CHAPTER 1

INTRODUCTION

1.1 Background and Rationale

For many years, the element mercury (Hg) has received special attention from the scientific community since it caused the outbreak of the Minamata disease in 1956. Today, arsenic (As) is the focus of public attention. This is mainly due to the almost epidemic-like health problems of hundreds of thousands of people in Bangladesh and west Bengal, India, caused by As-contaminated groundwater (Matschullat, 2000).

Arsenic is a trace element which has generated increased interest in recent years due to its toxicity and its possible essential character (Woolso, 1975 cited in Ruiz-Navarro *et al*., 1998). Arsenic can enter terrestrial and aquatic environments through both natural formation and anthropogenic activity (Zhang *et al*., 2002).

Natural pathways of arsenic include weathering, biological activity and volcanic activity. The primary anthropogenic input derives from combustion of municipal solid waste, fossil fuel in coal- and oil-fired power plants, release from metal smelters, and direct use of arsenic-containing herbicides by industry and agriculture (Zhang *et al*., 2002).

Arsenic is considered as a potentially toxic element in the environment. It is very well known that toxicity depends not only on the total concentration but also on the chemical species in which this analyte is present (Vassileva *et al*., 2001). Inorganic arsenic species are known to be more toxic than organic ones. Arsenite (As^{III}) and arsenate (As^{V}) are the most toxic species. As^{III} is reported to be at least 60 times more toxic than As^{V} (Cavicchioli *et al*., 2004). The methylated forms of arsenic monomethylarsonic acid (MMAA), dimethylarsinic acid (DMAA) and trimethylarsine oxide (TMAO) are less toxic, followed by arsenocholine (AsC) and arsenobetaine (AsB) and finally the arsenosugars, which are regarded as being non-toxic (Ellwood and Maher, 2003).

Furthermore, the inorganic compounds of arsenic (As^{III} and As^{V}) have been classified as carcinogenic whereas MMAA and DMAA have been identified as possible cancer

promoters (B'Hymer and Caruso, 2004). Long-term exposure to low concentrations of arsenic can lead to skin, bladder, lung, and prostate cancer. Short-term exposure to high doses of arsenic can cause other adverse health effects (Zhang *et al*., 2002).

There are a number of ways by which the human population can become exposed to arsenic. The most important one is through ingestion of arsenic in drinking water. Nowadays, it is apparent that arsenic contamination in drinking water has created health problems in a number of countries such as India, Taiwan, Chile, Argentina, Mexico and China (Piamphongsant, 1999 and Huerga *et al*., 2004).

In case of Thailand, Ron Phibun Sub-District in the Ron Phibun District of Nakhon Si Thammarat Province in the southern part of Thailand, formerly a major tin-mining location, has been recognized to have a problem of arsenic contamination since 1987 (Oshikawa *et al*., 2001). Human health problems occurred by heavily contaminated arsenic in this area include chronic arsenic poisoning (arsenicosis) and skin cancer which results from drinking arsenic-contaminated water. This problem has been plaguing local people in Ron Phibun District for more than 30 years (Piamphongsant, 1999).

Arsenic-contaminated soil is considered to be one of the major sources of arsenic in drinking water. Therefore the determination of arsenic species in soil is crucial. In addition, the determinations of arsenic species in plants grown on contaminated soils is considered to be an alternative way to monitor the critical risks occurred by arsenic because the concentration of arsenic in plants (vegetables and fruits) is directly related to the level of arsenic in contaminated soil (Zhang *et al*., 2002). Therefore, the determination of arsenic contents and their distribution in environment (soil, water, air, plants etc.) is of importance for awareness of environment and human health.

Previously, the determination of total concentration of the element arsenic was considered to be sufficient for critical and environmental considerations (Vassileva *et al*., 2001). Since arsenic forms own great differences related to their metabolism and toxicity, it is necessary to determine the individual species concentration (arsenic speciation analysis) for a reliable assessment on the environmental impacts and health risks.

Numerous instrumental methods for arsenic speciation are now reported in the literature. Most of them are based on chromatographic separation techniques coupled with

selective detectors such as AAS, HG-AAS, ICP-MS, and ICP-AES (Molénat *et al*.,1999). Although these techniques show great sensitivity and selectivity, they usually require important investing and high running costs, therefore limiting their uses in most laboratories. As a result, other inexpensive alternative speciation methods are needed.

Electroanalytical methods, particularly voltammetric methods, are considered as an alternative choice for arsenic speciation that combine excellent sensitivity with the availability of low-cost portable equipment. In addition, they are ideal for field analysis and feature an intrinsic unique ability of detection of distinct oxidation states.

This thesis has concentrated on the study and optimization of electrochemical technique (Cathodic stripping voltammetry, CSV) in the determination of inorganic arsenic species in edible plants grown on high risk arsenic-contaminated area, Ron Phibun Sub-District, Ron Phibun District, Nakhon Si Thammarat Province (Thailand), compared with atomic spectroscopic technique (flow injection-hydride generation-atomic absorption spectrometry, FI-HG-AAS).

1.2 Review of Literature

1.2.1 General description of arsenic

Arsenic is a ubiquitous element in nature and exhibits both metallic and nonmetallic properties (metalloid element). It is the third element in group V(A) of the periodic table with an atomic mass of 74.9216 g/mol. Arsenic can show either electro-positive or electro-negative valence in its compounds. The valencies of arsenic are 0, -3, +3 and +5. However, its trivalent and pentavalent forms are the most common oxidation states. Arsenic was firstly discovered by Albertus Magnus, in 1250 AD, in the name of orpiment before it was called arsenic shortly afterwards (Nriagu, 1994; Matschullat, 2000).

The main uses of arsenic compound in antiquity were medical and pharmaceutical. Moreover, preparations as deadly poisons and pigments were also described. In the current technological age, human beings have found other beneficial properties of arsenic, thus

its exponentially increasing uses were found (Nriagu, 1994). The main current uses of arsenic compounds are summarized in Table 1-1.

Arsenic found in environment can be classified into three major groups namely inorganic arsenic compounds, organic arsenic compounds and arsine gases. Some of the common arsenic compounds found in the environment are listed in Table 1-2 and Appendix A-1.

