UNIVERSITI SAINS MALAYSIA

Analysis of Cadmium in Infant Milk Powder Using The Differential Pulse Anodic Stripping Voltammetry

Dissertation Submitted in Partial Fulfillment for The Degree of Bachelor of Science (Hons) in Forensic Science

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Siti Zaharah binti Md Isa

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LIST OF ABBREVIATIONS

AAS	- Atomic Absorption Spectrometry
ASV	- Anodic Stripping Technique
Cd	– Cadmium
CSV	– Cathodic Stripping Voltammetric
DPASV	- Differential Pulse Anodic Stripping Voltammetry
DPP	- Differential Pulse Polarography
EAAS	- Electrochemical Atomic Absorption Spectrometry
E_{acc}	- Deposition potential
E_f	- Final Potential
E_i	- Initial potential
GFAAS	- Graphite Furnace Atomic Absorption Spectroscopy
HMDE	- Hanging Mercury Drop Electrode
ICP-AES	- Inductively Coupled Plasma Atomic Emission Spectrometry
ICP-ES	- Inductively Coupled Plasma Emission Spectrometry
K	- Kelvin
T_{acc}	- Deposition time

LIST OF SYMBOLS

$\overline{X}$	- mean
%	- percentage
° C	- Celcius
µg/kg	- microgram per kilogram
µg/l	- microgram per Litre
µl	- mircolitre
mg	- miligram
mg/kg	- milligram per kilogram
ml	- militre
ppb	- part per billion
ppm	- part per million
R	- Correlation Coefficient
s	- second
SD	- Standard Deviation
V	- Volt
V/s	- Volt per second

ABSTRACT

An anodic stripping voltammetric (ASV) technique was used for determination of the cadmium in infant milk powder by using the hanging mercury drop electrode (HMDE). Ten samples of infant milk powder which are suitable from zero to twelve months (one year) from different brands and manufactures from the hypermarket and pharmacies in Kubang Kerian area were bought and analyzed. The samples were digested in nitric acid solution by using microwave high pressure digestion technique at 210 °C. The determination of the cadmium trace elements was made in acetate buffer at pH 4.6 and was stirred at 2000 rpm. The optimize parameters for the voltammetric determination of the cadmium are: initial potential, E_i = -0.70 V; final potential, E_f = 0.50 V; deposition potential, E_{acc} = -1.15 V; deposition time, t_{acc} = 90 s; equilibrium time, t_{eq} = 10 s; sweep rate = 0.06 V/s; pulse amplitude = 0.05 V; and purge time = 300 s. Cadmium was not detected in all samples except for samples 9 and 10 which showed the Cd contents were about 12.458 ± 2.899 and 31.517 ± 4.320 ppb respectively. This amount is under limit state by the World Health Organization 1972 about 1 ug/kg/day cadmium for children.

ABSTRAK

Teknik anodik pelucutan voltammetrik telah digunakan untuk menentukan kadmium dalam susu tepung bayi dengan menggunakan elektrod titisan raksa tergantung *(HMDE)*. Sepuluh sampel susu tepung bayi yang sesuai daripada usia daripada sifar hingga dua belas bulan (satu tahun) dari jenama dan pengeluar berbeza daripada pasar raya dan farmasi di kawasan Kubang Kerian telah dibeli dan dianalisa. Sampel telah dicerna dalam asid nitrik melalui pencernaan gelombang mikro tekanan tinggi pada suhu 210 °C. Penentuan unsur surih kadmium dibuat dalam larutan acetate buffer pada pH 4.6 dan dikacau pada kuasa 2000 rpm. Parameter optimum voltammetri bagi penentuan kadmium adalah: potensi awal, Ei = -0,70 V; potensi akhir = 0.50 V; potensi pemendapan, Eaac = -1,15 V; masa pemendapan, tacc = 90 s; masa keseimbangan, teq = 10 s; kadar imbasan = 0.06 V / s; denyut amplitud = 0.05 V; dan masa pembebasan oksigen = 300 s. Kadmium tidak dikesan dalam semua sampel kecuali sampel 9 dan 10 yang menunjukkan kandungan Cd kira-kira 12.458 ± 2.899 dan 31.517 ± 4.320 ppb masing-masing. Jumlah ini adalah di bawah kadar had oleh *World Health Organization* 1972 kira-kira 1 ug/kg/hari kadmium untuk bayi.

