH_4H_2PO_4$ were selected as the combined matrix modifier for the Cd and Pb determination.

3.1.4 The effect of utilizing matrix modifier and without matrix modifier on the determination of Cd and Pb in seafood samples

The modifier salt is added to the sample in high concentration transform the analyte element into a more well-define compound. This makes the thermal pyrolysis more predictable and reproducible for a variety of matrices. The use of a matrix modifier that converts the analyte to one common species may eliminate this type of problem with multiple peaks (AAnalyst 800, Perkin Elmer).

The absorbance of Cd and Pb in seafood sample when using optimum modifier and without modifier are shown in Table 3-6 to 3-7 and Figure 3-7 to 3-8. In addition, Figure 3-8 to 3-9 are shown the peak shape of Cd and Pb in seafood sample when using combined modifier and without modifier.

Table 3-6 The effect of matrix modifier (0.06% (w/v) $Mg(NO_3)_2$ + 1% (w/v) ($NH_4H_2PO_4$) on the absorbance of Cd in seafood sample.

Modifier type	Absorbance (A.s)
No modifier	0.0274
0.06% (w/v) $Mg(NO_3)_2$ + 1% (w/v) $NH_4H_2PO_4$	0.0913

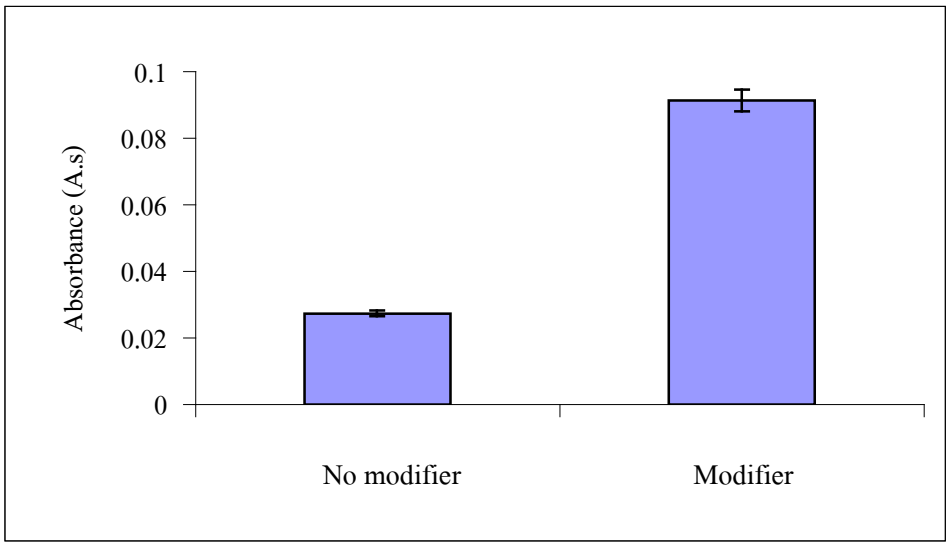


Figure 3-7 The effect of matrix modifier (0.06% (w/v) $Mg(NO_3)_2$ + 1% (w/v) ($NH_4H_2PO_4$) on the absorbance of Cd in seafood sample.

Table 3-7 The effect of matrix modifier (0.06% (w/v) $Mg(NO_3)_2$ + 1% (w/v) ($NH_4H_2PO_4$) on the absorbance of Pb in seafood sample

Modifier type	Absorbance(A.s)
No modifier	0.0514
0.06% (w/v) $Mg(NO_3)_2$ + 1% (w/v) $NH_4H_2PO_4$	0.0593

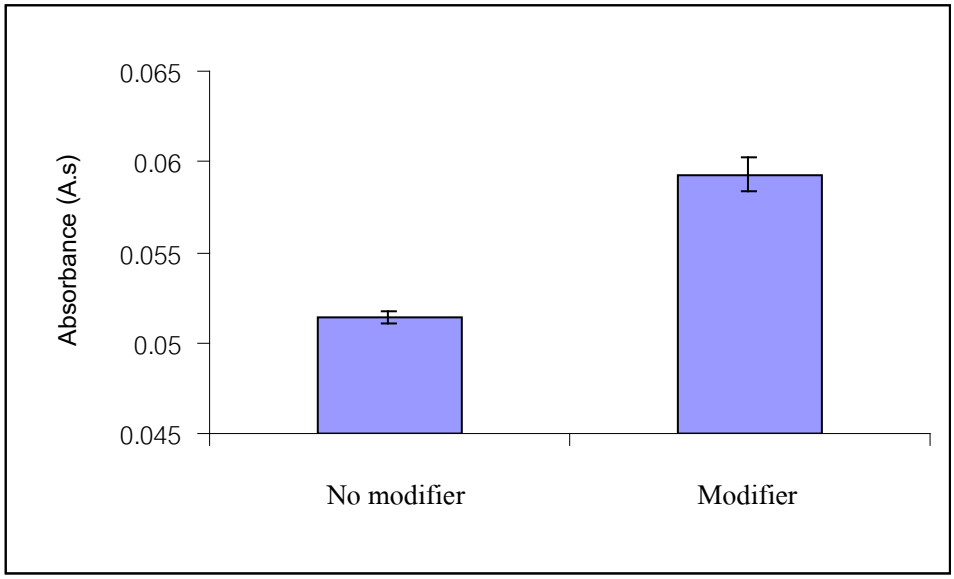


Figure 3-8 The effect of matrix modifier (0.06% (w/v) $Mg(NO_3)_2$ + 1% (w/v) ($NH_4H_2PO_4$) on the absorbance of Pb in seafood sample.

From the results in Table 3-6 to 3-7 and Figure 3-7 to 3-8 the optimum matrix modifier was the combined 0.06% (w/v) $Mg(NO_3)_2$ + 1% (w/v) $NH_4H_2PO_4$ for Cd and Pb determination in seafood samples. This matrix modifier gives the higher absorbance and eliminates the matrix interference from the sample.

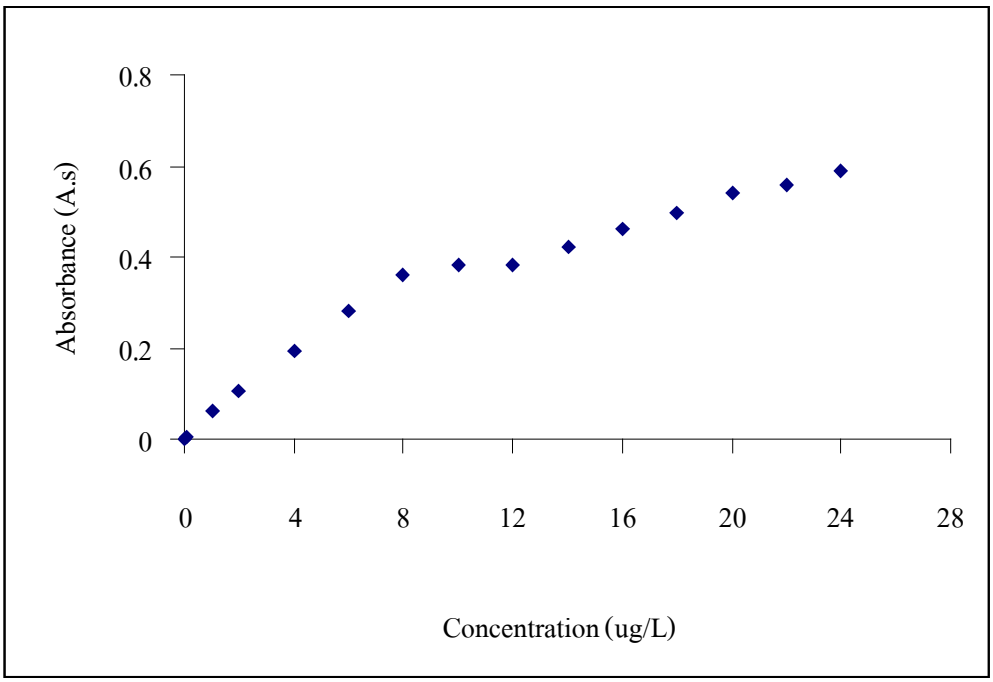
3.1.5 Linear range

The linear range is determined by plotting the integrated absorbance versus the concentration of standard solution. It is desirable to work within the linear region of the resulting calibration curve (AAnalyst 800, Perkin-Elmer).

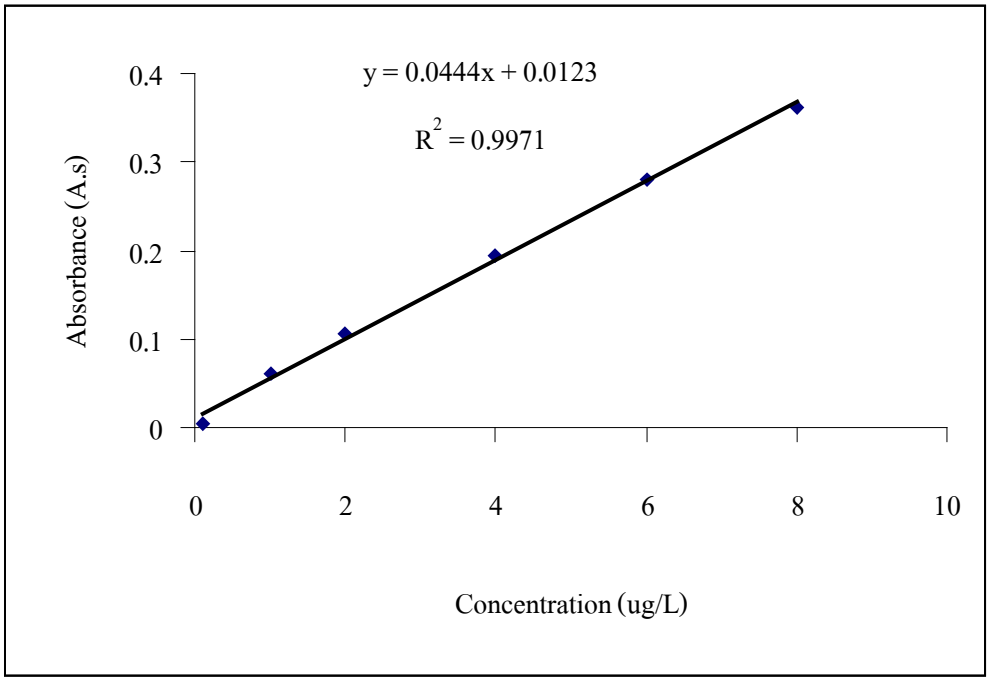
The calibration graph of Cd and Pb at various concentrations are shown in Table 3-8 to 3-9 and Figure 3-9 to 3-10. It was found that the linear dynamic range of Cd and Pb were from 0.1-0.8 µg L^{-1} and 0.1-160.0 µg L^{-1} with correlation coefficient 0.9971 and 0.9972, respectively.

