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VOLTAMMETRY DETERMINATION OF SELENIUM IN VARIOUS MILK SAMPLES

DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT FOR THE DEGREE OF BACHELOR OF SCIENCE (HONS) IN FORENSIC SCIENCE

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for my research. Their support and motivation are one of the important factor that lead me to the completion of this study.

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List of Abbreviations

DAN	-	Diamino-naphthalene
DLs	-	Detection limits
DPCSV	-	Differential Pulse Cathodic Stripping Voltammetry
DPASV	-	Differential Pulse Anodic Stripping Voltammetry
DRI	-	Dietary Reference Intake
ETAAS	-	Electrothermal atomic absorption spectrometry
FNB	-	Food and Nutrition Board
HG-AAS	-	Hydride generation atomic absorption spectrometry
HMDE	-	Hanging Mercury Dropping Electrode
ICP-AES	-	Inductively coupled plasma atomic emission spectrometry
ICP-MS	-	Inductively coupled plasma mass spectrometry
INAA	-	Instrumental neutron activation analysis
RDAs	-	Recommended Dietary Allowances
ULs	-	Tolerable Upper Intake Levels
UV	-	Ultraviolet
XRF	-	X-ray fluorescence

List of Symbols

mcg	-	microgram
µg/l	-	microgram per Litre
ng/g	-	nanogram per gram
ng/ml	-	nanogram per millilitres
kW	-	kilowatt
%	-	percent
N	-	Normality
µg	-	microgram
keV	-	kiloelectron volt
ml	-	millilitres
g/mol	-	gram per mole / Molar Mass
mol/L	-	mole per litre / Molarity
ppm	-	part per million
g	-	gram
M	-	Molarity
rpm	-	rate per minute
s	-	seconds
V	-	Volt
V/s	-	Volt per seconds
$\overline{X}$	-	Mean
SD	-	Standard Deviation
R	-	Correlation Coefficient

ABSTRACT

The interest concerning against selenium (Se) has been considerable arise in recent years as Se is an essential trace element for humans and it also exhibit toxicity effect in humans depending on its concentration. In this study, determination of the concentration of Se in various milk samples had been carried out using differential pulse cathodic stripping voltammetry (DPCSV) technique. This study provides a methodology that can be used to determine the concentration of selenium in various milk samples. Three types of raw fresh milks (goat's milk, cow's milk, and horse's milk) and seven types of processed commercial milks had been analysed using DPCSV technique. These milk samples were undergone sample preparation procedure before being analysis. The milk samples were digested in HNO_3 : $HClO_4$ (1:1) mixture by a wet digestion procedure. All forms of Se in milk samples were converted into Se(IV) by the addition of HCl. The prepared samples were undergone dilution before proceed to analysis. The diluted milk samples were then added with electrolyte constitutes of 3.3g ammonium sulphate, 0.1mol/L Na_2EDTA dehydrate, and 100 ppm Cu standard solution before the analysis starts. The DPCSV of milk samples in HCl solution showed a peak potential for selenium at -0.65V. The standard addition method was used to determine Se in the samples. The result for Se concentration in milk samples were successfully obtained in this study.

