

CHAPTER 1

INTRODUCTION

1.1 Overview of the research

Vinegar has long been used in the food industry. Vinegar is defined as a liquid fit for human consumption and contains a specified amount of acetic acid. Vinegar is a seasoning used in food preparation throughout the world. Generally, vinegar is produced by the fermentation of raw plant materials such as grapes, apples, pineapple and rice. Vinegar was used as a healthy food and medicines. However, some trace elements may contaminate vinegar through raw materials and production process and they are considered toxic to people's health [1-6]. Therefore, analytical techniques of high selectivity and sensitivity are required for determination of these elements. The traditional techniques for measurement of some trace elements in the vinegar including atomic fluorescence spectrometry (AFS)[7], inductively coupled plasma mass spectrometry (ICP-MS)[7, 8], flame atomic absorption spectrometry (FAAS)[8] and graphite furnace atomic absorption spectrometry (GFAAS)[9].

However, these techniques involve complicated sample pretreatment for removal of matrix interferences, high instrumental cost and required operator skill. Thus, it is necessary to develop a simple method for the analysis of trace elements in vinegar. Alternatively, voltammetric techniques offers high sensitivity and easy sample pretreatment procedures. Moreover, high selectivity, fast sample analysis, low operating cost would be its advantages [10-17]. Therefore the stripping voltammetry is promising technique for determination of the trace elements in vinegar. A more environmental friendly bismuth film electrode will be used. Furthermore, acetic acid solution is feasible to be used as an electrolyte in voltammetric analysis, since vinegar is a liquid consisting mainly of acetic acid. So, the novel method for determination of trace elements in vinegar is interesting to explore.

1.2 Introduction of vinegar

Vinegar commonly used as seasoning or as household purpose such as for cleaning. The acetic acid content in vinegar is about 4-5 %w/w. Distilled vinegar is obtained by distillation of fermented vinegar or distillation of ethanol followed by fermentation with vinegar bacteria. Fermented vinegar is derived naturally through the conversion of ethanol to acetic acid by bacteria called *Acetobacter spp.* Several kinds of fruit are used as a raw material for producing vinegar includes apple and pineapple. Value added of too ripped fruit by producing vinegar is an approach of choice. Since fruit may contain trace amount of metals depends on the agricultural process, the resulting vinegar may be at risk to have some toxic metals. The Ministry of Public Health, Thailand, has set the maximum allowance of some metals in vinegar. These are 1 mg/kg for arsenic (As) and lead (Pb) and 10 mg/kg for zinc (Zn), copper (Cu), and iron (Fe). Therefore it is necessary to control the quality of the resulting vinegar and also its raw material [18]. So, the standard methods for determination of metals in vinegar samples are shown in the Table 1.1[19] and the analytical methods which have been reported for determination of metals in vinegar are shown in Table 1.2.

Table 1.1 The standard method for determination of metals in vinegar samples

Element	Standard Method	Regulation limits
Arsenic	Colorimetry (diethyldithiocarbamate) (AOAC 952.13)	1 mg/kg
Copper	Atomic absorption spectrophotometry (AOAC 971.20)	10 mg/kg
Iron	Photometry (IFU method No.15,1964)	10 mg/kg
Lead	Atomic absorption spectrophotometry (AOAC 972.25)	1 mg/kg
Zinc	Atomic absorption spectrophotometry (AOAC 969.32)	10 mg/kg

Table 1.2 Literature review on techniques for the determination of metals in vinegar

Element	Analytical Technique	LOD (µg/L)	Ref.
Mercury	Atomic fluorescence spectrometry	0.08	[7]
Lead	Graphite furnace atomic absorption spectrometry	0.3	[9]
Lead	Inductively coupled plasma mass spectrometry	10-300	[20]
	Graphite furnace atomic absorption spectrometry		
Lead	Graphite furnace atomic absorption spectrometry	0.4	[21]
Arsenic	Atomic absorption spectrometry		[22]
Calcium Copper Iron Magnesium Manganese Zinc	Flame atomic absorption spectrometry		
Potassium Sodium	Flame emission spectroscopy		

