UNIVERSITI SAINS MALAYSIA

VOLTAMMETRIC DETERMINATION OF LEAD IN VEGETABLE JUICE SAMPLES

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

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ACKNOWLEDGEMENTS

First of all I would like to thanks to the Almighty God, Allah because with His blessing I had completed this research.I would like to thanks to my parents and my family which always with me through my hard time to finish this project.

I would like to express my greatest gratitude to my supervisor, Prof Madya Dr Mohammad Hadzri b. Yaacob for the interesting and challenging research project, for providing advice and the knowledge he shared over the duration of this project. Without his patience guidance, I would not have succeeded to develop my understanding and gain knowledge from this study. I am taking this opportunity to express my gratitude to Dr. Nur Haslindawaty Abd.Rashid as course coordinator to this paper.

I also would like to thanks all PPSK lecturers especially lecturers in Forensic Science programme, science officers and staff particularly Mr. Zulhairi Othman for his assistance and guide to use voltammetry instrument. Without his technical support my project would not finish complete in time. I also would like to thanks to Nur Syamimi Zainuddin, who helped me in the analysis of samples.

Lastly, I to thank you all my friends forensic science final year student especially to Oo Kee Lian, Rosila Omar, Nurzahirah Mohd Rashid and Siti Zaharah Md Isa for their support and encouragement to me.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATON

ASV: Anodic stripping voltammetry

HMDE: Hanging mercury drop electrode

DPV: Differential pulse voltammetry

DPASV: Differential pulse anodic stripping voltammetry

ICP-MS: Inductively coupled plasma-mass spectroscopy

LODs: Limit of detections

DME: Dropping mercury electrode

ICP-AES: Inductively coupled plasma atomic emission spectrometry

ICP-OES: Inductively coupled plasma - optical emission spectrometry

FAAS: Flame atomic absorption spectroscopy,

AAS: Atomic absorption spectroscopy

STAT: Slotted tube atom trap

EDTA: ethylenediaminetetracetic acid

DTPA: diethlenetriamine pentacetic acid

NaOH: Sodium Hydroxide

HCl: Hydrochloric Acid

ABSTRACT

This research investigated lead concentrations in vegetables juices were investigated as they are toxic to our body. The average daily intake of lead in vegetables juices is only 0.5 ppm due to its toxicity to our body. Lead can be adsorbed into vegetables through the water that use for watering the vegetables. It also may come from the water that use to make juice. In this work, the presence of lead levels in vegetables juices such as tomato, pumpkin, winter chestnut, winter melon, carrot, spinach, kale, cabbage, mustard, and eggplant were determined by Differential Pulse Anodic Stripping Voltammetry (DPASV) after wet digestion of the organic matter. Standard addition method was used for this analysis. Parameters used for DPASV were as follows, start potential: -0.599 V, end potential: -0.199 V, deposition time: 60 s, equilibrium time: 10 s, voltage step: 0.005951 V, voltage step time: 0.15 s, sweep rate: 0.0397 V/s, pulse amplitude: 0.05005 V, pulse time: 0.04 s.The result of lead concentration in tomato, pumpkin, carrot, spinach, kale, cabbage, mustard, and eggplant were 24.455 ppb, 18.755 ppb, 11.420 ppb, 0.440 ppb, 2.850 ppb, 6.155 ppb, 5.392 ppb and 3.164 ppb respectively. Lead was not detected in winter chestnut and winter melon juices.

ABSTRAK

Kepekatan plumbum di dalam jus sayuran dikaji kerana kandungan plumbum boleh memberi kesan yang tidak baik kepada badan manusia. Kandungan plumbum yang boleh di ambil oleh pengguna dalam seharian adalah tidak melebihi 0.5 ppm. Plumbum yang terdapat di dalam jus sayuran didapati daripada air yang digunakan untuk menyiram sayur tersebut serta daripada air yang digunakan untuk membuat jus itu sendiri. Di dalam kajian ini, kandungan plumbum diuji ke atas jus sayuran seperti tomato, labu, sengkuang cina, kundur, lobak merah, bayam, kangkung, bayam, terung, dan kubis yang menjadi kegemaran pengguna.. Kaedah Voltammetri Perlucutan Anod Denyut Pengkamiran digunakan selepas proses pengasingan dilakukan. Kaedah penambah piawaian digunakan di dalam menganalisis kandungan plumbum di dalam jus sayuran. Parameter yang digunakan untuk adalah seperti berikut , mulakan berpotensi: -0,599 V , potensi akhir: -0,199 V , masa pemendapan : 60-an , masa keseimbangan : 10s , langkah voltan : 0,005951 V , masa langkah voltan : 0.15s , kadar menyapu : 0,0397 V / s , nadi amplitud : 0,05005 V , masa nadi 0.04 . Hasil daripada kajian didapati kandungan plumbum di dalam tomato ialah 24.455 ppb, labu 18.755 ppb, lobak merah 11.420 ppb, bayam 0.440 ppb, kangkung 2.850 ppb, kubis 6.155 ppb, sawi 5.392 ppb dan terung 3.164 ppb. Manakala, di dalam sampel seperti sengkuang cina dan kundur kandungan plumbum tidak dapat dikesan.