Arsenic is widely distributed in rocks, soils, waters, air, plants, and animals (Cullen *et al*., 1989). Although arsenic is found in the environment to a small extent in its elemental form, it occurs mostly in inorganic and organic compounds.

Table 1-1 Main modern uses of arsenic compounds

Sector	Uses
Agriculture	Pesticides, insecticides, defoliants, wood preservatives, debarking trees, soil sterilants
Livestock	Feed additives, disease prevention, algaecides
Medicine	Antisyphilitic drugs, amebiasis, sleeping sickness, treatment of trypanosomiasis
Electronics	Solar cell, optoelectronic devices, semiconductor application, light-emitting diodes (digital watches)
Industry	Glassware, electrophotography, pyrotechnics, ceramics, antifouling paints, dyes and soap, pharmaceutical substances
Metallurgy	Alloys, battery plates

Source: Nriagu and Azcue, 1990

For inorganic form, arsenic is usually combined with other elements such as cobalt ($CoAs_2$), nickel (NiAs), iron ($FeAs_2$), which form the arsenide. However, arsenic was found concentrated in magmatic sulfides and iron ores, leading to the fact that the most important ores of arsenic are arsenic pyrite or mispickel (FeAsS), realgar (As_4S_4), and orpiment (As_2S_3) (Nriagu, 1994). Furthermore, it may be found as arsenic oxide, especially in water, namely under environmental oxidizing conditions. The predominant form of arsenic is arsenate (As^V) which

exists as oxyanions of arsenic acid ($H_2AsO_4^-$, $HAsO_4^{2-}$, AsO_4^{3-}). Under mild reducing conditions, arsenite (As^{III}) is thermodynamically stable and exists as arsenious acid ($HAsO_3^{2-}$, $H_2AsO_3^-$, H_3AsO_3) (Nha, 2004).

Table 1-2 Arsenic species commonly found in the environment

Name	Abbreviation	Chemical formula
Inorganic compounds		
Arsenite (arsenous acid)	As^{III}	$As(OH)_3$
Arsenate (arsenic acid)	As^{V}	$AsO(OH)_3$
Organic compounds		
Monomethylarsonic acid	$MMAA^{V}$	$CH_3AsO(OH)_2$
Monomethylarsonous acid	$MMAA^{III}$	$CH_3As(OH)_2$
Dimethylarsinic acid	$DMAA^{V}$	$(CH_3)_2AsO(OH)$
Dimethylarsinous acid	$DMAA^{III}$	$(CH_3)_2AsOH$
Dimethylarsinoyl ethanol	DMAE	$(CH_3)_2AsOCH_2CH_2OH$
Trimethylarsine oxide	TMAO	$(CH_3)_3AsO$
Tetramethylarsonium ion	Me_4As^+	$(CH_3)_4As^+$
Arsenobetaine	AsB	$(CH_3)_3As^+CH_2COO^-$
Arsenobetaine 2	AsB-2	$(CH_3)_3As^+CH_2CH_2COO^-$
Arsenocholine	AsC	$(CH_3)_3As^+CH_2CH_2OH$
Arsenic-containing ribosides	Arseno-sugars	various sugar structures
Arsines		
Arsine	AsH_3	AsH_3
Methylarsine	$MeAsH_3$	CH_3AsH_2
Dimethylarsine	Me_2AsH	$(CH_3)_2AsH$
Trimethylarsine	TMA^{III}	$(CH_3)_3As$

Sources: Gong *et al*., 2002; B'Hymer and Caruso, 2004

Organic form can be encountered attributing to the biological methylation of inorganic arsenic by microorganism (biotransformation). Arsenic was found as methylated forms such as monomethylarsonic acid (MMAA), dimethylarsinic acid (DMAA), and trimethylarsine oxide (TMAO). These species were well known to be often found in living organisms (Nriagu, 1994). In addition, some marine organisms have been shown to transform inorganic arsenic into more complex organic compounds, such as arsenobetaine (AsB), arsenocholine (AsC) and arsoniumphospholipids (WHO Regional Office for Europe, 2000).

For the pathway of arsenic to environment, there are numerous natural sources as well as anthropogenic activities that may introduce arsenic into environment. The primary sources of arsenic include geological formations such as rocks, soil and sedimentary deposits, geothermal activity and volcanic activity. These contribute to the regional background base level of arsenic and to abnormal geochemical arsenic conditions in some local areas. The major present and past sources of arsenic distribution influenced by human activities include the use of arsenic in wood preservatives, agricultural uses, industrial uses, mining and smelting activities (Ng, 2002).

1.2.2 The toxicology of arsenic

Arsenic is considered to be a potentially toxic element that can create severe health risks to human and other living organisms. In general, it was found that the toxicity of arsenic varies greatly with its chemical species and its toxicity has a strong relationship with status of arsenic in the component, that is, inorganic arsenic compounds are believed to be more hazardous than organic arsenic compounds (Kaise and Fukui, 1992).

Toxicological studies of arsenic help confirm that the degree of toxicity for arsenic depends upon its chemical species [i.e., arsine > arsenite (As^{III}) > arsenate (As^{V}) > monomethylarsonic acid (MMAA) > dimethylarsinic acid (DMAA) > arsenobetaine (AsB) and arsenocholine (AsC)] (Vassileva *et al*., 2001; Cava-Montesinos *et al*., 2005). The LD_{50} (50% lethal oral dose) values in rats obtained by toxicological study for some arsenic species (in $mgkg^{-1}$) are shown in Table 1-3.

For human acute poisoning incidents, the LD_{50} of arsenic has been estimated to range from 1 to 4 $mgkg^{-1}$ (Vallee *et al*., 1960). At non-lethal, but high acute doses, inorganic

arsenic can cause gastroenterological effects, shock, neuritis (continuous pain) and vascular effects in humans (Buchanan, 1962). Furthermore, acute toxicity is considered to be dependent upon other factors including physical state (gas, solution, or powder particle size), the rate of absorption into cell, the rate of eliminations , the nature of the chemical substituents in the toxic compound and the general state of the patient (B'Hymer and Caruso, 2004).