Table 3-8 The absorbance of Cd at the different concentration

Cd conc. (µg L^{-1})	Absorbance (A.s)
0.0	0.0006
0.1	0.0048
1.0	0.0612
2.0	0.1073
4.0	0.1952
6.0	0.2803
8.0	0.3613
10.0	0.3820
12.0	0.3821
14.0	0.4208
16.0	0.4616
18.0	0.4970
20.0	0.5409
22.0	0.5604
24.0	0.5886



(a)

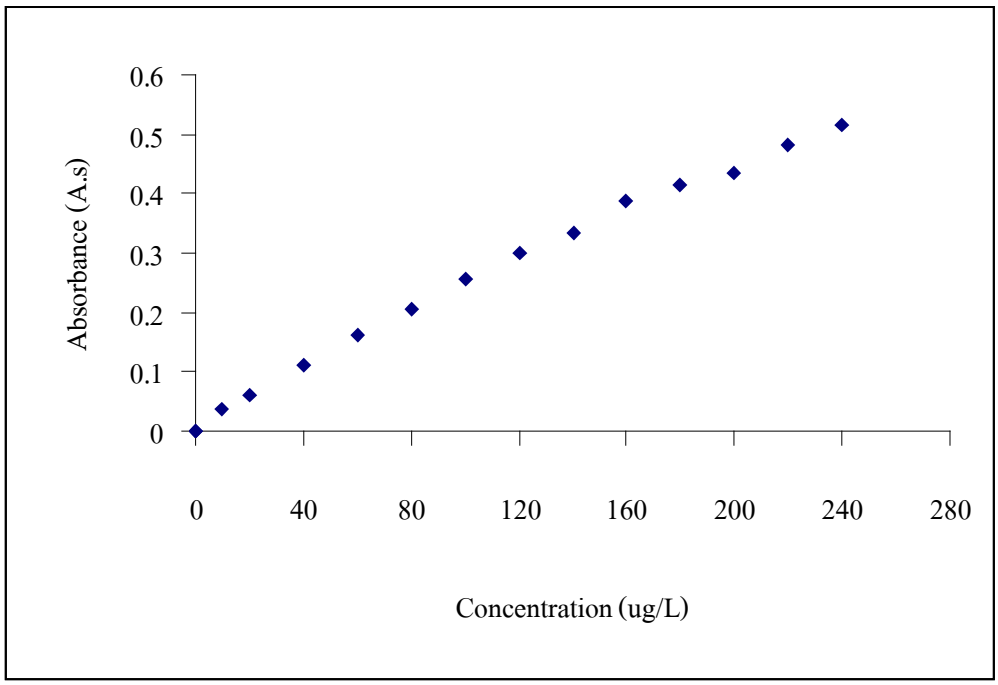


(b)

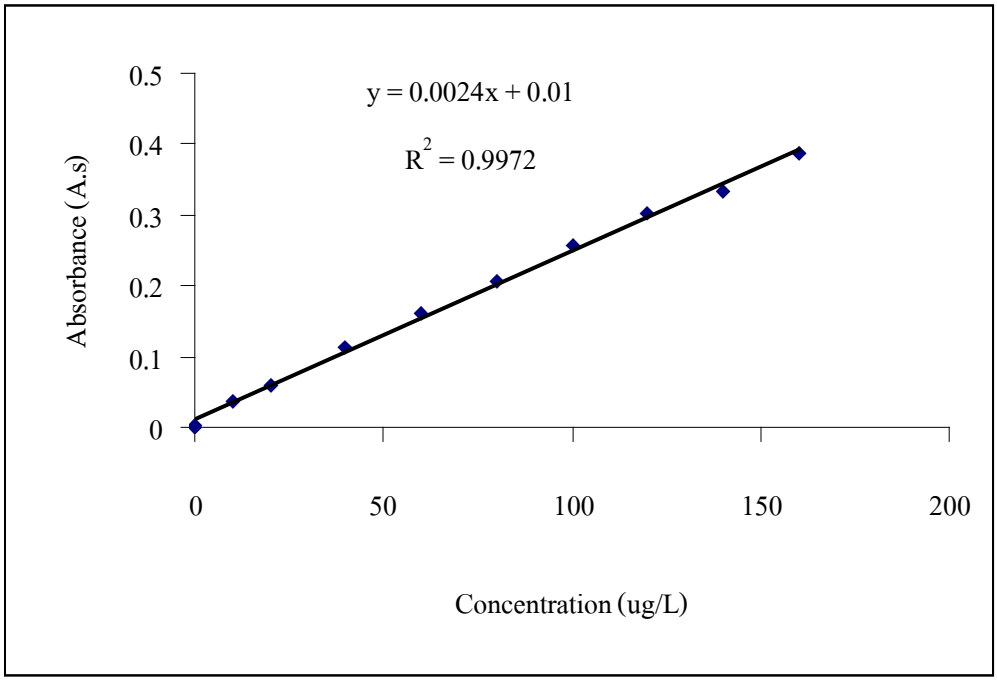
Figure 3-9 The calibration graph of Cd at the different concentration; (a) 0.0-24.0µg L^{-1}, (b) 0.1-8.0 µg L^{-1}

Table 3-9 The absorbance of Pb at the different concentration

Pb conc. ($\mu g.L^{-1}$)	Absorbance (A.s)
0.0	0.0004
0.1	0.0016
10.0	0.0358
20.0	0.0604
40.0	0.1121
60.0	0.1613
80.0	0.2055
100.0	0.2560
120.0	0.3014
140.0	0.3326
160.0	0.3884
180.0	0.4153
200.0	0.4350
220.0	0.4836
240.0	0.5168


0.6
0.5
0.4
0.3
0.2
0.1
0
Absorbance (A.s)
0
40
80
120
160
200
240
280
Concentration (ug/L)

(a)



(b)

Figure 3-10 The calibration graph of Pb at the different concentration; (a) 0.0 -240.0 µg L^{-1}, (b) 0.1-160.0 µg L^{-1}

3.1.6 Detection limit (DL)

The detection limits (DL) of Cd and Pb were studied by measuring the absorbance of ten replications of blank. The detection limits were calculated from the equation in section 2.3.3.6 (Ingle and Crouch, 1988). The equation are shown as follow.

$$\text{Detection limit} = (3*SD)/m$$

When, SD = standard deviation of blank

m = slope of calibration graph

The absorbance of blank was carried out for evaluating detection limit of Cd and Pb. The results are shown in Table 3-10 and Figure 3-11 to 3-12. The detection limit of Cd and Pb are summarized in Table 3-11.

Table 3-10 The absorbance of Cd and Pb in reagent blank (n = 10)

Replicate	Absorbance (A.s)	
	Cd	Pb
1	0.0208	0.0160
2	0.0256	0.0157
3	0.0228	0.0150
4	0.0237	0.0154
5	0.0217	0.0156
6	0.0209	0.0161
7	0.0238	0.0158
8	0.0203	0.0159
9	0.0225	0.0155
10	0.0234	0.0154
Mean	0.0226	0.0156
SD	0.0017	0.0003
%R.S.D	7.32	2.08
Calib. Slope	0.0703	0.0027

Table 3-11 The detection limits for Cd and Pb with optimum condition of GFAAS

Metals	Detection limit ($\mu g\ L^{-1}$)
Cd	0.073
Pb	0.332

3.1.7 Accuracy and precision

The accuracy of this investigation method was evaluated from the determination of certified reference material (DORM-2). The determined and certified values were compared in section 2.3.3.7 and the result is shown in Table 3-12.

Table 3-12 The comparison of the experimental and certified values for Cd and Pb determination in certified reference materials (n = 3) by using the investigation method.

Sample	Concentration of Cd (mg kg^{-1})		Concentration of Pb (mg kg^{-1})	
	Certified	Determined	Certified	Determined
DORM-2 (Dogfish muscle)	0.043 ± 0.008	0.048 ± 0.001	0.065 ± 0.007	0.068 ± 0.005

* 3 replication

From the results in Table 3-12, the determined concentration of Cd and Pb in DORM-2 by using the propose method were in good agreement with certified values. The recovery of Cd and Pb were 112.5 and 104.6% respectively

In addition, the precision of the solid phase extraction method was also evaluated as %RSD of five replication measurement. The %RSD of Cd and Pb was obtained from this method were 3.09 and 3.80%. The result is shown in Table 3-13.

Table 3-13 The absorbance of Cd and Pb for evaluating the precision.

Replicate	Cd Absorbance (A.s)	Pb Absorbance (A.s)
1	0.0412	0.0494
2	0.0439	0.0455
3	0.0405	0.0463
4	0.0417	0.0449
5	0.0413	0.0457
Mean	0.0417	0.0464
SD	0.0065	0.0088
%RSD	3.09	3.80

3.2 Sample preparation using solid phase extraction

3.2.1 The effect of pH of sample solution on adsorption of Cd and Pb on octadecyl silica membrane disk

The Cd (II) and Pb (II) ions are an intermediate Lewis acid that exhibits a high tendency to form complexes with ligands containing N donor atoms with intermediate base properties. On the other hand, the existence of N and O donating atoms in 8-hydroxyquinoline was expected to increase both the stability and selectivity of its complex over other metal ions, especially alkali and alkali earth cations. Most chelating ligands are conjugated bases of weak acid group which have a very strong affinity for hydrogen ions. Therefore, the pH will be a very important factor in the separation of metal ions by chelation, because it will determine the value of the condition stability constant of metal complexes on the surface of sorbent. Due to the presence of OH and NH group on the 8-hydroxyquinoline structures, it is expected that the extent of its complexation is sensitive to pH. Thus, the effect of pH on the extent of Cd (II) and Pb (II) ions were studied (A.R. Ghiasvand et al., 2004).

The pH of sample solution was varied as in 2.4.2 and the results are shown in Table 3-14 and Figure 3-11.