CHAPTER 1

INTRODUCTION

1.1 Milk

During early growing age, milk is the basic food for the infants that must fulfill the entire nutritional requirement (Zamir & Hussain, 2001). Infant formula is the artificial substitute for the human breast milk and it composition is as closely as possible must mimic human milk as closely as possible (Lamia & Amal, 2008). Milk powder for infants is very important and acts as the special kind of human nutrients that give source of major and trace essential elements for the normal biological growth of infants (Borkowska-Burnecka *et al.*, 1996). The infant milk powder composition will change according to the infant age. The composition is very important in infant milk due to children that have low weight and immunity is lower.

The infant formulas are manufactured in powdered, liquid concentrated and liquid ready-to-eat forms. There are about four types of infant's formulas types. Which are milk, soy-protein, protein hydrolysate and amino acid based formulas. The basic components in the infant formula are the carbohydrate, proteins, fats and their properties, vitamins, and minerals (Nasirpour *et al.*, 2006).

1.2 Heavy Metals

Group of heavy metals involve some metalloids, transition metals, basic metals, lanthanides and also actinides. By individual or compounds, the heavy metal is the toxic metals that will give negative effect towards people health (Business Education, 2015). Heavy metals like lead, mercury, arsenic and cadmium will cause poisoning to human health. Some of the heavy metals such as zinc, copper, chromium, iron and manganese are necessary for human life if consume it in small amount, but it will become toxic if use in large amount (Business Education, 2015). Some metals been called heavy metals due to their high density, high atomic number and high atomic weight in the nature and can cause damage to animals, human and plants even in low concentration (Helmenstine, 2015).

1.3 Cadmium

Cadmium or with a symbol of Cd is the heavy metals that can be found in environmental contaminant. It is the rare element that originates from natural occurrences and also from industrial and agricultural sources. It usually occurs in a small amount that associated with the zinc ore like sphalerite (ZnS). The cadmium can be obtained as a by-product of the zinc, copper and also lead ore refining operations.

It was first discovered by the Friedrich Stromeyer in 1817 as an impurity of the zinc oxide and it was discovered independently by the Karl Hermann, a German Chemist in 1818. The name of the cadmium is from *cadmia* and the latin name for *calamine*. Calamine is the old name for the zinc ore (Chemicool, 2015). It present as the complex oxides, sulphides, and carbonates in zinc, lead and ores when in the natural conditions (Kabir *et al.,* 2014).

Cadmium is a soft metal. It is malleable, ductile, a bluish-white metal that is easily cut with a knife, flexible and very fusible (Chemicool, 2015). It can acts as the excellence electrical conductor and good resistance to corrosion and chemicals. Cadmium is soluble in acids especially nitric acids and slowly soluble in hydrochloric acids, insoluble in alkalis and slightly soluble in water (Chemicool, 2015; Kabir *et al.*, 2014).

Cadmium is the solid state metal that is found in earth crust and has atomic number of 48 at the periodic table. Classification as the transition metal types with 112.4 g molecular weight. At 20°C, it has density of 8.642 g/cm^3 (Kabir *et al.*, 2014). The melting point for cadmium is about 321.1°C or 594.3 K and the boiling point is 765°C or 1038 K with relatively high vapour pressure (Chemicool, 2015; Kabir *et al.*, 2014). It always been used in batteries, alloys for bearing, and electroplating. In batteries, cadmium common use in rechargeable nickel cadmium (Nicad) batteries (Chemicool, 2015).

Human can be exposed towards the cadmium at work or in the environment itself. Once the cadmium been ingested, inhaled or by other route to human body, it will efficiently retained in the body (Bernard, 2008). It will accumulate throughout the life. This trace element will accumulate in the kidney or more precise it accumulates at the proximal tubular cells. This will become toxic to kidney. Bernard studied in 2008, showed that bone demineralization will happen by direct bone damage or indirectly as a result of renal dysfunction. Based on studied by Horton *et al.* (2013), because children and infant consume more food in daily to support their growth, they will absorb metal more readily than adults. By this circumstance, the children and infants will have high exposure to the metals.