1.3 Introduction of zinc, cadmium, lead, and copper

1.3.1 Zinc

Zinc is commonly found in the Earth's crust. It is found as a mineral (most commonly sphalerite, zinc sulphide), often associated with the ores of other metals (e.g. copper, lead and cadmium). The mineral zinc is present in all organs, tissues, fluids and secretions. About 90% of total body zinc is found in skeletal muscle and bone, with much smaller amounts in the liver, gastrointestinal tract, skin, kidney, brain, lung, prostate and other organs. It is generally assumed that zinc is non-toxic because of the strong homeostatic regulation of processes controlling the absorption and secretion of this mineral. However, if zinc is ingested in very high doses it may lead to gastrointestinal distress and vomiting. Zinc is an essential trace element for human body. It is necessary for the formation of various metal-dependent enzymes, such as carboxyl peptidase, collagenase, dipeptidase, alkaline phosphatase, carbonic anhydrase, phospholipase C and neutral protease. Zinc supports a healthy immune system, helps to maintain sense of taste and smell, and is needed for DNA synthesis. Zinc also supports

normal growth and development during pregnancy, childhood, and adolescence. Zinc is abundant in a wide variety of food such as oysters, cereal, meat, beans and nuts.

The risk factors of zinc deficiency are inadequate intake and digestive diseases. Zinc is a part of several liver enzymes, one of which is important in the oxidation of alcohol to less toxic substances. A high level of alcohol in the body may cause the enzyme to break down, thus producing toxic conditions in the liver. Alcoholics having cirrhosis of the liver show a high level of zinc in the urine. Zinc toxicity has been seen in both acute and chronic forms. Intake of 150 to 450 mg of zinc per day have been associated with low copper status, altered iron function, reduced immune function and reduced levels of high density lipoproteins (the good cholesterol). Zinc salt-mainly $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ is used as supplements to correct zinc deficiency. Level of zinc relates to change in levels of other minerals in the body, hence, the determination of zinc in supplements was considerable [23-26].

1.3.2 Cadmium and Lead

Cadmium and lead are very heavy element. They occur in nature (present in small concentrations within hard rocks) in small amounts of the Earth's crust. They are usually found in ore with zinc, silver and copper, which significant amount of them are recovered as a by-product or co-product from mining and smelting of zinc. The cadmium and lead are removed when zinc metal is purified in a refinery process. The major forms of cadmium and lead in natural sources are sulphide form. In addition, cadmium and lead can occur as an impurity in phosphate minerals used as fertilizers. Cadmium and lead are used mainly in metal and in a small number of other applications of processing industries such as batteries, electronics and communications, ammunition, television glass, construction, a stabilizing compound in plastics, paint pigments, protective coatings, and a wide variety of household products. Cadmium and lead are emitted from industrial and natural sources to contaminate into the environment, e.g., in air, water and soil. Heavy metals may enter the human body through food, respiratory and skin when they come in contact with humans in agriculture and in industrial or residential settings. These metals are heavy metals that cause adverse health effects at very low exposure levels. They are well known metals that seriously affect on health.

Cadmium affects many organ systems primarily. It is similar to zinc in structure and function and may replace zinc in many physiological and enzymatic functions. It is absorbed into the body in the stomach. Then spread to the liver, spleen and intestines and the cumulative increase in high doses can induces cancer and Vertebrate limb pain. It causes the kidneys impaired. Diseases caused by cadmium poisoning were called kidney disease - kidney I (Itai Itai disease). Lead poisoning is one of the most common environmental health problems affecting on health. Lead can cause serious harm. When lead enters the body, it is held in the red blood cells to reduce the hems which interfere with the functioning of various enzymes, mitochondria and a neurotransmitter in the cell. It affects multiple systems of the body such as the nervous system, kidneys, gastrointestinal tract, reproductive system and circulatory system [27-31].

