CHAPTER 2

METHODOLOGY

2.1 Chemical and material

All chemicals used in this work (Table A-1 in Appendix A) were analytical grade and prepared in deionized water (DW). All glasswares were cleaned by soaking in 10% (v/v) nitric acid (HNO_3) for 24 hours and then were rinsed with DW and stored in plastic bags. The detail of preparation for reductant, carrier solution, mercury solution for calibration curves and standard curve are given in Appendix A. All transferal processes and acid preservation were performed in a class 100 clean bench. Plastic gloves were used at all time.

2.2 Instrument; Mercury Analysis System FIMH 400 (Perkin Elmer)

A mercury analysis system FIMH 400 (Perkin Elmer) was used for mercury determination. The Hg^{2+} in the solution was reduced to be Hg^0 by reluctant and was carried to quartz cell by carrier gas. The quartz cell was heated at 100°C and mercury absorption was measured at 253.7 nm. The operating parameters are presented in Table A-2 and A-3 in Appendix A and the instrument is shown in Figure A-1.

2.3 Sample Collection

2.3.1 Hair samples

About two grams of hair were collected from the exposed persons and non-exposed Hat-Yai residents by cutting 1-2 cm of hair from the back park of the skull. One hundred and eighty-nine exposed dental personnel from Faculty of Dentistry, Prince of Songkla University and seventy-one Hat-Yai residents were collected during June 2003 to June 2004. The exposed person was divided into 5 groups:

Group	Number
Dentists (DT)	28
Dentist assistants (DT Ass)	17
Fourth year dentist students (4 DTS)	36
Fifth year dentist students (5 DTS)	54
Sixth year dentist students (6 DTS)	54

The samples were collected in zip lock plastic bags. The hair samples were cut to small pieces using stainless steel scissors. Contaminants or dirt on the hair surfaces were washed out prior to acid digestion.

2.3.2 Questionnaires

Each participating person filled out a questionnaire. The detail of questionnaire (in Thai) is shown in Appendix B. Questionnaires were assigned numbers in order to ensure confidentiality. It consisted of questions intended to provide information the exposure factors, a summary of the categories included in questionnaire is showed in Table 2-1. These categories were classified into four categories; life style, work habit, dental filling and precautionary measure for statistically analysis (SPSS).

Table 2- 1 Summary of the categories included in the questionnaire

Occupation
Sex
Age
Weight
Religion
Frequency of fish and seafood consumption
Hair dye, hair shampoo and conditioning
Number of amalgam filling each of participants has
Dentist personnel working hour per day, per week
Number of amalgam filling set per week
Number of composite filling set per week
Frequency of using masks, goggles and gloves
Place for work of dental personnel

2.4 Optimization of Analytical Procedures

The experiments for washing process, drying temperature, various acids for digestion and digestion time were carried out for optimization the analytical condition.

2.4.1 Reagent for pre-cleaned process

Eight different washing reagents were investigated. They were deionized water, acetone (an organic solvent), 1% w/v EDTA (a complex-forming agent), 1% v/v Triton X-100 (a nonionic detergent), 1% v/v SLS (an inorganic detergent), 1% v/v baby shampoo, 0.1 M HCl and 1%(v/v) HNO_3.

Hair samples were collected from Hat-Yai resident. All samples were homogenized by cutting it into small pieces and by mixing with a glass rod on a filter paper. The sample was placed in a beaker, pouring deionized water to cover hair sample and sonicating for two minutes before discarding the deionized water. Eight different washing reagents were investigated by adding the reagents to cover hair sample, sonicate for 2 minutes, discard the reagent, re-sonicate for 2 minutes, rinsed 5 times with deionized water and oven-dried at 60°C for one hour. After cooling, the samples were kept in the zip lock plastic bags until analysis.

2.4.2 Drying temperature

Cleaned hair sample was placed in a Petri dish and then dried in oven. The drying temperatures were varied: room temperature ≈25°C by left in desiccator overnight, 60°C and 90°C for 1 hour in the oven.