1.0 INTRODUCTION

1.1 Lead

Lead is one of the heavy metal that is harmful to human . Concentration of lead that allowable for human in vegetables juice is not more than 0.5 ppm (Food Act, 1983). Lead has caused extensive health problems in many parts of the world. Exposure of lead has been estimated to cause about 143 000 deaths of human for every year and 0.6% of global that was reported burden of disease (World Health Organization, 2010).

There are many sources exposures of lead contamination including industrial processes, food and smoking, drinking water and domestics sources. In this paper will focus on the vegetables juice. Lead is found at low level in Earth's crust mainly as lead sulfide (World Health Organization, 2006).

Largest contributions of intake of lead in daily life are from food, dirt and dust. The content of lead in food plants depends on the soil concentrations (World Health Organization, 2010). The contents of lead in plants also may be due to the water use for watering the plants. Lead in drinking water can come from household plumbing systems containing lead pipes, solders and fittings. Water is exposed for overnight in this way will have a higher concentration of lead (World Health Organization, 2003).

Lead will be distributed in the bones, liver, brain, and kidneys upon absorption into human body. It also will be stored in the teeth and bones. For the assessment of lead poisoning, it can be measured directly through the measurement of lead in blood, teeth and bones (World Health Organization, 2004).

Lead affects multiple body systems such as neurological, haematological, gastrointestinal, cardiovascular and renal systems. For children, even in low level of lead exposure can cause serious disease such as irreversible neurological damage (World Health Organization, 2010).

1.1.1 Characteristics of lead

Lead is a bluish-white lustrous. It is soft, highly malleable, ductile, and a relatively poor conductor of electricity. It is very resistant to corrosion but become dull when exposed to the air. Lead isotopes are the end products of each of the three series of naturally occurring radioactive elements. Table 1 show the general properties of lead.

Table 1: Properties of lead (Water treatment solution Lenntech,)

Atomic number	82
Atomic mass	207.2 g/mol
Electronegativity according to Pauling	1.8
Density	11.34 g/cm^3 at 20°C
Melting point	327 °C
Boiling point	1755 °C
Vanderwaals radius	0.154 nm
Ionic radius	0.132 nm (+2) ; 0.084 nm (+4)
Isotopes	13
Electronic shell	[Xe] $4f^{14}$ $5d^{10}$ $6s^2$ $6p^2$
Energy of first ionisation	715.4 kJ/mol
Energy of second ionisation	1450.0 kJ/mol

1.2 Voltammetry as general

In 1922 voltammetry was discovered by the Jaroslav Heyrovsky from the discovery of the polarography techniques which is actually the branch of the study of electrochemistry. At this time, this technique was not ideal for routine analytical use because this method experienced difficulties in analyse sample. Early 1960s, this technique is modified to enhance sensitivity and expand the repertoire of analytical methods (Kounaves, S.M., n.d).

Application of electrochemical techniques is important in the fundamental studies of oxidation and reduction processes to breakdown reaction mechanisms and also useful in studying the kinetics and thermodynamics of electron and ion transfer processes (Bagotsky, V.S., 2005). Electrochemical techniques also useful tools for the study of the adsorption and crystalline phenomena at the electrode surfaces (Bard, A.J., and Faulkner L.R., 2000). Among the electrochemical techniques that used in food analysis, voltammetric techniques and polarographic are the principal ones (Brainina K.Z *et al.* 2000).

Electrochemical techniques use electrodes include auxiliary electrode, working electrode and reference electrode as shown in Figure 1. Auxiliary electrode is the electrode which functions as the total balancing charge in the system. Working electrode is an electron conductor which is the reaction of the species takes place. Reference electrode is designed to have constant or reversible potential (Gulaboski, R. and Pereira, C.M, 2008).

