

# CHAPTER 1

## INTRODUCTION

### 1.1 Background and Problem Statement

Lead is one of the 80 metals that can be found in this world. Lead is a naturally occurring metal found deep within the ground. It occurs in small amounts in ore, along with other elements such as silver, zinc or copper. Even though it is found in small amount, there is an abundant supply of lead throughout the earth. Lead can be found in many products around the market such as cosmetics, dyes, pigments, paint, batteries, bullet, building constructions, toys and etc due to its widespread, easy to work with and easy to extract

Lead is an element in the carbon group with symbol Pb (from Latin: *plumbum*) and atomic number 82. Lead is a soft and malleable metal, which is regarded as a heavy metal and other metal. Metallic lead has a bluish-white color after being freshly cut, but it soon tarnishes to a dull grayish color when exposed to air. Lead has a shiny chrome-silver luster when it is melted into a liquid. It is also the heaviest non-radioactive element.

Environment Protection Agency (EPA) have stated that, for hundreds of years, lead has been mined, smelted, refined, and used in products (e.g., as an additive in paint, gasoline, leaded pipes, solder, crystal, and ceramics). Natural levels of lead in soil range between 50 parts per million (ppm) and 400 ppm. Mining, smelting, and refining activities have resulted in substantial increases in lead levels in the environment, especially near mining and smelting sites.

Lead is a naturally-occurring element that can be harmful to humans when ingested or inhaled, particularly to children under the age of six. Lead poisoning can cause a number of adverse human health effects, but is particularly detrimental to the neurological development of children. The possible route of lead exposure include ingestion of lead contaminated water, paint chips, inhalation of lead containing particles of soil or dust and ingestion of foods that contain lead from soil or water. In children, ingestion of foods or anything that contain lead is most usual route of exposure. They tend to eat or put anything on their reach in their mouth.

History of regulatory actions on lead start in 1975, Occupational Safety & Health Act (OSHA) lower the limit of lead from  $150 \mu\text{g}/\text{m}^3$  to  $100 \mu\text{g}/\text{m}^3$  and the current level now is  $50 \mu\text{g}/\text{m}^3$ . In 1978, leaded paint was banned by the Environment Protection Agency (EPA) while in 1987, lead pipe/solder/flux was prohibited by the Food & Drugs Act (FDA). EPA was first limit the use of leaded gasoline until it was finally banned on 1996. In 1993, FDA established a provisional tolerable total intake level (PTTIL) of lead based on the lowest level of lead exposure associated with adverse effect which were  $6 \mu\text{g}/\text{day}$  for children under 6,  $15 \mu\text{g}/\text{day}$  for children 7 years and older,  $25 \mu\text{g}/\text{day}$  for pregnant women and  $75 \mu\text{g}/\text{day}$  for adults and in 1994, FDA set 15 ppb as action level for bottled drinking water. Consumer Product Safety Commissioner (CPSC) policy banned content of lead in miniblinds, crayons, toy figurines and children jewellerys in 1998. After the implementation of Hazard Analysis Critical Control Point (HACCP) in juice industry, FDA established a maximum of 50 ppb content of lead in readymade drink juice. The regulation started to implement on 2002. Last but not least, the CPSC also, banned lead in candlewicks (2003) and in 2006, EU directive banned the use of lead in electrical, electronic products and their components parts.

Basically, lead is banned due to the toxic effect that was brought by exposure of lead to children and adults. Lead was particularly dangerous because once it gets into a person's

system, it is distributed throughout the body just like helpful minerals such as iron, calcium, and zinc. A blood lead level of 10 ug/dl in children and 80 ug/dl in adults can cause significant adverse health effect to children and adults respectively. It can be acute toxicity or chronic toxicity. Exposure to high lead level of in a short period of time is called acute toxicity. Exposure to small amounts of lead over a long period of time is called chronic toxicity.

The sign and symptoms of lead poisoning includes abdominal pain, weight loss, headaches, loss of appetite, nausea or vomiting, muscle and joint weakness or pain, constipation, metallic taste in mouth, sluggishness or fatigue, difficult to concentrate on something and as well as seizures. Each year in the United States, 310,000 1- to 5-year-old kids are found to have unsafe levels of lead in their blood, which can lead to a wide range of symptoms, from headaches and stomach pain to behavioral problems and anemia (not enough healthy red blood cells).

Rossol (2009) reported that, a study has shown a significant risk of mortality in adults associated even with rather low lead levels. The study reported in the *Journal of the American Heart Association*, 2006 was the Third National Health and Nutrition Examination Survey Mortality Follow-Up Study. It involved 13,946 adults whose blood lead levels were collected and measured between 1988 and 1994. When researchers studied those who died by December 31, 2000, they found that death from any cause, cardiovascular disease, heart attack and stroke increased progressively at higher lead levels. She also reported that, lead causes devastating effects on children including retardation, hearing loss, and kidney damage depending on the amounts the child takes in. The effects that occur at the lowest doses are subtle effects on mental acuity. The studies have shown a measurable reduction in IQ in children whose blood lead levels are at 10 µg/dl. For years, experts thought that reduction of

mental acuity probably occurs at even lower lead levels. In 2003, a study reported in the *New England Journal of Medicine* confirmed that significant effects occur below 10 µg/dl. The researchers found that for blood-lead concentrations between 1 and 10 µg/dl, the total decrease in IQ averaged 7.4 points, a drop of 0.82 points for each 1 µg/dl. However, the decrease in IQ associated each increase of 10 µg/dl at levels above 10 µg/dl was only 4.6 points, that is, 0.13 points lost for each additional 1 µg/dl.

The study done by Needleman et al. (1998) indicated that the exposure to lead even in children who remain asymptomatic, may have an important and enduring effect on the success in life such children and that early indicators of lead burden and behavioural deficit are strong predictors of poor school outcome. For the small group of 10 subjects who were diagnosed earlier as having plumbism, the outcome was especially dire; half of these young people have reading disabilities, and almost half left high school before graduation. According to Mudipalli A. (2007), extensive in vivo and in vitro laboratory animal experimental evidence clearly potential hepatotoxicity resulting from exposure to lead. These studies, ranging from simple biochemical and structural alterations to molecular characterization of hepatic hyperplasia or liver cell proliferation, have demonstrated pathologic changes indicatives of liver toxicity.

## **1.2 Wax Crayon**

Crayon is made from paraffin which is a type of wax and colour pigments. Paraffin is made from extracting chemicals from wood, coal or petroleum. Pigments come from natural or artificial made substances which mean they can be found in earth, minerals and or made in

factories. Paraffin wax is heated and cooled to achieve the correct temperature in which a usable wax substance can be dyed and then manufactured for use around the world.

Crayons as shown in Figure 1.0, which are available at a range of price points, are easy to work with, often less messy than paints and markers, blunt (removing the risk of sharp points present when using a pencil or pen), usually non-toxic, and are available in a wide variety of colors. These characteristics make them particularly good instruments for teaching small children to draw in addition to being used widely by student and professional artists.

Characteristic of wax crayon which is blunt make it the best choice by parents for their children compared to pencil colours, other drawing and colouring tools. Colouring is important both in lives of children and adults. One way of expressing their feeling is through colouring. The images that they draw, the colour that they use shows the feeling that they feel. A child who draws hearts, suns, and other cheerful objects may express satisfaction, content and love. Besides, many children receive their first exposure to the colour wheel and art through the use of crayon compared to other colouring tools due to ease of use of crayon.