Table 1-3 The LD_{50} values in rats for some arsenic species

Arsenic species	**Abbreviation**	**LD_{50} (mg/kg)**
Arsine (gas)	AsH_3	3
Arsenite	As^{III}	14
Arsenate	As^{V}	20
Monometylarsonic acid	$MMAA^{V}$	700-1800
Dimethylarsinic acid	$DMAA^{V}$	700-2600
Trimethyl arsine oxide	TMAO	10600
Arsenobetaine	AsB	>10000
Arsenocholine	AsC	>10000

Source: Ng., 2002

In case of long term exposure, arsenic results in chronic arsenic poisoning (arsenicosis). This has been reported for people who live in endemic areas with higher arsenic concentrations in drinking water or in burning coal. Arsenicosis has also been reported for people exposed to the workplace. Skin lesions are the typical symptom of chronic arsenic poisoning. Moreover, chronic arsenic poisoning can cause damages in the respiratory system, digestive system, circulatory system, neural system and renal system. The most significant consequence is that inorganic arsenic causes cancers in various organs especially the skin, lung and bladder (Ng, 2002; ATSDR, 2000; IPCS, 2001).

1.2.3 Arsenic in the environment and human exposure

Arsenic is introduced into the environment from a number of sources. The main sources for the environment are from geological sources, either from surface weathering or underground deposits. Human activities do also play an important role. Arsenic compounds have been used in herbicides and pesticides for many years; the run-off from agricultural activity has been a persistent problem. Industrial uses for arsenic include in the manufacture of semiconductors and as a wood preservative (B'Hymer and Caruso, 2004). Arsenic contamination in environment depends upon the level of human activity, the distance from the sources of pollution and the dispersion and fate of the arsenic that is released (US-EPA, 2000). Arsenic appears in nature primarily in the form of sulfides in association with the ores of sulfides of silver, lead, copper, nickel, antimony, cobalt and iron. Therefore, trace amounts of arsenic are found in various environmental media.

1.2.3.1 Arsenic in air

Arsenic is released to atmosphere from both natural and anthropogenic sources. The principal natural source is volcanoes, with minor contributions by exudates from vegetation and wind-blown dusts. Man-made emission to air arises from the smelting of metals, the combustion of fuels, especially of low-grade brown coal, and the use of pesticides (Merian, 1984).

Arsenic in air is present mainly in particulate forms as inorganic arsenic. It is assumed that methylated arsenic is a minor component in the air of suburban, urban and industrial areas, and that the major inorganic portion is a variable mixture of the trivalent and pentavalent forms, the latter being predominant (WHO Regional Office for Europe, 2000).

Particulate arsenic compounds may be inhaled, deposited in the respiratory tract and absorbed into the blood. Inhalation of arsenic from ambient air is usually a minor exposure route for the general population. Assuming a breathing rate of 20 $m^3 day^{-1}$, the estimated daily intake may amount to about 20-200 ng in rural areas and 400-600 ng in cities without substantial industrial emission of arsenic (WHO Regional Office for Europe, 2000).

1.2.3.2 Arsenic in water

Arsenic is mainly transported in the environment by water. In oxygenated water, arsenic usually occurs as arsenate, but under reducing conditions, for instance, in deep well-water, arsenite predominates (WHO Regional Office for Europe, 2000). Arsenic is found at low concentration in natural water. The unpolluted fresh water has a level of arsenic ranging from 1-10 μgl^{-1}, rising to 100-5000 μgl^{-1} in areas of sulfide mineralization and mining (Mandal and Suzuki, 2002; Willium *et al*., 1996).

Arsenic contamination of drinking water is also a concern and has been an important topic in the recent literature. Drinking water arsenic contaminations in Bangladesh, India and other regions around the world (Table 1-4) have spurred research in the development of analytical techniques to monitor arsenic in its various chemical forms. Drinking water may contribute significantly to oral intake in regions where there are high arsenic concentrations in well water or in mine drainage areas.

The current maximum contaminant level (MCL) for all forms of arsenic in groundwater is 50 μgl^{-1} (50 ppb), set by USEPA (United States Environmental Protection Agency) in 1975. On 22 January 2001, USEPA adopted a new standard for arsenic in drinking water at 10 μgl^{-1} (10 ppb), to be enforced by January 2006 (Melamed, 2005).

1.2.3.3 Arsenic in soil

Arsenic concentrations in uncontaminated soil are generally in the range of 0.2-40 $mgkg^{-1}$. However, levels of 100-2500 $mgkg^{-1}$ have been found in the vicinity of copper smelters. Arsenic in soil occurs mostly in inorganic form. In oxygenated soil, inorganic arsenic is present in the pentavalent forms. Under reducing conditions, it is in the trivalent forms. Moreover, there is an ample evidence of biomethylation in soil and of the release of methylarsines into air, leading to the fact that arsenic may also be found in organic form (WHO, 1981). Biomethylation of arsenic in soil is illustrated in Figure 1-1.

According to arsenic biomethylation process (Figure 1-1), arsenate is reduced by microbial activity to arsenite. Further transformations are performed by methylation from arsenite

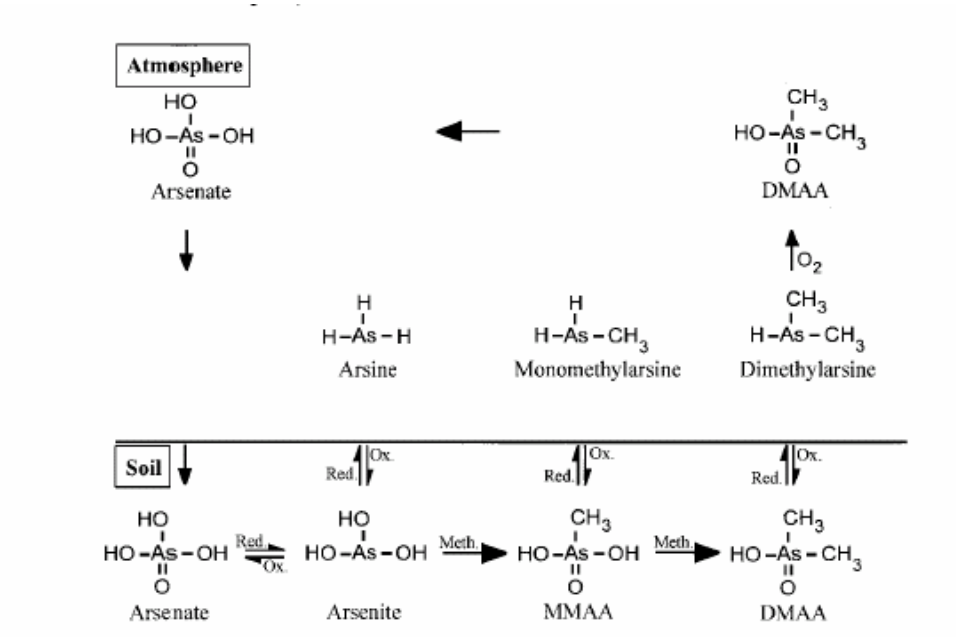
to MMAA and in another step to DMAA. The formed compounds can be reduced to corresponding arsines by aerobic microorganisms. These arsines, which are highly volatile, are transferred from the soil to the atmosphere where an oxidation process follows. As a consequence, the arsines are transformed to inorganic arsenic. The cycle is completed because atmospheric arsenate returns to the soil via dry deposition or rain (Pongratz, 1998).

