CHAPTER 3

RESULTS AND DISSCUSION

3.1 Analytical characteristics of the method

The flame atomic absorption spectrometers (AAS Analyst 800, PerkinElmer, Germany) was used for Cr, Ni and Pb determination. The hollow cathode lamp (PerkinElmer) was the light source. The operating parameters of Cr, Ni and Pb were set as recommended by the manufacturer in **Table 3.1** [48].

Table 3.1 Operating parameters and analytical conditions of Cr, Ni and Pb in FAAS

Metal / Parameters	Cr	Pb	Ni
1. Wavelength (nm)	357.9	283.3	232
2. Slit width (nm)	0.7	0.7	0.2
3. Characteristic concentration check (mg/l)	4.0	20.0	7.0
4. Linear range (mg/l)	5.0	20.0	2.0

3.1.1 Precision

The precision of the FAAS instrument was studied by repeating the measurement of standard solutions for ten times. The concentrations of Cr, Ni and Pb standard solution for studied precision were 2, 3 and 10 μg ml^{-1}, respectively. The results obtained are shown in **Table 3.2**. The precision with replicated injections of FAAS instrument can expressed as the relative standard deviation (%RSD). The results of %RSD for Cr was 1.27 %, Ni was 1.59 % and Pb was 0.81 %. This indicated that the FAAS instrument provided good repeatability (less than 5% RSD).

Table 3.2 The precision of the FAAS instrument for Cr, Ni and Pb analysis

No.	Abs. of Cr (2 μg ml^{-1})	Abs. of Ni (3 μg ml^{-1})	Abs. of Pb (10 μg ml^{-1})
1	0.041	0.032	0.1
2	0.041	0.033	0.101
3	0.042	0.033	0.102
4	0.041	0.033	0.101
5	0.042	0.032	0.100
6	0.041	0.032	0.101
7	0.041	0.032	0.102
8	0.042	0.033	0.101
9	0.042	0.032	0.102
10	0.042	0.032	0.100
Mean	0.042	0.032	0.101
SD	0.001	0.001	0.001
%RSD	1.27	1.59	0.81

3.1.2 Limit of detection (LOD) and Limit of quantitation (LOQ)

The Limit of detection of the proposed method for determination of Cr, Ni and Pb were investigated by analyzing blank solution of 1% HNO_3 for ten times. The results are shown in **Table 3.3**. The detection limits of Cr, Ni and Pb were 0.077 and 0.162 and 0.249 μg ml^{-1}, respectively and the quantitation limits were 0.256 0.538 and 0.831 μg ml^{-1}, respectively.

Table 3.3 Absorbance obtained from blank solution

No.	Abs. of 1% HNO_3 for		
	Cr	**Ni**	**Pb**
1	-0.001	0.000	0.001
2	0.000	-0.001	0.000
3	0.000	0.001	0.000
4	0.000	0.000	0.001
5	0.001	0.000	0.001
6	0.000	-0.001	0.002
7	0.000	0.000	0.000
8	0.000	0.001	0.000
9	0.001	-0.001	0.002
10	0.000	0.000	0.001
Mean	0.000	0.000	0.001
SD	0.001	0.001	0.001
Slope	0.021	0.013	0.009
Detection limit (μg ml^{-1})	0.077	0.162	0.249
Quantitation limit (μg ml^{-1})	0.256	0.538	0.831

3.1.3 Accuracy

The accuracy of the proposed procedure, the assay of the known added amount of analyte in the sample. The obtained results are given in **Table 3.4 – 3.6**, the values given represent the average and standard deviation of the three determinations of each sample. Accuracy was calculated as the percentage of recovery. The recoveries were found in the range of 91.90 – 99.69%, 90.69 – 95.59 and 98.89 – 101.48% for Cr, Ni and Pb, respectively.

Table 3.4 The recoveries of Cr analysis

Sample	Concentration of Cr (μg ml^{-1})		% Recovery
	Added	Found*	
1	-	ND	
	1.00	0.86 ± 0.05	91.90
	2.00	1.83 ± 0.03	94.24
2	-	ND	
	1.00	0.89 ± 0.03	93.46
	2.00	1.87 ± 0.03	95.79
3	-	ND	
	1.00	0.94 ± 0.03	99.69
	2.00	1.86 ± 0.03	95.79

*Mean ± SD (N=3)

Table 3.5 The recoveries of Ni analysis

Sample	Concentration of Ni (μg ml^{-1})		
	Added	**Found***	**% Recovery**
1	-	0.27 ± 0.04	
	2.00	2.08 ± 0.04	90.69
	3.00	3.14 ± 0.04	95.59
2	-	0.32 ± 0.04	
	2.00	2.16 ± 0.04	91.91
	3.00	3.09 ± 0.07	92.32
3	-	0.34 ± 0.04	
	2.00	2.21 ± 0.00	93.14
	3.00	3.19 ± 0.04	94.77

*Mean ± SD (N=3)

Table 3.6 The recoveries of Pb analysis

Sample	Concentration of Pb (μg ml^{-1})		
	Added	**Found***	**% Recovery**
1	-	ND	
	10.00	9.85 ± 0.17	101.48
	20.00	19.48 ± 0.06	98.89
2	-	ND	
	10.00	9.74 ± 0.06	100.74
	20.00	19.56 ± 0.11	99.44
3	-	ND	
	10.00	9.67 ± 0.11	100.37
	20.00	19.48 ± 0.17	99.26

*Mean ± SD (N=3)

3.2 Optimization of ultrasonic acid digestion (UAD)

Ultrasonic acid digestion is based on mechanical and chemical effects. The chemical effects result from the reactivity of the chemical agents (oxidants or reductants) promoted by the radicals generated by sonolysis of the solvents in the liquid phase. The radicals act as promoters of the chemical reactions involved in matrix decomposition. The mechanical effects result from negative pressure. This negative pressure breaks up the fluid, creating many thousands of small cavitations (bubbles). When these cavitations subsequently encounter sound waves, they begin to vibrate. These vibrations make the cavitations expand until they become unstable and

collapse. These implosions create small but strong jets in the ultrasonic fluid. Because this does not involve only one, but many thousands of implosions taking place simultaneously, a powerful reaction is created in ultrasonic fluid.

Therefore, ultrasonic acid digestion (UAD) can be considered as an alternative for solid sample digestion because this technique provides intense and high frequency ultrasound energy to sample through liquid. In addition, samples are mixed effectively and then efficient chemical and physical reactions are provided. Variables influencing ultrasonic acid digestion were optimised for human hair sample preparation. The first parameter was solvent systems, HNO_3 and a mixture of HNO_3 and H_2O_2 were studied. The second was presonication time (without ultrasonic stirring) after adding acid mixture. The third was sonication time to ultrasound at a fixed frequency of 53 kHz and the last step was temperature of the ultrasonic bath. The effect of the experimental conditions on the digestion has to be thoroughly evaluated and optimized.