1.3.3 Copper

Copper is found naturally in a wide variety of mineral salts and organic compounds, and in the metallic form. The metal is sparingly be soluble in water, salt or mildly acidic solutions, but can be dissolved in nitric and sulfuric acids as well as basic solutions of ammonium hydroxide or carbonate. Copper possesses high electrical and thermal conductivity and resists corrosion. The copper metal has a specific gravity of about 8-9, and melts at about 1,100 °C. Copper is one of the first metals, which have been used by humans. Its usefulness is accounted by its combination of chemical, physical, electrical, mechanical properties and its fairly abundant supply. Anthropogenic emissions include smelters, iron foundries and power station sources such as municipal incinerators. The major release of copper to land is from tailings and overburdens from copper mines and sewage sludge. Agricultural use of copper products accounts for 2% of copper released to soil. Copper ores are mined, smelted and refined to produce many industrial and commercial products. Copper is widely used in cooking utensils and water distribution systems, as well as fertilizers, bactericides, fungicides, algacides and antifouling paints. It is also used in animal feed additives and growth promoters, as well as for disease control in livestock and poultry. Copper is used in industry as an activator in froth flotation of sulfide ores, production of wood preservatives, electroplating, azo-dye manufacture, as a mordant for textile dyes, in petroleum and the manufacture of copper compounds.

Acute toxicity due to ingestion of copper is infrequent in humans and is usually a consequence of the contamination of beverages or from accidental or deliberate ingestion of high quantities of copper salts. Numerous case reports of single oral exposures to high levels of copper have been reported. Such exposures, including suicide attempts with copper sulfate, have occurred in youths and adults at doses ranging from 0.4 to 100 g Cu. Symptoms including vomiting, lethargy, acute hemolytic anemia, renal and liver damage, neurotoxicity, increased blood pressure and respiratory rates. In some cases, coma and death followed. There are also a number of reports showing copper toxicity of high dose copper ingestion in beverages [32-34].

1.4 Anodic Stripping Voltammetry

Anodic stripping voltammetry is a voltammetric method for quantitative determination of specific ionic species. Anodic stripping analysis is the most sensitive of all commonly used electroanalytical techniques. Analyzes can be performed at the trace level and are applicable to solutions containing metal ions in the low concentration. Other advantageous features of stripping voltammetry include the capability for simultaneous multi-element determination and relatively inexpensive instrumentation compared to that required for the spectroscopic techniques.

1.4.1 Principle of stripping voltammetry

Anodic stripping voltammetry is an electrochemical method, which current is measured while voltage is applied to an electrochemical cell containing (1) electroactive analytes, (2) a supporting electrolyte solution, (3) a reference electrode (RE), (4) an indicator or working electrode (WE), and (5) an auxiliary electrode (AE) [35] (Figure 1.1). A current versus voltage curve is so called a voltammogram.

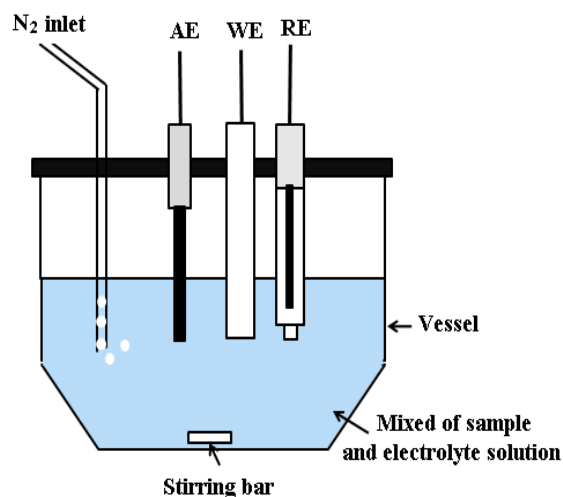


Figure 1.1 Diagram of an electrochemical cell for a voltammetric system

The diffusion current (i_d) produced by the desired reaction is proportional to the concentration of analyte (C_A) under ideal conditions,

$$i_d = k \cdot C_A \quad (1.1)$$

Where k is a constant[36]. Thus, the plot of i_d versus concentration can be used for calibration.