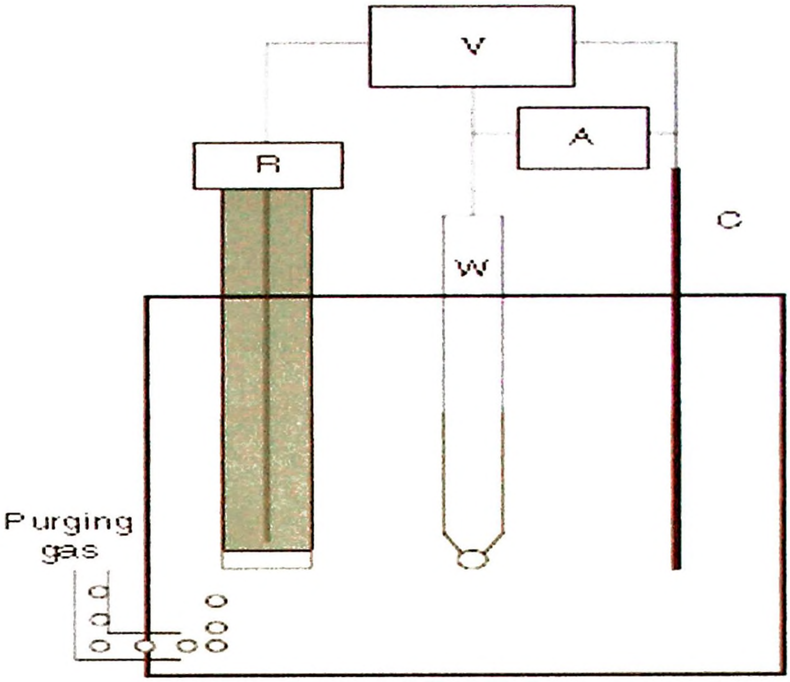

Figure 1: Schematic representation of a common electrochemical cell contain of:R: reference electrode, W: working electrode, C: counter electrode, V: voltmeter A: amperemeter (Gulaboski, R., and Pereira, C.M. 2008).

1.2.1 Principles of voltammetric technique

Voltammetry is the branch of the electro-analytical techniques family which is designed to measured current cell as function of the electrode (E). This technique is measures the amount of the chemical change occurring at the electrodes. Voltammetry exhibits a wide range, from sub-ppb to ppm or higher. For the lower concentration, pre-concentration step should be combined which is referred to stripping techniques. For the higher concentration, direct measurement by differential pulse voltammetry or square-wave voltammetry can be done (Jignesh, S. et al, 2012).

Voltammetry is a method which is capable to detect multi elements either trace elements or essential elements in product. It can be defined as the method which a voltage scan is applied to an electrode and resulting current is recorded. The purpose of this technique is to measure the current with that can be used to determine the amount of concentration of an electrochemically active species (Ewing, G.W., 1997).

Electrochemical cell is the platform where the voltammetric experiment is carried out. When electroactive species present in the electrochemical cell, the applied potential will provoke the changes of the concentration of the species at the surface of electrode by the oxidizing or reducing them. (Kounaves, S.M., n.d). According to Protti, P. (2001), electric current flowing through electrode can be measured when element is discharging itself. Figure 2 shows the process of oxidation and reduction occur on the mercury drop electrode.

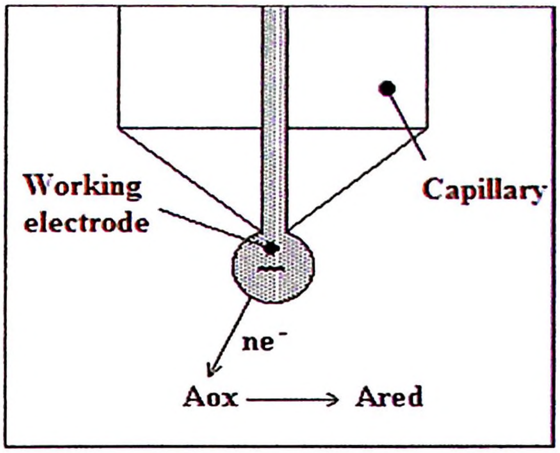

Figure 2: Discharging process of A_{ox} on mercury drop electrode.

For discharging to occur, it must follow some condition as shown below:

$A_{ox} + ne \rightarrow A_{red}$

Example:

$Pb^{2+} + 2e \rightarrow Pb$

A_{ox} can be reducible which is able to gain an electron at the electrode as shown above. Reducible means a lower or more negative potential is imposed to the electrode respect to the reduction potential of the compound A_{ox}. Then, the discharge of the A_{ox} to the surface of the electrode is fulfilled. The process is controlled by two kinetics factors:

i. The speed of the chemical compound reach to the electrode from the solution

ii. The speed of the electronic exchange between the electrode and the solution.