3.2.1 Effect of solvent systems

The influence of solvent systems of concentrated HNO_3 and mixture of HNO_3 and H_2O_2 were studied by fixing the other variables at their optimal values. Normally, the digestion is incomplete with only single strong acid. In addition, the digestion depended on the usage of an oxidizer. H_2O_2 can usefully be employed in conjunction with nitric acid as a mean of improving the quality of digestion.[1] As a result of heating, H_2O_2 dissociates to hydroxyl radicals ($OH^{\bullet}$) which attack to protein, carbohydrate and polyunsaturated fatty acid in biological sample and improved the efficiency of the extraction of metals from sample. As shown in **Figure 3.2**, using only concentrate HNO_3 for digestion, a lot of yellow gas (NO_2) occurred in test tube

and the color of sample solution was brown. Digestion system using mixture of HNO_3 and H_2O_2 showed that small amount of gas was observed and the digestion solution was colorless and transparent. It showed that the sample was digested completely [49-52].

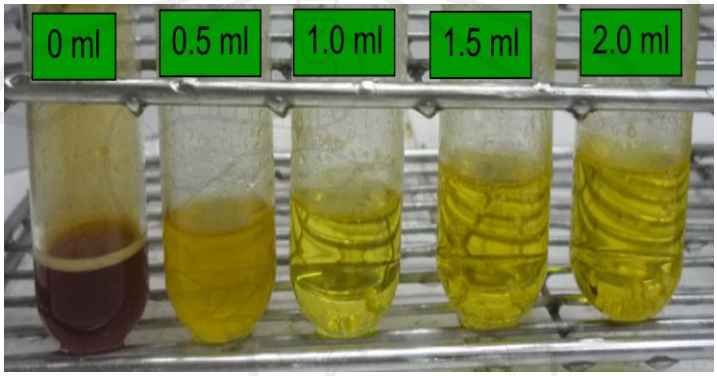

Figure 3.2 The sample solution with various volume of H_2O_2

The results of sample solution from various volume of H_2O_2 optimization are shown in **Table 3.7** and **Figure 3.3**.

Table 3.7 The effect of solvent system on the analytical signal of 3 μg ml^{-1} Ni, 2 μg ml^{-1} Cr and 10 μg ml^{-1} Pb

Condition HNO_3 : H_2O_2 (ml)	Absorbance*		
	Pb	Cr	Ni
2 : 0	0.098± 0.002	0.035± 0.002	0.026± 0.003
2 : 0.5	0.101± 0.003	0.038± 0.002	0.031± 0.002
2 : 1.0	0.102± 0.0021	0.042± 0.003	0.035± 0.002
2 : 1.5	0.102± 0.002	0.042± 0.002	0.035± 0.002
2: 2.0	0.101± 0.002	0.041± 0.001	0.035± 0.003

*Mean ± SD (N=3)

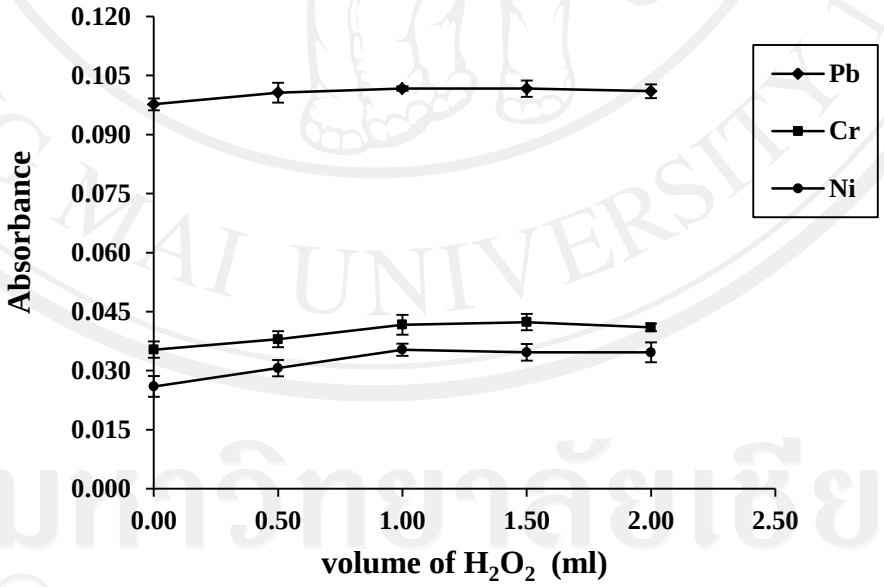

Figure 3.3 Effect of volume of H_2O_2 on the analytical signal of 3 μg ml^{-1} Ni, 2 μg ml^{-1} Cr and 10 μg ml^{-1} Pb

From these results, mixture of HNO_3 and H_2O_2 was chosen for the oxidizer of digestion. Ratio volume of H_2O_2 was studied, as shown in Figure 3.3. The results showed that there were no significant difference in the ratio volume of H_2O_2 of 0.5 - 2.0 ml. However, at 0.5 ml of H_2O_2 cloudy solution was observed. Therefore, an acid mixture of 2 ml concentrated HNO_3 and 1 ml H_2O_2 was chosen for this study.

3.2.2 Effect of presonication time

After the treatment with acid–oxidant mixtures human hair samples were kept at room temperature for different time intervals 0–60 min before being subjected to the ultrasonic bath, denoted as presonication time (PST). **Figure 3.4** shows human hair samples digested with acid–oxidant mixtures. The reaction tube was still occurring but sample solution was not complete. Then, the sample tubes were placed inside the ultrasonic bath and were sonicated (ultrasound energy remaining at 53 kHz) for 10 min at 60 ^{o}C. The determination of Cr, Ni and Pb were analysed by FAAS. The results of presonication time optimization are shown in **Table 3.8** and **Figure 3.5**.

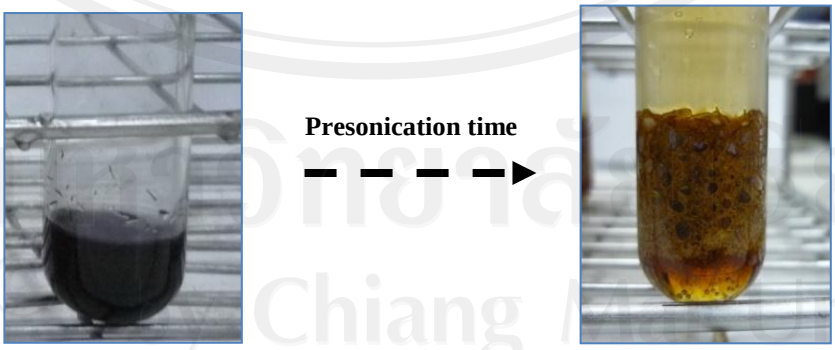

Figure 3.4 The human hair sample was added with acid mixtures and kept at room temperature

Table 3.8 The effect of presonication time on the analytical signal of 3 μg ml^{-1} Ni, 2 μg ml^{-1} Cr and 10 μg ml^{-1} Pb

Presonication time (min)	Absorbance*		
	Pb	Cr	Ni
0	0.098 ± 0.002	0.039 ± 0.002	0.031 ± 0.003
5	0.099 ± 0.002	0.039 ± 0.002	0.033 ± 0.001
10	0.102 ± 0.002	0.042 ± 0.003	0.035 ± 0.002
15	0.101 ± 0.002	0.042 ± 0.002	0.034 ± 0.003
20	0.101 ± 0.002	0.041 ± 0.003	0.034 ± 0.004
30	0.102 ± 0.001	0.040 ± 0.001	0.033 ± 0.002
40	0.103 ± 0.003	0.041 ± 0.002	0.034 ± 0.002
50	0.102 ± 0.002	0.041 ± 0.003	0.033 ± 0.003
60	0.101 ± 0.002	0.040 ± 0.002	0.034 ± 0.001