The technique consists of two steps of operation (Figure 1.1). The first step is a preconcentration or deposition step, metal ions are deposited onto an electrode which is held at a suitable potential by reduction at the surface of WE electrode. The solution is stirred during this step to maximize the amount of metal deposited. The period of preconcentration is the so called deposition time, t_d .



The second step, the stripping step, the deposited metal are stripped or oxidized from the electrode by scanning the potential toward the anodic (positive) direction. The ASV method is applied for cation determination. The current measured during the stripping step is related to the amount of the metal in the solution [35].



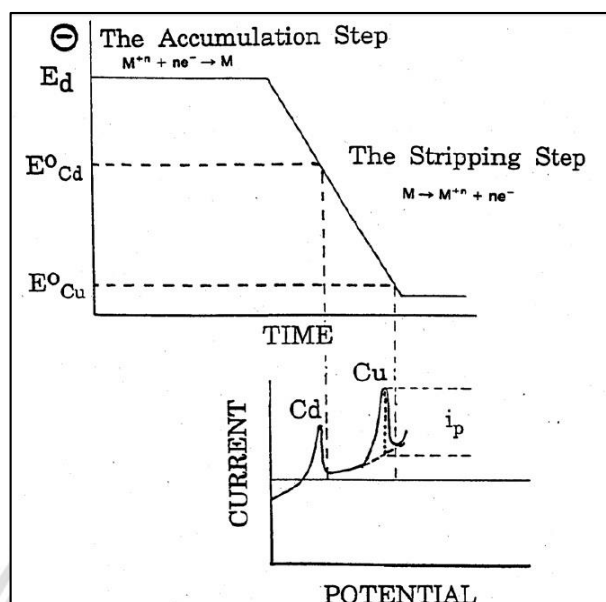


Figure 1.2 Operating step in anodic stripping voltammetric (ASV) method[37].

ASV is a sensitive electroanalytical technique. It can be used to in-situ preconcentrate trace metal ions, and thus it provides a low detection limit, i.e. ppb levels[36-38]. It can be used for the analysis of various metal ions simultaneously. This method can be used for quantitative and qualitative approach.

1.4.2 Square Wave Voltammetry

Square-wave stripping voltammetry is another approach to discriminating against the charging current, based on the different current decay rate following potential step. The square-wave potential-time waveform is shown in Figure 1.3. It involves the superposition of small square-wave potential amplitude on a staircase ramp. By sampling the current just before the square wave changes polarity, the charging current is corrected. The total current is plotted versus the potential. The high frequency of the square-wave cycles requires solutions of high supporting electrolyte content to allow rapid decay of the charging current. Aside from the charging current correction, the analytical current is enhanced by the opposite current associated with the metal repeating.

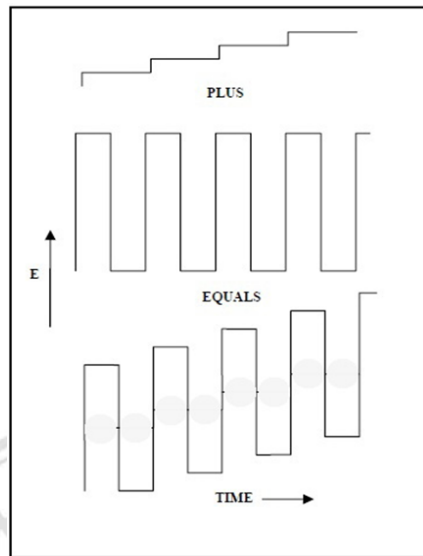


Figure 1.3 Potential-time waveforms used in square-wave stripping voltammetry [39]

The timing and applied potential parameters for square wave voltammetry are depicted in Figure 1.4. τ is the time for one square wave cycle or one staircase step in seconds. The square wave frequency in Hz is $1/\tau$. E_{sw} is the height of the square wave pulse in mV, where $2 E_{sw}$ is equal to the peak-to-peak amplitude. E_{step} is the staircase step size in mV. The scan rate for a square wave voltammetry experiment can be calculated from the equation

$$\text{Scan Rate (mV/Sec)} = \frac{E_{\text{step}} \text{ (mV)}}{\tau \text{ (sec)}}$$

