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Voltammetric Determination of Cadmium in Infant Milk

Dissertation submitted in partial fulfilment for the Degree of Bachelor of Science (Health) in Forensic Science

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ABBREVIATIONS

ASV	- Anodic Stripping Voltammetry
AAS	- Atomic Absorption Spectrometry
DPASV	- Differential Pulse Anodic Stripping Voltammetry
AES	- Atomic Emission Spectrometry
WHO	- World Health Organization
DHHS	- Department of Health and Human Services
EPA	- Environmental Protection Agency
FDA	- Food and Drug Administration
OSHA	- Occupational Safety and Health Administration
HMDE	- Hanging Mercury Drop Electrode
IUPAC	- International Union of Pure and Applied Chemistry
ppm	- parts per million
ppb	- parts per billion

ABSTRACT

Among the various environmental pollutants, heavy metals like cadmium was considered the most important due to high stability for bioaccumulation they were extremely dangerous for all biological organisms and especially for humans. This is a great importance to analyze and quantify this toxic agent in the environment. Various methods have been done such as atomic absorption spectrometry (AAS), atomic emission spectrometry (AES) in determination of cadmium in matrix. Often these techniques require complex sample preparation and expensive instrumentation. In contrast, voltammetric methods particularly anodic stripping voltammetric (ASV) had some advantages such as little or no sample pretreatment is required, detection limit is as low as 10^{-10} M and low cost instrumentation.

For this work, cadmium was studied in various brand of infant milk. Besides, aim of this work was to improve anodic stripping voltammetric (ASV) method for cadmium detection by optimizing voltammetric condition (pH) and voltammetric parameters such as initial potential (E_i), final potential (E_f), scan rate (v), deposition potential (E_{acc}), deposition time (t_{acc}), and equilibration time (t_{eq}) in ASV. The samples preparation was done by extraction using acid digestion and filtration using filter paper. The samples were analyzed under optimized voltammetric condition and parameters. Cadmium was not detected in any of studied under this project work.

Key Words: Anodic Stripping Voltammetry (ASV), Cadmium, Heavy metal, Infant Milk

INTRODUCTION

i. Brief description of infant milk

Infant formula was a modern artificial substitute for human breast milk. Formulas were designed for infant consumption, and usually based on either cow milk or soy milk (http://en.wikipedia.org/wiki/Infant_formula). Modern scientific research had established that breastfed babies had lower rates of medical problems and hospital admissions, and most major medical and health organizations strongly advocate breastfeeding over the use of infant formula except in unusual circumstances (World Health Organization (WHO), Executive Board, "Infant and Young Child Nutrition", 2001).

Infant formula is available in powder, liquid concentrate and ready-to-feed forms, which were prepared by the caregiver or parent in small batches and fed to the infant, usually with either a baby bottle or cup. It is very important to measure powders or concentrates accurately to achieve the intended final product. It was advisable that all equipment that comes into contact with the infant formula be cleaned and sterilized before each use. Proper refrigeration was essential for any infant formula which was prepared in advance, since infant formula was especially susceptible to bacterial growth. Powdered, cow's milk-based infant formulas were not recommended for premature or sick infants or for infants under one month of age due to lack of nutrition and antibody that help to build the immunization in their body.

In daily quality control of milk and milk products required fast, sensitive, accurate and precise analytical methods. The importance of elements such as Cu, Cr and Fe is related to lipid oxidation involved in storage and processing (H.Jonsson et al., 1976). Serious attention is paid to toxicological effects of other heavy metals such as Cd and Ni in view of the importance of milk and its by-products in diet of infant and children (J.Koops et al., 1978).

ii. Brief description of heavy metal

Heavy metal was the one of 23 chemical elements that has a specific gravity (a measure of density) at least five times that of water. As many other metal, they can be found in the Earth's shell. Human body also contain small amount of these substances. Mainly they get to organisms via food, drinking water and air.

The heavy metals most often implicated in human poisoning are lead, mercury, arsenic, and cadmium. Some heavy metals, such as zinc, copper, chromium, iron, and manganese, were required by the body in small amounts, but these same elements could be toxic in larger quantities.