1.4 Determination of Cadmium

The analytical techniques that are suitable and sensitive are needed for the detection of the toxic and essential trace elements in food (Muntean *et al.*, 2013). The modern techniques with capable multi element that require for determinations of the elements are flame atomic absorption spectrometry (FAAS), capillary zone electrophoresis, inductively coupled argon plasma emission spectroscopy, inductively coupled plasma optical emission spectrometry (ICP OES), atomic flourescene, and differential pulse anodic stripping voltammetry technique

(Amponsah, 2014). Muntean *et al.* (2013) also state that, the heavy metals like cadmium can be detected using the electrochemical atomic absorption spectrometry (EAAS), inductively coupled plasma-atomic emission spectrometry (ICP AES) and microchip. Inam and Somer study in 2000 state that except for the voltammetric technique, the other technique like ICP AES and X ray fluorescence need cost. Those techniques also do not give enough sensitivity required in food, biological and environmental sample in determination for the most elements at the trace to ultra trace concentration (Jannat *et al.*, 2009).

The study by Alghamdi (2010), conclude that voltammetric techniques can be used in determination of food contaminations like toxic metals, pesticides, fertilizers, and veterinary drugs residuals; trace essentials elements, food additives dyes and also other significances biological organic compounds. Stripping voltammetric has been used widely in nowadays various chemical analysis fields. Application of voltammetric technique gives highly effective result in analyze of molecules from high biological significance such as vitamins, alkaloids and natural organic acids. The voltametric technique such as the anodic stripping voltammetric (ASV), cathodic stripping voltammetric (CSV), differential pulse polarography (DPP), and adsorption voltametry are highly sensitive, selective, inexpensive, high accuracy, high precision and easy to run (Inam and Somer, 2000; Alghamdi, 2010 and Sadeghi *et al.*, 2014).

In this experiment, differential pulse anodic stripping voltammetric (DPASV) been used in determination of the cadmium in infant milk powder. Differential pulse anodic stripping voltammetry (DPASV) is the voltammetry technique that use with the hanging mercury drop electrode (HMDE) (Jannat *et al.*, 2009). It mainly applied to trace analysis of heavy metals ions such as lead, mercury, arsenic and cadmium. ASV basis for the analysis of metals is the electrolytic dissolution of metal which before that had been deposited on the mercury electrode.

Objective of this study generally is to analyze cadmium in infant milk powder by using the voltammetric technique. In specific, this study objective is to analyse the cadmium in infant milk powder samples using the differential pulse anodic stripping voltammetric technique. Secondly, the specific objective is to determine level concentration of cadmium that present in various brands of infant milk powder samples.

CHAPTER 2

LITERATURE REVIEW

Studied by Sadeghi *et al.* in 2012 reported that cadmium will enter the body through food chain naturally and it cannot be avoided. If exposed towards it, kidneys become dysfunction and cause estrogenic properties. The differential pulse voltammetry is the best technique to determine the trace elements due to it inexpensive, sensitivity and selective characteristics. The direct method for determination of cadmium, zinc, copper and lead in baby weaning food and powder milk from Tehran market by the differential pulse anodic stripping voltammetry (DPASV) been carried out by Sadeghi *et al.* (2012) using the hanging mercury drop electrode (HMDE). About 120 samples of each brand from Tehran market were measured three times; the result of the concentration was analyzed and compared with the data from WHO and UNICEF. In this study, cadmium had been detected in baby food type I and was higher than baby food type II. Cadmium level showed highest in infants formula than the other two baby food types, but, the amount of Cd in infant food and formulas does not exceed the limit or standard levels of $P<0.01$. The different in amount of the Cd in baby food type I or wheat type and type II or rice type maybe from ability and capability of plants to intake the trace elements from water, soil and air. Other than that, difference level of trace elements in infant's formula due to the different manufacturing practices, quality of raw materials, and packaging containers used.

In 2010, a voltammetric technique was carried out by De Castro *et al.* (2010). Whereby simple of anodic stripping voltammetric (ASV) technique with the hanging mercury drop electrode (HMDE) was used for determination of the toxic metals (Pb and Cd) and their respective antagonist (Ca and Zn) in infant formulas and milk marketed in Brasilia, Brazil. The concentrations of the trace elements were successfully detected in 55 samples of infant formulas and milk in Brasilia. In powdered samples, Pb and Cd concentrations were 0.109 mg/kg and 0.033 mg/kg respectively; this was below the limit of detection and references safety levels. The fluid samples also show the result of Cd low the limit of detection but the Pb level concentration show high amount with 0.084 mg/kg. Cd and Pb can exposed towards the child and infants from atmospheric deposits, continuous application of large amounts of fertilizer, disposal waste sludge and vehicle emissions. Both studies by the Sadeghi *et al.* (2012) and De Castro *et al.* (2010) stated that packaging and technological process can bring the infants food and formula contaminate with Cd and Pb. Polluted environment and accidental contamination will transfer the heavy metals to the cow's milk to be consume by the infants.