Figure 1.0: Wax Crayon

Furthermore, institutions across the globe use colouring to help people escape their feelings and frustrations, as well as other emotions through colouring since for many people, colouring is therapeutic. As for example, a child who is angry might choose to scribble over a picture of a sun with a black crayon until the picture is not visible or just roughly scribble over outside the lines to de-stress after a busy morning of school work, wind down, and calm down, after the stresses of a day at school.

In terms of child's grip/control, coordination and building motor skills, crayon does help a lot in developing these three skills. For many children, crayon is the first object that they have to 'grip' in a certain manner, in order to grip it. Since, crayon is blunt and attractive, it is a good tool in learning session for children to develop proper grip and control over a crayon to help them properly grip and control other writing instruments in the future. Then, when colouring activities is done, it takes a lot of hand-eye coordination to colour in a colouring page. In fact, this activity develops motor skills at a very basic, simple level that they will expand on later life. Colouring with crayons, learning to print, pencil grasp, playing with Play Doh, beading, lacing, crumpling paper, tearing paper, using stamps, and wiggling fingers all are activities that help to strengthen and develop hand muscles.

Pigment is used to make crayons because it supplies the uncooked colour to crayon. When this solution is mixed with binder material and wax, then it gives the right colour. The amount of pigment in crayons varies from bright colour to attractive colour. There are many types of pigments available such as organic, biological, metal based pigment and inorganic pigments. The problem arises when crayon was detected to contain certain amount of heavy metals which could bring significant health effect to the children and adult. The main concern is the exposure of this heavy metal to children since they work quite well with crayon and

they tend to eat the crayon as well. The bright and colourful colours of crayon made it look delicious to eat.

Each colour represents pigments that give them colour. As for example, there were 11<sup>th</sup> types of pigments that make up red colour. The pigments were Alizarin crimson, Brazilwood, Cadmium Red, Carmine or Crimson Lake, Dragonsblood, Lake Colors, Madder Lake, Minium or Saturn Red or Red Lead, Realgar, Sinopia or Sinoper and Vermillion or Cinnabar. The main pigment related to this study is the Minium or Saturn Red or Red Lead. The Red lead pigment is produce by heating white lead in the presence of air. In producing blue colour, there were 12 types of pigment that can give blue colour such as Woad, Turnsole, Smalt, Prussian blue, Phthalocyanine blue, Lapis lazuli, French ultramarine, and etc.

### **1.3 Previous works on determination of lead in wax crayon**

Basically, there were only few study related to determination of lead in wax crayon. In 1994, the Consumer Product Safety Commission (CPSC) conducted a study of the total lead level of individual crayons which was measured after hot nitric acid digestion and inductively-coupled emission spectrometric analysis. Apart from this study, CPSC also had done analysis on determination of total lead in wax crayon by bock digestion DigiPrep.

## **1.4 Methods of determining lead in crayon**

### **1.4.1 Sampling**

Before an analysis is started, it is important to prepare a representative sample from the lot to be analysed. In a laboratory, there were few methods available for sampling representative sample such as grab sampling, coning and quartering, sample splitters, rotary riffle and etc. In this study, coning and quartering technique was choosing to prepare a representative sample for analysis.

#### **1.4.1.1 Coning and Quartering**

This technique is suitable for large lots of material and can be conveniently done using shovel of front-end loaders if the sample size is very large. In case of smaller scale sample size, the use of retort and conical flask can be applied. In this technique, the sample is mixed into a conical pile. The pile must be made so that the natural segregation in the cone is radially symmetrical. The cone is then spread from the center to form a flattened disk of material. After that, the disk is then divided into quarters using perpendicular boards. One pair of opposite quarters is removed, and the other pair is used as the sample. If the sample is too large, then it can be coned and quartered again until the desired sample size is obtained. Figure 1.1 and 1.2 illustrate the cone, flattened disk and as well as the use of perpendicular boards to divide the disk into quarters.

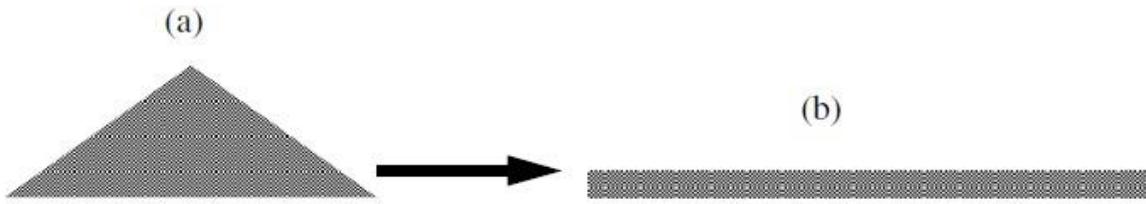


Figure 1.1: Side view of sample (a) in conical shape, and (b) after flattening

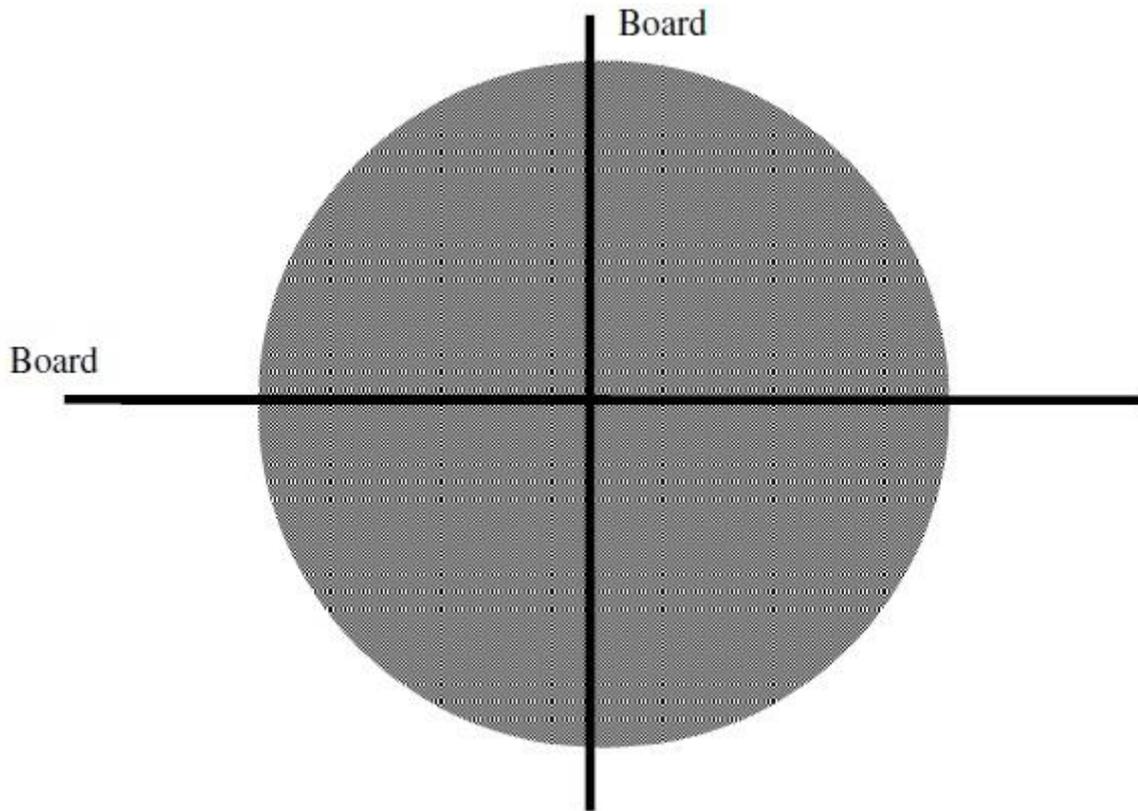


Figure 1.2: The sample was divided using board into quarters. Use boards to delineate them so that the sample can be divided precisely.