1.2.2 Anodic stripping voltammetry (ASV)

Anodic stripping voltammetry techniques can be used to measure the amount of trace metal in the environment, food analysis or pharmaceutical product. The results of this method are very precise and sensitive. Its precision is in the order 1 to 2% (Rouessac, F and Rouessac, A., 2000).

This technique also can be used to detect the concentration of the sample with low detection limits and minimal sample preparation. The sensitivity of this technique is improvised by use of pre-concentration or accumulation step, at which the element accumulated at the electrode. Metals are pre-concentrated by electro-deposition onto a small volume mercury electrode which is known as hanging mercury drop electrode (HMDE) (Jignesh, S. *et al.* 2012). Figure 3 shows the principle of anodic stripping method which pre-concentration can occur before the analysis of sample.

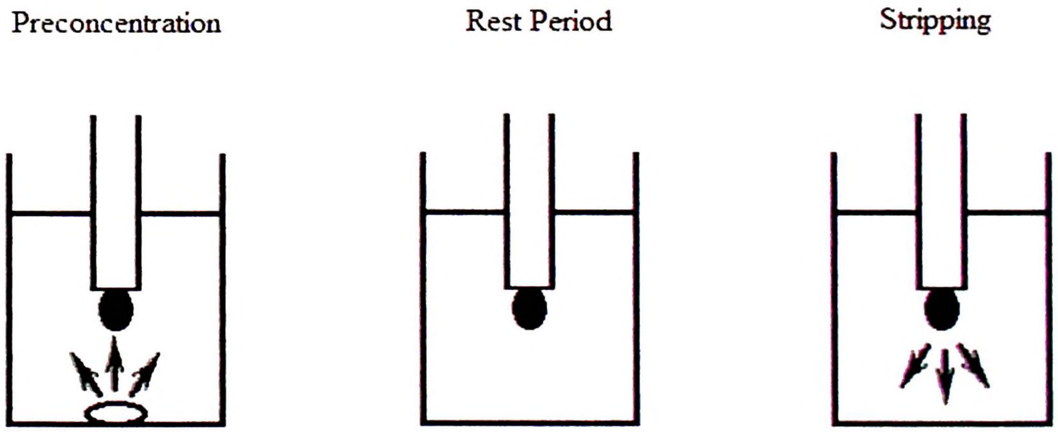

Figure 3: Principle of anodic stripping voltammetry (ASV).

Source: (Jignesh, S. *et al.* 2012).

The pre-concentrated analyte is then measured by the application of a potential scan. The species of interest is reduced into the mercury that forms amalgams (Kounaves, P.S., n.d).

When amalgamated, the metals are thereafter oxidised from the mercury electrode, when running the potential in anodic direction. Their dissolution process is depicted in voltammetric peaks, with position at the potential scale depending on the nature of the metals. The resulting peaks of the voltammetric responses are proportional to the metal ion concentrations in the solutions. This technique also can simultaneously determine several metal ions which is up to six if their standard redox potential differs for at least 200 mV (Gulaboski, R. and Pereira, C.M., 2008).

Hanging mercury dropping electrode (HMDE) is used. This is to enhance the reliability and convenience of stripping measurements. This is due to the automatic production of highly reproducible drops that hang at the capillary tip. Besides, built-in valve also is utilised to allowing the mercury flow to be stopped to produce the stationary electrode. This also improves the ability to hold the drop, over long deposition periods, in a stirred solution (Ewing, 1997).

1.2.3 Differential Pulse Voltammetry (DPV)

The differential pulse technique is one of the sensitive techniques for analysis of trace metal. There are many advantages of this technique, including information about the chemical form in which an analyte appears can be trace. Besides, acid-base chemistry can be characterised, the oxidation stated can be defined, and complexation can be detected (Rouessac, F. and Rouessac, A., 2000).

The potential form in differential pulse voltammetry consists of small pulse of constant amplitude 10-100 mV superimposed on a staircase-wave form. The current in this technique is measured at the beginning of the applied pulse and at the ending of the same pulse. It is measured twice in each pulse period. Differential pulse voltammogram as shown in Figure 4, referred to as the difference of the current measured for each single pulse (Gulaboski, R. and Pereira, C.M., 2008).