The simultaneous determination of lead, cadmium, copper and zinc in infant formula by anodic stripping voltammetry was conducted by the Jannat *et al.* in 2009. The differential pulse anodic stripping voltammetry (DPASV) with hanging mercury drop electrode (HMDE) was applied to analyze the concentration of trace elements in infant formula that available in Iran. DPASV is inexpensive, selective and most sensitive technique in determination of the trace metals. The limits of detection of cadmium, lead, copper and zinc are 0.005, 0.005, 0.01, and 0.05 mg/kg respectively. From the analysis done by the Jannat *et al.* (2009), the concentration of the cadmium, lead, copper and zinc in one of the infant formulas available in Iran were 0.359 ± 0.215, 0.384 ± 0.222, 4.436 ± 0.811 and 37.426 ± 8.951 mg/kg (mean SD), respectively.

Graphite furnace atomic absorption spectrophotometer was used in determination of lead (Pb) and cadmium (Cd) in some baby foods and cereal products (Muntean *et al.*, 2013). 85% from the samples of cereal and baby foods showed the cadmium detection ranged from 0.3 to 10.37 ug/kg and for less than 15% of samples cadmium under LOD. About 22 milk powder samples analyses in this study, the cadmium concentration were ranged from 0.1 to 4.75 ug/kg and 18% samples having the cadmium levels under the limit of detection. Even though the amount of cadmium obtained from analysis, but the levels is not higher than the maximum allowed according to the Commission Regulation (EC) No 1881/2006 where 100 ug.kg of cadmium in cereals and 20 ug.kg in infants formula for lead and 200 ug/kg for lead in cereals.

The studied from Zamir and Hussain in 2001 showed the determination of the lead and cadmium level in powdered milk in Quetta (Pakistan) using the atomic absorption spectrometry (AAS) (Zamir and Hussain, 2001). AAS was used due to a better time saving, more sensitivity and high specific. The samples of different brands of powdered and infants formula powdered were collected from the local market. The analyses showed the detection of cadmium and lead but still to be within the safe limits as recommended by the World Health Organization (WHO). The daily permission intake (DPI) in children by WHO for Pb is 5 ug/kg /day and 1 ug/kg/day for Cd. The concentration of cadmium and lead in infant formula milks that was analyzed were ranged from 0.003-005 ppm and 0.1-0.2 ppm respectively. Even though lead intake is quite higher than cadmium concentration from infant baby formula and whole cream milk but these value are much lower than the tolerance level of 0.429 mg of lead and 0.0555-0.07 mg of cadmium by WHO standard.

Randomly collected 11 samples of infant formula (powdered milk) from the pharmacies representing many brands for infants from birth to second year of their life and 16 samples of cow's milk were collected from 8 dairy shops (Lamia and Amal, 2008). The samples in this study were analyzed using the atomic absorption spectrometry after been digested by trichloroacetic acid extraction method. In infant formula, the mean level for Cd is 0.0012±0.0011 ug/ml and Pb is 0.1563±0.0624 ug/ml. Lamia and Amal (2008) analysis showed that two samples out of eleven did not detect the present of cadmium. The estimated weekly intake (EWI) was calculated in all samples for both Pb and Cd according to feeding table for a normal healthy baby for each type of milk. The EWI for both Pb and Cd showed lower result than 7 ug/kg BW/week in Provisional Tolerable Weekly Intake (PTWI) according to the WHO expert group.

A study by Winiarska-Mieezan (2009) stated that amount of cadmium is 0.01 mg/kg as accepted by the Journal of Laws 2003. The assessment of the infant exposure towards the cadmium (Cd) and lead (Pb) content in infant formulas that purchased in different groceries in Lublin were successfully determined by atomic absorption spectrometry. The six samples out of eight samples showed the level of cadmium under the references standard level by Journal of Laws and two samples did not satisfy the the requirements regarding the cadmium level. This will become dangerous to infant because they do not have the effectively functioning regulatory mechanism and can easily absorb mineral elements including toxic elements than in older children and adults (Winiarska-Mieezan, 2009).