Danger of heavy metals was in their ability for bioaccumulation. Bioaccumulation was a deposition of a chemical substance in a biological organism with an increased of its concentration in the organism every time this substance was emitted to the environment independently from its amount. Accumulation of compounds in living things is faster

than its decomposition and happens at any time they were exposed to the substance (Ed. Anthony S. Fauci, et al., 1997).

iii. Brief description of Cadmium

Cadmium (atomic number 48; relative atomic mass 112.40) is a metallic element belonging, together with zinc and mercury, to group IIb of the periodic table. Some cadmium salts, such as the sulfide, carbonate, and oxide, are practically insoluble in water; these could be converted to water-soluble salts in nature. The sulfate, nitrate, and halides are soluble in water. The speciation of cadmium in the environment is of importance in evaluating the potential hazard.

Cadmium had no constructive purpose in the human body. This element and solutions of its compounds are toxic even in low concentrations, and will bioaccumulate in organisms and ecosystems. Chronic poisoning by cadmium is called Itai-itai disease (http://www.eoearth.org/article/Health_effects_of_cadmium). One possible reason for its toxicity is that it interferes with the action of zinc-containing enzymes. Zinc is an important element in biological systems, but cadmium, although similar to zinc chemically in many ways, apparently did not substitute or "stand in" for it well at all. Cadmium may also interfere with biological processes containing magnesium and calcium in a similar fashion. Pathways of human contact include soil contamination from industrial releases or landfill and associated leachate processes.

Inhaling cadmium laden dust quickly leads to respiratory tract infection and kidney problems which can be fatal (often from renal failure). Ingestion of any significant amount of cadmium causes immediate poisoning and damaged to the liver and the kidneys. The US Department of Health and Human Services (DHHS) had determined that cadmium and cadmium compounds may reasonably be anticipated to be carcinogens. Cadmium and cadmium compounds are known to be human carcinogens based on sufficient evidence of carcinogenicity in humans, including epidemiological and mechanistic information that indicate a causal relationship between exposure to cadmium and cadmium compounds and human cancer. In addition to human cancer, it can induce the kidney damage and patients also suffered from osteoporosis and osteomalacia.

iv. International Standard Level of Cadmium in Food

Cadmium was not considered to be an essential element in human nutrition. Food was the main source of cadmium intake for humans that were not occupationally exposed. Because it was difficult to reduce cadmium intake from food, the intake from water should be as low as possible.

a. A joint FAO/WHO expert committee has estimated a provisional tolerable weekly intake of cadmium to be between 0.4 and 0.5 mg. Daily consumption of 1.5 L of water containing cadmium at a concentration of 0.005 mg/L would result in the ingestion of about 12 percent of the provisional permissible intake.

b. The maximum acceptable concentration of cadmium in drinking water was therefore 0.005 mg/L. (World Health Organization. Guidelines for drinking water. Vol. 2. Health criteria and other supporting information. Geneva (1984).)

c. The U.S. Environmental Protection Agency (EPA) had set a limit of 5 ppb of cadmium of drinking water. EPA does not allow cadmium in pesticides.

d. The Food and Drug Administration (FDA) limits the amount of cadmium in food colors to 15 parts per million (15 ppm).

e. The Occupational Safety and Health Administration (OSHA) limits workplace air to 100 micrograms cadmium per cubic meter (100 $\mu g/m^3$) as cadmium fumes and 200 μg cadmium/m^3 as cadmium dust.

v. Brief Description of Stripping Voltammetry

Anodic stripping voltammetry was an electrolytic method in which a mercury electrode was hold at a negative potential to reduce metal ions in solution and form an amalgam with the electrode. The solution was stirred to carry as much of the analyte metal(s) to the electrode as possible for concentration into the amalgam. After reducing and accumulating the analyte for some period of time, the potential on the electrode was increased to reoxidize the analyte and generate a current signal. The ramped potential usually uses a step function, such as in normal-pulse polarography (NPP) or differential-pulse polarography (DPP).

The concentration of the analyte in the Hg electrode, C_{Hg}, was given by:

$$C_{Hg} = \frac{i_l \, t_d}{n \, F \, V_{Hg}}$$

Where,

i_l = the limiting current during reduction of the metal.

t_d = the duration of accumulation.

n = the number of moles of electrons transferred in the half reaction.