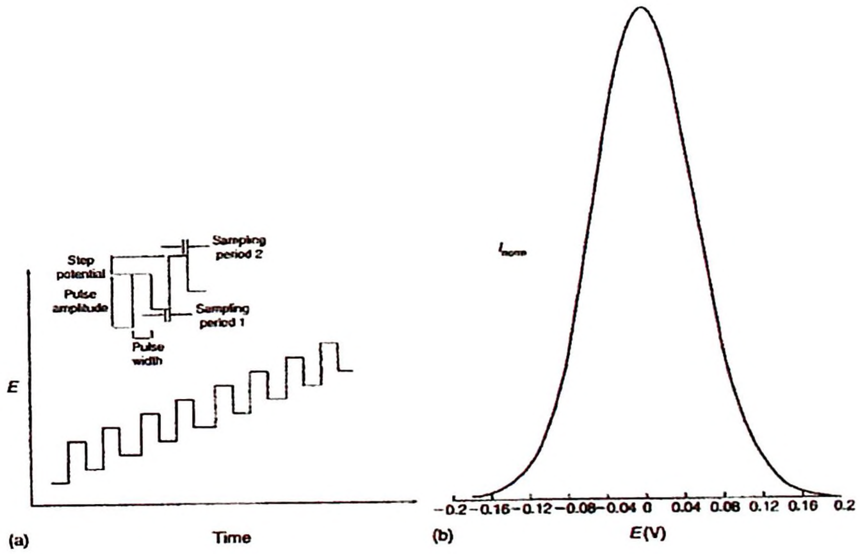

Figure 4: (a) Potential form and (b) Result of stimulated voltammogram in differential pulse voltammetry (Gulaboski, R. and Pereira, C.M. 2008).

2.0 LITERATURE REVIEW

Many techniques can be used to analyse lead such as stripping voltammetric technique, plasma emission spectroscopy, atomic absorption spectroscopy, and x-ray. Stripping voltammetric technique was the famous technique in analyse trace metal concentration over the years. Stazger and colleagues (1982) have reported the simultaneous determination of ultratrace levels of lead and cadmium in selected agriculture crops can be determined by differential pulse anodic stripping voltammetry.

Based on Matloob (2004), anodic striping voltammetric (ASV) method was a suitable method for analyze lead in foodstuffs such as leafy vegetables. Besides, it could analyse lead in wheat and rice sample (Ogorevc *et al.* 1987).

In 2000, Queirolo F and workers conduct an experiment to determine lead and cadmium in vegetables cultivated at northern Chile. The concentration of lead and cadmium were determined simultaneously by differential pulse anodic stripping voltammetry (DPASV). They determined concentration of lead and cadmium after two standard additions. They found no significant difference when compare result with inductively coupled plasma-mass spectroscopy. Concentration lead which determined by DPASV was 0.048 ppb and using ICP-MS was 0.045 ppb.

Abbasi Sh and coworkers (2009) studied the concentration of lead in edible oils and water sample using high selective adsorptive stripping voltammetry with 4,5-Dihydroxy-3-(p-Sulfophenylazo)-2,7-Naphthalene Disulfonic Acid Trisodium Salt (SPADNS). SPADNS act as complexing agent at hanging mercury drop electrode (HMDE). This method were found to be more accurate and precise and provide very low detection limits. It also showsed excellent selectivity against the other co-existing ionic species.

Besides, stripping voltammetry also has been used to detect lead in child blood (Mahajan R.K *et. al.* 2005). Differential pulse anodic stripping voltammetry also could be used to detect lead in pollen. According to Silveira *et al.*(2013) this technique was considered as a good alternative for pollen analysis because low operating cost and having scanning of parts per trillion (PPT).

Determination of lead also has been done on the infant formula by anodic stripping voltammetry. This technique was chosen because this technique is one of the most sensitive techniques with low cost (Jannat B. *et. al.* 2009).

Most of the voltammetry used mercury as the working electrode because mercury can be exchanged between experiments which can be used either hanging mercury drop electrode (HMDE) or dropping mercury electrode (DME). Therefore, this technique produced result without any bias because it provides a clean, reproducible surface for each determination. Mercury electrode also can access to wider range of potentials than other electrode surfaces (Brown R.J.C and Milton M.J.T, 2005).

Mercury also can be placed for the questioned elements to be absorbed and formed amalgam. The other techniques of determining trace metal elements by merely adsorbing it to surface. Mercury can be configured either in hanging drop or thin film. Thin film can detect the lower limit of detections (LODs) while hanging drop can avoid contamination (Brown R.J.C and Milton M.J.T, 2005). As shown in Table 2 the application of stripping voltammetry in the determination of lead in food samples.