2.4.3 Acid for digestion

In the present work, the efficiency of microwave digestion with a mixture of concentrated nitric acid and oxidant reagent for hair samples was investigated. They were HNO_3 (2 mL), $HNO_3 + H_2O_2$ (2+1), $HNO_3 + HClO_4$ (2+0.2), $HNO_3 + H_2SO_4$ (2+0.1). The reagents are mixed in volume by volume ratio.

2.4.4 Digestion times

The digestion times for heated the Teflon bomb which have the human hair and concentrated acid. A Teflon bomb is heated in a domestic microwave oven. Heating program of full power for 90 seconds and half power for 5, 10, 15, 20, 25, 30 minutes were examined.

2.4.5 Digestion procedure

The dry-cleaned hair samples were acid digested using Teflon bomb and domestic microwave oven as described in Loring and Rantala (1995). In brief: 0.2 grams of hair sample (weighted accurately) was placed in LORRAN Teflon bomb, added 2 mL of HNO_3 and 1 mL H_2O_2, placed in the domestic microwave oven for full power 90 seconds and half power for 5 minutes. After cooling, adjust the solution to 10 mL in volumetric flask with deionized water. The detail of sample digestion by using domestic microwave oven is in Appendix C.

2.5 Optimization the mercury analysis system

The mercury concentration in sample was determined by an automated cold vapor mercury/hydride generation system equipped with flow injection. The instrumental condition was presented in Table A-2 and A-3 in Appendix A.

A continuously flowing carrier (3%v/v HCl) of a flow rate 10 $mLmin^{-1}$ transported the sample from the injection loop to the manifold, where its was mixed with the reductant (0.2% (w/v) $NaBH_4$ in 0.05% (w/v) NaOH) by using flow rate at 6 mL min^{-1}. The reaction mixture was then merged with an argon gas flow rate at 45 mL min^{-1} and transported to the gas/liquid separator, where the liquid was separated from the gaseous components. The mercury vapor is transported to heated quartz cell. The chemical reaction is shown below. The peak heights in absorbance were used for a quantitative analysis.

$$\begin{aligned} NaBH_4 + 3H_2O + HCL &\rightarrow H_3BO_3 + NaCl + 8H^{\bullet} \\ Hg^{2+} + 2H^{\bullet}(excees) &\rightarrow Hg^0 + H_2(excess) \end{aligned} \qquad (3)$$

The optimized parameters were performed by changing one parameter and keeping other parameters constant and then the optimum value was selected for all experiments. The optimization tests were carried out by using 20 µg L^{-1} mercury stock solution. Carrier gas (Ar) flow rate of this instrument is fixed at 45 mL min^{-1}. Minimum flow rate of this instrument is 40 mL min^{-1} and if flow rate is higher than this value liquid phase will be transported through the gas-liquid separator membrane and will damage to quartz cell. The cell temperature is 100°C. The following parameters were performed including:

2.5.1 Effect of $NaBH_4$ concentration

The effect of the $NaBH_4$ concentration was investigated at 0.1, 0.2, 0.3, 0.4, 0.5 and 1.0 % (w/v). Three replicates were performed for each concentration**.**

2.5.2 Effect of HCl concentration

The effect of HCl concentration was investigated at 0.5, 1, 2, 3, 4, 5, 7 and 10 % (v/v). Three replicates were performed for each concentration.

2.6 Comparison of the standard addition method and calibration method to quantification of the analyses

Standard addition method is used to quantify the mercury concentration in this study. The matrix in the sample has unknown constituents that one could not incorporate into standard solutions to make a calibration curve.

2.7 Analytical performance characteristics

The analytical performance characteristics were evaluated including detection limit, linearity, accuracy, precision and recovery test of measurements.