F = Faraday constant (96,487 coulombs/mole of e^-)

V_{Hg}= volume of the electrode

The expression for current produced by anodic stripping depends on the particular type of Hg electrode, but was directly proportional to the concentration of analyte concentrated into the electrode. The main advantage of stripping analysis was the preconcentration of the analyte into the electrode before making the actual current measurement and make very low detection limit as low as 10^{-10} M.

vi. Other Method

Besides, there have other method that can be used for detection of cadmium. Table 1 shows the other methods, the detection limit and their matrix of sample:

Method	Detection limit	Matrix
Atomic Absorption spectrometry (AAS)	1 to 5 mg/litre 0.1 mg/kg	Water Biological samples
Neutron activation analysis samples/fluids	0.1 to 1 mg/litre	Biological samples
X-ray atomic Fluorescence	17 mg/kg	Biological samples

From: Friberg et al. (1986)

Table 1: The other methods for detection of cadmium in certain matrices

REVIEW OF LITERATURE

The detection of cadmium was important whether the technique was different. Since the effect of cadmium was vulnerable to produce harmless to our body, it may reasonably be anticipated to be carcinogens that can induce many types of cancer (http://www.cancer.wisc.edu/uwccc/article_cadmium.asp) and it no constructive purpose in the human body. Due to that research/studies had been conducted in order to determine the concentration of cadmium in certain medium.

In present paper by M. M. Ghoneim, the scheme analysis was done to determine ultratrace of 11 elements (Cd, Pb, Cu, Sb, Bi, Se, Zn, Mn, Ni, Co, Fe) in water sample using different modes of differential pulse stripping voltammetry at a hanging mercury drop electrode (M. M. Ghoneim et al., 2000). The determination of cadmium in various sample like milk, cheese and chocolate was published in determine cadmium contain was conducted by using instrumental determination-differential pulse cathodic stripping voltammetry (DPCSV) or electrothermal atomic absorption spectroscopy (ETAAS)-as well as a direct analysis method-slurry sampling ETAAS. This instrument was used to determination of Cd, Co, Cr, Cu, Fe, Ni, and Pb in milk, cheese and chocolate. The reliability of the procedure had been verified by analyzing standard reference material. Results obtained were in good agreement with certified values and relative standard deviations (for those results) were in range 5-10 % DPCSV or ETAAS and 3-9 % for slurry sampling ETAAS in range of 2 μg / g (Karadjova et al., 2000).

The investigation of trace element level in body by analyze the Cadmium (Cd), lead (Pb), copper (Cu) and zinc (Zn) levels in khat and 6 leafy vegetables commonly consumed in the Republic of Yemen were determined by differential pulse anodic stripping voltammetry after wet digestion of the organic matter with the hanging mercury drop electrode (HMDE) mode. A platinum rod and a saturated Ag/AgCl electrode were used as auxiliary and reference electrodes respectively for detection of that element (Matloob MH, et al., 2001).

Cadmium is element could be in some other state due to speciation. Anodic stripping voltammetry with a rotating disk electrode was used to investigate the kinetic speciation of cadmium in freshwaters (Lam, M. T. et al, 2001) and in aqueous solutions containing dissolved organic matter (Lam, M. T. et al., 1997). Another technique like gel-integrated Hg-plated-Ir-based microelectrode array in combination with anodic stripping voltammetry was suitable to discriminate between mobile and colloidal metal species in natural waters at nanomolar or subnanomolar levels (Pei, J. et al., 2000). Organic chelates were the dominant chemical ligands of cadmium (73 to 83%) in filtered estuarine water samples from a high-salinity region determined by differential pulse anodic stripping voltammetry (DPASV) (Kozelka et al., 1998).

In the nineteen-fifties, several polarographers such as Drs. Vladimir Cermak and Vogel in Prague, and Dr. Zenon Kublik and Professor Wiktor Kemula in Warsaw, started using the hanging mercury drop electrode instead of the dropping mercury electrode. That meant that they recorded the whole curve with one single drop of mercury (like in