*Mean ± SD (N=3)

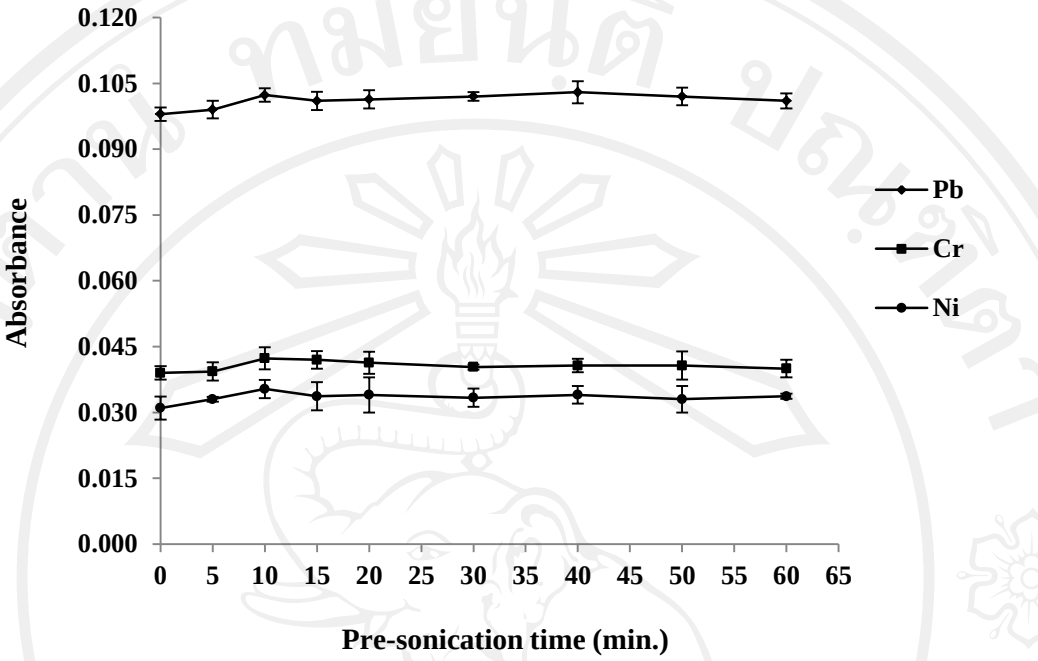

Figure 3.5 Effect of presonication time on the analytical signal of 3 μg ml^{-1} Ni, 2 μg ml^{-1} Cr and 10 μg ml^{-1} Pb

Results of presonication time showed no significant difference. Therefore, presonication time for 10 minute was enough for acid digestion of human hair samples.

3.2.3 Effect of sonication time

The extraction time necessary for achieving total solid–liquid extraction depends on analyte–matrix interaction, composition of the liquid media and ultrasonic device being used [60]. In the present study, sonication time was varied between 5 and 60 min by keeping constant ultrasound frequency at 53 kHz. **Figure 3.6** shows the solution sample from the beginning of sonication and the

solution sample after sonication. Therefore, the ultrasonic acid digestion efficiency increased with the increasing of sonication time, as shown in **Table 3.8**.

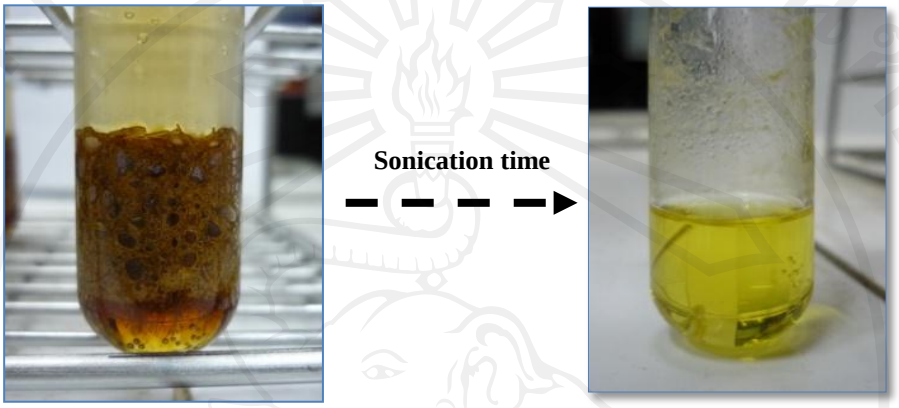

Figure 3.6 Presonication of digested hair sample for 10 minute (left) and sonicattion sample for 30 minute (right)

The determination of Cr, Ni and Pb were analysed by FAAS. The results of sonication time optimization are shown in **Table 3.8** and **Figure 3.7**.

Table 3.9 The effect of sonication time on the analytical signal of 3 μg ml^{-1} Ni, 2 μg ml^{-1} Cr and 10 μg ml^{-1} Pb

Sonication time (min)	Absorbance*		
	Pb	**Cr**	**Ni**
5	0.097 ± 0.002	0.037 ± 0.003	0.030 ± 0.003
10	0.098 ± 0.002	0.037 ± 0.004	0.031 ± 0.003
15	0.098 ± 0.003	0.038 ± 0.003	0.031 ± 0.003
20	0.100 ± 0.004	0.040 ± 0.002	0.032 ± 0.002
25	0.101 ± 0.003	0.042 ± 0.003	0.033 ± 0.002
30	0.102 ± 0.002	0.042 ± 0.002	0.034 ± 0.002
40	0.102 ± 0.002	0.042 ± 0.001	0.034 ± 0.002
50	0.102 ± 0.002	0.041 ± 0.002	0.033 ± 0.001
60	0.101 ± 0.003	0.041 ± 0.003	0.033 ± 0.003

*Mean ± SD (N=3)

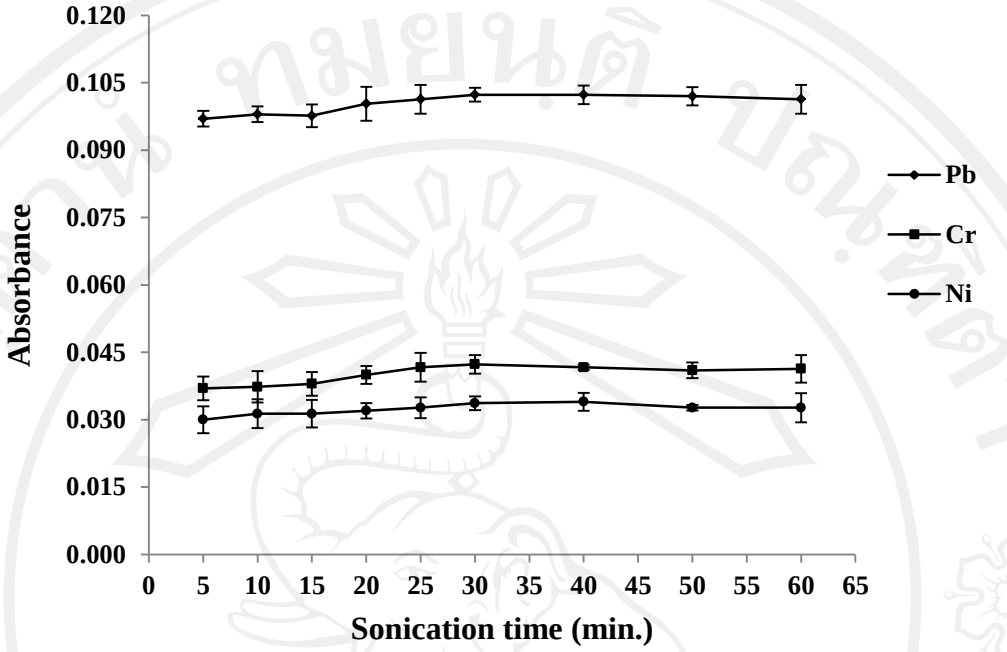

Figure 3.7 Effect of sonication time on the analytical signal of 3 μg ml^{-1} Ni, 2 μg ml^{-1} Cr and 10 μg ml^{-1} Pb