Table 1-4 Episodes of arsenic caused poisonings and areas of potential arsenic contamination around the world

Country/area	**Population at risk**	**Sources of As exposure**	**Reported year**	**References**
Bangladesh	50,000,000	Drinking water	1980s	Saha, 1984
West Bengal, India	1,000,000	Drinking water	1980s	Chowdhury *et al*., 1999
Xinjiang, China	100,000	Drinking water	1980s	Wang, 1994
Inner Mongolia, China	600,000	Drinking water	1990s	Cheng, 1998
Taiwan	200,000	Artisan well	1950s	Tseng *et al.*,1968
Ron Phibun, Thailand	1,000	Drinking water	1980s	Choprapwon *et al*., 1995; 2001
Mexico	400,000	Drinking water	1983	Cebrian *et al*., 1983
Chile	437,000	Drinking water	1971	Sancha *et al*., 2001
Bolivia	20,000	Drinking water	1997	Sancha *et al*., 2001
Vietnam	Millions	Drinking water	2001	Berg *et al*., 2001
Hungary	220,000	Drinking water	1974	Sancha *et al*., 2001
Romania	36,000	Drinking water	2001	Gurzau *et al*., 2001

Source: Ng, 2002

The knowledge of the arsenic concentrations in soils is considered to be necessary because these concentrations are mostly responsible for groundwater contamination that can enter the aquatic environment and effect human health (Vassileva *et al*., 2001).



Source: Pongratz, 1998

Figure 1-1 Biological transformation of arsenic in soil

1.2.3.4 Arsenic in plants

Generally, in unpolluted environments, ordinary plants (vegetables and fruits) do not accumulate enough arsenic to be toxic to humans. However, in arsenic contaminated soil, the uptake of arsenic by the plant tissue is significantly elevated, particularly in vegetables and edible crops (Larsen *et al*., 1992). Therefore, it can be mentioned that arsenic concentrations in terrestrial plants, the widest varieties of arsenic species have been found in these sample matrices, depend upon soil arsenic-contaminations, the ability of the plant to accumulate and the types of plants (Frank *et al*., 2005).

Table 1-5 presents a compilation of arsenic concentrations in some kinds of plants. The values cited give normal concentrations without any known additional accumulation of arsenic from contaminated water, soil, fertilizer, or irrigation systems.

Table 1-5 Natural arsenic concentration in plants ($\mu g g^{-1}$)

Terrestrial plants	**Concentration ($\mu g g^{-1}$)**	**References**
Grass	0.020-0.160	Fergussun, 1990
Wheat grain	0.010-0.070	Fergussun, 1990
Oat grain	0.010	Fergussun, 1990
Barley grain	0.003-0.018	Fergussun, 1990
Clover	0.280-0.330	Fergussun, 1990
Carrots	0.040-0.080	Fergussun, 1990
Lettuce	0.020-0.250	Fergussun, 1990
Potatoes	0.030-0.200	Fergussun, 1990
Mushrooms	1.2-2.5	Bowen, 1979
Ferns	1.3	Bowen, 1979
Vegetables	0.01-1.5	Bowen, 1979
Kale	0.12	Bowen, 1979
Mosses and lichens	0.26	Reimann and Caritat, 1998
Shurb	1.2-80	Visoottiviseth *et al*., 2002
Herb	0.4-76	Visoottiviseth *et al*., 2002
Galanga	ND-2.6	Khoomrung, 2006
Curcuma	1.1-2.0	Khoomrung, 2006
Papaya	ND-1.0	Khoomrung, 2006
Guava	ND-0.5	Khoomrung, 2006
Holy basil	ND-4.5	Khoomrung, 2006
Sweet basil	1.8-7.4	Khoomrung, 2006
Polyscias leaves	ND-1.0	Khoomrung, 2006
Lemongrass	ND-1.0	Khoomrung, 2006
Water morning glory	1.0-2.3	Khoomrung, 2006
Citrus leaves	0.2-0.3	Khoomrung, 2006

Uncontaminated terrestrial plants commonly contain 0.2-0.4 $mgkg^{-1}$. However, in plants from contaminated sites arsenic concentrations, up to several thousand $mgkg^{-1}$ (dry mass) of arsenic have been observed (B'Hymer and Caruso, 2004; Frank *et al*., 2005). Furthermore, it was found that only soil arsenic concentrations far above average (> 200-300 $mgkg^{-1}$) will lead to elevated arsenic concentrations within the plant of 1 $mgkg^{-1}$ (wet weight). The highest arsenic concentrations occur in the root parts of plants (Bhumbla and Keefer, 1994).

Due to the fact the amount of arsenic ingested daily by humans via food is greatly influenced by the amount of and the kinds of food in the diet, it is necessary to pay attention to the accumulation of arsenic in agricultural crops and vegetables grown in the arsenic-affected areas.