the single-sweep polarography); after the curve was completed, the used drop was knocked off and a new one was produced from the capillary. As this time a stationary electrode has been used, according to the official of International Union of Pure and Applied Chemistry (IUPAC) nomenclature the method thus introduced is not "polarography" any more, it had to be called "voltammetry". The curves were still reproducible, however, unlike the dropping electrode; the hanging drop carries its whole "history" along. This simple fact had been utilized to produce a considerable increase of sensitivity in electroanalysis: using a selected constant potential and controlled stirring, a product of the electrolytic process was quantitatively accumulated at the electrode surface, either in the form of an amalgam or in the form of an adsorbed layer; after an exactly measured time of electrolysis, a linear voltage scan was applied and the current-voltage curve was recorded, displaying the electrode reaction in reverse. This voltammetric operation is known as "stripping", of which three modifications are distinguished: anodic stripping (usually dissolution of a metal from the previously formed amalgam), cathodic stripping (usually reduction of compounds with mercury, previously formed at positive potentials) and adsorptive stripping (usually desorption of species previously accumulated by controlled adsorption). The voltammetric stripping methods allow analytical determinations of species in dilutions as low as one tenth of one billionth molar concentration (M) and lower (Michael Heyrovsky, 2005).

There were several methods of measuring plant-available cadmium (Cd) were compared using soils that had accumulated Cd under normal New Zealand agricultural practices (low total Cd concentrations, and phosphatic fertiliser as the dominant Cd

source). The study encompassed 9 New Zealand soils with different Cd input histories. Cadmium was extracted from these soils by demineralised water, 0.05 M disodium-ethylenediaminetetraacetic acid (Na_2EDTA), 1 M ammonium acetate (NH_4OAc) (pH 7), 0.01 M calcium chloride ($CaCl_2$), and 0.05 M $CaCl_2$ and quantified by differential pulse anodic stripping voltammetry (DPASV) and graphite furnace atomic absorption spectrophotometry (AAS). The DPASV measures the free Cd ion and that associated with labile complexes, but not large organic Cd complexes. Extractable Cd levels were compared with those which are plant-available, as determined by pot studies (lettuce). The 0.01 M $CaCl_2$-extractable Cd measured by AAS and 0.05 M $CaCl_2$-extractable Cd measured by DP-ASV gave the best estimate of plant availability of Cd (P Andrews, 1996).

In a research article by M. Praveen Kumar, the concentrations of heavy metals such as Pb, Cd, Cu, and Zn had been determined by using differential pulse anodic stripping voltammetry (DPASV) in air particulates, diet, and children's blood residing at different locations of Tirupati. The reliability of the procedure for estimation of Pb, Cd, Cu, and Zn in environmental and biological samples by DPASV technique was checked by analyzing various standard reference materials (M. Praveen Kumar et al., 2005).

OBJECTIVE OF THE STUDY

1. To gain an understanding of Anodic Stripping Voltammetry (ASV).
2. To describe basic properties of cadmium and its (Cd) effects on human.
3. To provide other technique for detection of cadmium.
4. To determine cadmium qualitatively and quantitatively in various brands of infant milk.
5. To optimize voltammetric parameters for detection of cadmium in infant milk by using Differential Pulse Anodic Stripping Voltammetry (DPASV) technique.
6. To compare the results obtained by DPASV to the results obtained by Atomic Absorption Spectrometry (AAS).

1. Instrumentation

The experimental set include computer and Metrohm 757 VA Computrace Voltammetric Analyser combined with Multimode Electrode (MME) System.