#### 1.4.2 Extraction method

Sample for analysis of metal usually was done by using atomic spectroscopic techniques such as flame atomic absorption and inductively coupled plasma atomic emission

which required the sample to be presented as a dilute aqueous solution, usually in acid. There are few methods for dissolving samples for metals analysis such as wet digestion and dry ashing. In wet digestion, the most common methods for dissolving samples for metal analysis are digestion in open flask, digestion in a pressurized, sealed container, and microwave assisted decomposition. Nitric acid is the strong acids commonly use to digest the matrix since there is no chance of forming insoluble salts as might happen with HCL or H<sub>2</sub>SO<sub>4</sub>. In this study, microwave assisted digestion had been utilized to dissolve the sample for metals analysis.

#### **1.4.2.1 Microwave digestion**

In 1975, microwave was first applied to acid digestion for metal analysis by Abu-Samra et al. Since then, much work has been done on microwave acid-digestion, and it has gained widespread acceptance and approval by regulatory agencies as standard method. Microwave assisted digestion was approved by the EPA as a standard method for the extraction of semivolatile and non-volatile compounds from solid samples in year 2000.

Microwave sample preparation is a proven technique that has been utilized for many years for a wide variety of sample types. The advantages of microwave digestion over open container dissolution method include eliminating losses of more volatile metal species and the chances of airborne dust contamination. Besides, the sealed, pressurized containers reduce evaporation, so that less acid digestion solution is required, reducing blanks. Furthermore, the electronic controls on modern microwave digesters allow very reproducible digestion conditions.

The basic components of microwave system include a microwave generator (magnetron), a waveguide for transmission, a resonant cavity, and a power supply. In the oven cavity, there is a carousel (turntable or rotor) that can hold multiple extraction vessels. The carousel rotates 360° during extraction so that multiple samples can be processed simultaneously. The vessels and the caps are constructed of chemically inert and microwave transparent materials such as TFM (tetrafluoromethoxyl polymer) or polyetherimide. The inner liners and cover are made of Teflon PFA (perfluoroalkoxy). The vessels can hold at least 200 psi of pressure. Under elevated pressures, the temperature in the vessel is higher than the solvent's boiling point, and this enhances digestion efficiency. The vessels need to be cooled down and depressurized after extraction.

### **1.4.3 Analytical techniques for determination of lead**

#### **1.4.3.1 Atomic Absorption Spectrometry**

Several analytical techniques such as atomic absorption spectrometry (AAS), inductively coupled atomic emission spectrometry (ICP-AES) and inductively coupled plasma mass spectrometry (ICP-MS) are available for the determination of trace metals with sufficient sensitivity for most of applications (Teo, K.C. et al, 2001). In this study, flame atomic absorption spectrometry (FAAS) is utilized to determine the lead in wax crayon.

Flame atomic absorption spectrometry is currently the most widely used of all the atomic techniques because of its simplicity, effectiveness, and relatively low cost. The technique was introduced in 1955 by Walsh in Australia and by Alkemade and Milatz in Holland (A.Walsh, 1995). The first commercial atomic absorption (AA) spectrometer was introduced in 1959, and use of the technique grew explosively after that (Skoog, 2004).

Flame atomic absorption provides a sensitive tool of determining 60 – 70 elements. This method is well suited for routine analysis by relative unskilled operators. The major drawback of atomic absorption is its single element at a time nature, imposed by the need for a different lamp for each element.

In this technique, it involved the study and measurement of radiant energy by free atoms. Radiant energy absorbed by atoms is in form of very narrow absorption lines with wavelengths in visible or ultraviolet region of energy spectrum. Atomic spectroscopy begins with atomizing the sample. Sample in liquid form is atomized by atomizer devices which either continuous or discrete. Continuous are in the form of plasmas and flame while discrete are in the form of electrothermal. Nebulizers are the method to introduce samples into the atomizer as shown in Figure 1.3. Direct nebulizer creates fine droplets by aerosol. Flame atomizer contains a pneumatic nebulizer, which converts the sample solution into a mists or aerosol. When nebulized sample is carried into a flame, desolvations of the droplets occur in the primary combustion zone, located in the tip of the burner. The fine solid particles are carried to a region in the center of the flame called the inner core.

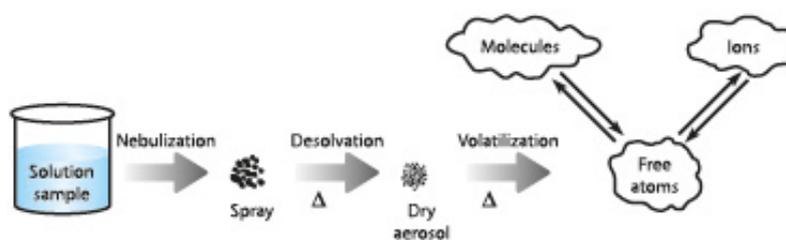


Figure 1.3: Sample Introduction

The component of the technique includes a radiation source, burner, monochromator, detector, amplifier and read out. This component can be lined up in two optical systems which are single beam and double beam instrumentation as presented in Figure 1.4 – 1.5.

The radiation from light sources passes through sample atoms generated in atomizer. The light then proceeds to a monochromator. The function of the monochromator is to disperse radiation using slit system so that only light of desired wavelength allowed to proceed along light path. The light passes to detector and intensity is measured. The signal is read out on a recorder which is usually a computer. In this study, the radiation source is from electrodeless discharge lamps (EDLs) and the instrumentation is double beam instrumentation. EDLs provide much higher light output and longer lifetime when compared to conventional hollow cathode lamps.