ABSTRAK

Keprihatinan terhadap selenium (Se) semakin meningkat pada masa kini ini memandangkan Se merupakan unsur yang penting untuk manusia dan ia juga mempunyai kesan toksik kepada manusia bergantung kepada kepekatan Se. Dalam penyelidikan ini, pengesanan kepekatan Se dalam pelbagai sampel susu telah dilakukan dengan teknik voltammetri perlucutan kathodik denyut kebezaan (DPCSV). Penyelidikan ini menggunakan satu metodologi tentang pengesanan kepekatan Se dalam pelbagai sampel susu. Tiga jenis susu mentah segar (susu kambing, susu lembu, dan susu kuda) dan tujuh jenis susu komersial telah dianalisis dengan teknik DPCSV. Sampel-sampel susu ini telah menjalani beberapa langkah penyediaan sebelum dianalisis. Sampel-sampel susu tersebut telah dicernakan dalam campuran HNO_3 : $HClO_4$ (1:1) dengan prosedur pencernaan basah. Semua bentuk Se dalam sampel susu telah ditukar kepada Se(IV) selepas penambahan HCl. Sampel-sampel yang disediakan telah dicairkan sebelum dianalisis. Sampel-sampel yang telah dicair ditambah dengan 3.3g ammonium sulfat, 0.1mol/L Na_2EDTA dehydrate, dan 100 ppm larutan piawai kuprum untuk analisis. DPCSV untuk sampel susu di dalam HCl menunjukkan puncak keupayaan untuk Se pada -0.65V. Kaedah tambahan piawai telah digunakan untuk menentukan Se dalam Sampel. Keputusan menunjukkan penentuan kepekatan Se dalam sampel-sampel susu telah diperoleh dengan jayanya dalam penyelidikan ini.

CHAPTER I

INTRODUCTION

1.1 Selenium

Selenium (Se) is an essential trace element that can be found in environment which is also a vital micronutrient for biological systems. Se can be presented as organic and inorganic species in the environment. Inorganic Se can present as selenide (-II), elemental Se (0), selenite (IV), selenite (VI); Species like selenite (SeO_3^{2-}) and selenite (SeO_4^{2-}) also found in most oxygen rich environmental matrices (Munoz Olivas *et al.*, 1994), while the organic forms of Se are generally encountered as selenoamino acids and methylated compounds as dimethyl selenide.

Minerals are single inorganic elements which are widely distributed in foods and in living bodies. These minerals carry out their functions like building body tissue; activate, regulate and control metabolic process in their ionized form. In 1957, Schwarz and Foltz first recognized Se to be important in normal metabolism. It was found that Se prevented necrotic degeneration of the liver in the vitamin E deficient rat (Combs & Combs, 1984). Se plays an important role as part of an antioxidant enzyme that protects cells and their lipid membranes against oxidative damage (Zingaro & Cooper, 1974). It also functions as an essential part of fatty acid metabolism, where the nutritional interrelationship between the actions of Se and vitamin E also affects the oxidation of fatty acids which involved vitamin E.

The interest concerning against Se has been considerably arise in recent years as Se is an essential trace element for humans and it also exhibit toxicity effect in humans depending on its concentration. Research by Neve *et al.* (1987) stated that the safety margin between its essential level and toxicity is very narrow. The difference of

concentration range between the level of Se at which it is considered toxic and at which it is considered essential is very small (Burk, 1977). Food and Nutrition Board (FNB) at the Institute of Medicine of the National Academies (formerly National Academy of Sciences) had developed a Dietary Reference Intakes (DRIs) that provides intake recommendations for selenium. DRI is the general term for a set of reference values used for planning and assessing nutrient intakes of healthy people and these values are vary by age and sex.

Table 1.1 Recommended Dietary Allowances (RDAs) for Selenium

Age	Male	Female	Pregnancy	Lactation
Birth to 6 months	15 mcg	15 mcg		
7-12 months	20 mcg	20 mcg		
1-3 years	20 mcg	20 mcg		
4-8 years	30 mcg	30 mcg		
9-13 years	40 mcg	40 mcg		
14-18 years	55 mcg	55 mcg	60 mcg	70 mcg
19-50 years	55 mcg	55 mcg	60 mcg	70 mcg
51+ years	55 mcg	55 mcg		

Note. Adapted from Institute of Medicine, Food and Nutrition Board. Dietary Reference Intakes: Vitamin C, Vitamin E, Selenium, and Carotenoids. National Academy Press, Washington, DC, 2000.