The ultrasonic acid digestion efficiency increased with increasing sonication time from 5 to 30 min. There was no significant difference between 30 and 60 min sonication periods for all metals. It was observed that optimum signal of Ni, Cr and Pb required 30 min. This fact offers an important practical advantage because the time for the acid digestion can be shortened, and our results are consistent with other study [24]. This optimized condition was applied for further study.

3.2.4 Effect of temperature of the ultrasonic bath

The high temperature and pressure within a collapsing cavitation bubble produced by ultrasound irradiation causes the formation of free radicals, to accelerate the reactions involved in sample digestion. In addition, temperature of ultrasonic bath can increase oxidative power of acid solution. Therefore, the high temperature was necessary for optimum recoveries of heavy metals from all samples as compared to the work reported at room temperature [55]. The water bath temperature has a highly significant effect, as shown in **Figure 3.8** and **Table 3.10**.

Table 3.10 The effect of temperature of the ultrasonic bath on the analytical signal of 3 μg ml^{-1} Ni, 2 μg ml^{-1} Cr and 10 μg ml^{-1} Pb

Temperature (^{o}C)	Absorbance*		
	Pb	Cr	Ni
30	0.093 ± 0.003	0.034 ± 0.002	0.027 ± 0.002
40	0.097 ± 0.002	0.038 ± 0.002	0.031 ± 0.004
50	0.100 ± 0.002	0.040 ± 0.003	0.032 ± 0.004
60	0.103 ± 0.002	0.043 ± 0.003	0.035 ± 0.001
70	0.102 ± 0.002	0.042 ± 0.002	0.033 ± 0.003
80	0.102 ± 0.001	0.041 ± 0.002	0.033 ± 0.003

*Mean ± SD (N=3)

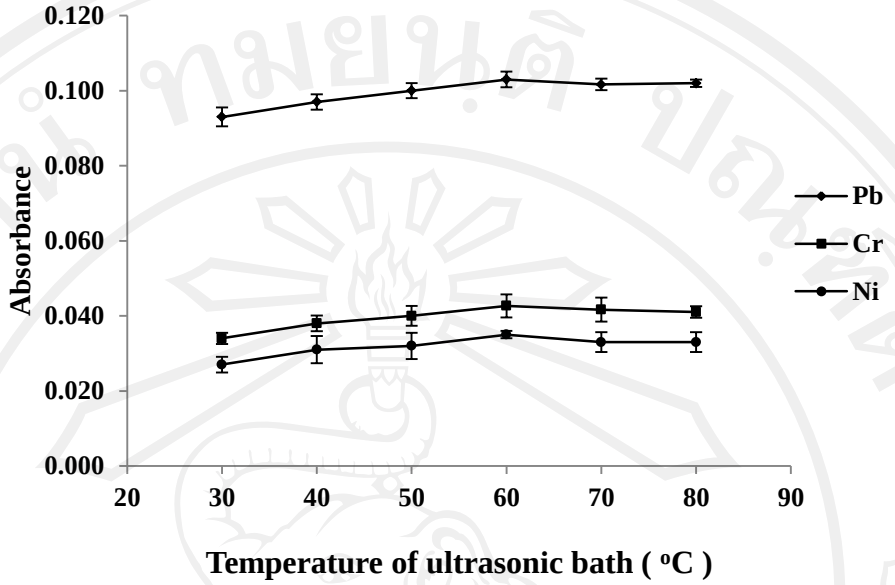

Figure 3.8 Effect of ultrasonic bath temperature on the analytical signal of 3 μg ml^{-1} Ni, 2 μg ml^{-1} Cr and 10 μg ml^{-1} Pb

The obtimum temperature of ultrasonic bath at 60 ^{o}C was obtained because of higher absorbance value. When increased temperature of the ultrasonic bath more than 60 ^{o}C, the analytical signals was not different. Therefore, temperature of ultrasonic bath at 60 ^{o}C was chosen in order to achieve the highest possible digestion efficiency.

3.2.5 Optimum conditions of ultrasonic acid digestion

The optimum conditions of ultrasonic acid digestion for the determination of Cr, Ni and Pb in human hair samples are shown in **Table 3.11**.

Table 3.11 Optimum condition of UAD for digestion human hair samples

Parameters	**Optimal conditions**
Solvent systems HNO_3 : H_2O_2 (ml)	2:1
Presonication time (min)	10
Sonication time (min)	30
Temperature (ºC)	60

The results in **Table 3.11** indicated that optimum conditions of the proposed method was improved the efficiency of ultrasonic acid digestion for Cr, Ni and Pb determination in human hair samples. This research used HNO_3 : H_2O_2 as a acid mixture for sample. The use of HNO_3 and H_2O_2 rarely reported to determine the concentration of Cr, Ni and Pb in UAD, especially the application for human hair samples. Clear solutions were produced after using a mixture solution of 2 ml HNO_3 and 1 ml H_2O_2. Moreover, the optimum of presonication time, temperature of ultrasonic bath and sonication time were studies. The proposed procedure is more attractive due to a minimum reagents, used reduced time (less than 60 min compared with acid digestion process, approximately 2–3 hours).

3.3 Optimization of cloud point extraction (CPE)

In cloud point extraction (CPE), separation of the surfactant–rich phase and the aqueous supernatant phase requires an appropriate experimental condition. Extraction of metal ions can be performed in the absence of a chelating agent. The efficiency of the CPE depends on the hydrophobicity of the ligand and the complex, their apparent equilibrium constants in the micellar medium and kinetics of the complex formation and their transference between the phases. Various organic ligands used in cloud point extraction such as 1-(2-pyridylazo)-2-naphthol (PAN) and 1-(2-thiazolylazo)-2-naphthol (TAN) which is pyridylazo compounds have been widely applied as reagents for the determination of metallic elements. This work used ammonium pyrrolidine dithiocarbamate (APDC) as a chelating agent. APDC has a dithiocarbamates which is highly versatile ligands toward main group metals [27]. They can stabilize a variety of oxidation states and coordination geometries, and seemingly small modifications to the ligand can lead to significant changes in the structure behavior of the complexes formed. Surfactant is one of the key reagents. The surfactants which are used in cloud point extraction are mostly of nonionic type such as Triton X-114, Triton X-100 or PONPE. This research used Triton X-114 as a non-ionic surfactant. Triton X-114 has been the most applied reagent because of its low cloud-point temperature (30 ^{o}C) and high density of the surfactant rich phase, as well as low cost, commercial availability and lower toxicity.