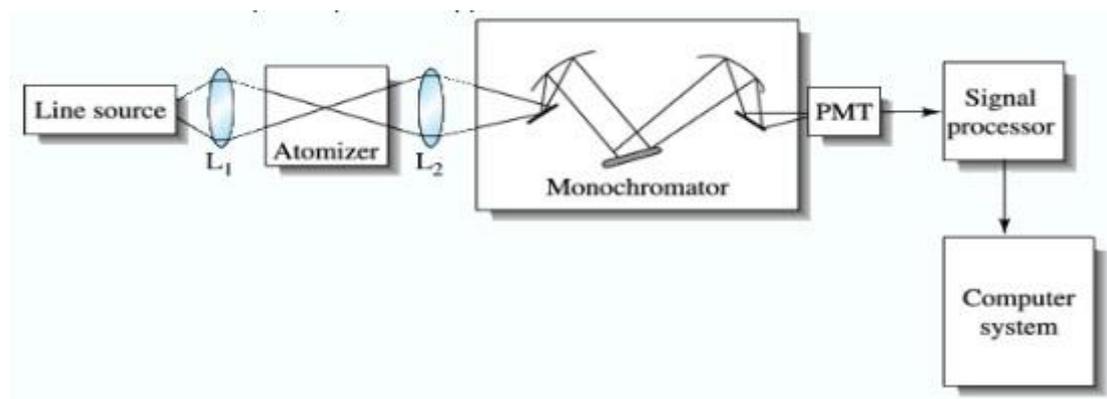


Figure 1.4: Block diagram of single-beam atomic absorption spectrometer

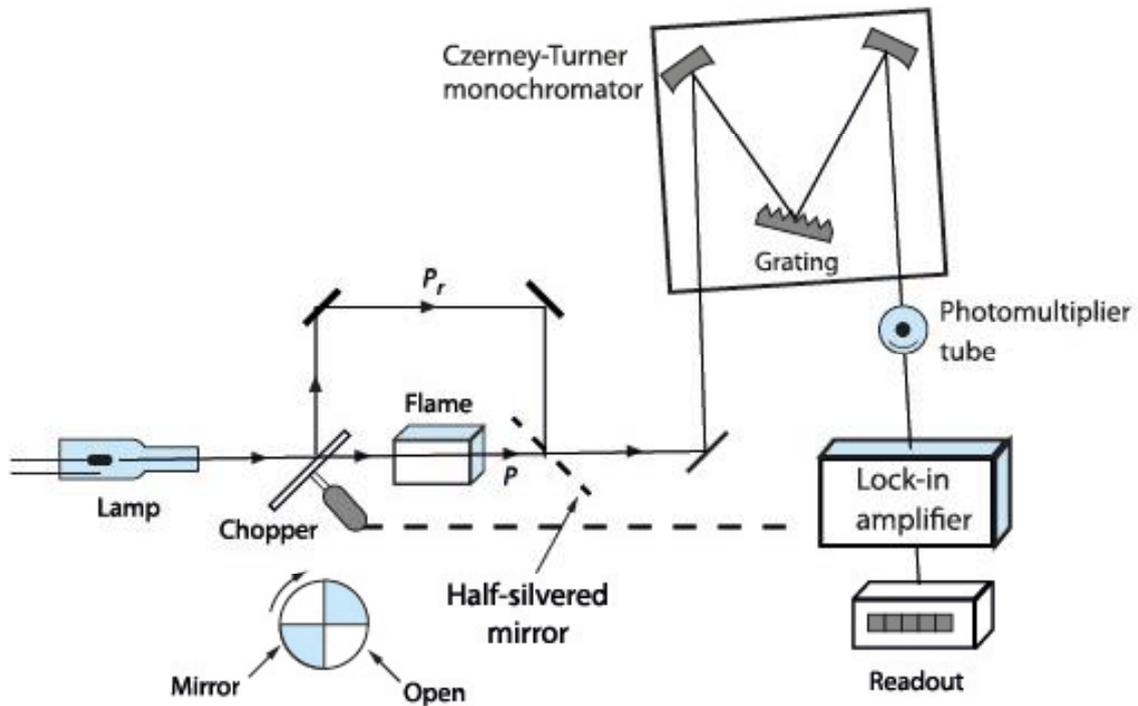


Figure 1.5: Block diagram of double-beam atomic absorption spectrometer

The advantages of AAS includes, it is virtually independent of temperature. Besides, the AAS system is independent of wavelength of absorption line. Another advantages of AAS is majority of the free atoms exist in ground or unexcited state and directly contribute to atomic absorption signal. Furthermore, in atomic absorption, it is only necessary to measure intensity ( $I_I$  &  $I_0$ ) in order to measure absorption. Frequently, it is easier to measure absolute quantities, particularly when effect of interferences must be corrected to obtain accurate results.

## **1.5 Significance of study**

The importance of this study is to determine the presence of lead in wax crayon and to develop in-house method for analysis of wax crayon using microwave digestion by Flame Atomic Absorption Spectroscopy. Comparison between different colours of wax crayon was done to study the significant content of lead in blue, red and yellow colour wax crayon. Parents and child care taker tend to allow children use crayon for their colouring activities due to the blunt shape of crayon compared to pencil colour and other stationary tools. But, children have tendency to eat crayon since they are bright in colour, attractive and look so yummy. So the major route of entry of most metals (lead) in crayon into the body is through the diet.

This study is significant to provide information and knowledge to people about the hazard of wax crayon due to the content of lead which could bring significant health effect to their children. It is important to choose wax crayon that contain no lead as one of the ingredient in producing the wax crayon.

## **1.6 Objectives of study**

The aims for this study are:

- 1) To adopt and verify the method for determining lead contents in wax crayon using microwave digestion.
- 2) To develop in house method for determining lead contents in wax crayon using microwave digestion and analysed by Flame Atomic Absorption Spectroscopy (FAAS).
- 3) To compare quantitatively the amount of lead in red, orange and blue colour wax crayon.

## CHAPTER 2

### METHODOLOGY

#### 2.1 Background

All the laboratory analysis was conducted at Level 5, Research Laboratory, Department of Chemistry (new building) and Atomic Absorption Spectrometer Room, Faculty of Science, University Malaya.

#### 2.2 Reagents and Materials

Concentrated nitric acid (65% HNO<sub>3</sub>, analytical reagent, Fisher Scientific) was used as the reagent solutions throughout the study. Ultrapure water (Milli-Q water purification system, Millipore, Billerica, MA, USA) with resistivity of 18.2 Ω cm<sup>-1</sup> was used as distilled deionised water together with diluted HNO<sub>3</sub> (5% v/v) throughout the experiments. All the plastic containers, glassware and microwave digestion vessel were cleaned by soaking in diluted HNO<sub>3</sub> (5% v/v) overnight and were rinsed with distilled water prior to use. The volumetric flask used in this experiment includes 50 mL, 100 mL and 2000 mL.

Distilled water was used to mark up the samples volume after the sample decomposition procedure. All standards were freshly prepared before the analysis started. All the reagents solutions and samples were kept in the refrigerator before the measurements. A series of standard solution of lead was prepared from 1000 mg L<sup>-1</sup> lead standard stock solution (Merck, Darmstadt, Germany) and diluted HNO<sub>3</sub> (5% v/v) was used to mark up the volume.

## **2.3 Methods**

### **2.3.1 Glassware cleaning**

5% v/v concentrated nitric acid,  $\text{HNO}_3$  was prepared by diluting 25 ml of concentrated nitric acid into a 500 ml volumetric flask. Ultrapure water (Milli-Q water purification system, Millipore, Billerica, MA, USA) with resistivity of  $18.2 \Omega \text{ cm}^{-1}$  was used to mark up the volume. All the glassware, plastic bottle containers and microwave vessel were soaked in 5% v/v of concentrated nitric acid,  $\text{HNO}_3$  overnight and rinsed with distilled water for several times.

### **2.3.2 Preparation of Lead Standard Solution**

A  $100 \text{ mg L}^{-1}$  lead standard solution was prepared from  $1000 \text{ mg L}^{-1}$  lead standard stock solution (Merck, Darmstadt, Germany). 5 ml of  $1000 \text{ mg L}^{-1}$  lead standard solution was diluted with 5% v/v  $\text{HNO}_3$  in a 50 ml volumetric flask. From  $100 \text{ mg L}^{-1}$  lead standard solution, an intermediate lead standard solution was prepared which was  $10 \text{ mg L}^{-1}$ . 5 ml of  $100 \text{ mg L}^{-1}$  lead standard solution was diluted with 5% v/v  $\text{HNO}_3$  in a 50 ml volumetric flask until the calibration mark to prepare  $10 \text{ mg L}^{-1}$  lead standard solution. All standard solutions were freshly prepared immediately prior to analysis of sample to prevent any interference contribute to systematic error. All standard prepared were transferred into a plastic container for the analysis.