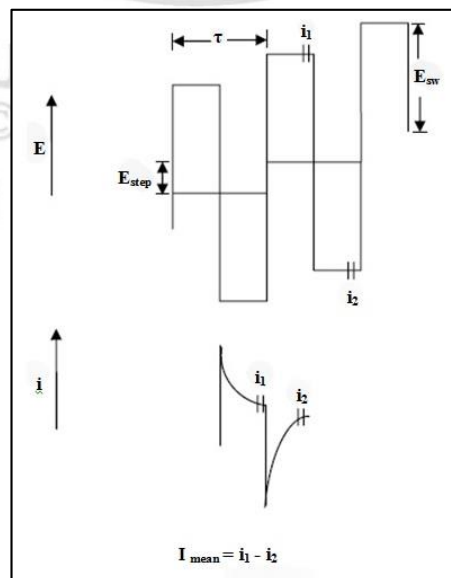


Figure 1.4 Square-wave stripping voltammetry timing relationships [39]

1.4.3 Working electrodes

The working electrode is an important component of the ASV. The analyte is reduced or oxidized here. There are several materials which can be used as a working electrode such as mercury, glassy carbon, gold and platinum, etc.

Mercury such as hanging drop mercury electrode (HDME) is commonly used as an electrode material because it is chemically inert in most aqueous solutions and hydrogen was evolved only at very negative potentials and the wide redox working range[40-42](Figure 1.5). It is useful because amalgams are formed with the mercury by the metals produced when the cations are reduced. Mercury electrode is a perfect electrode for ASV. Due to mercury is toxic to the environment; we are interested in using environmental friendly electrode materials such as bismuth film electrode.

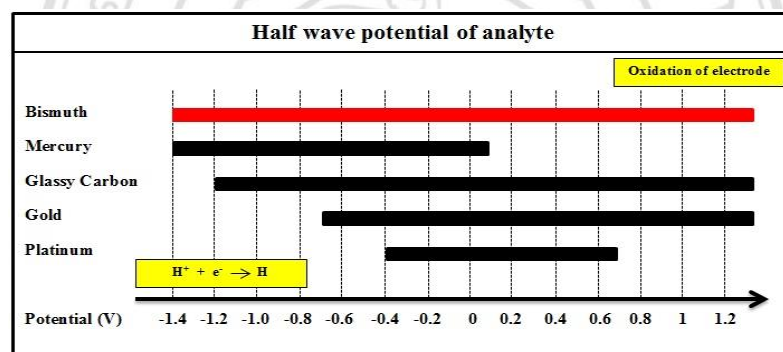


Figure 1.5 Potential ranges of the different working electrodes

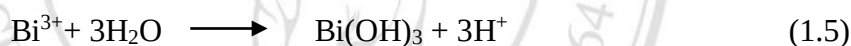
1.4.4 Bismuth Film Working Electrode

Bismuth film electrode (BiFE) is an alternative electrode for voltammetric analysis. It composes of metallic bismuth on a conductive supporting substrate similar to that of the conventional mercury film electrode that is being used commonly for anodic stripping voltammetric analysis [15-17, 43-46]. However, bismuth is negligible toxic comparing to the mercury. The high sensitivity of the electrode is due to the property of bismuth to from fused alloys with heavy metals, which is analogous to the amalgams that mercury forms according to equation (1.4). Bismuth can form fusible alloys with numerous heavy metals, including lead, cadmium, thallium, antimony, indium, gallium, etc. It has been deposited on several materials, such as glassy carbon, carbon paste, screen-printed carbon ink, carbon- fibers and gold.



The advantages of this electrode are simplicity of preparation, high sensitivity, good mechanical stability, well defined and highly reproducible stripping signal, good resolution between neighboring peak, low background characteristic, less affected by potential interferences, not required de-oxygenation step and more environmentally friendly. There are three methods of coating the electrode substrate with bismuth to produce a BiFE as listed below [47-49].