Experimental scheme of formation between metals, chelating agent and surfactant in CPE is shown in **Figure 3.9**.

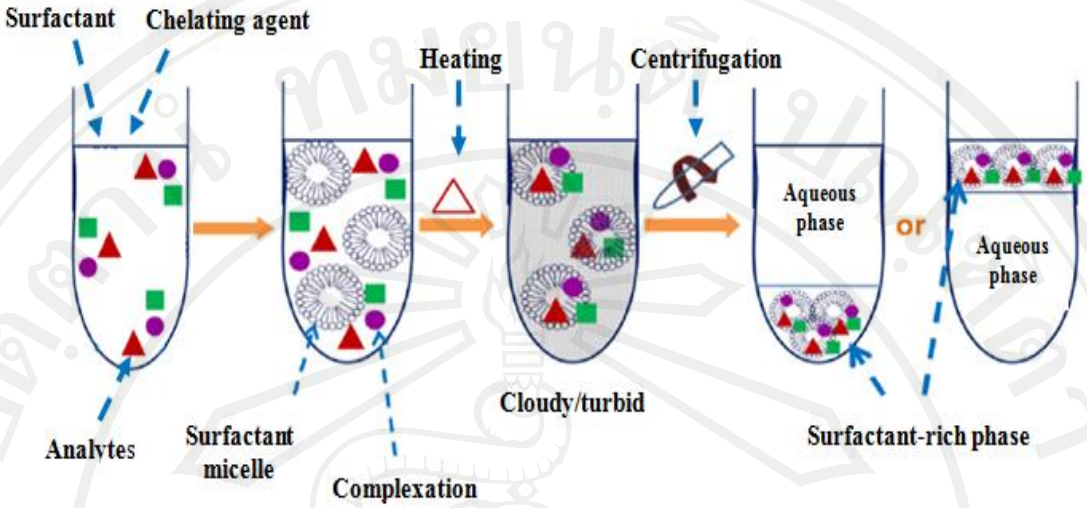

Figure 3.9 The prior formation of metal ions and chelating agent in CPE [56]

Usually, the experimental procedure is as follows: [57]

- The metal reacts with a suitable ligand to form a hydrophobic complex
- Clouding is generated by increasing temperature above the cloud point
- The micelles formed entrap the metal complexes inside their hydrophobic core
- The surfactant-rich phase is subsequently separated from the bulk aqueous one by centrifugation.

Optimizations of the procedure of CPE were investigated to gain maximum extraction efficiencies of metals. The extracted solutions were subjected to analysis of Cr, Ni and Pb by FAAS. The optimum conditions in this study were obtained by the maximum value of analytical signal. In this work, the effect of the experimental

variable parameters on the cloud point extraction procedure had been preliminary studied.

3.3.1 Effect of pH

The formation of metal complexes and its chemical stability are important influence factors for the CPE, and the pH plays a unique role on metal chelate formation and subsequent extraction. In any other case, the role of pH is the same as in traditional pH-selective fractional precipitation, where the separation of several metal ions was made feasible by repeatedly adjusting the pH. Therefore, the CPE of Ni, Cr and Pb ions was performed in the pH ranging from 2 to 8. **Figure 3.10** and **Table 3.12** show the effect of pH on the absorbance of Ni, Cr and Pb.

Table 3.12 The effect of pH on the analytical signal for Cr, Ni and Pb

pH	Absorbance		
	Pb	**Cr**	**Ni**
2	0.056 ± 0.004	0.025 ± 0.004	0.020 ± 0.003
3	0.090 ± 0.004	0.038 ± 0.003	0.027 ± 0.003
4	0.102 ± 0.002	0.042 ± 0.003	0.036 ± 0.002
5	0.098 ± 0.004	0.039 ± 0.002	0.033 ± 0.002
6	0.087 ± 0.003	0.035 ± 0.003	0.030 ± 0.002
7	0.078 ± 0.004	0.032 ± 0.002	0.030 ± 0.001
8	0.065 ± 0.006	0.032 ± 0.003	0.029 ± 0.003

*Mean ± SD (N=3)

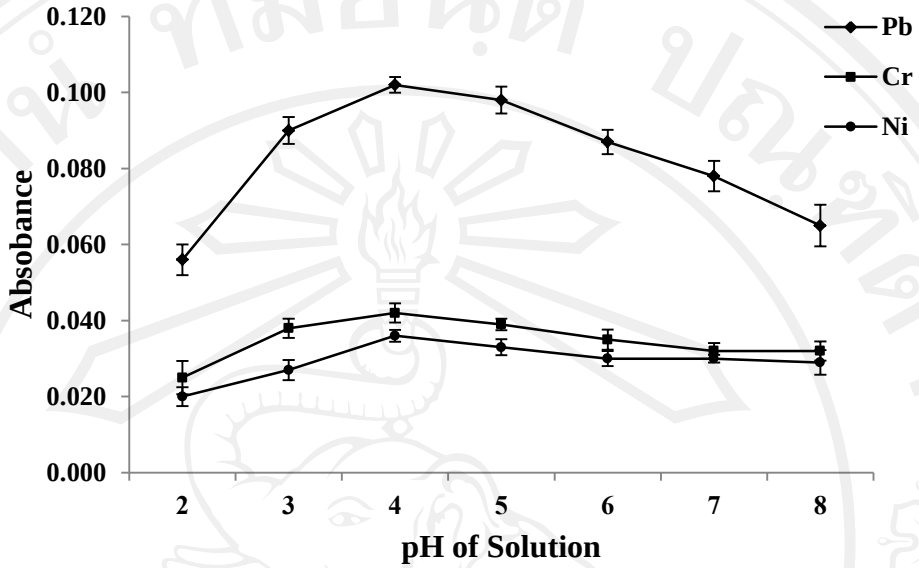

Figure 3.10 Effect of pH on the cloud point extraction of 3 μg ml^{-1} Ni, 2 μg ml^{-1} Cr and 10 μg ml^{-1} Pb

Figure 3.10 shows that the maximum sensitivity isobserved at pH 4 and an indicationof maximum extractions efficiency could be obtained. At lower pH, the ligand is protonated and its ionic characteristics increase and lead to decrease in its solubilization in the hydrophobic micelles. At higher pH, the ligand is deprotonated and it behaves like a hydrophilic molecule and easily gets solubilized in the micelles. In addition, at pH > 4 the precipitation of $M(OH)_2$ or $M(OH)^+$ ions is in the form of hydroxides. Hence the optimum pH value of 4 was chosen for the study.

3.3.2 Effect of APDC concentration

Ammonium pyrrolydine dithiocarbamate (APDC) is a non-specific chelating agent, which reacts with metallic ions forming a very stable complex with

the majority of the transition metals [58]. It reacts with a large number of di- and trivalent metals which can be separated from a large excess of alkali and alkaline earth elements. This chelating agent ideally suited for the preconcentration of heavy metals from environmental samples. The structure of M- APDC complex is shown in **Figure 3.11**.