### **Lead Standard Solution for Calibration Curve**

A series of lead standard solutions with concentration ranging from 2 – 8  $\text{mg L}^{-1}$  (2, 4, 6, 8) were prepared from  $10 \text{ mg L}^{-1}$  working solution. 1 mL, 2 mL, 3 mL, 4 mL of  $10 \text{ mg L}^{-1}$

lead standard solution were diluted in a 50 mL volumetric flask with 5% v/v HNO<sub>3</sub> to the calibration mark in order to prepare 2 mg L<sup>-1</sup>, 4 mg L<sup>-1</sup>, 6 mg L<sup>-1</sup> and 8 mg L<sup>-1</sup> of lead standard solutions.

### **Lead Standard Solution for Quality Control**

A lead standard solution of 5 mg L<sup>-1</sup> were also prepared for Quality Control purpose. 10 mg L<sup>-1</sup> lead standard solution were diluted in a 50 ml volumetric flask with 5% v/v HNO<sub>3</sub> to the calibration mark.

### **Lead Standard Solution for Spiked Samples**

Three (3) series of concentration were prepared from 10 mg L<sup>-1</sup> working standard solution. 5 ml, 15 ml and 25 ml of 10 mg L<sup>-1</sup> working standard were transferred into digestion vessel respectively prior to microwave digestion procedure.

## 2.4 Sample Preparation

### 2.4.1 Sample pretreatment

Ten (10) boxes of wax crayon were bought from a local supermarket in Kuala Lumpur. 10 pieces of chosen colour (red, orange and blue) wax crayon were grated separately as shown in Figure 2.1. About 36.0 g of each grated wax crayon was weighted and cone and quartering technique was applied to accomplish homogenised sample of about 3.6 g for each analysis. Triplicate measurements were prepared for each sample.



Figure 2.1 : Sample of Wax Crayon

### 2.4.2 Sample decomposition

In this experiment, triplicates of sample were prepared weighing about 3.0 g of wax crayon. The wax crayon was placed in a digestion vessel together with 7.5 mL of 65% nitric acid. Blank sample was represented by 7.5 mL of 65% nitric acid. 5 mL, 15 mL and 25 mL of 10 mg L<sup>-1</sup> of lead working standard were pipetted into different vessel containing sample to prepared mg L<sup>-1</sup>, 3 mg L<sup>-1</sup> and 5 mg L<sup>-1</sup> of spiked samples respectively. All blank, spiked and unspiked samples were closely tight and then subjected to microwave digestion under specific condition as shown in Table 2.1. CEM MARS Microwave oven was employed in this

experimental study. The obtained clear solution were allowed to cool until the temperature reached below 40 °C. Whatman No. 1(110 mm pores size) filter paper together with funnel was used to filter the solution in a 50 ml volumetric flask. The method was repeated for other colour of wax crayon involved in this experiment. Distilled water was used to mark up the obtained solution to the calibration mark throughout this work. All the samples were transferred to plastic container for analysis.



Figure 2.2: CEM MARS Microwave Oven

Table 2.1: Microwave Digestion Operating Condition

<b>Method : Crayon</b>				<b>Number of Vessels : 8</b>			
<b>Control Style : Ramp to temperature</b>				<b>Sample Weight : 0.3 g</b>			
				<b>Acid : HNO<sub>3</sub>, 65%</b>			
<b>Stage</b>	<b>Maximum Wattage</b>	<b>Power</b>	<b>Ramp Time</b>	<b>Pressure Control</b>	<b>Maximum Temperature</b>	<b>Stirrer</b>	<b>Hold Time</b>
<b>1</b>	<b>800 W</b>	<b>100%</b>	<b>20 min</b>	<b>400 psi</b>	<b>210°C</b>	<b>0</b>	<b>10 min</b>



Figure 2.3: Sample Decomposition

### 2.4.3 Instrumentation

Flame Atomic Absorption Spectrometer from Perkin Elmer Model Aanalyst 400 AAS which utilized acetylene-air burner with deuterium background was used to determine the concentration of lead in wax crayon in this analysis. The operating conditions for flame Atomic Spectrometer in this experimental study is shown in Table 2.2.

Table 2.2 : Atomic Absorption Spectrometer Operating Conditions

AAS System	Parameter
Light source	Electrodeless discharged lamps (EDLs)
Flame :	
Fuel	Acetylene
Oxidant	Air
Wavelength (nm)	283.3
Acetylene Flow (L/min)	2.5
Oxidant Flow (L/min)	10



Figure 2.4: Flame Atomic Absorption Spectrometer from Perkin Elmer Model Aanalyst 400

AAS

#### **2.4.4 Method Verification Procedure**

The analytical method employed in this experimental study was first verified. The method was verified for linearity, accuracy, precision, limit of detection and limit of quantification.

## CHAPTER 3

### RESULTS & DISCUSSION

#### 3.1 Method Verification

In this study, the method for determination of lead in wax crayon was adopted from Health Canada Safety Program, Product Safety Laboratory, 'Determination of Total Lead in Wax Crayon by Closed Vessel Microwave Digestion' and verified based on parameters such as accuracy, precision, limit of detection, limit of quantification and linearity.