Table 3-14 The effect of pH of sample solution on adsorption of Cd and Pb on the modified octadecyl silica membrane disks.

pH	% Recovery of Cd	% Recovery of Pb
1.0	7.50	49.17
2.0	17.09	70.20
3.0	40.21	77.12
4.0	58.12	90.54
5.0	72.06	94.16
6.0	105.18	103.22
7.0	91.80	98.48
8.0	72.06	60.23

* 3 replications, RSD $\leq$ 7%

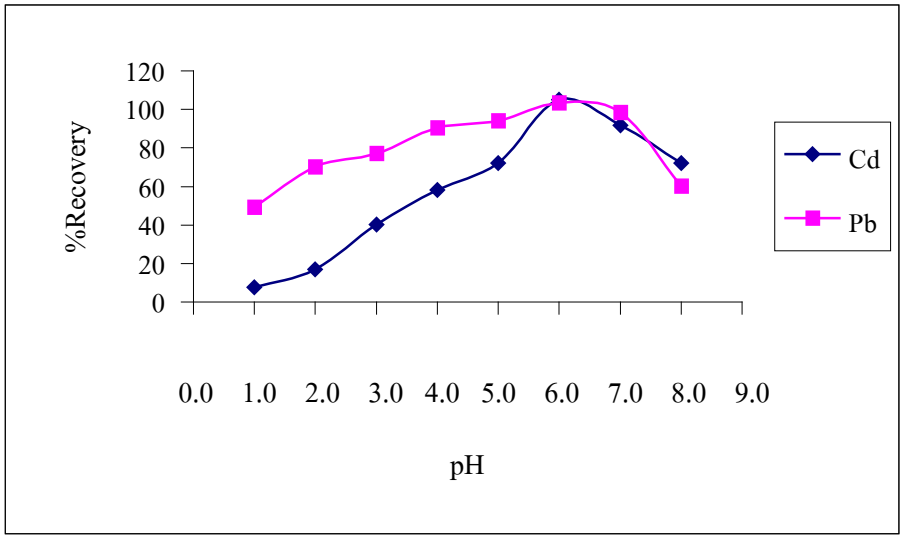


Figure 3-11 The effect of pH of sample solution on adsorption of Cd and Pb on the modified octadecyl silica membrane disks.

The results are shown in Table 3-14 and Figure 3-11 indicate that Cd (II) and Pb (II) ions can be retained in the pH range 6.0-7.0 and the best recovery of Cd (II) and Pb (II) ions were found at pH 6.0. The pH values higher than 8.0 were not studied because of the possibility of hydrolysis of octadecyl silica and consequently decrease the active life time of the disks. (Hashemi et al., 2001)

At very low pH, the protonation of -NH groups (in 8-hydroxyquinoline) and at high pH, deprotonation of -OH groups will occur. Therefore, at low and high pH the complexation of 8-hydroxyquinoline with Cd (II) and Pb (II) ions will decrease.

It can be concluded from the results in Table 3-15 and Figure 3-11 that the pH of sample solution that gives the maximum percent recovery was 6.0. Consequently, this pH was selected for the preconcentration in the next experiment.

3.2.2 The effect of amount of 8-hydroxyquinoline on adsorption of Cd and Pb on octadecyl silica membrane disks.

The effect of difference amount of 8-hydroxyquinoline on adsorption of Cd and Pb on octadecyl silica membrane disks are shown in Table 3-15 and Figure 3-12.

Table 3-15 The effect of amount of 8- hydroxyquinoline on adsorption of 1.0 0 µg L^{-1} Cd and 20.00 µg L^{-1} Pb mixed standard solution on octadecyl silica membrane disks.

8-Hydroxyquinoline (mg)	%Recovery of Cd	%Recovery of Pb
1.00	22.31	32.03
5.00	72.54	105.97
10.00	106.72	100.84
15.00	105.26	107.04
20.00	106.18	100.92
25.00	108.88	104.69
40.00	106.73	109.52

*2 replications, RSD ≤ 7%

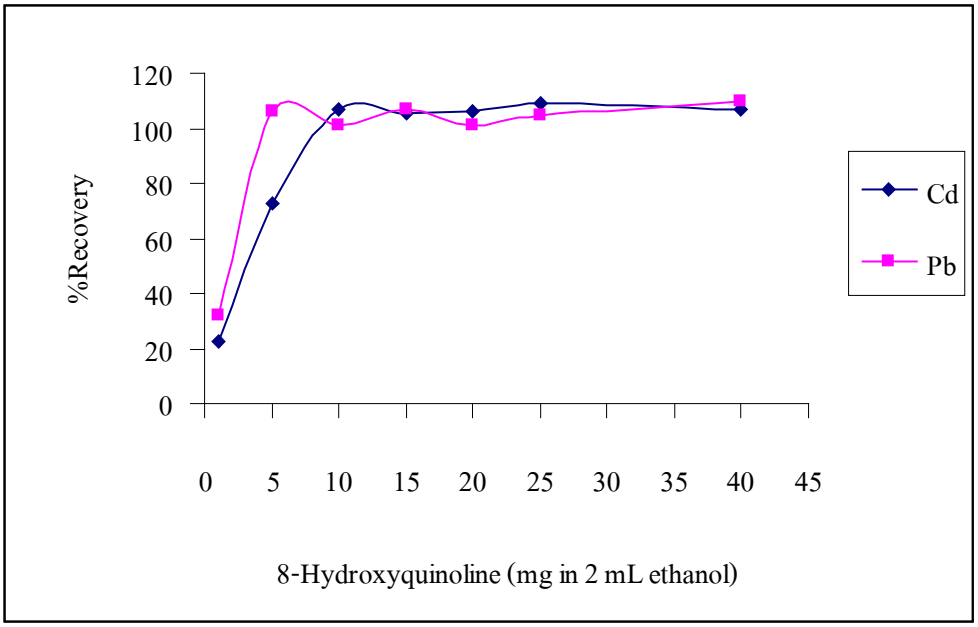


Figure 3-12 The effect of amount of 8- hydroxyquinoline on adsorption of Cd and Pb on octadecyl silica membrane disks.

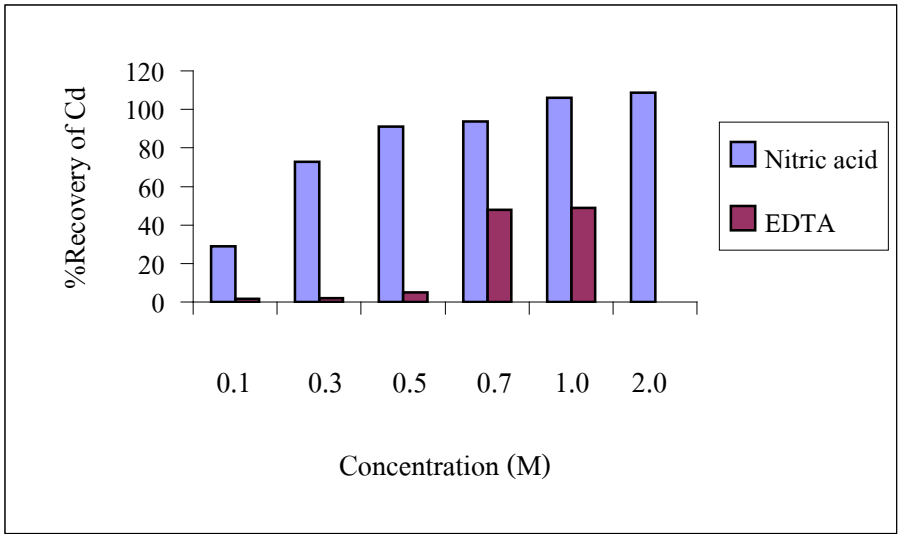
It can be concluded from the results in Table 3-15 and Figure 3-12 that the amount of 8-hydroxyquinoline that gives the good percent recovery was in the range 10.00 to 40.00 mg (in 2 mL etanol). However the 10.00 mg of 8-hydroxyquinoline was selected for the next experiment to reduce the cost effective.

3.2.3 The effect of eluent type and concentration on desorption of Cd and Pb on the modified octadecyl silica membrane disks.

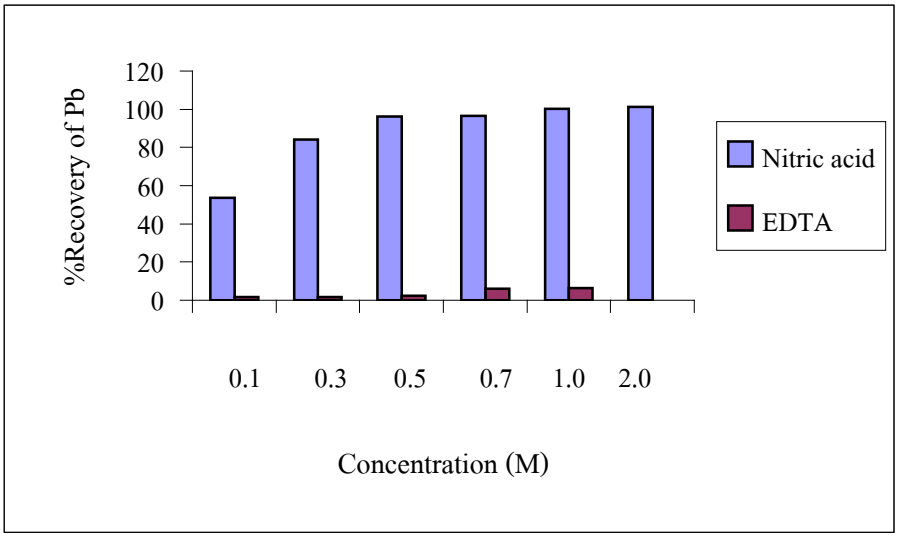
The selection of a suitable eluent is important for the analytical performance of solid phase extraction. In this experiment the various concentrations of nitric acid and EDTA were studied for Cd and Pb desorption. The eluent type and concentration for preconcentration of these metals were varied as in 2.4.4 and the results are shown in Table 3-16 and Figure 3-13.

Table 3-16 The effect of eluent type and concentration on desorption of Cd and Pb on the modified octadecyl silica membrane disks.

Concentration (M)	%Recovery of Cd		%Recovery of Pb	
	HNO_3	EDTA	HNO_3	EDTA
0.1	28.83	1.60	53.65	1.54
0.3	72.88	2.06	84.02	1.70
0.5	91.08	5.15	96.35	2.26
0.7	93.84	47.83	96.57	6.02
1.0	105.93	48.86	100.18	6.42
2.0	108.58	-	101.14	-



(a)



(b)

Figure 3-13 The effect of eluent type and concentration on desorption of Cd (a) and Pb (b) on the modified octadecyl silica membrane disks.

The results in Table 3-16 and Figure 3-13 indicated that 1.0 M nitric acid solution was efficient for quantitative elution of the adsorbed Cd (II) and Pb (II) ions. In contrast, for the elution of the adsorbed Cd (II) and Pb (II) ions using EDTA was less effective than nitric

acid. Therefore nitric acid was selected for this experiment. It was found that EDTA was less effective due to the stability constant of EDTA with Cd and Pb was less than the stability constant of 8-hydroxyquinoline. Table 3-17 is shown the equilibrium constant of 8-hydroxyquinoline and EDTA complexing with Cd and Pb.