Excessive intake of Se can cause pronounced toxic symptoms (Roekens *et al.*, 1985). For example, severe irritations of the respiratory system, a metallic taste in the mouth, pulmonary oedema, and the characteristic smell of garlic in the breath and

sweat due to dimethyl selenide (Bedwal *et al.*, 1993). Acute selenium toxicity can result in severe gastrointestinal and neurological symptoms, acute respiratory distress syndrome, myocardial infarction, hair loss, muscle tenderness, tremors, lightheadedness, facial flushing, kidney failure, cardiac failure, and, in rare cases, death (Sunde, 2006). The Food and Nutrition Board (FNB) has established upper intake levels (ULs) for selenium from food and supplements based on the amounts of selenium that are associated with hair and nail brittleness and loss.

Table 1.2 Tolerable Upper Intake Levels (ULs) for Selenium

Age	Male	Female	Pregnancy	Lactation
Birth to 6 months	45 mcg	45 mcg		
7-12 months	60 mcg	60 mcg		
1-3 years	90 mcg	90 mcg		
4-8 years	150 mcg	150 mcg		
9-13 years	280 mcg	280 mcg		
14-18 years	400 mcg	400 mcg	400 mcg	400 mcg
19+ years	400 mcg	400 mcg	400 mcg	400 mcg

Note. Adapted from Institute of Medicine, Food and Nutrition Board. Dietary Reference Intakes: Vitamin C, Vitamin E, Selenium, and Carotenoids. National Academy Press, Washington, DC, 2000.

On the other side, a deficiency of Se will also cause many health problems. The lack of this element is found to be related to a large number of diseases of different aetiologies, such as a severe congestive cardiomyopathy – Keshan disease (Zhou & Yu, 1992), muscle (Orndahl *et al.*, 1994) and blood disorders (Meydani, 1992), certain

types of cancer (Taylor *et al.*, 1994), neurological diseases (Alvarez Prieto *et al.*, 1994), etc. Se also plays a role as enzymatic cofactor of glutathione peroxidase in the elimination of peroxide radicals from the organism (Sunde & Hoekstra, 1982). According to Institute of Medicine, Food and Nutrition Board (2000), Keshan disease areas like countryside in China, had average intakes of no more than 11 mcg per day; It was also found that intakes of at least 20 mcg/day protect adults from Keshan disease (Institute of Medicine, Food and Nutrition Board, 2000).

Without being affected by the regular intake of Se, its level is generally low in human tissues and body fluids. Se concentration ranges of 4.8 – 46 $\mu g/l$ in urine, 57 – 320 $\mu g/l$ in whole blood, 260 – 410 ng/g in liver and 100 – 630 ng/g in kidney have been reported (Iyengar *et al.*, 1978). Foods that high in protein such as meat, sea foods, eggs and milk are good sources of Se (Chaney, Ross & Witsch, 1979). The intake of this element by human is normally comes from these protein foods (Stacchini *et al.*, 1989). The accumulation of Se in human body is usually found in higher concentrations in kidney and liver (Jaffar & Ashraf, 1988).

The amount of Se in foods is influenced by the concentration of Se of the soil. Plants grow on the soil will absorb the minerals, and the minerals were taken by the animals when they eat the plants. Thus, it has been found that the concentration of Se in human milk samples obtained from vegetarian women was much greater (22.2 ng/ml) than that from a non-vegetarian women (16.8 ng/ml) (Debski *et al.*, 1989). As Se also has its unique photoelectrical and semi-conducting properties, Se also used in mining, fuels production and photocopying technology. The release of inorganic Se species into the environment is due to corresponding industrial activities. These inorganic species including selenite, Se(IV) and selenite, Se(VI), which are bioconverted to organometallic species through biomethylation processes. Se that present in

environment and several living organisms often exist in four different oxidation states like +6, +4, 0, and -2.

Since milk and milk products are one of the most important foods to human due to their nutritional value, protein and mineral contents, it is vital to ensure that all the commercial milk products in the market as well as the freshly harvested milk's selenium level are tested and its concentration is within the safe range. The toxicity and bioavailability of Se is controlled by not only its concentration but also its chemical forms, thus, it is vital to have selective and sensitive methods to identify them and determine their levels. There are many methods have been developed for the determination of Se such as X-ray fluorescence (XRF), (Sotirios *et al.*, 1980), instrumental neutron activation analysis (INAA), (Y.Shi *et al.*, 1999), hydride generation atomic absorption spectrometry (HG-AAS), (Kazuo *et al.*, 1988), inductively coupled plasma atomic emission spectrometry (ICP-AES), (Martinez L.D. *et al.*, 1997), inductively coupled plasma mass spectrometry (ICP-MS), (Forrer. *et al.*, 1998), or electrothermal atomic absorption spectrometry (ET-AAS). (F Čuparigova, 2011).