Table 2: Application of stripping voltammetry for the determination of lead in food samples. (Alghamdi A.H., 2009)

Lead	Stripping voltammetric technique	Working electrode	Food sample	References
Pb(II)	ASV	HMDE	Cow's milk	Imam and Somer (2000).
Pb(II)	ASV	Mercury microelectrode	Honey	Sanna et al., (2000).
Pb(II)	ASV	Modified silver electrode	Canned juice, stewed fruit	Fomintseva et al., (1997).
Pb(II)	ASV	Think-film modified electrode	Wines	Brainina et al., (2004).
Pb(II)	ASV	Modified glassy carbon electrode	Edible oils,beer,honey,apple drink	Buckova et al., (1996) Gutierrez et al., (1999) Wahdat et al., (1998)
Pb(II)	ASV	Modified ultra microelectrode	Alcohol drink	Matysik et al.,(1994)
Pb(II)	ASV	Thick film epoxy graphite composite	Canned fruit, drink and juices	Faller et al., (1997).

Notes:

HMDE: Hanging Dropping Mercury Electrode

ASV: Anodic Stripping Voltammetry

The sensitivity of this voltammetric can be increased by pre-concentration step at which the element was accumulated at the electrode by either anodic or cathodic or absorption process. Then, the process was continuing to the voltammetric stripping step (Jignesh, S. *et. al.* 2012). This technique could detect very low elements concentrations by requiring long accumulation times to make sure that the material is enough at the electrode before it was measured. It also ensures that the material is accurately measured. In that way, stripping voltammetry could detect the lower LODs because the relative repeatability of the blank measurement is improved. The ability of use long accumulation stage as desired make the voltammetry technique could achieved lower LODs than the others technique (Brown R.J.C and Milton M.J.T, 2005). However, pre-concentration step required more time and involved rigid control of conditions (Abbasi 2009).

Besides voltammetric technique, flame atomic absorption spectroscopy (FAAS), X-ray fluoresce spectrometry, indirect methods such as the application of chemical ligand, plasma emission spectroscopy such as inductively coupled plasma atomic emission spectrometry, (ICP-AES), inductively coupled plasma - optical emission spectrometry, (ICP-OES), and inductively coupled plasma mass spectrometry, (ICP-MS), are applicable for lead determination.

Flame atomic absorption spectroscopy, (FAAS) is used in determination of lead in root vegetables after carbon activated enrichment. This technique is widely used in determine amounts of lead either in environment or biological matrixes because it is fast and sensitive. The purpose of enrichment step is to enhance the sensitivity of FAAS for trace metal analysis. However, this technique is need complex reagent (anthranilic acid) which is important parameter for determine lead using activated charcoal enrichment procedure. Without this reagent, it may effect pH, contact time, matrix component, accuracy and precision (Niazi S., et al., 1999).

Trace of lead in food also can be determined by X-ray fluoresce spectrometry. Based on Ali M and workers studies (2014) an energy dispersive x-ray fluorescence method using X-ray emitting isotopes in combination with pre-concentration by carbonization was developed to determine the levels of lead accumulated in foods. Compare to others techniques this techniques is more sensitive. However, this technique suffers from serious matrix effects and need special methodology develop to overcome this problem. It is also expensive, have a relatively low throughput and difficult to calibrate (Brown R.J.C and Milton M.J.T, 2005).

Concentration of lead also can be determined by using indirect methods such as the application of chemical ligands such as ethylenediaminetetracetic acid (EDTA), diethlenetriamine pentacetic acid (DTPA) and ammonium acetate or water and hydrochloric acid (Fargo and Mehra, 1992). However, there are few factors to be consider such as pH, temperature, redox reactions and chemical composition (Anyanwu Ec *et al.* 2000).

A study conducted by Allen L.B in 1997 had used for determination of arsenic and lead in sucrose, corn syrup, and high-fructose corn syrup. Before the samples undergo ICP-AES analysis, the sweeteners were digested (Allen L.B., 1997). The prominent lines of arsenic will overlap with carbons which make the chemical interferences limit the determination of lead (Allen L.B., 1997).

The other disadvantage of this technique is it products biased results low concentration because of interferences. It also suffer of possible loss of volatile lead species during ashing, contamination in the course of the digestion or chelating process, or non qualitative recoveries especially when numerous extraction steps are involved (Karadjova *et al.* 1998).

The advantage of ICP-AES is it has the ability to determine elements with high sensitivity, good accuracy and precision, wide dynamic ranges of working curves, multi element detection capability and extensively useful in analytical method (Brown R.J.C and Milton M.J.T, 2005).

Inductively coupled plasma - optical emission spectrometry, (ICP-OES) is other instrument used to detect lead in eggplant combined with microwave digestion procedure. This instrument is better than the other instrument because of charge transfer device with a detector, the possibility for the development of flexibility, consumers programmability, increasing spectral information and sample analyses (Dospatliev L, *et al.* 2012). However, it is complex and inter-element interferences are possible to occur especially when wavelength of the interest closely with another elements such as Pb and Nickel (Eag. 2014).