#### 3.2 Standard Calibration Curve of Lead by FAAS

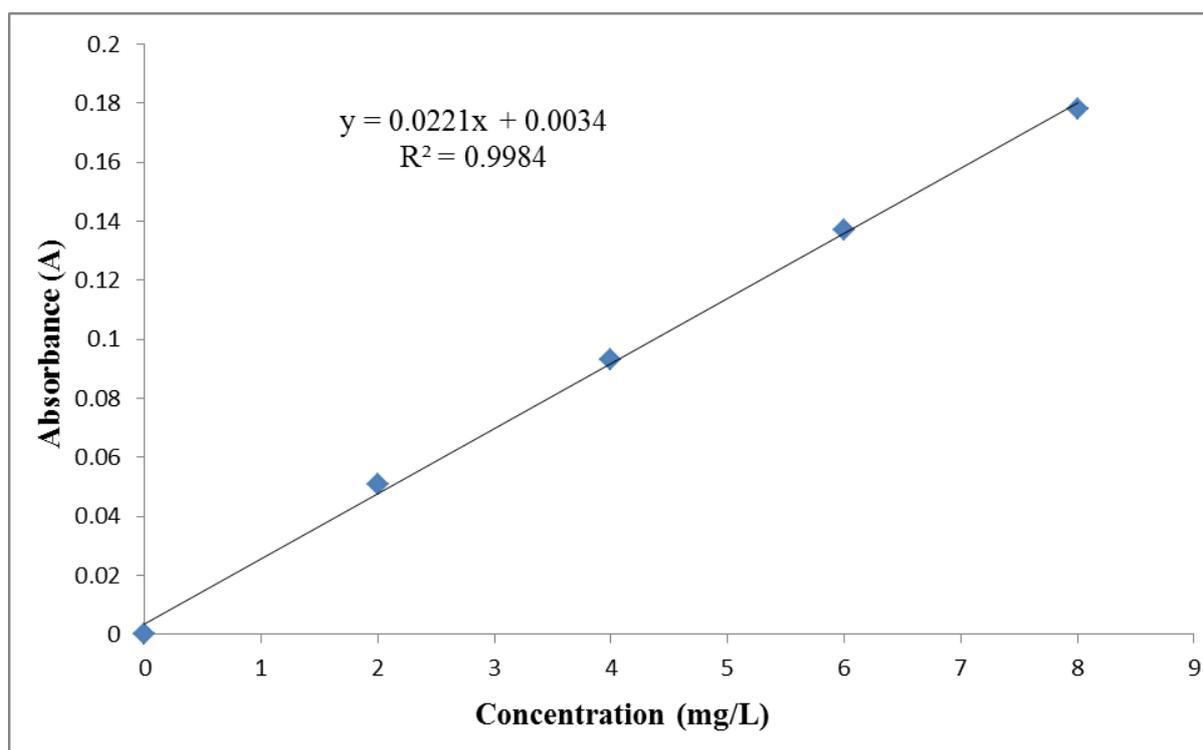


Figure 3.1 : Calibration Curve for Lead in Red Colour Wax Crayon

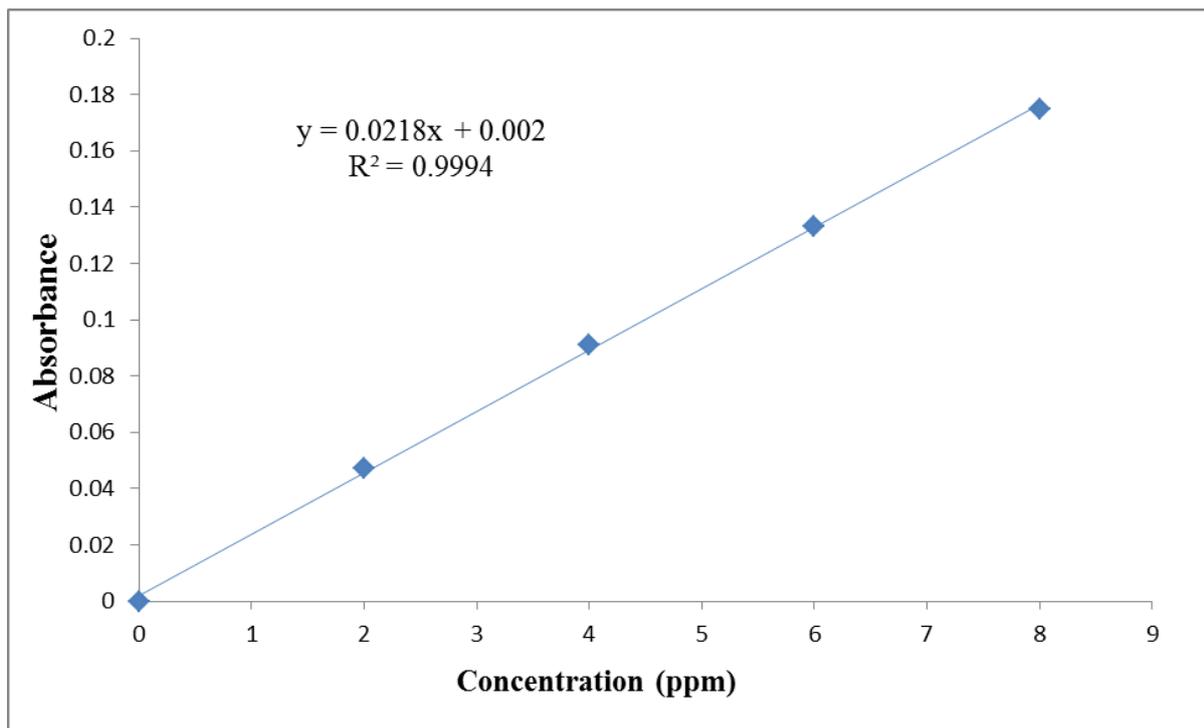


Figure 3.2 : Calibration Curve for Lead in Blue Colour Wax Crayon

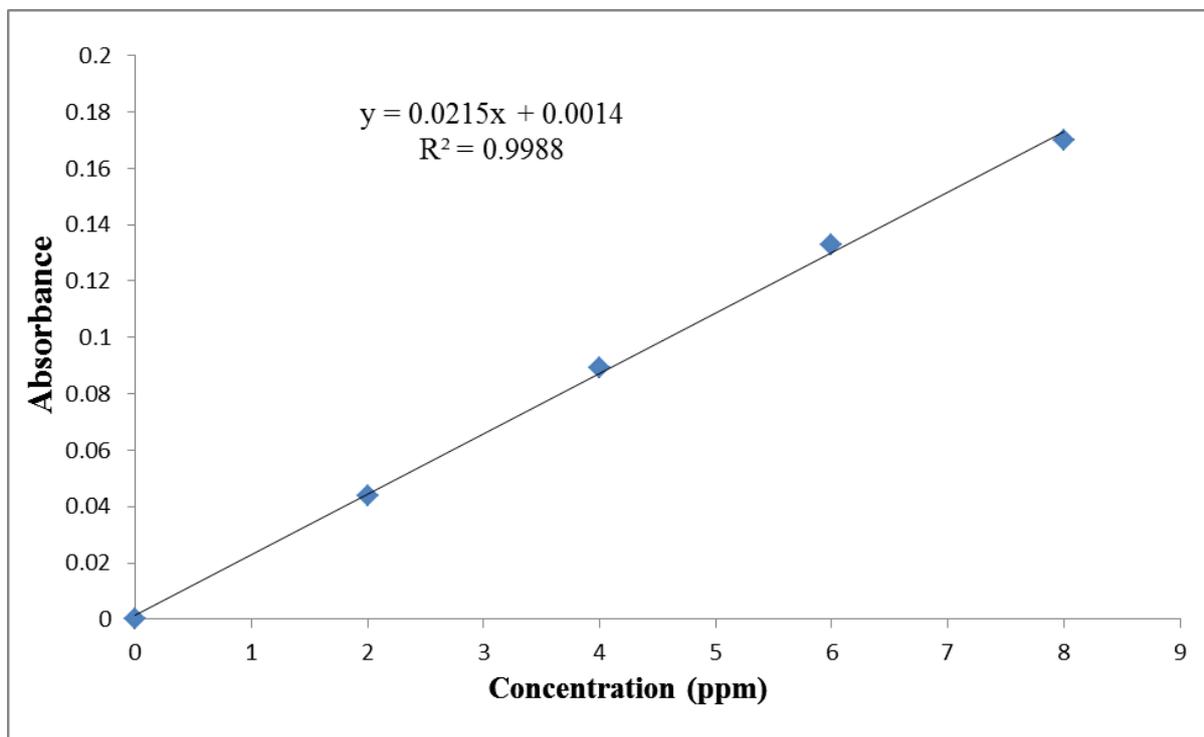


Figure 3.3 : Calibration Curve for Lead in Orange Colour Wax Crayon

Based on Figures 3.1- 3.3, all of the calibration curves showed a good linearity between absorbance intensity and concentration. The correlation coefficient obtained were in the range of 0.99 – 1.0.