Table 3-17 Equilibrium constant of 8-hydroxyquinoline and EDTA (Abollino *et al*., 2004)

Ligand	Equilibrium constant
8-hydroxyquinoline (OX)	$pKa_1 = 4.94$, $pKa_2 = 9.66$ $\log \beta (CdOX^+) = 8.22$, $\log \beta (CdOX_2) = 15.22$ $\log \beta (PbOX^+) = 10.03$, $\log \beta (PbOX_2) = 17.34$
EDTA	$pKa_1 = 2.0$, $pKa_2 = 2.69$, $pKa_3 = 6.18$, $pKa_4 = 10.15$ $\log \beta (CdHEDTA^-) = 9.07$ $\log \beta (PbHEDTA^-) = 9.68$, $\log \beta (PbH_2EDTA)) = 6.22$
Nitric acid	pKa = -1.44

K_a = Acid dissociation constant; β = cumulative stability constant.

It can be concluded from the results in Table 3-16 and Figure 3-13 that the eluent type and concentration that gives the good percent recovery was 1.0 M of nitric acid. Consequently, this eluent was selected for the next experiment.

3.2.4 The effect of eluent volume on desorption of Cd and Pb on the modified octadecyl silica membrane disks

The effect of different eluent volume on desorption of Cd and Pb on the modified octadecyl silica membrane disks are shown in Table 3-18 and Figure 3-14.

Table 3-18 The effect of eluent volume on desorption of Cd and Pb on the modified octadecyl silica membrane disks

Nitric acid volume (mL)	%Recovery of Cd	%Recovery of Pb
3.0	11.57	11.99
5.0	102.01	102.37
8.0	100.29	101.27
10.0	107.04	108.61
15.0	102.45	101.59
20.0	116.20	101.84

*2 replications, RSD ≤ 6%

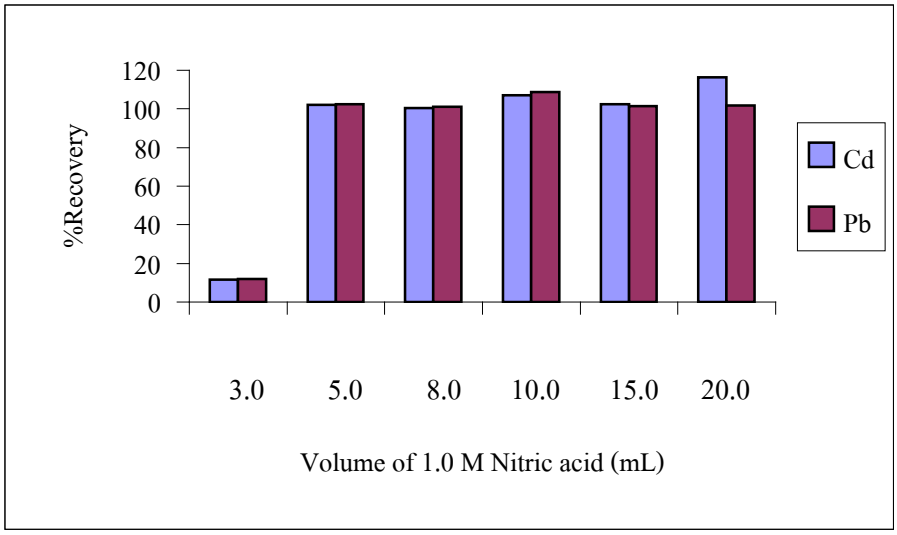


Figure 3-14 The effect of eluent volume on desorption of Cd and Pb on the modified octadecyl silica membrane disks

From the results obtained in Table 3-18 and Figure 3-14, it can be concluded that the optimum eluent volume was 5.0 mL, therefore this eluent volume was selected for the next experiment.

Preconcentration factor for a given of the sample solution passed through the modified membrane disk depend upon the original sample volume and the volume of eluent solution required to quantitatively eluting the metal sorbed onto the disks. In this experiment the 25.0 mL of sample volume was passed through the disk and eluted using 5.0 mL of 1 M nitric acid therefore an enrichment factor of five times could be achievable.

The optimum conditions for Cd and Pb preconcentration using the 8-hydroxyquinoline modified octadecyl silica membrane disk are shown in Table 3-19.

Table 3-19 The optimum conditions for solid phase extraction using 8-hydroxyquinoline modified octadecyl silica membrane disk.

Parameters	Optimum conditions
pH range	6.0–7.0
8-hydroxyquinoline	10.00 mg/ 2 mL ethanol
Eluent type	Nitric acid
Eluent concentration	1.0 M
Eluent volume	5.0 mL

3.2.5 The comparision of the calibration and standard addition method for determination of Cd and Pb in seafood samples

The experiment was performed to compare standard method between calibration and standard addition for determination of Cd and Pb in seafood samples after preconcentration using octadecyl silica membrane disks modified by 8-hydroxyquinoline. The results are shown in Table 3-20 to 3-21 and Figure 3-15 to 3-16.

Table 3-20 The comparison of absorbance using calibration and standard addition method for Cd determination in seafood sample

Cd Conc. (μg L^{-1})	Absorbance (A.s)	
	Calibration	Standard addition
0.0	0.0000	0.2863
2.0	0.1046	0.3657
4.0	0.2014	0.4543
8.0	0.3973	0.5774

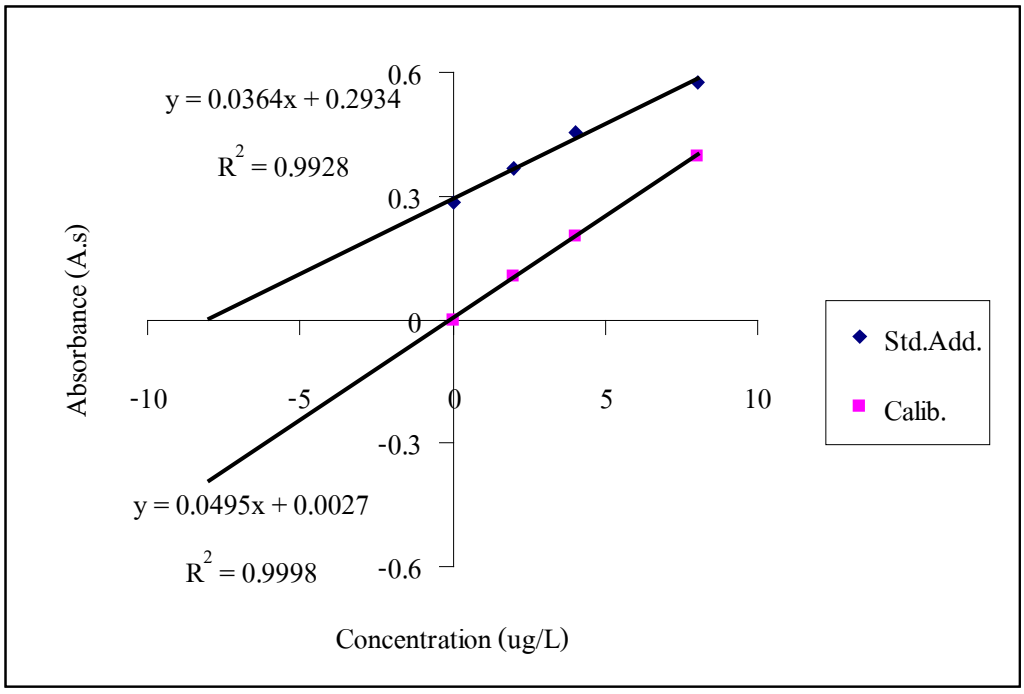


Figure 3-15 The comparison of calibration curve and standard addition curve for Cd determination in seafood sample

Table 3-21 The comparison of absorbance using calibration and standard addition method for Pb determination in seafood sample

Pb Conc. ($\mu g\ L^{-1}$)	Absorbance (A.s)	
	Calibration	Standard addition
0.0	0.0000	0.2258
40.0	0.0972	0.3058
80.0	0.2003	0.3901
160.0	0.3819	0.5042

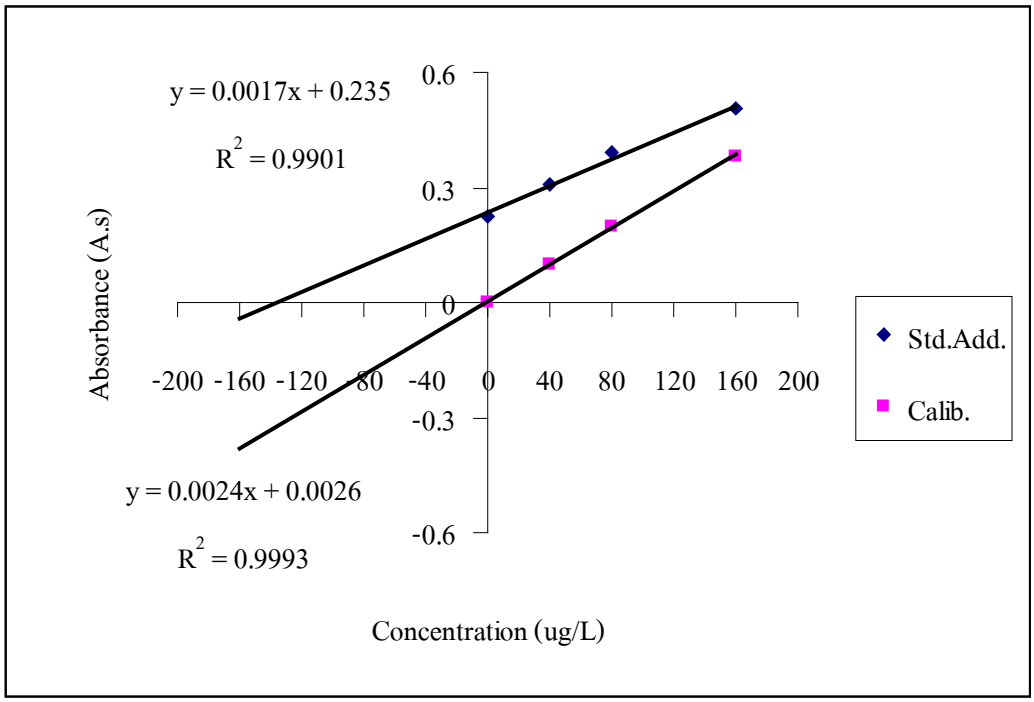


Figure 3-16 The comparison of calibration curve and standard addition curve for Pb determination in seafood sample

From the results in Figure 3-15 to 3-16 it can be concluded that the calibration curve and the standard addition curve for Cd and Pb were not parallel. The slopes of standard

addition and calibration curve for Cd and Pb were compared using two-way ANOVA (F-test). From the statistical evaluation found that slopes of both methods were significant difference at the 95% confidential level ($P < 0.05$). It was presented that the matrix effect can affect the analysis. Therefore the standard addition was the suitable method for Cd and Pb determination in seafood samples.