Voltammetry techniques are among the techniques which are inexpensive, sensitive and selective and also be used for speciation analysis. Voltammetry is an electrochemical technique in which the current is measured as a function of applied potential in electrochemical cell. The potential is varied in some systematic manner to cause electroactive chemical species to be reduced or oxidized at the electrode. The current produced is proportional to the concentration of the chemical species (Princeton Applied Research).

Stripping voltammetry is a two-step technique which involved the electrolytic deposition of a chemical species onto an inert electrode surface at a constant potential

and the application of a voltage scan to the electrode that causes an electrolytic dissolution, or stripping, of the various species in the amalgam or film back into solution at characteristic potentials. Mercury is usually the general choice of electrode in striping voltammetry; it is used to retain chemical species in voltammetry by forming either an amalgam or an insoluble mercurous salt. The hanging mercury drop electrode (HMDE) is the best working electrode for stripping voltammetry due to its extremely reproducible surface. The entire stripping voltammetry experiment will be performed on one mercury drop, and it is imperative that this mercury drop should be dispensed with an area that is reproducible to within 1%. The measured current in an electrochemical experiment is proportional to the electrode area (Princeton Applied Research).

Among the types of voltammetry techniques, stripping analysis is the most selective electro analytical technique combining low detection limits (DLs), high sensitivity, multi-element capabilities, required least sample preparation and low cost, and it is especially useful for trace analysis. Thus, stripping voltammetric technique will be chosen to analyse the Se in milk samples. As mentioned in the previous text, it is important to control the concentration of Se in the consumer's products as the concentration range between toxicity and essential level is very narrow. Therefore, this study is carried out to determine the concentration of selenium in various milk samples using differential pulse stripping voltammetry technique (Princeton Applied Research).

1.2 Objectives

1.2.1 General Objective

To determine the concentration of selenium in various milk samples using voltammetry determination.

1.2.2 Specific Objectives

i. To determine the concentration of selenium in raw cow's milk.

ii. To determine the concentration of selenium in raw goat's milk.

iii. To determine the concentration of selenium in raw horse's milk.

iv. To determine the concentration of selenium in various commercial processed milks.

1.2.3 Significance of study

Selenium is a trace element that exists in most foods including milk, and it behaved two ways in human body as it can be either toxic or nutritious depending on its concentration. This study was carried out to provide a methodology that can be used to determine the concentration of selenium in various milk samples.

CHAPTER II

LITERATURE REVIEW

During recent years, the public has raised the interest against Se due to its double behaviour it can have in human body, it can be either toxic or a nutritious element depending on its concentration. Therefore, several studies had been carried out to determine the concentration of Se in various types of foods and drinks. These studies were carried out by researchers all over the world using several types of instrument including differential pulse stripping voltammetry (DPSV), hydride generation atomic absorption spectrometry (HG-AAS), inductively coupled plasma mass spectrometry (ICP-MS), fluorometric method, X-ray fluorescence (XRF), instrumental neutron activation analysis (INAA), inductively coupled plasma atomic emission spectrometry (ICP-AES), electrothermal absorption spectrometry (ET-AAS) and etc.