### 3.3 Accuracy

Accuracy is defined as the closeness of agreement between a test result and the accepted reference value or true value of the property being measured. According to International Conference for Harmonization (ICH), accuracy of a method can be accessed by several ways including:

1. Comparing the results of the method with a result from an established reference method. This approach assumes that the uncertainty of the reference method is known.
2. If the certified reference material is not available, a blank matrix of interest can be spiked with a known concentration by weight or volume
3. Analyse a sample of known concentration and comparing the measured value to the true value as supplied with certified reference material

As the accuracy assessment measures the effectiveness of sample preparation, so the sample preparation must be carry out as good as possible to get a good recovery which close to actual value. In this study, the second technique has been chosen as a tool to access the accuracy. The accuracy was reported as a percent recovery ( % recovery). A good recovery value shows that the method applied is accurate. The formula to calculate the recovery is shown below:

$$\text{Accuracy} = \frac{(\text{Spiked sample} - \text{Unspiked sample})}{\text{Concentration of standard}} \times 100\%$$

Table 3.1 : Analysis of spiked standard

Element	Colour	Concentration of spiked standard		
		1 mg L <sup>-1</sup>	3 mg L <sup>-1</sup>	5 mg L <sup>-1</sup>
Lead	Red	71	78	80
	Blue	88	96	98
	Orange	78	92	92

In this study, the recovery obtained were between 72 – 98 % which are in good range as shown in Table 3.1. Good recovery shows that the method utilized in this study is accurate. A perfect 100% recovery is probably not achievable in the real world for most analytes in complex matrices. Whether a certain percentage recovery is satisfactory depends on the analyte, the sample matrix and the method. For example, the acceptable surrogate standard limit for volatile organic compounds in water and soil samples is approximately 85 – 115% and 80-120%, respectively. The same limits for base/neutral semi volatiles compounds are approximately 30-120% and 20-125% for water and soil samples respectively (Popek, 2003).

### 3.4 Precision

Precision is defined as the degree of agreement between replicate measurements of the same quantity. ISO 3534-1(2006) describes precision as a measure of the closeness (degree of scatter) between independent test results obtained under stipulated conditions (stipulated conditions can be, for example, repeatability, intermediate precision, or reproducibility). Precision is usually expressed numerically by measures of imprecision, such as standard deviation (less precision is reflected by a larger standard deviation) or relative

standard deviation (co-efficient of variance) of replicate results. In this study, coefficient of variance is calculated. Higher degree of precision, gave greater chance of obtaining the true value. Precision can be determined by accessing the repeatability and reproducibility of the method. Repeatability or intra-day precision is a series of results obtained using the same method, on the identical test material under same conditions (same operator, same apparatus, same laboratory) and the results were obtained from the same day. ICH recommends repeatability to be assessed using minimum of nine determinations covering the specified range for the procedure. In this study, ten (10) measurements were taken. Reproducibility or the inter-day precision is a series of results obtained using the same method on identical test material under different condition and in this study the results were obtained from three different days. The repeatability (intra-day precision) and reproducibility (inter-day precision) were measured based on their coefficient of variation (CV) or sometimes called relative standard deviation.

Coefficient of variation (CV) is calculated using the following formula;

$$\text{Coefficient of variation} = \frac{\text{standard of deviation}}{\text{mean}} \times 100\%$$

Table 3.2: Coefficient of variation (CV) of the measurements (n=10)

Elements	Colour	Coefficient of Variation (%)	
		Intra-day Precision	Inter-day Precision
Lead	Orange	1.16	1.93

The precision of the method was achieved with the coefficient of variation was less than 5% for both intra-day and inter-day precision. A good coefficient of variation shows that

the method utilized in the study can provide reliable results. The lower the value of the CV the higher the precise of the data and precise data represent a good analytical method.

### **3.5 Limit of Detection (LOD) and Limit of Quantification (LOQ)**

The detection limit of an analytical method tells how low a concentration can be said to be measured. Limit of detection is defined as the smallest concentration or absolute amount of analyte that can be reliably detected. According to IUPAC, detection limit is defined as the smallest concentration or amount of analyte that has significantly larger than the signal arising from a reagent blank. Limit of quantification is described as the smallest concentration or absolute amount of analyte that can be reliably determined at a known confidence level. The limit of detection and limit of quantification is measured to determine the sensitivity of the method employed in this study.

In this study, a total of 10 replicates (n=10) of blank sample were analysed and the mean values and its standard deviation was determined. The formula used to calculate the limit of detection (LOD) and limit of quantification (LOQ) is as follows:

$$\mathbf{LOD = Mean + 3SD}$$

$$\mathbf{LOQ = Mean + 10SD}$$

**\*SD : Standard deviation**

The result showed that the limit of detection (LOD) and limit of quantification (LOQ) of lead in wax crayon was 0.062 mg L<sup>-1</sup> and 0.061 mg L<sup>-1</sup> respectively. Therefore, the analytical method adopted was very sensitive to all the analyses studied.

### 3.6 Concentration of Lead in Red, Blue and Orange Wax Crayon

The analysis of lead in different colour of wax crayon by using Atomic Flame Spectroscopy is reported in ppm ( $\text{mg kg}^{-1}$ ) unit. The concentration ( $\text{mg kg}^{-1}$ ) of lead by weight of total dry solids in the test sample is calculated according to following equation:

**Concentration of metal ( $\text{mg kg}^{-1}$ )**

$$= \frac{\text{Concentration (mg/kg)} \times \text{Dilution factor} \times \text{volume of solution (L)}}{\text{Mass of sample (kg)}}$$

Table 3.3 represent the concentration of the lead in different colour of wax crayon in this study.

Table 3.3: Concentration of Lead in different colour of Wax Crayon

Concentration of metals ( $\text{mg kg}^{-1}$ )	Different Colour of Wax Crayon		
	Red	Blue	Orange
Lead	$25.23 \pm 3.26$	$21.21 \pm 3.44$	$32.15 \pm 0.35$