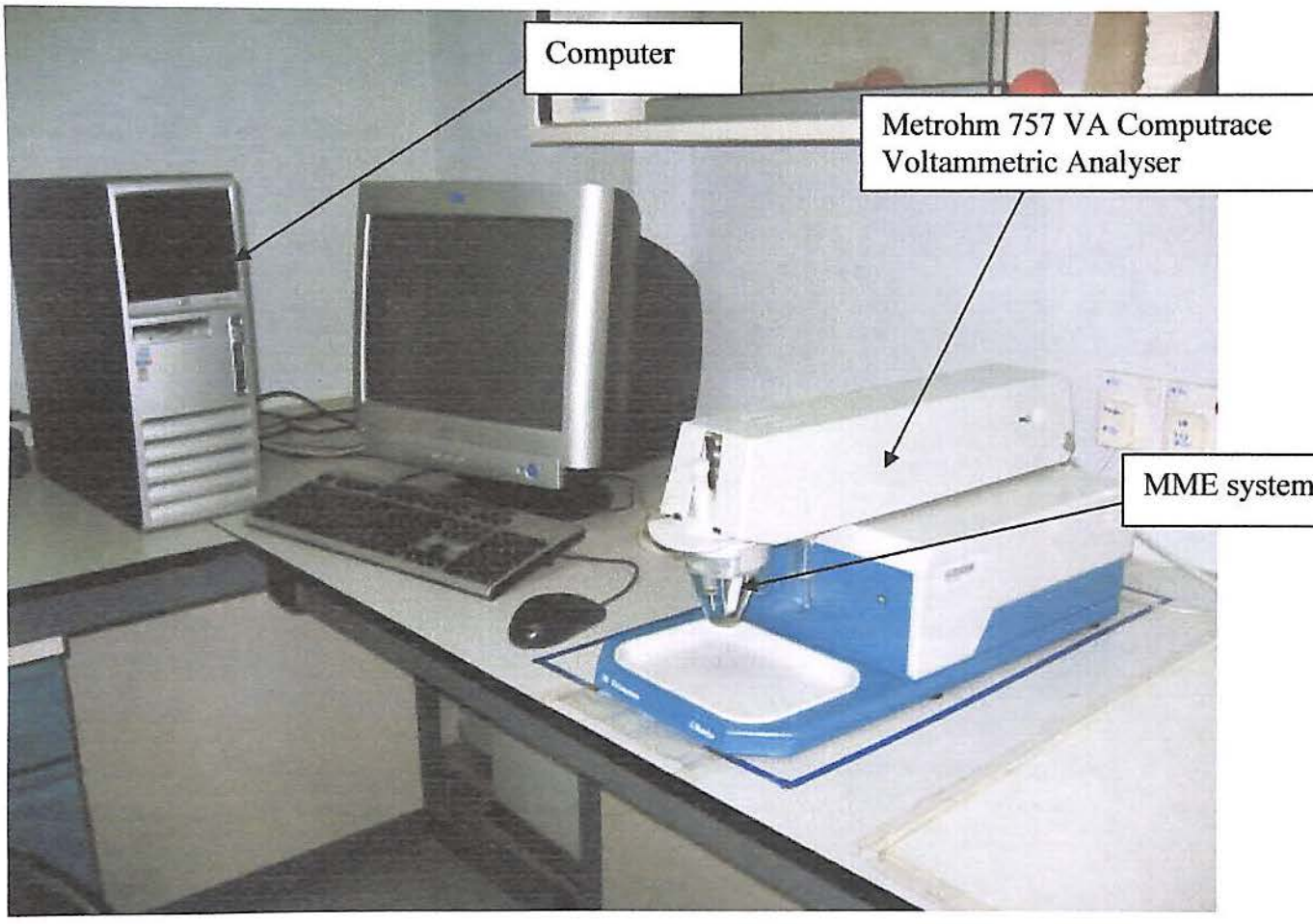

Figure 2: Experimental set of voltammetry instruments.

The electrode system consists of:

i. Working electrode: Multi Mode Electrode (MME)

ii. Reference electrode : Ag / AgCl / KCl 3 mol/L (double junction)