Recai and Somer (1999), carried out a direct method for the determination of Se and lead in cow's milk by differential pulse stripping voltammetry. In their study, they used a hanging mercury drop electrode (HMDE) and differential pulse cathodic stripping voltammetry (DPCSV) and differential pulse anodic stripping voltammetry (DPASV) to determine Se and lead contents in milk samples from Turkey respectively. The standard addition method has been used to determine the concentration of Se and lead in the sample. In this method, a simple and suitable procedure had been used for the determination of trace amounts of Se and lead, and there is no need for sophisticated instruments and tedious separation procedure. The Se and lead contents of milk samples from three distinct regions of Turkey were obtained between 21.5 – 69.4 and 22.1 – 59.2 μg/l (n=4 – 5), with the relative standard deviations of 10.3 – 10.7 and 6.8 – 9.9%, respectively.

A study of the determination of inorganic and organic Se species in natural waters by cathodic stripping voltammetry was carried out by Elleouet *et al.*, (1994). In their study, a Se speciation model was proposed by them for the studies of natural waters based on Se(IV) voltammetric determination. The separation of the organic and inorganic forms was realized by a rapid ion exchange method and total Se was determined after UV irradiation. In this method, it only permits the determination of Se(IV), so it is necessary to separate and/or transform the other chemical forms into Se(IV). In this work, Se(-II) was oxidised and Se(VI) was reduced into Se(IV) in order to propose a speciation model based on the electrochemical properties of Se(IV). The concentration of Se(IV) was directly determined by cathodic stripping voltammetry which is also corresponding to the total concentration of Se in the sample.

Maria and Fotis (2001), developed a speciation analysis of Se using voltammetric techniques. A simple and accurate method has been developed for the separation and determination of Se(IV), selenocystine and dimethyldiselenide based on differential pulse cathodic stripping voltammetry (DPCSV) at a hanging mercury drop electrode (HMDE) and an extraction procedure for the prior separation of the Se species. For the optimization of the procedure, several parameters of the voltammetric determination, such as the deposition time and voltage, the kind of electrolyte and the acidity of the sample as well as the kind of extractant for the effective separation of the Se species were investigated in the aqueous and organic phase. The quantitatively transformation of the electrochemically inactive Se(VI) to Se(IV) was achieved by testing different reducing agents.

In the study of Maria and Fotis (2001), the study of the electrochemical behaviour of Se(IV), Se(IV), as well as Se-Cyst and Se-Met and S-Cyst was performed in the aqueous phase using HCI as electrolyte. Since the methyl-groups Se is insoluble in water, their voltammetric determination has to be performed in suitable organic media. The water

soluble Se compounds, Se(VI) and Se-Met were found to be electrochemically inactive, whereas Se(IV) and Se-Cyst can be determined using DPCSV. Although Se-Cyst is found electrochemically active, however, its sensitivity in voltammetric determination is very poor and has no significant effect so its sensitivity must be increased by using HCl. For the reduction of Se(VI) to Se(IV), a wide range of reducing agents was tested. No Se(IV) was detected using $SnCl_2$, KI, $Na_2S_2O_3$, $FeSO_4$ and $N_2H_6SO_4$ as reducing agents or UV photolysis at a wide range of pH value (3 – 8.5), but it was found after the 60% reduction of Se(VI) by using concentrated HCl (6M) at 95 °C for 4 hour. A 100% quantitative reduction was achieved using HCl (5M) in combination with strong UV photolysis, by means of a high power 1 kW Xe lamp. It was also found that dichloromethane was successfully separates quantitatively inorganic Se (Se(IV) and Se(VI)) and Se-Cyst remaining in the aqueous phase due to their ionic form, from $(CH_3)_2Se_2$ which is extracted in the organic phase. Thus, Se(IV), Se-Cyst and Se(VI) after reduction can be determined simultaneously in the aqueous phase using HCl as electrolyte and $(CH_3)_2Se_2$ determined in the (acidified) organic phase as above.

The determination of Se in sediments by hydride generation atomic absorption spectrometry (HG-AAS) was carried out by Kazuo *et al.*, (1988). This method with an automated flow system is useful for the routine analysis of Se in environmental samples. This method, however, is sensitive to interferences from transition metal ions and other hydride forming ions such as copper(II) and nickel(II), precious metal such as silver(I), hydride forming ions such as arsenic(III), antimony(III), bismuth(III) and tin(IV), and anions such as nitrite and nitrate. The interferences of transition metals were minimized by decrease of tetrahydroborate and increase of acid concentration, especially that of hydrochloric acid, and by adding iron(III), if necessary.