3.2.6 The study of percent recovery of Cd and Pb in seafood sample

The percent recovery of Cd and Pb in seafood samples was studied in section 2.4.7 and the results are shown in Table 3-22 to 3-23.

Table 3-22 Recovery test for the studied method using spiked 1.0 μg L^{-1} Cd and 20.0 μg L^{-1} Pb in seafood sample (Preconcentration 5 times)

Experiment	Cd concentration (μg L^{-1})			SD	%RSD	%Recovery
	Rep.1	Rep.2	Mean			
1	6.994	7.626	7.310	0.4469	6.11	106.17

Table 3-23 Recovery test for the studied method using spiked 1.0 μg L^{-1} Cd and 20.0 μg L^{-1} Pb in seafood sample (Preconcentration 5 times)

Experiment	Pb concentration (μg L^{-1})			SD	%RSD	%Recovery
	Rep.1	Rep.2	Mean			
1	101.73	109.19	105.46	5.2750	5.00	105.45

3.3 Analytical performance of the modified octadecyl silica membrane disks

3.3.1 Maximum capacity of the modified octadecyl silica membrane disks on adsorption of Cd and Pb

The maximum capacity of the 10.0 mg 8-hydroxyquinoline modified membrane disk was studied in section 2.5.1. The results are shown in Table 3-24

Table 3-24 Maximum capacities of the 10.00 mg 8-hydroxyquinoline modified octadecyl silica membrane disks on adsorption of Cd and Pb.

Metals	Maximum capacity ($\mu g\ L^{-1}$)
Cd	30.67 ± 3.77
Pb	168.82 ± 11.13

3.3.2 Breakthrough volume of the modified octadecyl silica membrane disks on adsorption of Cd and Pb

The maximum sample volume passed through the 10.00 mg 8-hydroxyquinoline modified octadeyl silica membrane disks was studied in section 2.5.2. The results are shown in Table 3-25.

Table 3-25 Maximum sample volume passed through the 10.00 mg 8-hydroxyquinoline modified octadecyl silica membrane disks for Cd and Pb extraction in this study

Sample volume (mL)	%Recovery		Eluent volume (mL)	Preconcentration factor
	Cd	Pb		
50	118.72	104.4	5	10
100	104.55	89.73	5	20

* Preconcentration factor = Sample volume / Eluent volume

3.3.3 Interference of coexist ions on adsorption of Cd and Pb using the modified octadecyl silica membrane disks

The effect of coexist ions interferences was studied in section 2.5.3. The results are shown in Table 3-26 and Figure 3-17.

Table 3-26 Interferences of coexist ions on adsorption of Cd and Pb using the modified octadecyl silica membrane disks.

Metal	Absorbance (A.s)	
	Without coexist ions	With coexist ions
Cd	0.1219	0.1202
Pb	0.1062	0.0972

*3 replications, RSD $\leq$ 5%

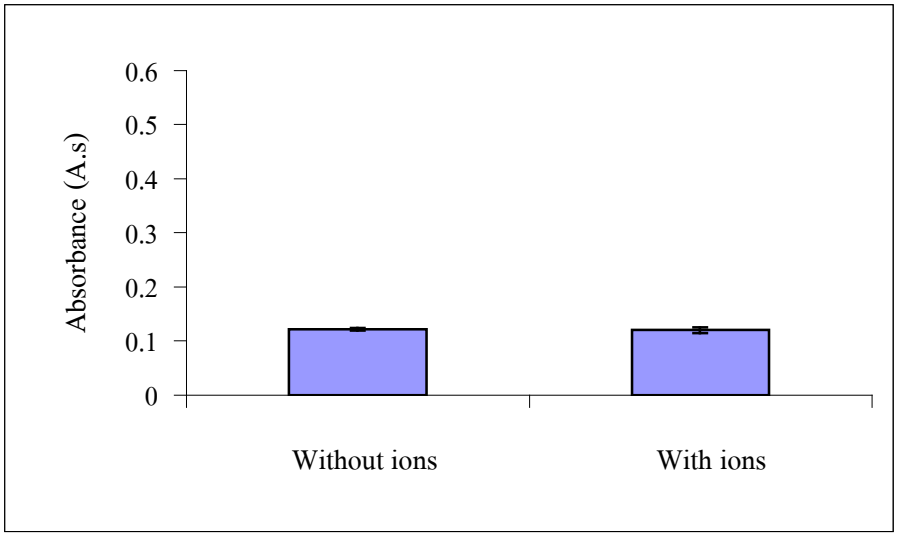


Figure 3-17 Interferences of coexist ions on adsorption of Cd using the modified octadecyl silica membrane disks.

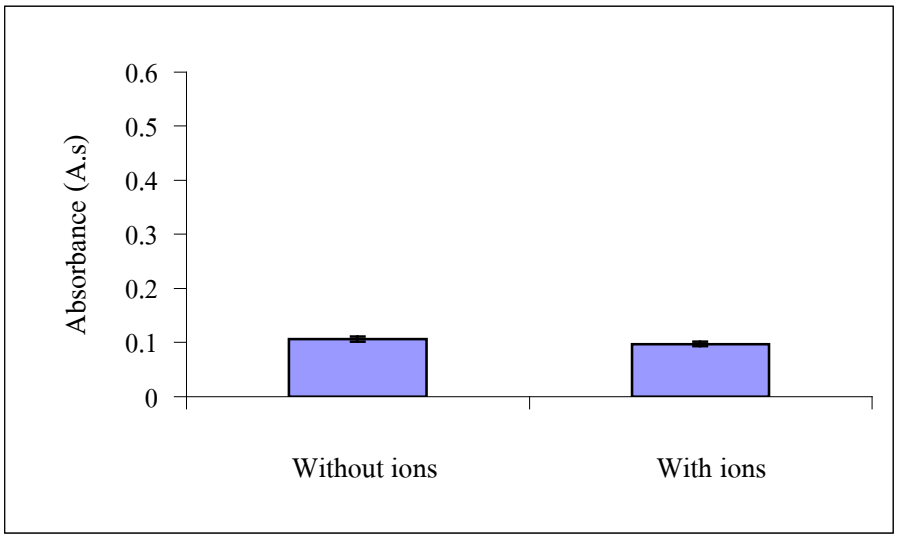


Figure3-18 Interferences of coexist ions on adsorption of Pb using the modified octadecyl silica membrane disks.

From the results obtained in Table 3-26 and Figure 3-17 to 3-18, it can be concluded that the interference of coexist ions such as Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}, Zn^{2+} and Cl^{-} on the extraction of Cd (II) and Pb (II) ions by using the modified membrane disk were not much affect. The retention of other ions by the modified membrane disk was very low then the interference effect of coexist ions were not the serious effect for Cd and Pb determination with the proposed method.

The coexist ion interferences on the studied method were evaluated by statistical analysis using paired sample Student t-test. From the statistical results, the absorbance of Cd and Pb with and without coexist ions was no significant difference at the 95% confidential level ($P > 0.05$) then the interference of coexist ions was not affect on the extraction by using the studied method.

3.4 Application of the studied method in seafood samples

3.4.1 The study of sample digestion method for Cd and Pb determination in seafood samples

3.4.1.1 Hot plate digestion method

Hot plate digestion method was studied in section 2.6.3.1. The results are shown in Table 3-27.

Table 3-27 Analytical results (mg kg^{-1}, Mean ± SD, n = 3) for the determination of trace Cd and Pb in the certified reference material using hot plate digestion.

Sample	Concentration of Cd (mg kg^{-1})		Concentration of Pb (mg kg^{-1})	
	Certified	Determined	Certified	Determined
DORM-2 (Dogfish muscle)	0.043 ± 0.008	0.047 ± 0.001 (%RSD =2.72)	0.065 ± 0.007	0.071 ± 0.006 (%RSD = 7.77)

3.4.1.2 Water bath digestion method

Water bath digestion method was studied in section 2.6.3.2. The results are shown in Table 3-28.

Table 3-28 Analytical results (mg kg^{-1}, Mean ± SD, n = 3) for the determination of trace Cd and Pb in the certified reference material using water bath digestion.

Sample	Concentration of Cd (mg kg^{-1})		Concentration of Pb (mg kg^{-1})	
	Certified	Determined	Certified	Determined
DORM-2 (Dogfish	0.043 ± 0.008	0.048 ± 0.001 (% RSD = 1.29)	0.065 ± 0.007	0.068 ± 0.005 (% RSD = 6.80)

muscle)				

3.4.1.3 Dry ashing method

Dry ashing method was studied in section 2.6.3.3. The results are shown in Table 3-29.

Table 3-29 Analytical results (mg kg^{-1}, Mean ± SD, n = 3) for the determination of trace Cd and Pb in the certified reference material using dry ashing method.

Sample	Concentration of Cd (mg kg^{-1})		Concentration of Pb (mg kg^{-1})	
	Certified	Determined	Certified	Determined
DORM-2 (Dogfish muscle)	0.043 ± 0.008	0.045 ± 0.001 (% RSD = 2.45)	0.065 ± 0.007	0.071 ± 0.003 (% RSD = 4.49)

From the results in Table 3-27 to 3-29 it can be concluded that sample digestion methods such as dry ashing, hot plate digestion and water bath digestion were efficient for sample digestion but the simple and rapid method was preferred. Therefore, the water bath digestion method was more effective than another method due to the digestion time only 3 h. The digestion was carried out in the polypropylene vessels which can reduce the amount of the contamination. The water bath digestion method was less time consuming and many replicate of sample can be done. In this study, the water bath digestion method was selected to digest the seafood samples before analysis.

3.4.2 Determination of Cd and Pb in seafood samples using the studied method (GFAAS)

The studied sample preparation method was applied to determination of Cd and Pb in several seafood samples such as tunafishes, squids, cuttlefishes, octopuses and prawn from the frozen seafood companies in Trang, Pattani and Songkhla provinces which supported from Central Equipment of Science Faculty, Prince of Songkla University. The results are shown in Table 3-30 to 3-32. The results presented that the concentration of Cd and Pb in seafood samples

were at the trace levels. The studied sample preparation method was used to extraction, clean up and preconcentration of Cd and Pb in seafood samples before analysis using GFAAS.

Table 3-30 The concentration of Cd and Pb in frozen tunafishes from the seafood companies in the South of Thailand.