1.2.4 Arsenic speciation analysis

Arsenic is commonly found throughout the environment in a wide array of chemical species whose toxicity and mobility vary. These species can be readily transformed by such events as biological activity and changes in redox potential or pH. This creates the possibility of a wide variety of unstable arsenic species that can transform with subtle changes in the environment. To determine the potential transformation and risk of arsenic in the environment for remedy decisions, the analysis of environmental samples should include identifying and quantifying both the total quantity of arsenic present and the specific chemical forms present, a procedure known as speciation (Melamed, 2005). In analytical chemistry, the term "speciation" refers to the determination of different oxidation states of an element that prevail in a certain specimen. This knowledge could help explain the mobility, storage, retention and toxicity of the different species in different environments including the human body (Burguera *et al*., 1997)

The techniques used for the detection of arsenic species in environmental and biological samples should be sensitive and selective. The rapid analysis of samples to prevent species conversion is also important (Gong *et al*., 2002).

Several techniques have been developed to determine the concentrations of the different arsenic compounds. The most widely analytical methods used for element speciation are hyphenated techniques, favored for both the efficient separation and elemental-specific detection (Villa-Lojo *et al*., 2002). Most suitable method currently used for arsenic speciation are based on

the combination of HPLC with very sensitive, element specific detection methods such as HPLC/AAS (Hansen *et al*., 1992), HPLC/AFS (Woller *et al*., 1995), or HPLC/ICP-MS (Wrobel *et al*., 2002).

To improve sensitivity and eliminate matrix effects, hydride generation (HG) technique has been employed as a convenient link between the chromatographic separation (González *et al*., 2003). Therefore on-line combinations such as HPLC/HG/AAS (Suner *et al*., 2001), HPLC/HG/AFS (Slejkovec *et al*., 2004), or HPLC/HG/ICP-MS (Wrobel *et al*., 2002) are now well described.

Hydride generation technique is the most popular sample derivertization method used for arsenic detection, since Holak first report in 1969 (Hung *et al*., 2004). It was a developed method in which sodium or potassium tetrahydroborate ($NaBH_4$ or KBH_4) has been used for various arsines production (Table 1-6), followed by the separation of arsenic from the liquid products of reaction. These arsines subsequently were detected by a variety of detectors. However, the formation of these arsines is pH dependent. The pH requirements of the reduction reaction indicate that the arsenic species must be fully protonated before they can be reduced to the corresponding arsines (Carrero *et al*., 2001). The reactions taking place in the production of arsines when using $NaBH_4$ as derivertization reagent are shown in following equations.

$$R_nAs(O)(OH)_{3-n} + BH_4^- + H^+ \rightarrow R_nAs(OH)_{3-n} + H_2O + BH_3 \quad (1)$$

$$R_nAs(OH)_{3-n} + (3-n)BH_4^- + (3-n)\,H^+ \rightarrow R_nAsH_{3-n} + (3-n)H_2O + (3-n)BH_3 \quad (2)$$

$$BH_3 + 3H_2O \rightarrow H_3BO_3 + 3H_2 \quad (3)$$

Where R is methyl group

n is number of 0-3

In reaction (1) arsenic in the +5 oxidation state is reduced to arsenic +3. Subsequent reaction with the tetrahydroborate (III) takes the arsenic compound to the corresponding arsines in reaction (2). In reaction (3) the borane generated in reactions (1) and (2) react further through hydrolysis to yield boric acid and gaseous hydrogen.

Table 1-6 Molecular forms and boiling points of arsines

Compound	pK	Volatile product	Boiling point (^{o}C)
Arsenous acid (As^{III})	9.23	AsH_3	-55
Arsenic acid (As^{V})	2.25	AsH_3	-55
Monomethylarsonic acid (MMAA)	2.60	CH_3AsH_2	2
Dimethylarsinic acid (DMAA)	6.19	$(CH_3)_2AsH$	35.6

Source: Burguera *et al*., 1998

It has been previously reported that arsenic compounds are reduced by $NaBH_4$ to corresponding arsines only when they are under acidic form (arsenic species must be fully protonated). The pKa values (Table 1-6) are 9.23, 2.25, 2.60 and 6.19 (1^{st} acidity) for H_3AsO_3 (As^{III}), H_3AsO_4 (As^{V}), $CH_3AsO(OH)_2$ (MMAA) and $(CH_3)_2AsO(OH)$ (DMAA) respectively. Therefore, adjustment of pH in the hydride generation allows a selective reduction of these compounds. Nowadays, this procedure is considered to be a commonly used method to obtain the speciation of various arsenic compounds (Molénat *et al*., 1999). However, for samples containing only inorganic arsenic (i.e., As^{III} and As^{V}), the chromatography separation part is unnecessary. Hydride generation –atomic absorption spectrometry (HG-AAS) technique based on arsine generation under controlled pH conditions or different reaction media can be directly applied to determination of both oxidation states of inorganic arsenic. For the determination of these species of arsenic using HG-AAS, the experiments were divided into two stages, namely, (i) selective determination of As^{III} under controlled pH condition; (ii) determination of total inorganic arsenic (TAs_{inorg}; i.e. sum of As^{III} and As^{V}) after pre-reduction of As^{V} to As^{III} with any reducing agents. The As^{V} content was estimated by the difference of both measurements (González *et al*., 2003).

In 1988, Glaubig and Sabine have developed a simple HG-AAS technique to analyze aqueous inorganic arsenic in the μgl^{-1} range. In their experiments, total arsenic (As^{III}+As^{V}) was determined by reducing As^{V} to As^{III} using KI then generating the hydride in 6 M HCl. As^{III} was determined by generating the hydride at pH 4-4.5 using an oxalate buffer. Detection limits for the total inorganic arsenic and As^{III} were less than 0.4 μgl^{-1}. As^{V} was calculated by difference between total inorganic arsenic and As^{III}. Moreover, they also found that spontaneous oxidation of

As^{III} to As^{V} may occurred on spiked samples mixed 3 hours before analysis (Glaubig and Sabine, 1988).

In 1997, Nielsen and Hansen have developed a volume-based flow injection-hydride generation-atomic absorption spectrometry (FI-HG-AAS) for determination and speciation of trace inorganic arsenic. The total inorganic arsenic was determined by on-line reduction of As^{V} to As^{III} by means of 0.5% (m/v) ascorbic acid and 1.0% (m/v) KI in 4 M HCl. The combined sample and reducing agent is initially heated by flowing through heating coil at 140 ^{o}C, and subsequently, flowing through cooling coil at 10 ^{o}C. As^{V} can be completely reduced to As^{III} by these processes, then arsine gas can be formed and carried to atomizer (quartz tube) by argon gas. For the determination of As^{III} using FI-HG-AAS system without the heating and cooling coil with mild hydrochloric conditions (0.03 M), As^{V} is not converted to arsine, thereby allowing the selective determination of As^{III} (Nielsen and Hansen, 1997).