In 1997, a direct determination of Se and other trace elements in serum samples by using inductively coupled plasma mass spectrometry (ICP-MS) was carried out (Forrer *et al.*, 1997). This paper described a simple sample preparation method designed to overcome the matrix effects and most of the problems for the direct determination of Se and other trace elements in serum, without the need of standard addition of sample digestion. This method allowed the sample volume to be small enough to be used in neonatology and with small animals. Part of the procedure includes the optimization of the instrument settings such as nebulizer flow rate and sampling depth. Despite recent advances in speciation analysis, direct determination of trace Se in complexes matrix is still not easy, thus, separation or pre-concentration techniques are often required before any analytical determination. Moreover, various ion chromatographic separation modes can be coupled with ICP-MS, but factors such as separation mechanisms, mobile phase, pH, and the steps involving sample preparation must be carefully considered to avoid the inter-conversion of species and to ensure accurate characterization of sample (Wang *et al.*, 2010).

Fluorimetric determination has been used by most of the researchers to determine Se in biological samples using the fluorescence of Se-DAN complex. In this method, all the Se-compounds in the sample must be converted to selenite. So a previous step of mineralization of samples and reduction to selenite is required for the fluorimetric determination. Fluorimetry with 2,3-diamino-naphthalene (DAN) has been widely selected to be used in the determination of Se in biological samples due to its good sensitivity and precision (Iyengar, 1991). This method make used of the fluorescence properties of Se-DAN complex (4,5-benzopiazselenol) derived from selenite for the Se determination. This fluorometric determination of Se can be divided into four phases: digestion of samples,

reduction of Se(VI) to Se(IV), formation of Se-DAN complex, and extraction of the complex (Rodriguez *et al.*, 2008).

A method was developed to determine Se in the ng/g and μg/g range in organic and biological matrices using X-ray fluorescence determination (Sotlrios *et al.*, 1980). It is based on a two-step reduction using 5N HCl and NaI. The elementary Se was deposited subsequently on a small membrane filter. ^{75}Se was used as a tracer in this procedure and errors were eliminated. None of the interferences were found in the chemical preparation step or in the X-ray fluorescence measurements. A calibration curve was constructed and the linear range extends through 150 μg. The determination of Se in standard reference materials was accomplished with a calibration curve where Se was carried through the entire procedure including the decomposition to account for the 98.5% recovery determined before. In comparison with the atomic absorption hydride evolution technique, this method is less sensitive but more accurate with much better precision.

Se content of a variety of Canadian food items were also determined by using instrumental neutron activation analysis (INAA) and pseudocyclic INAA (PCINAA), (Shi *et al.*, 1999). The use of the 162-keV gamma ray of short-lived ^{77m}Se in INAA allowed relatively simple and rapid determinations and was suitable for many of the foods. A PCINAA method with three cycles was found to give lower detection limits and was used for the low-selenium food samples. Both internal and external quality assessments were used to produce result with good accuracy and precision.

In 2011, a method for direct Se determination in human blood serum by electrothermal atomic absorption spectrometry (ETAAS) was developed, (F Čuparigova, 2011). Total Se was determined by ETAAS employing 10 μg of palladium as matrix modifier in a graphite atomizer with pyrolytically coated tubes and Zaeman background correction. Blood serum was diluted before analysis. The measurements were confirmed

by the analysis of the standard reference material and by the method of standard additions. The precision of the method ranges from 2.06% to 5.95%. The obtained data from the Se concentrations in serum samples from 83 patients show that the content of Se is relatively low, ranging from 43.91 ± 4.80 μg/l for female to 45.21 ± 5.60 μg/l for male (F Čuparigova, 2011). Direct electrothermal atomic absorption spectrometry (ETAAS) produces high sensitivity and accuracy for selenium determination in biological fluids and requires little sample preparation, but is very sensitive to matrix interference and reliable methods have been validated only for blood plasma and serum (Li *et al.*, 1998).