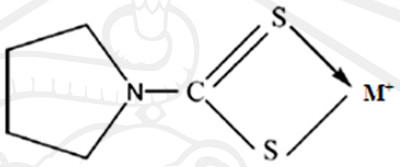

Figure 3.11 Structure of metal-APDC complex [65]

The extraction efficiency as a function of concentration of the chelating agent is presented in **Table 3.13** and **Figure 3.12**.

Table 3.13 The effect of APDC concentration on the analytical signal for Cr, Ni and Pb

APDC (mol l^{-1})	Absorbance*		
	Pb	**Cr**	**Ni**
$3.0x10^{-5}$	0.084 ± 0.005	0.031 ± 0.003	0.024 ± 0.003
$6.0x10^{-5}$	0.090 ± 0.003	0.035 ± 0.003	0.028 ± 0.002
$1.8x10^{-4}$	0.097 ± 0.004	0.038 ± 0.002	0.030 ± 0.003
$3.0 x10^{-4}$	0.100 ± 0.002	0.040 ± 0.003	0.032 ± 0.002
$6.0 x10^{-4}$	0.102 ± 0.002	0.043 ± 0.003	0.036 ± 0.002
$9.0 x10^{-4}$	0.102 ± 0.001	0.043 ± 0.003	0.036 ± 0.002
$1.2 x10^{-3}$	0.101 ± 0.002	0.043 ± 0.002	0.036 ± 0.003
$1.5 x10^{-3}$	0.102 ± 0.002	0.042 ± 0.001	0.036 ± 0.002

*Mean ± SD (N=3)

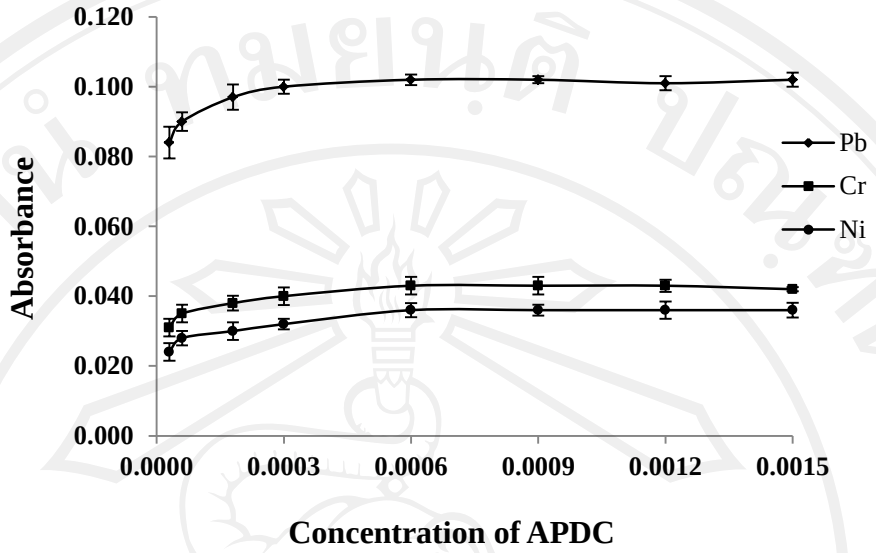

Figure 3.12 Effect of APDC concentration on the cloud point extraction of 3 μg ml^{-1} Ni, 2 μg ml^{-1} Cr and 10 μg ml^{-1} Pb

The CPE efficiency for metals increased as the concentration of APDC increased from $0.3x10^{-4}$ to $6x10^{-4}$ mol l^{-1} and then kept constant with the increasing of APDC concentration. The concentration of $6x10^{-4}$ mol l^{-1} APDC was sufficient for total complexation and the obtained responses were improved. 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 [27]. Therefore, an APDC concentration of $6x10^{-4}$ mol l^{-1} was employed for subsequent experiments.

3.3.3 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. The non-ionic surfactant Triton X-114 was chosen because of its commercial availability in a high purified homogeneous form, low toxicological properties and cost. Also, the high density of the surfactant rich phase facilitates phase separation by centrifugation. The phase separation mechanism can be described to ethylene oxide segments in the micelle that repel each other at low temperature. The structure of Triton X-114 and micelle formation of surfactant are shown in **Figure 3.13 – 3.14**.

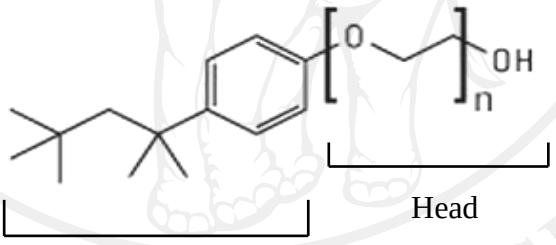

Figure 3.13 Structure of Triton X-114 surfactant [60]

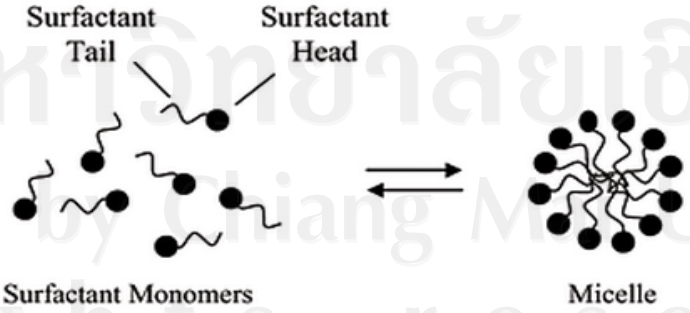

Figure 3.14 The micelle formation of surfactant [61]

The variations in the analytical signal as a function of the concentration of Triton X-114 in the range of 0.01 – 0.30 %v/v were investigated. The influences of the concentration of Triton X-114 are shown in **Table 3.14** and **Figure 3.15**.

Table 3.14 The effect of Triton X-114 concentration on the analytical signal for Cr, Ni and Pb

Triton X-114	**Absorbance**		
(%v/v)	**Pb**	**Cr**	**Ni**
0.01	0.094 ± 0.002	0.033 ± 0.001	0.023 ± 0.003
0.05	0.099 ± 0.002	0.040 ± 0.002	0.031 ± 0.002
0.10	0.102 ± 0.001	0.043 ± 0.001	0.035 ± 0.001
0.15	0.098 ± 0.003	0.040 ± 0.002	0.032 ± 0.002
0.20	0.096 ± 0.002	0.038 ± 0.004	0.030 ± 0.001
0.25	0.095 ± 0.002	0.036 ± 0.002	0.027 ± 0.002
0.30	0.090 ± 0.001	0.030 ± 0.002	0.022 ± 0.002

*Mean ± SD (N=3)