The others instrument that used in detect lead is inductively coupled plasma mass spectrometry (ICP-MS). ICP-MS is an instrument used for elemental and isotopic analysis, which combines the remarkable characteristics of ICP for atomizing and ionising samples with the sensitivity and selectivity of mass spectrometry (Beauchemin, D. 2006). This technique has also been employed for the determination of several metals such as Ag, As, Ba, Be, Cd, Co, Cr, Pb, Cu and Fe in oils, paying particular attention to the analysis of olive oils (Benincasa, *et, al.* 2007; Castillo, *et al.* 1999; Jiménez, *et al*. 2003; Jiménez, *et, al.* 2004).

ICP-MS is commonly used to detect the lead in oils due to the sensitivity, LODs, throughput and multi-element measurement (Brown R.J.C and Milton M.J.T, 2005). This instrument also can give result for both qualitative and quantitative in short time that independent on the number of isotopes. However, the lower duty cycle resulting from requirement to modulate the ion beam, it sensitivity and detection limits degraded, but this can disappear by monitor the isotopes (Beauchemin, D. 2006).

3.0 OBJECTIVES

3.1 General Objectives

To determine lead in the selected vegetables juice sample using anodic stripping voltammetric technique.

3.2 Specific Objectives

1. To apply the method for analysis of vegetables juice using anodic stripping voltammetric technique.
2. To apply standard addition calibration method for analysis of lead in commercial and raw vegetables juice samples.
3. To use voltammetric technique for analysing lead content in vegetable juice samples.

4.0 MATERIALS AND METHODS

4.1 Materials

4.1.1 Sample

Sample A, B, C, D and E were obtained from Tesco Supermarket at Kota Bharu, Kelantan. For the rest of samples were obtained at the market in Pengkalan Chepa, Kelantan and samples were divided into commercial samples and raw samples. Commercial samples were labelled as Sample A, B, C, D and E and the rest are the raw samples as shown in table 3.

Table 3: Label of vegetables samples

Sample	Sample types
Sample A	Tomato juice
Sample B	Pumpkin juice
Sample C	Winter chestnut juice
Sample D	Winter melon juice
Sample E	Carrot juice
Sample F	Spinach
Sample G	Kale
Sample H	Cabbage
Sample I	Mustard
Sample J	Eggplant

4.1.2 Chemicals

i. Potassium chloride, (Merck), Darmstadi, Germany.
ii. Sodium acetate, (Merck), Darmstadi, Germany.
iii. Lead, (Metrohm), Switzerland.
iv. Nitric acid 65%, (Merck), Darmstadi, Germany.
v. Hydrochloric acid fuming 37%, (Merck), Darmstadi, Germany.

4.1.3 Apparatus

i. Beaker, Pyrex. (50 mL)
ii. Volummetric flask, Pyrex. (10 mL, 25 mL, and 100 mL)
iii. Micropipette, Eppendorf. (100 μL and 1000 μL)
iv. Burrette, Hishmann EM. (5 mL, and 10 mL)
v. Mortar and pestle
vi. Burette bulb

4.1.4 Equipments

i. Weighing balance, Shimadzu ATX224, Uni bloc
ii. pH meter, pH211 microprocessor pH meter, Hanna instrument, Portugal
iii. Centrifuge machine, Hettich Zentrifuges, Universal 32 R, Germany.
iv. Printer, LBP 6000, Cannon, Vietnam.

4.1.5 Instrument

The main instrument in this experiment is voltammetry machine that was attached together with three electrode system, computer and stabilizer as shown in Figure 5.

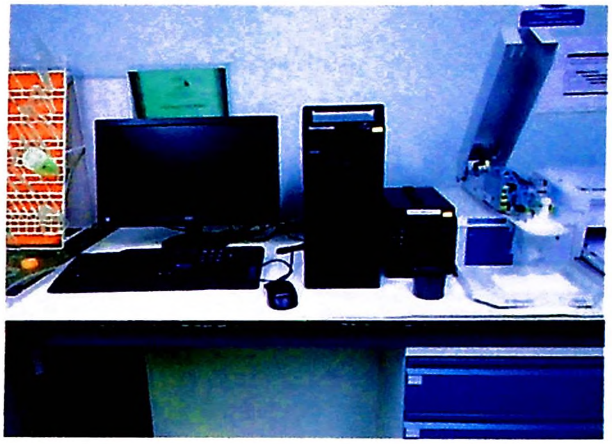

Figure 5: Voltammetric machine 797 VA computrace was attached together with three electrode system, computer and stabiliser.