In 1999, Samanta and co-worker have used a simple FI-HG-AAS system for the determination of arsenic in part-per-billion levels in water and biological samples. The organic matter in a biological sample was destroyed by acid digestion and dry ashing technique. The system was used for the determination and speciation of arsenic. As^{III} was determined in citrate/citric buffer at pH 3 and total inorganic arsenic was determined in 5 M HCl. Thus, As^{V} can be quantified from the difference (Samanta *et al*., 1999).

In 2002, Coelho and co-worker have developed a simple procedure for the direct determination of As^{III} and As^{V} in water samples by FI-HG-AAS, without pre-reduction of As^{V}. In this study, they used 0.1% (m/v) $NaBH_4$ to perform arsine selective generation from As^{III} and on-line arsine generation with 3.0% (m/v) $NaBH_4$ to obtain total inorganic arsenic concentration. As^{V} was calculated as the difference between total inorganic arsenic and As^{III}. Furthermore, the proposed system was tolerant to potential metal interferences such as Fe^{III}, Cu^{II}, Ni^{II}, Sb^{III}, Sn^{II} and Se^{IV}. It was found that these metals could, at an As^{III} level of 0.1 mgl^{-1}, be tolerated at a weight excess of 5000, 5000, 500, 100, 10 and 5 times, respectively. The method was also shown to be satisfactory for analysis, the detection limits of 0.3 $ngml^{-1}$ for As^{III} and 0.5 $ngml^{-1}$ for As^{V} were achieved (Coelho *et al*., 2002).

In 2005, Narcise and co-worker have developed a flow injection-column preconcentration-hydride generation-atomic absorption spectrometric (FI-column-HG-AAS)

method for determining μgl^{-1} levels of As^{III} and As^{V} in water samples, with simultaneous preconcentration and speciation. For the preconcentration step, the chloride-form anion exchange column was used and it was found that the enrichment factors (EFs) ranged from 5-40 and 4-24 for As^{V} and total arsenic, respectively. In this experiment, the As^{V} could be directly determined at the neutral pH and total inorganic arsenic (As^{III}+As^{V}) at pH 12. With As^{III} could be obtained from the difference between total arsenic and As^{V}. The proposed method provided detection limits of 0.03-0.3 and 0.07-0.3 μgl^{-1} for As^{V} and total inorganic arsenic (As^{III}+As^{V}), respectively (Narcise *et al*., 2005).

In the same year, Akter and co-worker have compared between HG-AAS technique with CE-UV (capillary electrophoresis-ultraviolet detector) technique and LC-ICP-MS (liquid chromatography-inductively coupled plasma-mass spectrometry) for the speciation of arsenic in groundwater samples. It was found that the results obtained by all three techniques are in good correlation ($r^2 = 0.996$; for total arsenic) (Akter *et al*., 2005).

Although HG-AAS technique is accepted to be able to accurately measure arsenic in environmental samples to parts per billion (ppb) concentration levels, some drawbacks have been reported for this technique including: (i) the method is limited to the materials that form volatile arsines; (ii) reaction conditions have to be strictly controlled; (iii) the presence of certain interfering elements can reduce the efficiency of HG; (iv) the method is laborious. Moreover, the development of this method requires important investing and high running costs, limiting their use in most laboratories (Molénat *et al*., 1999; Akter *et al*., 2005).

Electrochemical techniques are considered to be an alternative way commonly used to differentiate inorganic arsenic species. These techniques are well known as a simple, sensitive and inexpensive methods to speciate arsenic directly since As^{III} and As^{V} have different electroactivity (Li and Smart, 1996; He *et al*., 2004). Many kinds of electrochemical technique were applied to determine trace arsenic such as polarography, cyclic voltammetry (CV), anodic stripping voltammetry (ASV) and cathodic stripping voltammetry (CSV). However, recently stripping voltammetric techniques are perhaps the most popular voltammetric determination method for arsenic species in environmental samples. These methods are based on the preconcentration of the analyte on working electrode prior to the actual determination step during the potential scan (Cavicchioli *et al*., 2004).

In ASV (anodic stripping voltammetry), As^{III} or As^{V} is initially reduced to As^{0} (accumulation step) at a sufficiently negative potential and a film is formed on the surface of the working electrode (generally gold). In the subsequent reoxidation (stripping step) of arsenic, carried out by scanning with positive potential, leads to the anodic current recorded in the voltammogram (Cavicchioli *et al*., 2004) as follows:

Accumulation step : $As^{III} + 3e^{-} \rightarrow As^{0}$

Stripping step : $As^{0} \rightarrow As^{III} + 3e^{-}$

In CSV (cathodic stripping voltammetry), the accumulation step involves the use of an adsorption or deposition inducer (usually Cu or Se) to immobilize the analyte on the working electrode (generally hanging mercury drop electrode, HMDE). In the redissolution step, carried out by scanning to more negative potentials, arsine is formed according to the reaction below. (Cavicchioli *et al*., 2004)

Accumulation step : $2As^{III} + 3Cu(Hg) + 6e^{-} \rightarrow Cu_3As_2 + 3Hg$

Stripping step : $Cu_3As_2 + 12H^{+} + 3Hg + 12e^{-} \rightarrow 2AsH_3 + 3H_2 + 3Cu(Hg)$

Ferreira and Barros stated to the determination of inorganic arsenic species using anodic stripping voltammetric mode that As^{III} can be reduced to the element (As^{0}) at the potential of 0.25 V versus Ag/AgCl, in acidic solution onto a solid electrode such as gold, platinum or copper, and then stripped off using more positive potential. However, they found that there are often problems associated with As^{V} at solid electrodes, such as memory effect, limited sensitivity, and poor precision, which makes this approach inconvenient for routine analysis. (Ferreira and Barros, 2002)

In an attempt to avoid these above problems, cathodic stripping voltammetric mode at a hanging mercury drop electrode (HMDE) has been used to determine arsenic, utilizing the reaction between arsenic and copper or selenium to form an intermetallic compound (Cu_xAs_y) that can be preconcentrated on the HMDE and then cathodically stripped off (Cavicchioli *et al*., 2004). The application of cathodic stripping voltammetric techniques for the determination of arsenic in 1970-2005 periods that were listed in Table 1-7.