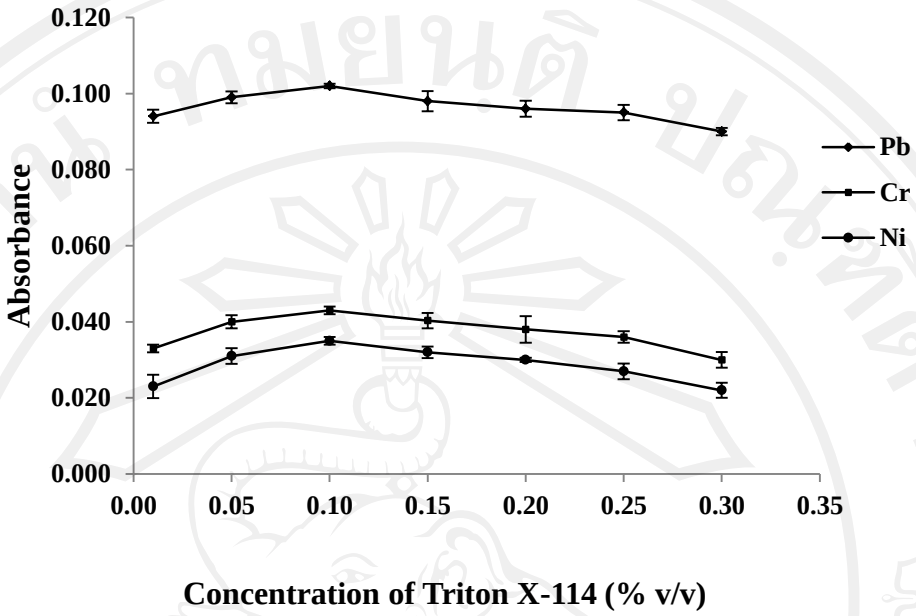

Figure 3.15 Effect of Triton X-114 concentration on the cloud point extraction of 3 μg ml^{-1} Ni, 2 μg ml^{-1} Cr and 10 μg ml^{-1} Pb

From the results in **Table 3.14** and **Figure 3.15**, the analytical signals decreased with the increasing of Triton X-114 concentration due to an increasing of the volume and the viscosity of the surfactant-rich phase. The preconcentration efficiency was evaluated using Triton X-114 concentrations ranging from 0.01% to 0.30 %(v/v). The highest signals of Ni, Cr and Pb were obtained with 0.10% (v/v) Triton X-114 and the signals decreased at a higher Triton X-114 concentration (0.15 %(v/v)). These results might be related to the presence of the high amount of surfactant, which cause of the viscosity of the surfactant-rich phase to increase and lead to poorsensitivity [20]. Therefore, an amount of 0.10% Triton X-114 was

selected in order to achieve the greatest analytical signal and thereby the highest extraction efficiency.

3.3.4 Effects of equilibration time

Optimal complexing time is necessary to complete the reaction and to achieve easy phase separation as efficient as possible. In addition, an important point, their reaction with chelating agents and their transportation inside the micelle are kinetically controlled (simulating the shift of equilibrium towards precipitation). It is therefore essential to maintain the reaction time above a minimum threshold for quantitative extraction [20]. In this work, the dependence of extraction efficiency upon complexing time for 10 to 60 min was thoroughly optimized. The results of complexing time optimization are shown in **Table 3.15** and **Figure 3.16**.

Table 3.15 The effect of complexing time on the analytical signal for Cr , Ni and Pb

Complexing time	**Absorbance**		
(min)	**Pb**	**Cr**	**Ni**
10	0.095 ± 0.003	0.034 ± 0.002	0.029 ± 0.002
20	0.099 ± 0.001	0.039 ± 0.002	0.032 ± 0.002
30	0.102 ± 0.001	0.044 ± 0.001	0.035 ± 0.001
40	0.099 ± 0.001	0.040 ± 0.001	0.031 ± 0.002
50	0.095 ± 0.004	0.036 ± 0.003	0.027 ± 0.003
60	0.092 ± 0.003	0.032 ± 0.002	0.025 ± 0.002

*Mean ± SD (N=3)

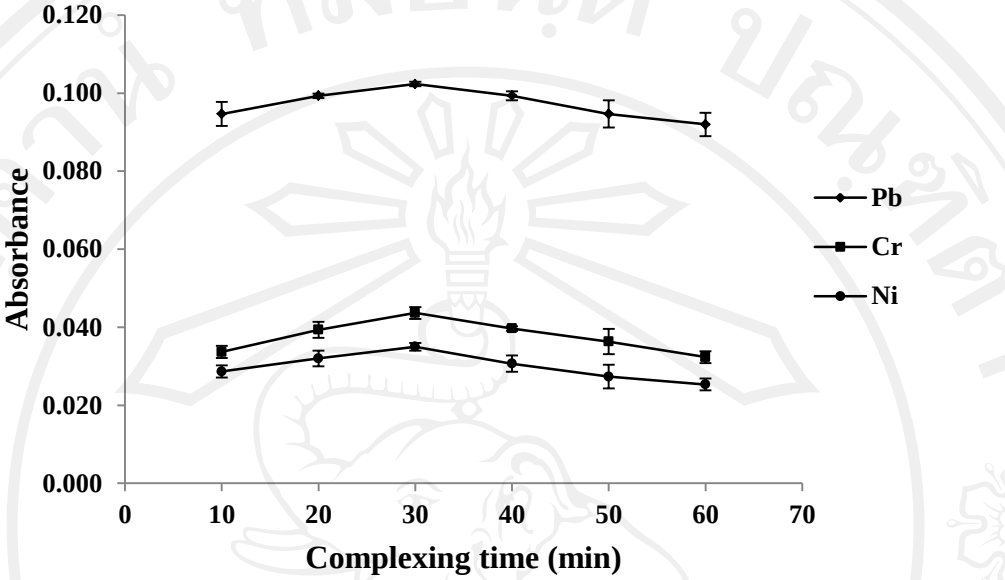

Figure 3.16 The effect of complexing time on the cloud point extraction of 3 μg ml^{-1} Ni, 2 μg ml^{-1} Cr and 10 μg ml^{-1} Pb

It was desirable to employ the shortest complexing time as a compromise between completion of extraction and phase separation efficiency. The results showed 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.

3.3.5 Effects of equilibration temperature

The very high temperatures are not proper for CPE, because they could reduce the stability of chelates and chelating agents. This research used Triton X-114 as a non-ionic surfactant. The cloud point temperature of Triton X-114 is 30 °C near room temperature which is preferred for cloud point temperature and analytical purposes. The effect of the equilibration temperature was studied with in a range of 30–50 ^{o}C. The results of equilibration temperature are shown in **Table 3.16** and **Figure 3.17.**

Table 3.16 The effect of equilibration temperature on the analytical signal for Cr , Ni and Pb

Temperature	**Absorbance***		
(^{o}C)	**Pb**	**Cr**	**Ni**
30	0.090 ± 0.001	0.030 ± 0.002	0.025 ± 0.002
35	0.095 ± 0.002	0.039 ± 0.003	0.030 ± 0.003
40	0.102 ± 0.003	0.042 ± 0.003	0.035 ± 0.002
45	0.100 ± 0.002	0.039 ± 0.002	0.031 ± 0.003
50	0.098 ± 0.001	0.038 ± 0.001	0.030 ± 0.002

*Mean ± SD (N=3)