Company	Sample Lot No.	Concentration level ($\mu g\ g^{-1}$) (dry weight)	
		Cd	Pb
1	1	0.015 ± 0.0010	0.129 ± 0.0221
	2	0.016 ± 0.0017	0.105 ± 0.0258
	3	0.015 ± 0.0047	0.115 ± 0.0090
	4	0.009 ± 0.0019	0.032 ± 0.0033
2	1	0.006 ± 0.0008	0.033 ± 0.0042
	2	0.001 ± 0.0003	0.020 ± 0.0127
	3	0.007 ± 0.0007	0.108 ± 0.0192
	4	0.008 ± 0.0021	0.041 ± 0.0217
	5	0.008 ± 0.0010	0.009 ± 0.0014
3	1	0.003 ± 0.0014	0.046 ± 0.0161
	2	0.006 ± 0.0007	0.032 ± 0.0104
	3	0.005 ± 0.0001	0.044 ± 0.0072
	4	0.002 ± 0.0006	0.041 ± 0.0083
	5	0.005 ± 0.0005	0.061 ± 0.0068
4	1	0.012 ± 0.0001	0.022 ± 0.0080
	2	0.006 ± 0.0017	0.015 ± 0.0014
	3	0.008 ± 0.0012	0.057 ± 0.0183
	4	0.003 ± 0.0006	0.035 ± 0.0160
	5	0.007 ± 0.0004	0.079 ± 0.0062

Table 3-31 The concentration of Cd and Pb in frozen squids, cuttlefishes and octopuses from the seafood companies in the South of Thailand.

Sample type	Sample Lot No.	Concentration level ($\mu g\ g^{-1}$) (dry weight)	
		Cd	Pb
Squid	1	0.004 ± 0.0004	0.165 ± 0.0100
	2	0.003 ± 0.00002	0.035 ± 0.0053
	3	0.006 ± 0.0001	0.089 ± 0.0092
	4	0.007 ± 0.0003	0.018 ± 0.0019
	5	0.001 ± 0.0005	0.037 ± 0.0182
	6	0.009 ± 0.0004	0.006 ± 0.0012
	7	0.002 ± 0.00004	0.005 ± 0.0001
Cuttlefish	1	0.003 ± 0.0003	0.003 ± 0.0002
	2	0.004 ± 0.00007	0.005 ± 0.0003
	3	0.003 ± 0.0001	0.003 ± 0.0002
Octopus	1	0.019 ± 0.0005	0.002 ± 0.0012
	2	0.028 ± 0.0001	0.005 ± 0.0005
	3	0.049 ± 0.0020	0.018 ± 0.0028
	4	0.036 ± 0.00001	0.012 ± 0.0015

Table 3-32 The concentration of Cd and Pb in frozen squids, cuttlefishes and octopuses from the seafood companies in the South of Thailand.

Sample Lot No.	Concentration level (μg g^{-1}) (dry weight)	
	Cd	Pb
1	0.002 ± 0.0001	0.013 ± 0.0042
2	0.004 ± 0.0003	0.005 ± 0.0007
3	0.002 ± 0.0003	0.013 ± 0.0002
4	0.002 ± 0.0002	0.007 ± 0.0008
5	0.002 ± 0.00004	0.022 ± 0.0012
6	0.003 ± 0.00003	0.025 ± 0.0045

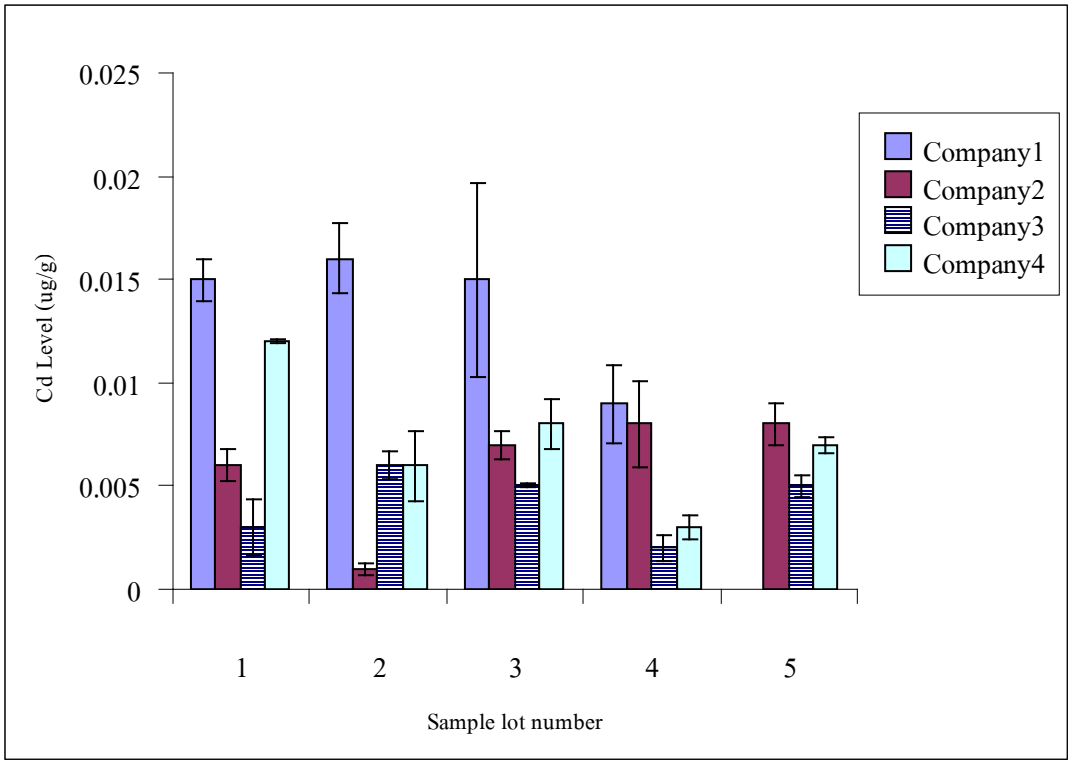


Figure 3-19 The concentration of Cd in frozen tunafishes (dry weight) from the seafood companies in the South of Thailand.

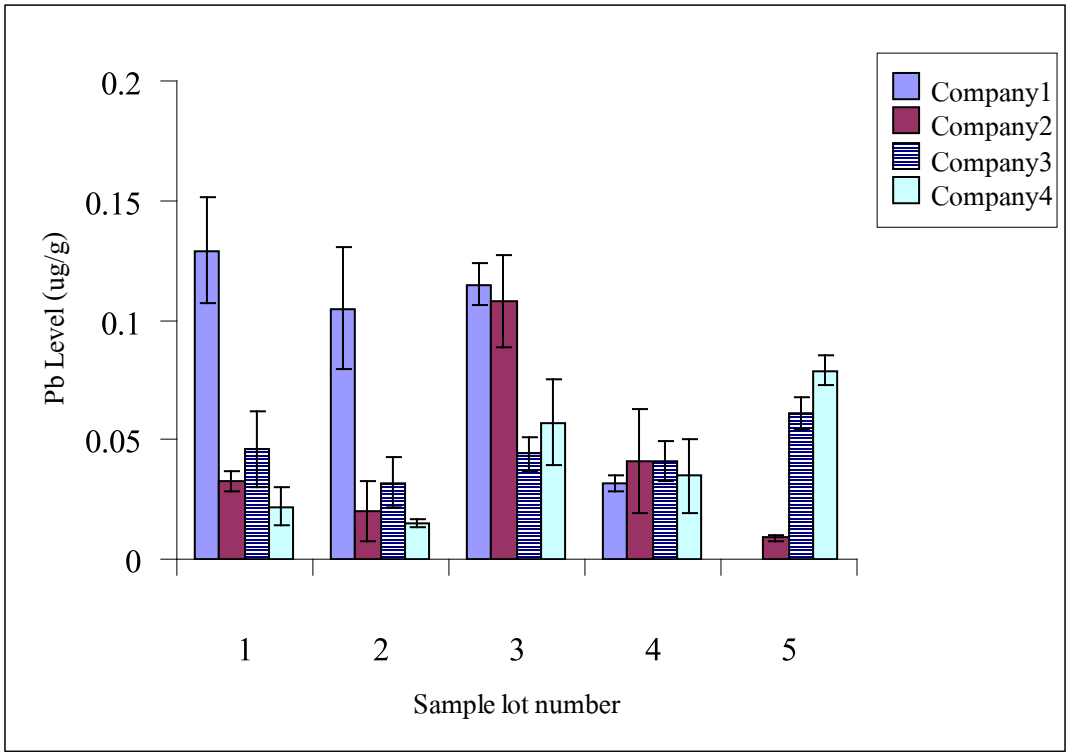


Figure 3-20 The concentrationtion of Pb in frozen tunafishes (dry weight) from the seafood companies in the South of Thailand.

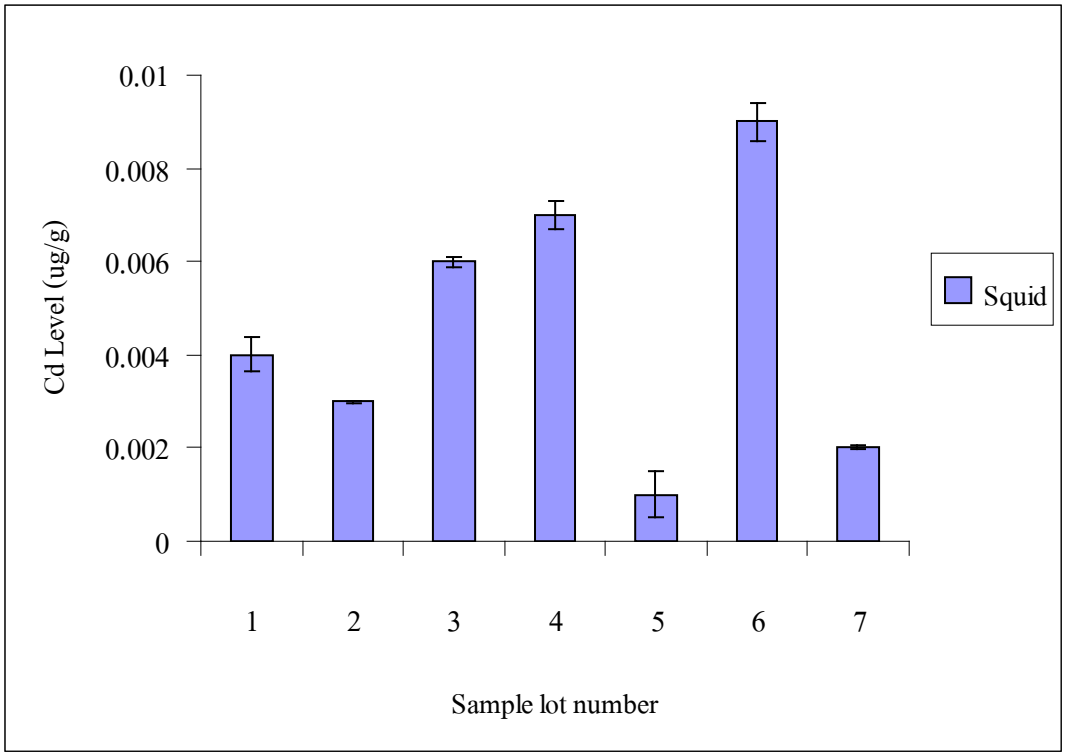


Figure 3-21 The concentration of Cd in frozen squids (dry weight) from the seafood company in the South of Thailand.