1. In situ plating BiFE. Bismuth film electrode can be electrochemically formed by in situ plating, by adding Bi(III) ions directly into the sample solution and the bismuth film is deposited together with other metals on the electrode surface during the analysis. Generally, the Bi(III) concentration must be at least 10-fold higher than the expected analyte concentration to avoid saturation effects. In situ plating method has some limitations in the accessible pH range of the sample solution. Bi(III) ions are very susceptible to hydrolysis in neutral and alkaline media according to the reaction (1.5).



In situ plating does not require separated bismuth plating solution, this method of coating the electrode substrate is simple and shortens the experimental procedure[47-51]. In this work, the preparation of BiFE by in situ plating on glassy carbon was investigated.

2. Ex situ plating or pre-plating can be prepared by electroplating of the substrate material with the bismuth from a solution containing Bi(III) ion separately before transferring the electrode to the sample solution for analysis. A concentration of Bi(III) can be in arrange of 5-200 mg/L with a deposition potential in the negative potential and a deposition time of about 1-8 min under condition of force convection (stirring or electrode rotation). The ex situ deposition of bismuth film was also proved to be a better option of film electrode preparation in the study of metal complexion where the presence of Bi(III) ions in the solution could interfere with the speciation of other metals [52-54].

3. Bi₂O₃ modified electrode. In this method, the electrode is prepared by modifying the substrate electrode with Bi₂O₃. It is prepared by mixing Bi₂O₃ in carbon paste to integrate it into the electrode surface. Bi₂O₃ is reduced to metallic bismuth on the surface of electrode by applying a sufficiently high negative potential [55-58] according to the reaction (1.6).

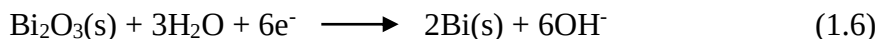


Figure 1.5 shows the potential window of bismuth film electrode which is wider than glassy carbon electrode. Bismuth film as working electrode because of a highly sensitive and accurate [59]. Many analytical methods based on voltammetry at bismuth film electrode have been proposed for determination of metal ions as shown in Table 1.3. Therefore, in this work it is of interest to develop an analytical method based on voltammetry using bismuth film electrode for metal analysis in vinegar.

Table 1.3 Anodic stripping voltammetry for metals determination

No.	Element	Sample	Method	LOD (µg/L)	Ref.
1	Cadmium, Zinc, Copper, Lead	Glaze ceramic surface extract	ASV (HMDE)	0.07-2.7	[10]
2	Lead, Cadmium	River water	ASV (BiFE)	0.5-1	[12]
3	Gallium	Ground water	ASV (BiFE)	6.6	[14]
4	Cadmium, Lead, Zinc	Tap water	ASV (pencil-lead BiFE)	0.3-04	[15]
5	Copper	Tap water	ASV (BiFE)	1.4	[16]
6	Zinc	Organic compounds	ASV (MFE)	0.1	[41]
7	Cadmium, Zinc, Copper, Lead	Water	ASV (HMDE)	0.02-0.2	[42]
8	Copper	Food	GCE	0.1-50	[60]

1.5 Research aims

According to the literature review, the determination of zinc, cadmium, lead and copper using anodic stripping voltammetry has enough sensitivity ($\mu\text{g/L}$ concentration level) with short analysis time. The use of bismuth film electrode for anodic stripping voltammetry is proper suit for sensitive measurement and environment safety.

In addition, the sample matrix which is mainly acetic acid can be acted as an electrolyte. The development of method for fast and sensitive determination of zinc, cadmium, lead and copper in vinegar is of interest.

The aims of this study are as follows;

1.5.1 To develop an analytical method based on voltammetry at bismuth film electrode for determination of traces elements in vinegar

1.5.2 To develop analytical method for trace metal analysis in vinegar with less sample pretreatment.

1.5.3 To apply the method for vinegar analysis