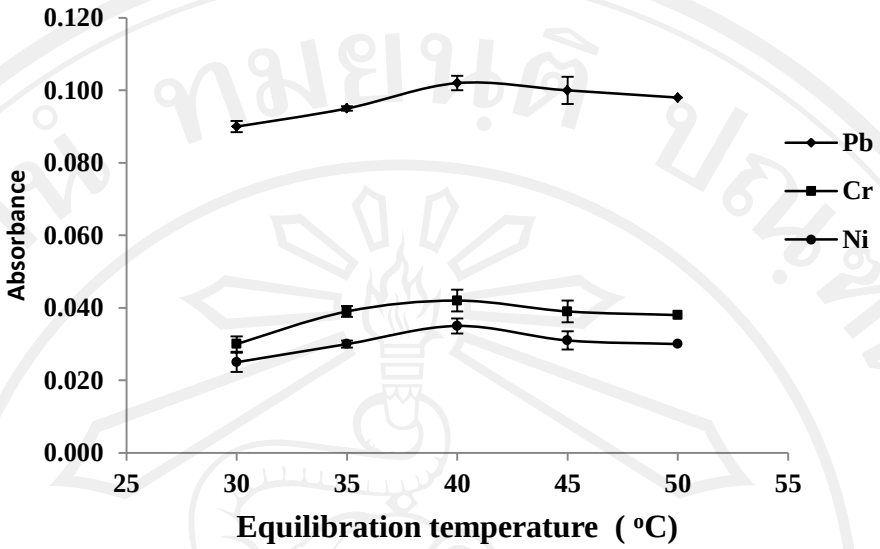

Figure 3.17 The effect of equilibration temperature on the cloud point extraction of 3 μg ml^{-1} Ni, 2 μg ml^{-1} Cr and 10 μg ml^{-1} Pb

It was found that an equilibration temperature of 40 ˚C was adequate to achieve quantitative extraction. 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. Hence, the cloud point temperature at 40 °C was selected to perform in the experiment for avoiding the loss of extraction efficiency.

3.3.6 Optimum conditions of cloud point extraction

Summary of the optimum conditions of cloud point extraction for the determination of Cr, Ni and Pb in human hair samples are shown in **Table 3.17**.

Table 3.17 Optimum conditions of cloud point extraction

Parameters	Optimum value
1. APDC concentration (mol l^{-1})	$6x10^{-4}$
2. Triton X-114 concentration (%v/v)	0.1
3. Complexing time (min)	30
4. Temperature (^{o}C)	40
5. pH of solution	4

When the temperature of Triton X-114 solution was greater than the cloud point temperature, centrifugal operation could accelerate the phase separation, which led the complex of M-APDC into the Triton X-114 surfactant-rich phase. The centrifugal time was shorter than 5 min, the two phases could not separate completely. However, the centrifugal time was longer than 15 min, the surfactant Triton X-114 partly dissolved in the aqueous phase, which led to the reversal of the phase separation [23]. Therefore, to ensure the full separation of the two phases, the centrifugal time of 10 min was chosen for the subsequent studies.

After phase separation it is necessary to decrease the viscosity of the surfactant-rich phase to facilitate handling and introduction into the atomizer. The

addition of a diluting solution in the surfactant-rich phase is always indispensable in order to obtain a clear and homogenous solution of low viscosity compatible with the requirements of flame and plasma nebulizer [28]. 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. 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 FAAS. Whereas for larger volumes of acidified methanol, dilution was clearly predominated resulting in a gradual absorbance reduction, as also observed by other workers [21]. 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.

For biological sample digestion techniques, microwave acid digestion has been widely used for total metal determination. However, microwave digestion system gives some drawbacks such as expensive microwave oven, short lifetime of digestion vessel, long time required for cooling digestion vessel and cause of explosion because of high temperature and pressure [26]. In this research, the method of sample digestion was improved using ultrasonic acid digestion instead of classical digestion technique. Ultrasonic acid digestion (UAD) can be considered as an alternative for solid sample digestion because this technique provides intense and high frequency ultrasound energy to sample through liquid. In addition, samples are mixed effectively and then efficient chemical and physical reactions are provided. Moreover, there are many

advantages of this technique which are short digestion times without applying high temperature and pressure, simple to use, low cost and not special vessel requirement**.** The determination of very low concentration of trace elements usually requires separation and preconcentration steps. The 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.

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3.4 Determination of Cr, Ni and Pbin human hair samples

The optimized method was applied to the determination of Cr, Ni and Pb in human hair samples. The human hair samples including untreated hair sample (A), hair sample from drug abuse person (B), hair sample from dyed color hair person (C-1 and C-2), hair sample from smoking person (D-1 and D-2) and hair sample from industry worker (E-1 to E-3) were studied. The 200 mg of each human hair sample was digested and preconcentrated following the proposed method and the procedure explained in **Section 2.5** and **Section 2.**6. Then, the concentrations of Cr, Ni and Pb were detected by flame atomic absorption spectrophotometry (FAAS). The results are shown in **Table 3.18**.

Table 3.18 The amount of Cr, Ni and Pb in human hair samples

Samples	Cr content (μg/g) *	Ni content (μg/g)*	Pb content (μg/g) *
A	ND	ND	ND
B	2.34 ± 0.00**	ND	ND
C-1	ND	ND	ND
C-2	2.34 ± 0.00**	27.13 ± 17.75	ND
D-1	ND	ND	5.15 ± 0.00**
D-2	ND	ND	5.15 ± 0.00**
E-1	ND	ND	ND
E-2	14.02 ± 0.00	ND	5.15 ± 0.00**
E-3	2.34 ± 0.00**	36.82 ± 0.00	ND

*Mean ± SD (N=3) ** Detectable but below LOD ND = Not detectable

The determination of Cr in human hair samples can be found in E-2, Whereas and A, C-1, D-1, D-2 and E-1 could not be detected the concentration of Cr by FAAS. The determination of Ni in human hair samples can be ordered as following: E-3 > C-2, Whereas sample A, B, C-1, D-1, D-2, E-1 and E-2 could not be detected the concentration of Ni by FAAS. The determination of Pb in human hair samples could not be detected the concentration of Pb but the content is below than LOD.

The high concentrations of Cr, Ni and Pb were found in hair samples from the workers who worked in electronic and lens industry. Exposure to these metals is common in industry, where the metals are used in a wide range of manufacturing processes [4]. Moreover, the electronic components contain high amount of certain toxic trace elements and heavy metals, such as As, Cd, Ni and Pb [62]. In addition, nickel are used in modern industry with other metals to form alloys to produce coins, lens, and stainless steel as well as for nickel plating and manufacturing Ni–Cd batteries. The most important way of human exposure to nickel is inhalation. The lower concentrations of Cr, Ni and Pb were found in hair sample from the person who dyed color hair and hair sample from the person who smoking. Some permanent hair dyes contain toxic metallic preparations based on cobalt (Co), copper (Cu), cadmium (Cd), iron (Fe), lead (Pb), nickel (Ni) [63]. Tobacco smoke particulate matter is harmful, containing high concentrations of toxic and carcinogenic compounds. Particulate matter contains a large compounds of heavy metals such as, hexavalent chromium, arsenic, lead, mercury and nickel [64]. The minimum concentration of Cr and Ni was found in hair sample from the person who drug abuse. Drugs or chemicals enter hair by passive diffusion from blood capillaries into growing cells over a length of 1.2 to 1.5 mm between the level of matrix cells and end of the keratinization zone of the hair follicle [1].

The results indicated the concentrations of Cr, Ni and Pb are difference. Metal enter human hair or human body by various mechanisms in a variety of locations, times and sources. Hair grows from follicles 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 [1]. Therefore, metals can be transferred and accumulated within hair for a long time. And the concentration of metals in hair increased with the increasing of exposure time.