Table 1-7 Summary of cathodic stripping voltammetry methods for the determination of arsenic

Voltammtric mode	Electrode	Supporting electrolyte	Operative parameter	References
DPCSV (As^{V})	HMDE	$NaClO_4$=2 M ; NaCl=0.3 M ; Mannitol=0.5 M ; Cu^{II}= 2 mM	E_{dep}-0.550 V ; E_f -1.050 V ; $E_{1/2}$-0.880 V ; t_{dep} 60 s	Greulach and Henze (1995)
SWCSV (As^{III})	HMDE	HCl=2 M ; Cu^{II}= 0.8 mM ; $N_2H_4H_2SO_4$=0.04 mM	E_{dep}-0.400 V ; E_f -1.000 V ; $E_{1/2}$-0.760 V ; t_{dep} 60 s	Li and Smart (1996)
DPCSV (As^{V})	HMDE	H_2SO_4=0.4 M ; Mannitol=0.22 M ; Cu^{II}= 10 ppm ; Se^{IV}= 70 ppm	E_{dep}-0.550 V ; E_f -0.850 V ; $E_{1/2}$-0.690 V ; t_{dep} 240 s	Henze *et al*. (1997)
DPCSV (As^{III} and Se^{IV})	HMDE	NH_4OH(0.2M)/ NH_4Cl(0.4M); pH 9	E_{dep}-1.050 V; E_f -1.650 V ; $E_{1/2}$-1.308 V; t_{dep} 270 s	Locatelli and Torsi (1998)
SWCSV (As^{III})	HMDE	HCl=1 M ; Cu^{II}= 45 ppm	E_{dep}-0.39 V ; E_f -1.00 V ; $E_{1/2}$ -0.82 V ; t_{dep} 40 s	Ferreira and Barros (2002)
DPCSV (As^{III})	HMDE	HCl=1 M; Cu^{II}=4.6 ppm Se^{IV}=3.7 ppm	E_{dep}-0.44 V; E_f -0.9 V ; $E_{1/2}$-0.68 V ; t_{dep} 60 s	He *et al*. (2004)
SWCSV (As^{III})	HMDE	HBr=0.45 M; Cu^{II}=50 ppm	E_{dep}-0.4 V; $E_{1/2}$-0.8 V; t_{dep} 40 s	Profumo *et al*. (2005)

DPCSV differential pulse cathodic stripping voltammetry; SWCSV square wave cathodic stripping voltammetry; E_{dep} electrodeposition potential; E_f final scanning potential; $E_{1/2}$ stripping peak potential; t_{dep} electrodeposition time

In 2002, Ferreira and Barros developed method for determination of As^{III} and total inorganic arsenic in natural spring and mineral waters using square wave cathodic stripping voltammetry (SWCSV) at a hanging mercury drop electrode (HMDE). In the determination of As^{III}, preconcentration was carried out on the electrode from a solution of 1 M HCl in the presence of 45 mgl^{-1} of Cu^{II} at a potentail of -0.39 V versus Ag/AgCl, and the deposited intermetallic compound was reduced at a potential of about -0.82 V versus Ag/AgCl. For the determination of total inorganic arsenic, after As^{V} was reduced to As^{III} using thiosulfate, preconcentration was carried out in 1 M HCl in the presence of 400 mgl^{-1} of Cu^{II} at a potential of -0.40 V versus Ag/AgCl, and the intermetallic compound deposited was reduced at a potential of about -0.76 V versus Ag/AgCl. Furthermore, it was found that in the presence of thiosulfate, in the determination of total inorganic arsenic, Cu^{II} was less efficient in promoting the accumulation of As at the electrode, leading to a situation that required the use of a much higher concentration of Cu^{II}. As^{V} was calculated as the difference between total inorganic arsenic and As^{III}. For the determination of As^{III} the limit of quantification (LOQ) was 0.2 μgl^{-1} for a deposition time of 40 s. For total arsenic, the limit of quantification was 2 μgl^{-1} for a deposition time of 3 min. In addition, the developed SWCSV technique was compared with OES-ICP-HG (optical emission spectrometry with inductively coupled plasma coupled to hydride generation) technique. It was observed that the results, total inorganic arsenic, obtained by two methods were in good correlation (Ferreira and Barros, 2002).

In 2004, He and co-worker had developed a sensitive speciation method for inorganic arsenic in water at the μgl^{-1} levels based on differential pulse cathodic stripping voltammetry (DPCSV). In their study, only As^{III} is deposited on a HMDE in the presence of Cu and Se in HCl medium. Determination of total inorganic arsenic was performed by reducing As^{V} to As^{III} using sodium *meta*-bisulfite/sodium thiosulfate reagent stabilized with ascorbic acid. As^{V} was calculated by the difference between total inorganic arsenic and As^{III}. The DPCSV was performed using a deposition potential of -0.44 V versus Ag/AgCl, applied for 60 s with stirring. After that the deposited intermetallic compound ($Cu_xSe_yAs_z$) was stripped by scanning the potential from -0.4 to -0.9 V versus Ag/AgCl with 25 mVs^{-1} scan rate. The As^{III} peak appeared at about -0.68 V. For this technique, the limit of detection was 0.5 μgl^{-1} (He *et al*., 2004).

In 2005, Profumo and co-worker have described a voltammetric method for the determination of As^{III} and total inorganic arsenic in thermal, spring and sea waters at HDME using SWCSV technique. This method is based on the formation of a Cu-As intermetallic compound at HDME during the preconcentration step. For the determination of As^{III} , preconcentration was carried out on the electrode from a solution of 0.45 M HBr in the presence of 50 mgl^{-1} of Cu^{II} at a potential of -0.4 V versus Ag/AgCl/KCl, and the deposited intermetallic compound was reduced at a potential of about -0.8 V versus Ag/AgCl/KCl. In the following stripping step a peak of As^{III} was obtained. In case of determination of total inorganic arsenic, sodium dithionite was used for the reduction of As^{V} to As^{III} and As^{III} was then determined as previously described for As^{III}. Moreover, it was also found that Cu^{II}, in the determination of total inorganic arsenic, should be added after gas purging, to avoid the reduction of Cu^{II} by dithionite. As^{V} was calculated by difference from total inorganic arsenic and As^{III}. For this experiment, the limit of quantification of 0.010 and 0.020 μgl^{-1} for As^{III} and As^{V} respectively were obtained (Profumo *et al*., 2005).