Types of electrodes such as reference electrode, working electrode, and auxilliary electrode in voltammetric cell shown in Figure 6.

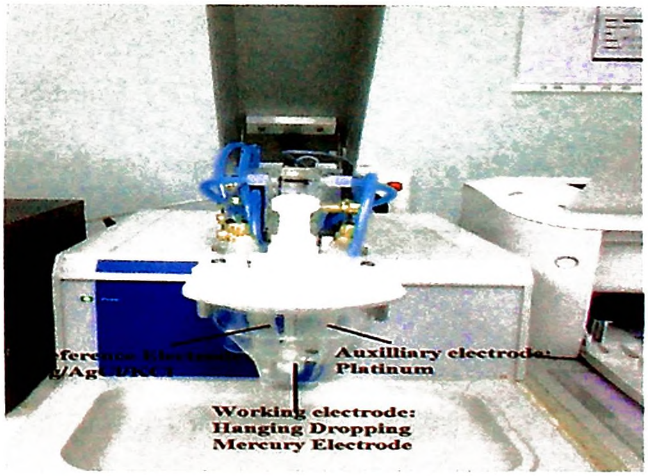

Figure 6: Voltammetric machine 797 VA computrace, Metrohm, Switzerland.

4.2 Methods

4.2.1 Sample collection

Sample of variety commercial vegetables juice from different brand were obtained from the Tesco supermarket in Kota Bharu. Sample of the varieties of raw vegetables were obtained from the market at Pengkalan Chepa area.

4.2.1 Reagent preparation

4.2.2.1 Acetate Buffer preparation (pH=4.64)

11.8 g of potassium chloride was added into 4.1 g of sodium acetate. Both are dissolved with deionized water. They were transferred to the 100 mL volumetric flask. Deionised water was added until mark of volumetric flask.

4.2.2.2 Lead standard preparation (5 ppm)

0.05 mL of stock solution of lead was transferred to the 10 mL of volumetric flask. Deionised water was added until mark of volumetric flask.

4.2.2.3 1M HCl

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

4.2.2.4 1M NaOH

Four g of NaOH was weighed and dissolved completely with deionized water. The solution was transferred into 100 mL volumetric flask. Then, deionised water was added to the mark of 100 mL.

4.2.3 Sample preparation

4.2.3.1 Commercial sample

Five milliliters of juice were taken in triplicate for digestion. Then, six ml of nitric acid and 0.5 mL of hydrochloric acid were added. The solution was evaporated to dryness on the hot plate at 40°C until clear solution was obtained and the solution left about 1 mL. The clear solution was transferred and diluted to 25 mL with deionised water. Before the samples were analysed, they have centrifuged for get homogenous solution and separating the residue with clear solution (Davidowski *et. al*. 2012). Clear solution was taken for the analysis.

4.2.3.2. Raw sample

Raw sample were grinding and one gram of sample were taken. Then, six ml of nitric acid and 0.5 mL of hydrochloric acid were added. The solution was evaporated to dryness on the hot plate at 40°C as until clear solution was obtained and the solution left about 1 mL. The clear solution was transferred and diluted to 25 mL with deionised water. Before the samples were analysed, they have centrifuged for get homogenous solution and separate the residue with clear solution (Davidowski *et. al.* 2012). Clear solution was taken for the analysis.

4.2.2.3 Stripping voltammetric analysis

Stripping voltammetric experiment was done with Metrohm 797 VA computrace using hanging mercury drop electrode (HMDE) mode. Mercury works as working electrode while platinum rod and a saturated Ag/AgCl electrode were used as counter and reference electrode respectively. A pH meter was used to measure pH of acetate buffer (pH=4.64).

Dissolved oxygen was removed from the sample by purging with purified nitrogen through the measuring vessel for 5 minutes. During the analysis, nitrogen was passed over the solution to prevent oxygen interference.

10ml of sample were put into electrochemical cell and 1ml of acetate buffer was added. The optimum experimental conditions were established as follows, start potential: -0.599 V, end potential: -0.199 V, deposition time: 60 s, equilibrium time: 10 s, voltage step: 0.005951 V, voltage step time: 0.15 s, sweep rate: 0.0397 V/s, pulse amplitude: 0.05005 V, pulse time: 0.04.

In the middle of the analysis the standard of lead (0.1 mL) was added three times manually to quantify lead concentrations in the sample. This method preferred as the sensitivity of the stripping voltammetry analysis could be varying between samples of ionic strengths.

After stripping analysis was done, the concentration that was shown in result was multiplied by dilution factor and minus with the concentration of lead content in water to get the actual concentration of lead in samples. Concentration of lead in deionized water is 0.145 ppb.