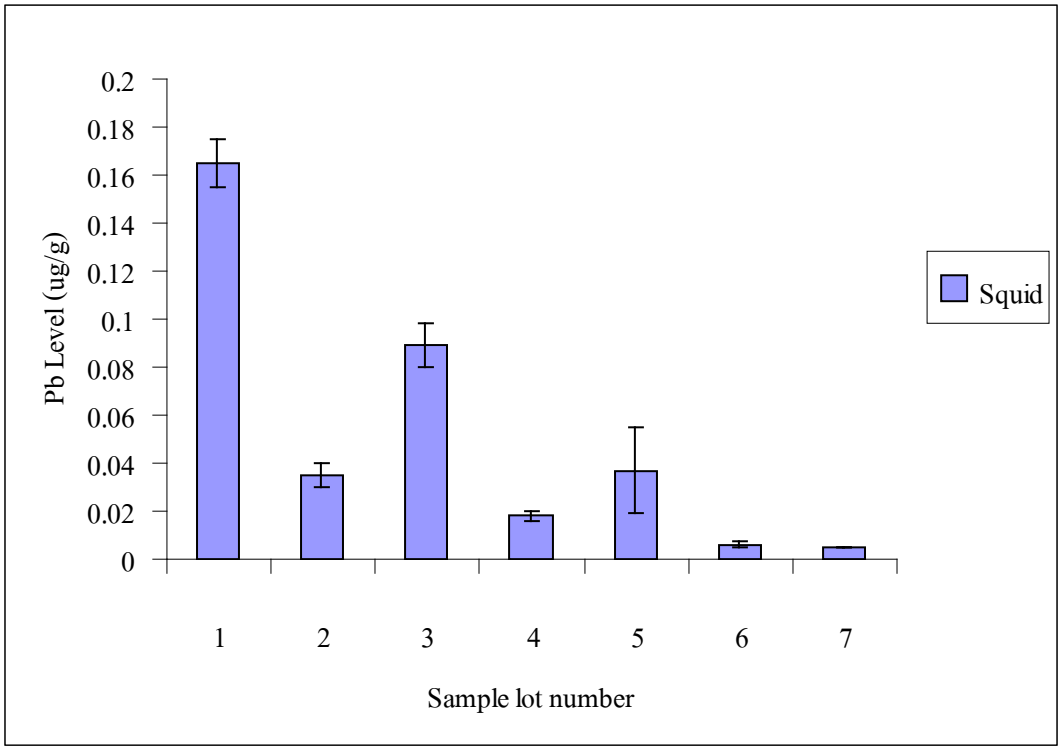


Figure 3-22 The concentration of Pb in frozen squids (dry weight) from the seafood company in the South of Thailand.

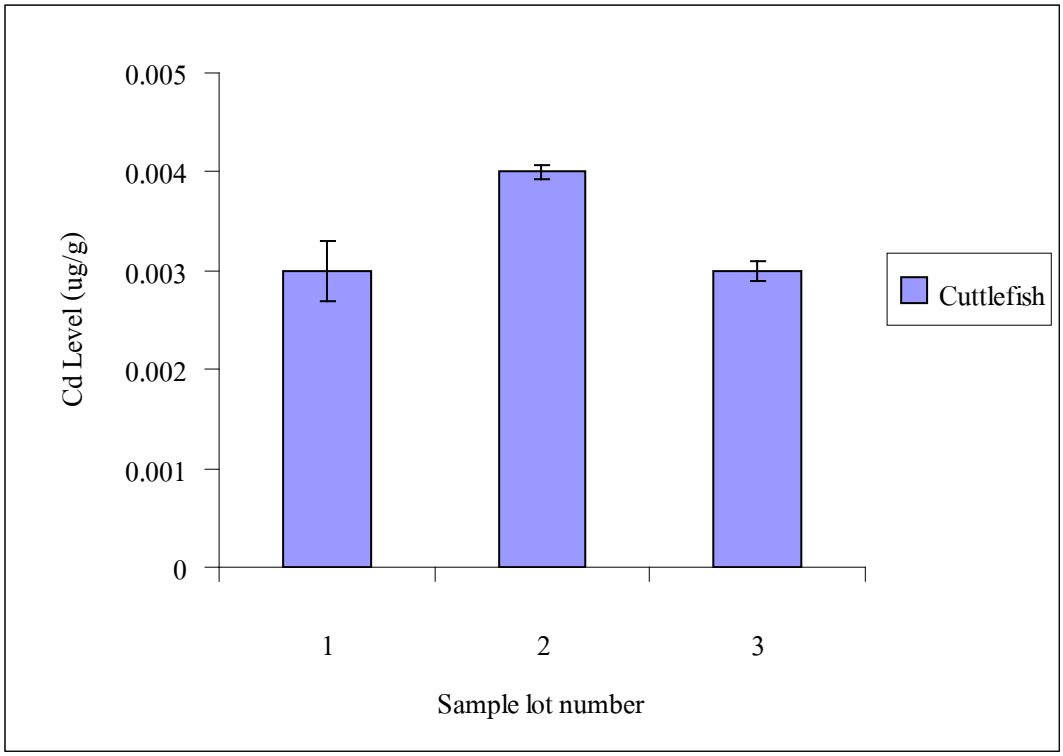


Figure 3-23 The concentration of Cd in frozen cuttlefishes (dry weight) from the seafood company in the South of Thailand.

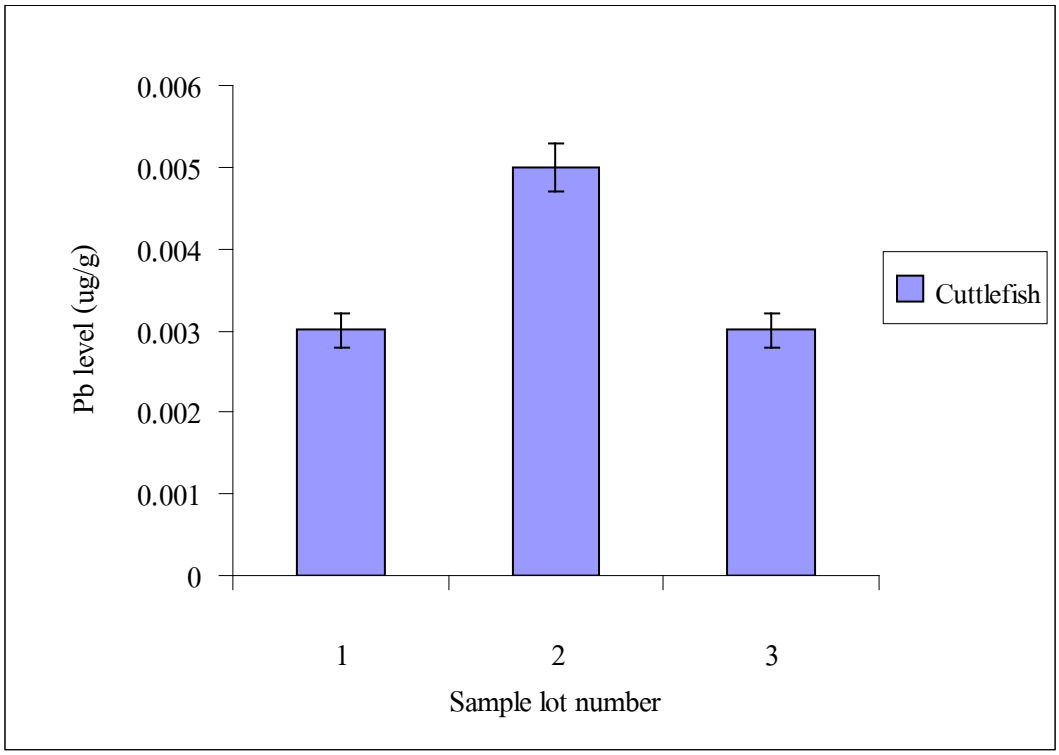


Figure 3-24 The concentration of Pb in frozen cuttlefishes (dry weight) from the seafood company in the South of Thailand.

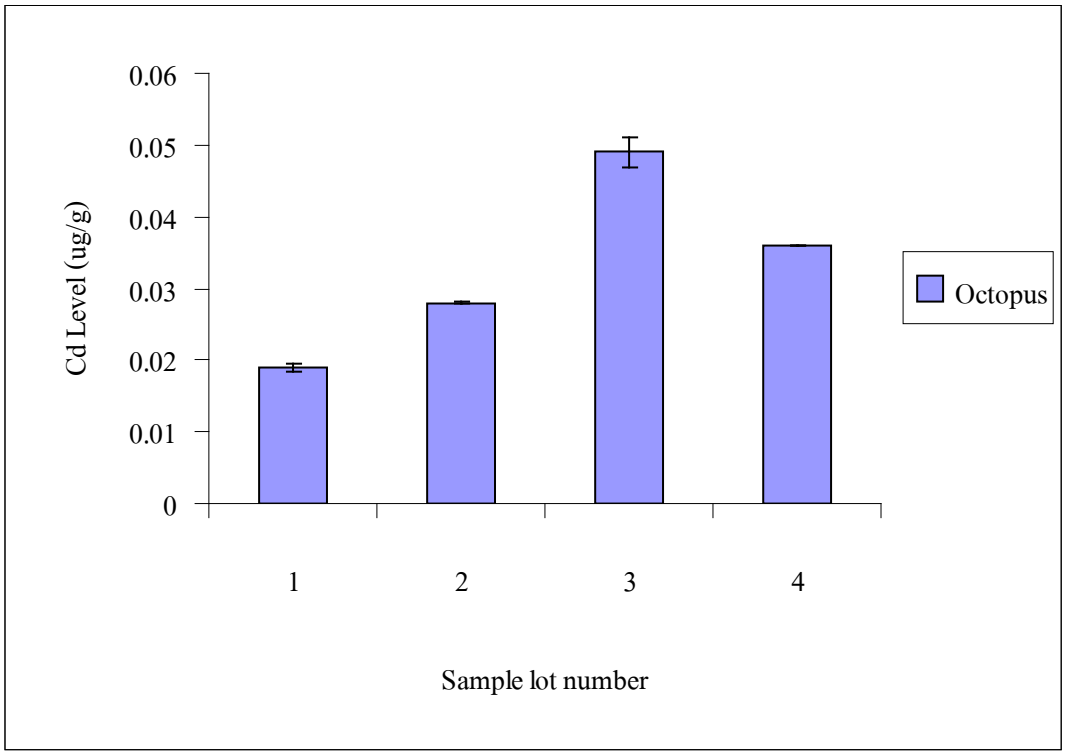


Figure 3-25 The concentration of Cd in frozen octopuses (dry weight) from the seafood company in the South of Thailand.