The other method to detect the cadmium is the inductively coupled plasma atomic emission spectrometry (ICP-AES). In 1996, the Borkowska-Burnecka *et al.* had used this method for determination of the major and trace elements in powdered milk. Eight samples of powdered milk had been studied and digested using the conventional hot plate and microwave high pressure procedure. The ICP-AES are quick and precise method for the various elements determination of the major and also trace elements in milk powder products. The result show more accurate by the using of digestion method of microwave high pressure digestion. Microwave digestion is quicker and effective for the milk powder treatment than the conventional wet digestion hot plate technique. Cadmium, chromium and lead were not detected in all eight samples.

In 2014, a study by the Amponsah using inductively coupled plasma emission spectrometry (ICP-ES) to determine the levels of heavy metals of arsenic (Ar), lead (Pb), cadmium (Cd) and mercury (Hg) in tin milk produced in Ghana. Cadmium showed the lowest level in milk in Ghana than mercury, arsenic and lead. Mercury gives the highest level detection in milk. From the statistical results, the examined samples in tin milk have Pb, Ar, Cd and Hg levels higher than the permitted limit. The ICP-ES was used for determination due to the sample size and the availability of the dissolved ash for analyzed the cadmium in sample.

The graphite furnace atomic absorption spectrometry (AAS) is the other technique that used in determination of lead (Pb) and cadmium (Cd) in raw cow's milk (Elatrash and Atoweir, 2014). Elatrash and Atoweir (2014) stated that the graphite furnace atomic absorption spectrometry (AAS) is a popular technique that is widely used for determination of Pb and Cd in foodstuffs. It is a direct technique for determination of trace elements in difficult matrices, give high sensitivity, high precision and accuracy. The various location taking the raw cow's milk give the different levels of the Pb and Cd. The mean concentration of the Cd in raw cow's milk is 1.24 ug/kg with a range of (0.64-2.74) ug/kg for 45 samples. This value is very low and below the maximum permission limit of 50 ug/kg w.w by European Commission (EC) Regulation 2001/466.

All analysis technique that can be used for the cadmium (Cd) determination in various samples is summaries in Table 3.0.

Table 3.0: The summary of the analysis techniques for determination of cadmium (Cd) in various samples.

No	Techniques	Samples	References
1	Differential Pulse Anodic Stripping Voltammetry (DPASV)	Baby weaning food and powder milk	Sadeghi *et al.*, 2012
2	Anodic Stripping Voltammetric (ASV)	Infant formulas and milk	De Castro *et al.*, 2010
3	Differential Pulse Anodic Stripping Voltammetry (DPASV)	Infant formula	Jannat *et al.*, 2009
4	Atomic Absorption Spectrometry (AAS)	Baby food and cereal products	Muntean *et al.*, 2013
5	Atomic Absorption Spectrometry (AAS)	Powdered Milk	Zamir and Hussain, 2001
6	Atomic Absorption Spectrometry (AAS)	Infant formulae	Lamia and Amal, 2008
7	Atomic Absorption Spectrometry (AAS)	Infant formulas	Winiarska-Mieezan, 2009
8	Inductively Coupled Plasma Atomic	Powdered milk	Borkowska-

	Emission Spectrometry (ICP-AES)		Burnecka *et al.*, 1996
9	Inductively Coupled Plasma Emission Spectrometry (ICP-ES)	Tin milk	Amponsah, 2014
10	Graphite Furnace Atomic Absorption Spectroscopy (GFAAS)	Cow's milk	Elatrash and Atoweir, 2014

CHAPTER 3

OBJECTIVES

3.1 General Objective

To analyze cadmium in infants milk powder by using voltammetric technique

3.2 Specific Objectives

1) To analyse the cadmium in infants milk powder samples using the differential pulse anodic stripping voltammetric technique.

2) To determine the level concentration of cadmium that present in various brands of infants milk powder samples.