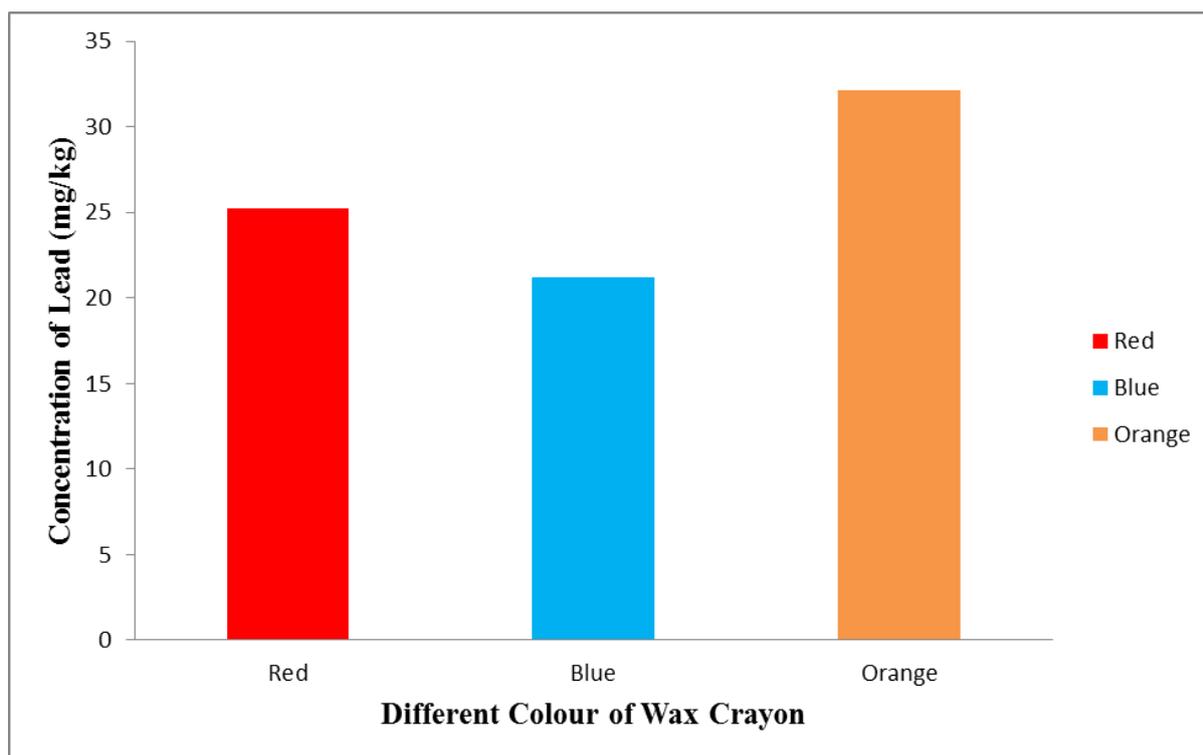


Figure 3.4 : Concentration of Lead in different colour of Wax Crayon

From the analysis that had been done, the results showed that the concentration of lead in orange ( $32.15 \pm 0.35 \text{ mg kg}^{-1}$ ) colour wax crayon was the highest compare to red ( $25.23 \pm 3.26 \text{ mg kg}^{-1}$ ) and blue ( $21.21 \pm 3.44 \text{ mg kg}^{-1}$ ) colour wax crayon. The least amount of lead content in wax crayon among the three colour studied was belonged to the blue colour wax crayon. The present of lead in wax crayon is probably due to the utilization of pigment containing lead for wax crayon colouring. It was found out in previous study done on water colour by Yee. (2010), showed that the highest concentration of lead was found in orange colour. The results are in accordance to the results found in this study.

Based on the study done by CPSC, the concentration of lead was in the range between 20 and  $33 \text{ mg kg}^{-1}$  which is considered safe. The hazard assessment is based on assumption that a child will ingest a total of 14 g of crayon per month consumed randomly from the package of crayons. 14 g of crayon is approximately 3 pieces of wax crayon of same size as

crayon used in this study. One kilogram of wax crayon is equal to 227 pieces of wax crayon (each weight approximately 4.4 g). Approximately, there was 0.132 mg of lead in each crayon. Based on assumptions of 3 pieces of crayon eaten for a month, it can be calculated that, a child is exposed to approximately 0.4 mg of lead/month (assuming the crayon a child ate was either blue, red or orange colour). In average, the amount of lead is about 13 ug/day. Directorate for Health Sciences staff estimated that a greater than 15 ug/day intake of available lead could cause a previously normal child to exceed a 10 ug/dl blood level. The calculation is based on the assumption that the child only ate the blue, red or orange which determined in this study to have content of lead. Therefore, the concentration of lead in blue, red and orange colour wax crayon found in this study is considered safe.

### **3.7 Statistical Analysis**

#### **3.7.1 *t*-test**

An independent sample *t*-test, two tails distribution assuming equal variance was performed to the red, blue and orange wax crayon. The confident interval in this statistical analysis is at 95%.

According to the results in this study, at 95% confident level, there is no significant difference between content of lead in red (M=0.152, SD=0.020) and blue (M= 0.128, SD= 0.020) wax crayon conditions;  $t(4) = 1.46$ ,  $p = 0.22$ . However, at 95% confident level, there is enough evidence to show that there is significant difference between content of lead in orange (M=0.193, SD= 0.002) and blue colour (M= 0.128, SD= 0.020) conditions;  $t(4) = 5.46$ ,  $p = 0.005$ . Therefore, at 95% confident level, there is enough evidence to show that there is significant difference between content of lead in orange (M=0.193, SD= 0.002) and red colour wax crayon (M= 0.152, SD= 0.020) conditions;  $t(4) = 3.65$ ,  $p = 0.02$ . Hence, the

results suggested that the concentration of lead in orange colour wax crayon varied from the concentration of lead in red and blue colour wax crayon.

### **3.8 The Recommended Daily Allowance of Lead**

The recommended daily allowance (RDA) of lead is zero. According to the Consumer Product Safety Commission (CPSC) Policy on toys/articles, lead was banned in miniblinds, crayons, toy figurines and children jewellerys since 1998. However, blood level of less than 10 ug/dl and 80 ug/dl were considered safe for children and adult respectively. World Health Organization (WHO) has established a Provisional Tolerable Weekly Intake (PTWI) for lead of 25 µg/kg of body weight (equivalent to 3.5 µg/kg of body weight per day). This level refers to lead from all sources and was set to protect all humans, including infants and children.

However, Directorate for Health Sciences staff estimated that a greater than 15 ug/day intake of available lead could cause a previously normal child to exceed a 10 ug/dl blood level. Considerations by the staff in developing the 15 ug/day level included estimated blood lead levels due to "normal" sources, increased lead absorption by young children compared to adults, and the relationship of intake and blood lead levels. A blood lead level above 10 ug/dl places a child at risk for serious, adverse health effects according to the Centres for Disease Control and Prevention, CPSC staff, and several other Federal agencies. Ten (10) ug/dl is not a threshold blood lead level for adverse health effects.

Intake levels below 15 ug/day contribute to the elevation of a blood lead level along with other lead sources. Contributions less than 15 ug/day are still of health concern. A child

with a marginal blood lead level could be pushed above 10 ug/dl when exposed to an additional lead source, such as leaded crayons. Exposure to crayons are most probably during the drawing and colouring activities. In some cases, children tend to eat the crayons and yet few studies have been done to estimate intake of crayons and hazard assessment by children in a month, (CPSC, 1994).

## CHAPTER 4

### CONCLUSION AND RECOMMENDATIONS

#### 4.1 Conclusion

From this study, the concentration of different colour (red, blue and orange) of wax crayon using Flame Atomic Absorption Spectroscopy was successfully determined and compared. The results obtained showed that the analytical method employed in this experimental study was reliable and accurate. Microwave digestions as sample decomposition method also proven to give a good recovery data. Therefore, the method used for the determination of lead in different colour of wax crayon can be concluded as simple and sensitive method which produced reliable results. The method is successfully verified as in house method for the particular analysis.

Orange colour wax crayon exhibits the highest concentration of lead among the three colour of wax crayon analysed in this study. The concentration of lead present in red, blue and orange colour wax crayon was  $25.23 \pm 3.26 \text{ mg kg}^{-1}$ ,  $21.21 \pm 3.44 \text{ mg kg}^{-1}$  and  $32.15 \pm 0.34 \text{ mg kg}^{-1}$  respectively. The concentration of lead of those three colour of wax crayon analysed in this study were not followed the allowable limit set by the Consumer Product Safety Commission (CPSC) which banned the content of lead in crayons. Based on the results obtained, the method applied in this study can be implemented as in-house method for the determination of lead in wax crayon using Flame Atomic Absorption Spectrometry.

## **4.2 Recommendations**

A study of the other colours of wax crayon which were not covered during this study should be done. The same methodology can be applied since the results provided were reliable. It is also recommended to do the same analysis but by different instrumentation such as inductively coupled plasma-mass spectrometry (ICP-MS) and graphite furnace atomic absorption spectrometry (GF-AAS) to compare the results obtained from this study.

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