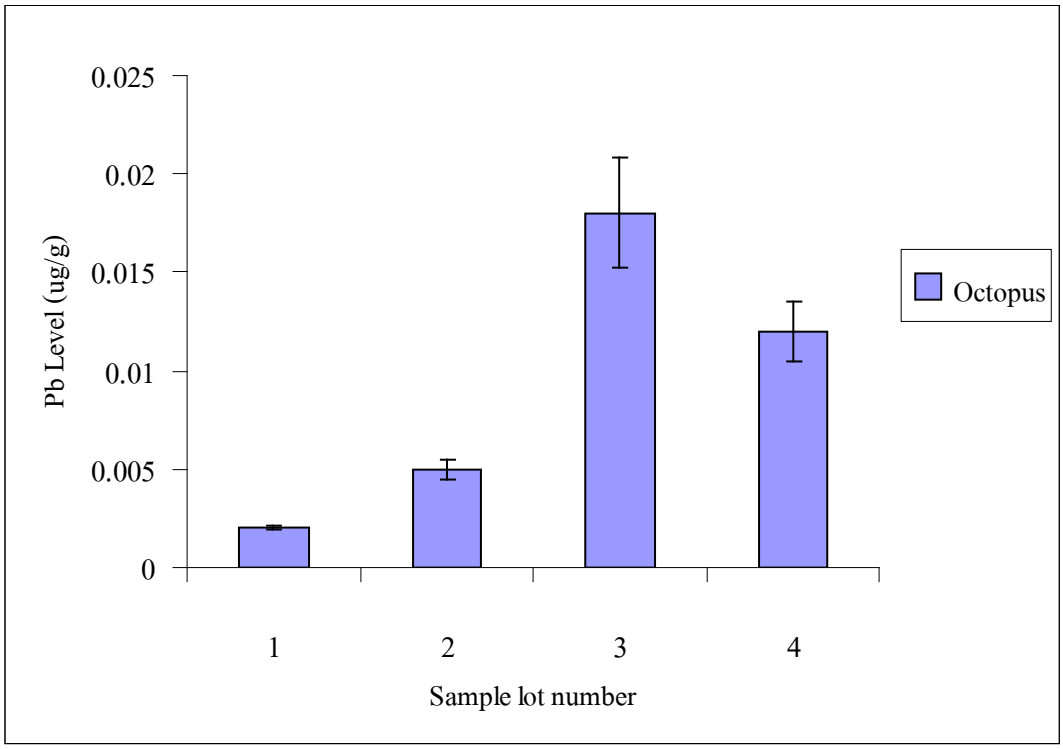


Figure 3-26 The concentration of Pb in frozen octopuses (dry weight) from the seafood company in the South of Thailand.

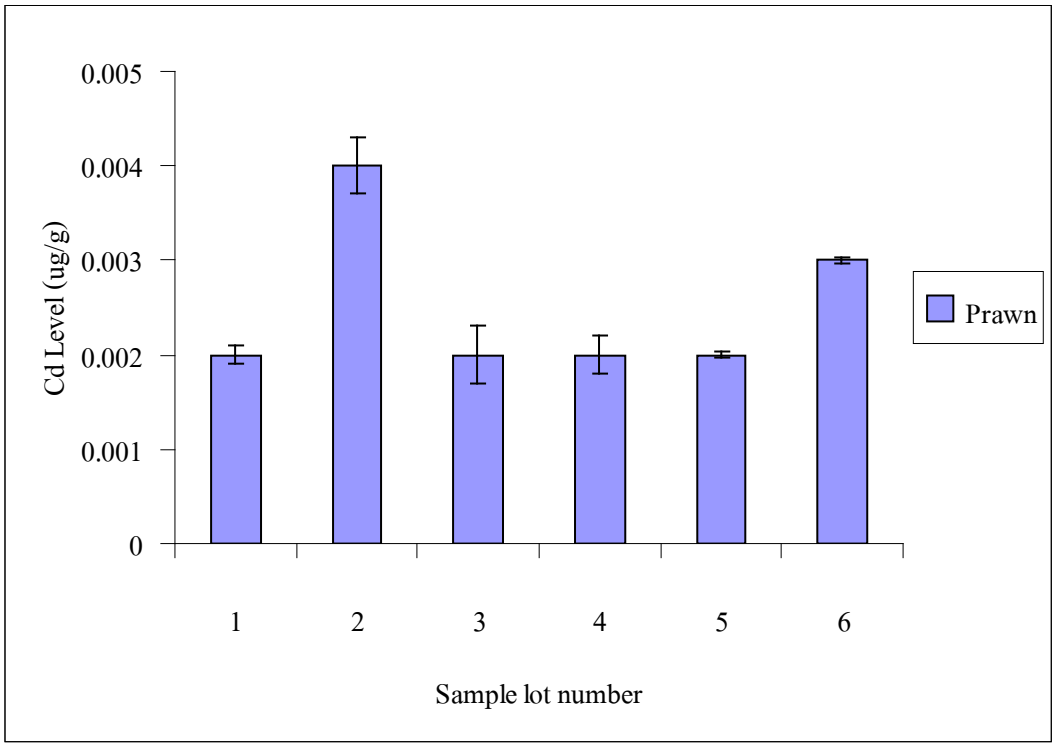


Figure 3-27 The concentration of Cd in frozen prawns (dry weight) from the seafood company in the South of Thailand.

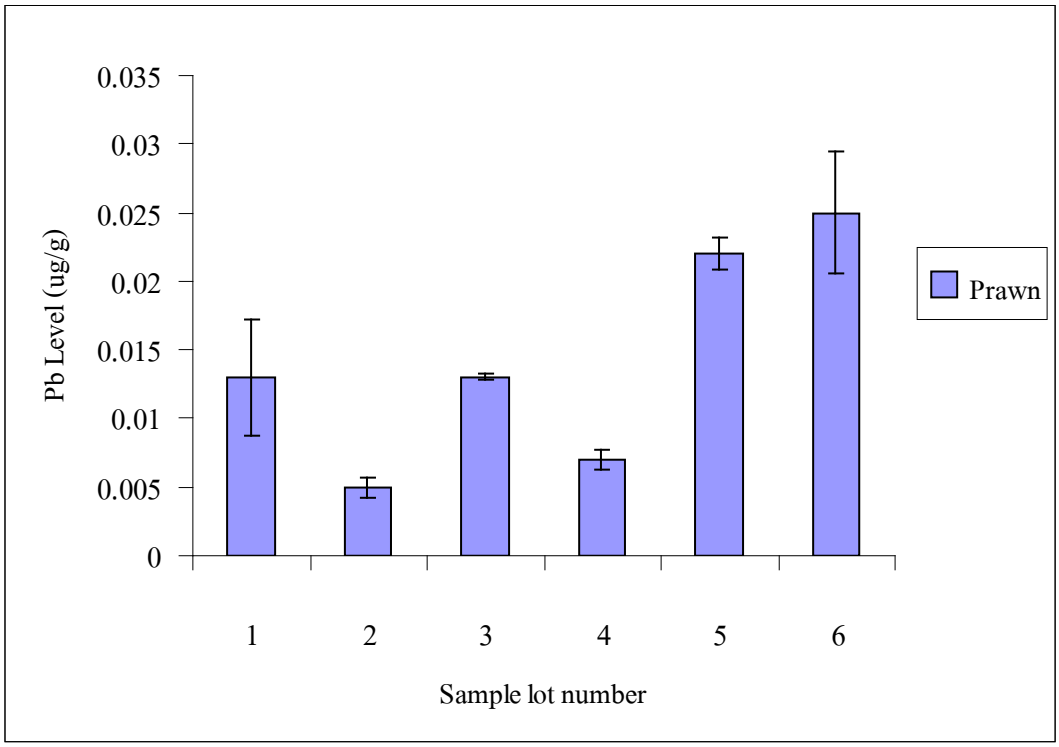


Figure 3-28 The concentration of Pb in frozen prawns (dry weight) from the seafood company in the South of Thailand.

The concentration of Cd and Pb in various frozen seafood samples (dry weight) were found in range as follows: tunafishes; 0.001-0.016 µg g^{-1} and 0.009-0.129 µg g^{-1}, squids; 0.001-0.009 µg g^{-1} and 0.005-0.165 µg g^{-1}, cuttlefishes; 0.003-0.004 µg g^{-1} and 0.003-0.005 µg g^{-}

[1], octopuses; 0.019-0.049 µg $g^{-1}$and 0.002-0.018 µg g^{-1} and prawns; 0.002-0.004 µg g^{1} and 0.005-0.025 µg g^{-1} respectively.

The standard concentration of Cd and Pb in food issued by the Ministry of Public Health of Thailand is less than 1.00 µg g^{-1}.

3.4.3 Comparison between the studied method and ICP-OES for Cd and Pb determination in seafood samples

The determination of Cd and Pb using the studied method (GFAAS) and ICP-OES are presented in Table 3-33. The comparison of the two methods was evaluated by using paired sample Student t-test at 95% confidential levels (P = 0.05). The study in this section was performed by comparing the mean of Cd and Pb concentration in seafood sample (0.2 g dry weight) which determined by using the studied method (GFAAS) and ICP-OES.

From the statistical evaluation, it was found that the determination of Cd and Pb by using two method was not significant difference at the 95% confidential levels (P > 0.05).

Table 3-33 The concentration of Cd and Pb in frozen tunafishes determined by using the studied method and ICP-OES.

Sample No.	Cd Conc. (µg g^{-1})		Pb Conc. (µg g^{-1})	
	GFAAS	ICP-OES	GFAAS	ICP-OES
1	0.005	0.006	0.058	0.033
2	0.010	0.012	0.062	0.041
3	0.005	0.002	0.040	0.032
4.	0.002	0.006	0.087	0.108
5	0.045	0.053	0.078	0.095

Mean	0.015	0.013	0.062	0.066
P-Value	0.23		0.68	

It can be concluded that the investigated method in this study has an effective to use for Cd and Pb determination in seafood samples similarly to ICP-OES.