CHAPTER 4

RESEARCH METHODOLOGY

4.1 Instruments

The instrument that was used to analyze the cadmium in infant milk powder is 797 VA Computerace Metrohm Voltammetric Analyzer with a Hanging Mercury Drop Electrode (HMDE) as shown in figure 4.0. This instrument functions as the current voltammetric measurements. Silver-silver chloride electrode (Ag/AgCl) filled with the 3M potassium chloride (KCl) is used as the reference electrode (RE). The platinum rod electrodes act as the auxillary electrode (AE) and the Hanging Mercury Dropping Electrode (HMDE) is the working electrode.

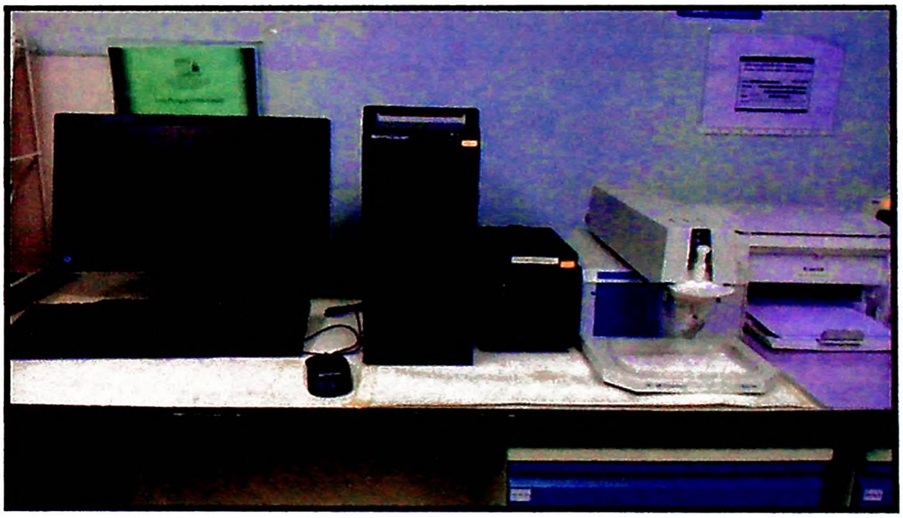

Figure 4.0: 797 VA Computerace Metrohm Voltammetric Analyzer with three electrode system

4.2 Apparatus

1) Volummetric flask 25.0 ml and 100.0 ml from Scoott Duran from Germany.

2) Pipette tube 10.0 ml from Hirshmann® Techcolor from Germany

3) Micropipette 10.0 µl, 100.0 µl and 200.0 µl from Eppendorf Research from US

4.3 Materials

4.3.1 Standard Solution and Cadmium Stock

The 1000 ppm standard solution of the Cd is supplied by the Merck KGaA, Damstadt, Germany. The stock solution of Cd was prepared to concentration of 0.1 ppm by diluting 10.0 µl of Cd standard of 1000 ppm with the 99.99 ml of deionised water in 100.0 ml volumetric flask.

4.3.2 The Infant Milk Powder Samples

About 0.2 gram of each ten samples of infant's milk powder which are suitable for zero to twelve months (one year) from different brands and manufactures labeled as 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 were selected to be analyzed for the presence of cadmium.

4.4 Reagents

All the reagents used were analytical grade. Nitric acid, HNO_3 (65%) from Merck KGaA, Germany were used in sample digestion using microwave digestion machine. Potassium chloride, KCl from Merck KGaA, Germany and sodium acetate, NaAc from Merck KGaA, Germany were used for the preparation of pH 4.6 acetate buffer. 1M hydrochloride acid, HCl from Merck KGaA, Germany and 1M sodium hydroxide, NaoH from Merck KGaA, Germany were used to adjust the acetate buffer for maintaining pH of 4.6. All reagents and apparatus that used were washed using the deionised water obtained from the Ultrapure Water SASTECTM.

4.4.1 Acetate Buffer

11.18 g of the KCl and 4.1 g of NaAc were weighed separately using the analytical balance. Then, the compounds were mixed and dissolved completely using dionised water. Then, the solution was transferred into 100 ml volumetric flask. Deionised water was added until the volume is 100 ml.

4.4.2 1M HCl

Measure 8.3 ml of concentrated HCl and pour into 100 ml volumetric flask. Then, the deionised water was added to make up the volume to 100 ml.