2.7.1 Detection limit

A measurement is acceptable only when the signal measured is larger than the uncertainly associated with the measurement. The detection limit is defined as the lowest concentration or the weight of analyte which can be measured at a specific confidence level. So, as the detection limit approaches, the signal generated by the instrument approaches that of the blank. Therefore, the smallest distinguis hable signal, Sm, is the calculation of detection limit is given in Equation 4 (Kebbekus and Mitra, 1998).

$$DL = 3\delta \quad (4)$$

Where DL = Detection limit

δ = Standard deviation

2.7.2 The linear dynamic range (Linearity)

The linear dynamic range is the range of concentration that can be obtained from a linear calibration curve. Usually, a 5% deviation from the linearity is considered the upper limit. The deviation from the linearity is usually found at the high concentration due to non-ideal detector responses or chemical effects (Skoog *et al.*, 2004). The standard stock solutions of Hg were diluted with 3 % HCl to various concentrations in the range of 0 to 80 $\mu g\ L^{-1}$. The linear dynamic range obtained from plotting the absorbance versus the concentration. The linearity of response was considered by the correlative coefficient value of the linear curve.

2.7.3 Accuracy

The accuracy term is the measurement of exactness value of the analyte concentration or agreement between measured value and certified value or an accepted reference value. Normally, the accuracy value is expressed as the relative percent error term. The following calculation of relative percent error is given Equation 5 (Skoog *et al.*, 2004):

$$\% \text{ Error} = \frac{\text{Measured value} - \text{Real value}}{\text{Real value}} \times 100\% \quad (5)$$

The accuracy from this research was studied by using Certified Reference Material (CRM) human hair (BCR- 397) from theInstitute for Reference and Measurement, Belgium and determination (three replicates). The difference in values between the measured value and certified value were compared and the relative percent error was also calculated.

2.7.4 Precision

Precision is the measure of the degree of an analytical method under the same conditions. Normally is always expressed as a percentage of the relative standard deviation (%RSD) for a statically significant number of samples. The calculation of %RSD is given Equation 6 (Skoog *et al.,* 2004):

$$\%\,RSD = \frac{s}{\bar{x}} \times 100\% \qquad (6)$$

In this research, the precision were investigated for measurement of the degree of repeatability and used for analyses of homogeneous samples were repeated 10 times. The relative standard deviation is the parameter of choice for comparing the precision of data of different units and magnitudes and is used extensively in analytical science. In order to check the reproducibility of the analysis, 10% of samples were analyzed in duplicate.

2.7.5 Recovery test

The terms recovery (*R*) is used to indicate the yield of an analyte in a pre-concentration or extraction stage in an analytical method. Actually, the recovery value is presented as a percent recovery (%*R*) and it can be calculated from the equation given Equation 7 (Rubinson, 1987).

$$\%R = \frac{Measured\ value}{\mathrm{Re}\,al\ \ value}\ X\ 100 \qquad (7)$$

When %R = Percent recovery

For the % recovery the cleaned real sample (0.2 gram) was placed in a Teflon bomb and then spiked with 0.1, 0.2, 0.3, 0.4 μg mercury. For spiked recovery test, an appropriate amount of mercury was added to the hair sample by a

micropipette 100 μL for each concentrated. The screw cap Teflon bomb tightened by hand and spiked sample was allowed to stand at room temperature overnight for the mercury remain on the hair, digested in the same way of human hair sample and analysis of mercury as the method described. Two replicates were performed at each concentration.

2.8 Statistical analyses

The information collected from each questionnaire was coded and analyzed using the statistical software SPSS 10.0 along with the hair mercury concentration for that particular participant. The variables collected from the questionnaire were taken as independent, while mercury concentration was taken as the dependent variable after it was log transformed since it was not normally distributed.

One-way ANOVA and independent student *t*-test were used in order to compare the mean mercury concentration levels among the different categories of the independent variables. The continuous independent variables were changed into discrete ones so that it is possible to perform the ANOVA or *t*-test. *P* values less than 0.05 denoted a significant difference in mercury level concentrations among the different categories of the independent variable.