CHAPTER III

METHODOLOGY

3.1 Instrument

A Metrohm 797 VA Computrace as shown on Figure 4.1 was used to analyse the trace selenium in milk sample. An analytical balance model ATX 224 as shown on Figure 4.2 was used for weighing ammonium sulphate, $(NH_4)_2SO_4$, Na_2EDTA dehydrate, and powdered milk sample. A hot plate model EMS-HP-7000 as shown on Figure 4.3 was used for heating and evaporation of milk samples. A pH211 Microprocessor pH meter was used to measure the pH of the stock solution.

Figure 3.1 Metrohm 797 VA Computrace

Figure 3.2 Weighing balance ATX 224

Figure 3.3 Hot plate model EMS-HP-7000

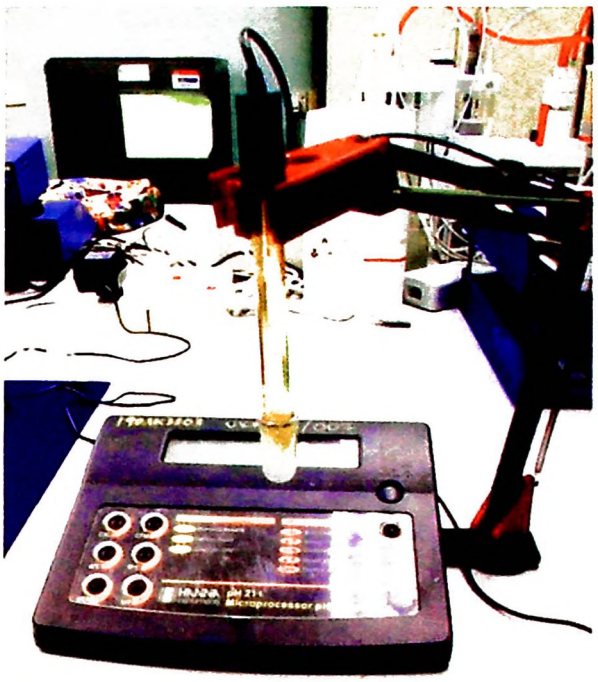

Figure 3.4 pH 211 Microprocessor pH Meter

3.2 Apparatus and Glassware

300 ml Erlenmeyer flask, beaker, dropper, micropipette P-1000 and pipette tips, 100 ml measuring cylinder, 5 ml Pipette, 25 ml volumetric flask, 100 ml volumetric flask, and 250 ml volumetric flask, were used.

3.3 Chemicals and Reagents

All the reagents used were of analytical grade. 65% Nitric acid (HNO_3), 70% perchloric acid ($HClO_4$), 37% hydrochloric acid, (HCl), were used in samples digestion procedure. 95% sulphuric acid (H_2SO_4), 30% sodium hydroxide solution (NaOH), 132.14 g/mol ammonium sulphate ($(NH_4)_2SO_4$), 0.1 mol/L Na_2EDTA dehydrate, 1000 ppm Copper standard stock solution, 1000 ppm Se standard stock solution, and E Pure deionized water were used.

3.4 Samples for analysis

Raw goat's milk, raw cow's milk, raw horse's milk and seven different brands of commercial processed milks were used.

3.5 Reagents Preparation

0.1 mol/L Na_2EDTA solution

0.1 mol/L of Na_2EDTA solution was prepared by weighing 3.72 g of Na_2EDTA and dissolved with E Pure deionized water. The dissolved EDTA solution was transferred into a 100 ml volumetric flask and added with deionized water until its volume reached 100 ml.

0.01 mol/L sulphuric acid, H_2SO_4

0.01 mol/L H_2SO_4 solution was prepared from 95 – 98 % H_2SO_4 acid stock solution which is approximately 18 M. 0.14 ml of H_2SO_4 stock solution was taken and added into beaker contained deionized water (to avoid extreme reaction from concentrated H_2SO_4 acid). The H_2SO_4 solution in deionized water was then transferred to 250 ml volumetric flask and added with deionized water until 250 ml was reached. The diluted H_2SO_4 was measured with pH meter and added with NaOH until the pH reached 2.2.