In the case of interferences, all well known 90 or so naturally-occurring elements found in normal plant tissue are possible candidates. Only 16 or so elements (Nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, boron, chlorine, iron, manganese, zinc, copper, molybdenum, nickel, silicon, sodium, cobalt, selenium, chromium, vanadium, and titanium) are truly essential for plants growth (http://www.soils.wisc.edu). Therefore, These elements are of great concern in terms of interfering. Since the methods of FI-HG-AAS and SWCSV are used to determine arsenic have their own merits, especially high selectivity, so the effect of interfering ions commonly found in samples can not deteriorate the analysis. Moreover, previous several studies suggested good confirmations that almost interferences can be tolerated by both methods namely;

In 1995, Greulach and Henze used CSV technique to analyze As^{V} in standard stream sediment and in water samples. The effect of interferences, i.e., the metal ions Pb^{II}, Bi^{III}, Sn^{IV}, Sb^{V} and Se^{IV} at a 100-fold mass excess on the stripping peak for arsenic was tested. It was found that only Sb^{IV} and Se^{IV} showed a large suppression of the arsenic peak, but when present in equimolar amounts, these two elements did not interfere (Greulach and Henze, 1995).

In 2000, Gòmez-Ariza and co-worker compared coupled HPLC-(UV)-HG-AFS and HPLC-(UV)-HG-ICP-MS and they also studied the possible matrix effect using both external

calibration and standard addition techniques in different environmental samples (water, sediment and biota). They found that the presence in some of these samples of elements such as Cu and Fe, which could interfere in hydride generation step, did not produce any significant difference in the results obtained with both methods or the calibration (Gòmez-Ariza et al., 2000).

In 2002, Coelho and co-worker studied the effects of potential interference that are known to interfere in the hydride process. They found that for 0.1 mgl^{-1} of arsenic a serious signal depression was only observed for Se^{IV} at a 5-fold excess. Interference was also caused by Sn^{II} at 10-fold higher level. Sb^{III} can be tolerated without interference up to a 100-fold excess. Ni^{II}, Cu^{II} and Fe^{III} can be tolerated up to a 500-, 5000- and 5000-fold excess, respectively (Coelho *et al*., 2002).

In 2004, He and co-worker used CSV technique to determine trace level inorganic arsenic in natural water samples and also investigated interference from Fe^{II}, Mn and phosphate. For solution containing 45 μgl^{-1} of As^{III} or As^{V} and different levels of Fe^{II} up to 300 μM and Mn up to 100 μM, these two ions were found to have negligible interference. In the case of phosphate, concentrations ranging from 5 to 100 μM had no effect on a 45 μgl^{-1} of As^{III} solution (He *et al*., 2004).

In 2005, Akter and co-worker optimized HG-AAS method to the analysis of ground water samples. After sample matrix and ionic effect (Fe, Mn, Al, P, S, Ca and Mg) on absorption signal were evaluated, they observed that the analysis was free of interferences (Akter *et al*., 2005).

In 2005, Profumo and co-worker described the use of CSV technique to determine As^{III} and total inorganic arsenic. They reported that heavy metals (Zn, Cd, Pb, Ni, Fe and Co) did not interfere at concentration $\leq$10 mgl^{-1}. Se, when Se/As $\geq$100 interfered because of the competitive accumulation process between As-Se and As-Cu. Moreover, they also reported that no interference or signal decrease was observed with alkaline and alkaline earth metals, halide, nitrate and sulfate even at concentration greater than 100 mgl^{-1}(Profumo *et al*., 2005).

In 2006, He and co-worker used CSV technique to determine arsenic species in environmental water. They also studied potential interferences caused by ions commonly found in environmental water, especially Mn^{II}, Fe^{II}, Cr^{III}, Zn^{II}, Cd^{II}, Ca^{II}, Mg^{II} and phosphate. They found that, for samples containing 50 μgl^{-1} of As^{III} or As^{V}, no interferences were found for As^{III} or As^{V}

samples with up to 120 μM Mn^{II}, 80 μM Fe^{II}, 10 μM Cr^{III} and Cd^{II}, 200 μM Zn^{II}, Ca^{II}, Mg^{II} and phosphate, respectively (He *et al*., 2006).

From all literature reviews mentioned above, it was revealed that almost interferences can be tolerated by both methods. Therefore, the study of effect of interference in this work was not considered and tested. Moreover, the literature reviewed above also indicates that CSV is considered to be an alternative way for determination of inorganic arsenic species (As^{III} and As^{V}). Therefore, this thesis has an objective to study (development and optimization) and to compare the recently attractive method, electrochemical technique (Cathodic stripping voltammetry, CSV), and the commonly used method, atomic spectroscopic method (flow injection-hydride generation-atomic absorption spectrometry, FI-HG-AAS), for determination of two inorganic arsenic species and to be applied to environmental samples which are edible plants grown on high risk arsenic-contaminated areas, Ron Phibun sub-district, Ron Phibun district, Nakhon Si Thammarat province (Thailand).

1.3 Objectives

1. To optimize some operating conditions of a cathodic stripping voltammetric technique for the determination of inorganic arsenic species (As^{III} and As^{V}) in edible plant samples.

2. To compare the performance of cathodic stripping voltammetry (CSV) and flow injection-hydride generation-atomic absorption spectrophotometry (FI-HG-AAS) for the speciation of inorganic arsenic (As^{III} and As^{V}) in edible plant samples.

1.4 Anticipated outcome

1. The optimized technique can be used to the determination of inorganic arsenic species in environmental samples.

2. The efficiency of CSV and HG-AAS techniques will be compared.

3. The amount of accumulated arsenic species in some edible plant samples grown on high contaminated arsenic areas will be obtained.