4.4.3 1M NaOH

About 4 g of the NaOH was weighed and dissolved completely with deionised water. The solution was transferred into 100 ml volumetric flask. Then, the deionised water was added until reach the mark of 100 ml.

4.5 Equipments

1) Analytical Balance Shimadzu ATX 224 by Uni Bloc from Japan as shown in Figure 4.1 was used for weighing the powder samples of each infants milk powder.

Figure 4.1: Analytical Balance Shimadzu ATX 224

2) Microwave Digestion machine of Anton Paar Multiwave 3000 Microwave Reaction System by Alpha Analitycal (M) Sdn Bhd from Malaysia as shown in Figure 4.2 was used for sample preparation of the infant milk powder.

Figure 4.2: Anton Paar Multiwave 3000 Microwave Reaction System

3) pH 211 Microprosessor pH Meter by Hannainstruments from Portugal as shown in Figure 4.3 used for determination of the pH reading of the acetate buffer reading 4.6 for every time it will be used before run the sample.

Figure 4.3: pH 211 Microprosessor pH Meter

4.6 Sample Preparation

About 0.2 gram from each 10 samples of infants milk powder were weighed in weighing plate using the Shimadzu ATX 224 analytical balanced. About 2.0 ml of 65 % nitric acid was measured by using the pipette tube. Then, the infant's milk powder samples were transferred into the PTFE vessel with 2.0 ml of the 65% nitric acid (HNO_3). The prepared PTFE vessel were put into the rotor HF 100 and digested. The digestion was done by high pressure of power at 600 Pa. It was heated at temperature of 210 °C for about 20 minutes and cooled for 40 minutes which completed within 1 hour. The pressure hole on the cover was opened carefully to release the gas pressure in the PTFE vessel. After that, the PTFE vessel with liquid was transferred into the 25.0 ml volumetric flask by using the filter funnel and filtered. Then, the deionised water was added until reach the mark 25.0 ml of volumetric flask.

4.7 The Analytical Technique

The determination of Cd in infant milk powder samples involved measuring solution of 10.0 ml diluted sample with 1.0 ml of acetate buffer. The pH of the acetate buffer has to been adjusted to 4.6 ± 0.2. About 0.1 ml of 0.1 ppm of Cd standard was spiked into the voltammetric cell. This 0.1 ppm Cd standard was spiked in triplicate. The parameters used to record the voltammogram result of Cd are shown in the Table 4.0. This is an established method developed by metrohm Ltd., 1984.

Table 4.0: The parameters used to record the voltammogram of cadmium in infant milk powder samples

Working Electrode	HMDE
Drop Size	4
Stirrer/RDE	2000 rpm
Measurement mode	Differential Pulse
Purge time	300 s
Pulse amplitude	0.05 V
Deposition potential	-1.15 V
Deposition time	90 s

Equilibration time	10 s
Start potential	-0.70 V
End potential	-0.50 V
Voltage step	0.006 V
Voltage step time	0.1 s
Sweep rate	0.06 V/s
Peak potential of cadmium	-0.56 V

The concentration of cadmium in sample is determined by internal standard calibration curve.

CHAPTER 5

RESULTS

5.1 Result of Samples in ppb

The results for cadmium (Cd) determination in various brands of infant milk powder samples are shown in Table 5.0.

Table 5.0: Result for cadmium content in various brands of infant milk powder samples labeled 1 to 10 tested in triplicates.

Samples	Test	Concentration (ppb)	Dilution factor	Actual concentration (ppb)	Mean conc ± SD (ppb) (n=3)
1 to 8	1	-	25x	-	-
	2	-	25x	-	-
	3	-	25x	-	-
9	1	0.409	25x	10.225	12.458 ± 2.899
	2	0.424	25x	10.600	
	3	0.662	25x	16.550	
10	1	1.070	25x	26.750	31.517 ± 4.320
	2	1.407	25x	35.175	
	3	1.305	25x	32.625	

(-) = not detected

Table 5.0 above showed the result for cadmium (Cd) content in various brands of infant milk powder samples labeled 1 to 10 in triplicates. Samples 1 to 8 do not show any detection of cadmium content. Samples 9 and 10 show small detection of cadmium content which are 12.458 ± 2.899 and 31.517 ± 4.320 ppb (mean ± SD), respectively. From the table, sample 10 give higher Cd content than sample 9.