iii. Auxilliary/counter electrode : platinum

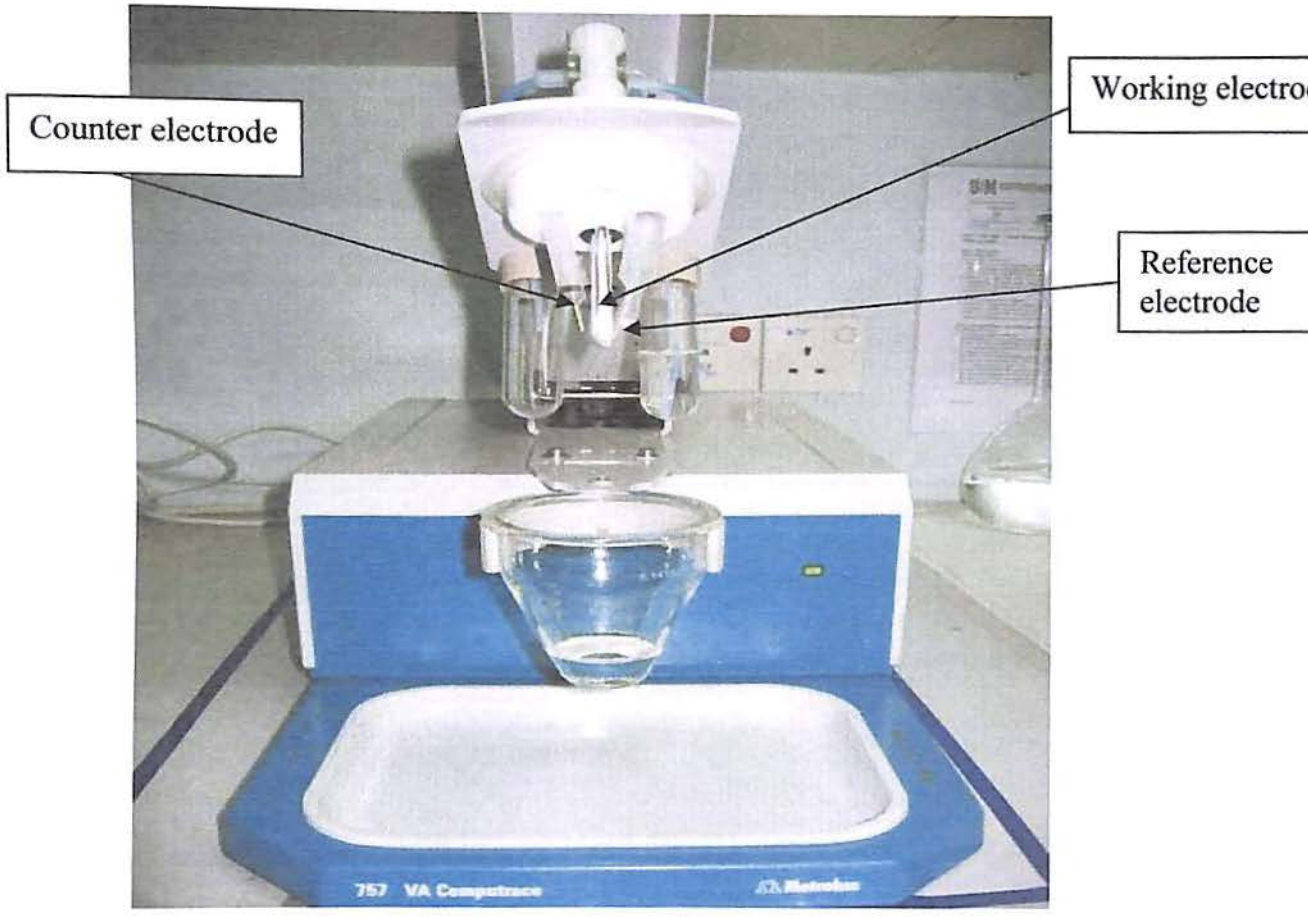

Figure 3: Three electrode of Metrohm 757 VA Computrace Voltammetric Analyser.

The voltammetric parameter for sample analysis was optimized first. These voltammetric parameters include:

Parameter	**Unit**
Initial potential, E_i	Volt
Final potential, E_f	Volt
Deposition potential, E_{acc}	Volt
Deposition time, t_{acc}	second
Scan rate, ν	Volt/second
Equilibration time, t_{eq}	second

Table 4: Parameters of voltammetry analysis

These parameters could be manipulated just after pH optimization. The optimizations have been carried out to obtain the highest and significant cadmium peak.

2. Procedure:

2.1) Samples of infant milk (brand):

1. Dutch Lady (A)
2. Lactogen (B)
3. Dumex (C)

2.2) Chemicals used:

i. For preparation of Britton-Robinson Buffer (BRB)

   a. Boric acid (Fluka)

   b. Glacial acetic acid (Merck)

   c. Orthophosphoric acid (Merck)

ii. Cadmium stock solution

iii. Nitric acid (HNO_3) (67%)

iv. Acid hydrochloric (HCl), 1 M

v. Sodium hydroxide (NaOH), 1 M

3. Sample preparation:

3.1) The samples were homogenized and weighed to 5 g (dry state).

3.2) The 20 mL of concentrated nitrite acid (HNO_3) was added into each sample. The samples were heated using hot plate to 95°C ± 5°C and evaporated.

3.3) It was continued by adding 10 mL of concentrated HNO_3 to produce the colorless solution of samples.

3.4) Then, 10 ml 1:1 HCl was added into the solution of samples and evaporated until clear.

3.5) After that, 10 mL of concentrated HNO_3 was kept added until the solution become colorless.

3.6) When the colorless solution was obtained, it is evaporated until residue left and cooled

3.7) When the samples were in room temperature, the particulates in solution of samples were removed by filtration using filter paper.

3.8) The solution was transferred into the volumetric flask and diluted to 25 mL with deionize water and it was ready to analyze.