Cu standard solution

Cu standard solution was prepared from 1000 ppm Cu stock solution by taking10.0 ml of Cu stock solution and added into 100 ml volumetric flask. Deionized water was added into volumetric flask until its volume reach 100 ml.

Se standard solution

Se standard solution was prepared from 1000 ppm Se stock solution by taking 0.1 ml of Se stock solution and added into 100 ml volumetric flask. Deionized water was added into volumetric flask until its volume reach 100 ml.

3.6 Sample Preparation

25.0 ml of milk, 5.0 ml of nitric acid (HNO_3) and 5.0 ml of perchloric acid ($HClO_4$) were transferred into a 300 ml Erlenmeyer flask and kept overnight. On the next day, it was evaporated and heated until its volume reached approximately one-third of the original volume. Heating was continued until the evolution of brown fumes of nitrogen oxides ceased. There was a danger of explosion when the digestive sample turned from yellowish to deep dark, so 2.5 ml of HNO_3 and 2.5 ml of $HClO_4$ were added, cooling the flask for about 2.0 minutes before addition. Heating was proceeding until the nitrogen oxide fumes were completely given off. The digestion was completed when approximately 4 – 5 ml solution remained. Finally, 1.0 ml of hydrochloric acid was added and heated for at least 20 minutes to convert all Se to Se(IV). During the digestion period, care was taken to make sure that no charring of the sample occurred, since it can cause loss of Se as volatile $SeCl_4$ and SeO_2. 1.0 ml of the final solution was transferred into a 25.0 ml calibrated flask which was made up to the mark with triply distilled water.

3.7 Voltammetric determination

1.0 ml of (diluted) sample or digestion solution was taken and added with 9.0 ml deionized water, 3.3 g of ammonium sulphate, 1.0 ml EDTA solution and 1.0 ml Cu solution. The pH of the solution is adjusted with sulphuric acid to pH 2.2 ± 1.0. If necessary, allow to cool.

The voltammogram were recorded using the following parameters:

Table 3.1 Optimum parameters used for Se analysis

Working electrode	HMDE
Stirrer speed	2000 rpm
Drop size	4
Mode	DP
Purge time	300 s
Deposition potential	-0.4 V
Deposition time	90 s
Equilibration time	10 s
Pulse amplitude	0.08 V
Start potential	-0.40 V
End potential	-0.85 V
Voltage step	0.004
Voltage step time	0.1 s
Sweep rate	0.04 V/s
Peak potential Se	-0.65 V

3.8 Atomic Absorption Spectroscopy

A comparison test with atomic absorption spectroscopy (AAS) on one of the sample – raw goat's milk had been carried out. The sample used for AAS test is the same as the sample prepared for DPCSV test. Three Se standards were prepared with the concentration of 1.5 ppm, 3.0 ppm and 6.0 ppm respectively. The parameters for AAS are shown in Table 3.2.

Table 3.2 Standard Atomic Absorption Conditions for Se

Wavelength (nm)	**Slit (nm)**	**Relative Noise**	**Characteristic Concentration (mg/L)**	**Characteristic Concentration Check (mg/L)**	**Linear Range (mg/L)**
196.0	2.0	1.0	0.59	30.0	200.0

CHAPTER IV

RESULTS AND DISCUSSION

The determination of the concentration of Se in raw fresh milks and also various commercial processed milks were successfully performed using differential pulse cathodic stripping voltammetry analysis. All the samples before analysis were undergone several preparation steps before being analysed to ensure that an accurate and verified result will be achieved. All the Se in the samples was converted into Se(IV) by the addition of HCl, because Se(IV) is the most stable oxidation states of Se. Se(IV) in the samples were detected at the peak potential of -0.65V± 0.1 and the resultant current is proportional to the concentration of the Se(IV).