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Determination of Arsenic in Water by Potentially Portable Methodology

Chengbei Li
University of Massachusetts - Amherst, chengbeili@ymail.com

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DETERMINATION OF ARSENIC IN WATER BY POTENTIALLY PORTABLE METHODOLOGY

A Dissertation Presented

By

CHENGBEI LI

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Approved as to style and content by:

Julian F. Tyson, Chair

Peter C. Uden, Member

Edward G. Voigtman Jr, Member

David A. Reckhow, Outside Member

Craig T. Martin, Department Head
Department of Chemistry

DEDICATION

To my parents

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First of all, I would like to give my special thanks to my advisor Dr. Julian Tyson. Thank him for not only giving me the best guidance in my research area, but also teaching me how to be a scientist, how to balance between science and personal life.

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ABSTRACT

DETERMINATION OF ARSENIC IN WATER BY POTENTIALLY PORTABLE METHODOLOGY

FEBRUARY 2013

CHENGBEI LI, B.S., XIAMEN UNIVERSITY

Ph.D., UNIVERSITY OF MASSACHUSETTS AMHERST

Directed by: Professor Julian F. Tyson

Arsenic contamination in groundwater is a worldwide problem. The existing portable field test kits can not provide accurate results when the arsenic concentration is around 10 μg L^{-1} or lower. This research first was focused on the development and validation of methods in which portable instrumentation, such as electrochemistry instruments or quartz crystal microbalances, could be used to accurately determine arsenic concentrations in water even when the concentration is below 10 μg L^{-1}. A modified anodic stripping voltammetry (ASV) and cathodic stripping voltammetry (CSV) method with measurement at a microarray electrode manufactured by TraceDetect Inc. was developed. When the ASV method with a gold electrode was applied for real water analysis, the detection limit of arsenite was 2.2 μg L^{-1}, and for arsenate was 0.13 μg L^{-1}. In the CSV method the more commonly used hanging mercury drop electrode was replaced with a mercury film array electrode. Under the optimum condition, this method had a detection limit for arsenite of 0.58 μg L^{-1} and for arsenate of 2.7 μg L^{-1}. A method for the determination of arsenic using a quartz crystal microbalance was developed in which the crystal surface was modified in situ by dithiolthreitol, an arsenite-selective ingand. The method was applied to real water sample analysis with a limit of 0.6 μg L^{-1}.

The second was concerned with an investigation of the kinetics of the reactions that are the basis of several currently available field test kits (as exemplified by the Hach Kit) using inductively coupled plasma mass spectrometry (ICP-MS) with the goal of improving the performance of the test kit. The time for arsine gas reaches to the maximum concentration in the headspace of the vessel was about 60 min without continuous stirring and only 20% of arsenic was absorbed on the test strip. To speed up the arsine generation, continuous stirring condition can be applied. It also made more arsine absorbed on the test strip. The SEM study proves the structure of the darker colored compound. For the lighter colored compounds, the information is not enough to make a conclusion.

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INTRODUCTION

1.1 The Overview of Arsenic Contamination over the World

In the natural environment, arsenic exists in four oxidation states: As(V), As(III), As(0), and As(-III).[1] The main species of arsenic found in the environment are the arsenites and arsenates. The toxicity of arsenic is different among different arsenic species and conforms to the following order (highest to lowest): arsine > inorganic arsenites > organic trivalent compounds > inorganic arsenates > pentavalent compounds > arsonium compounds > elemental arsenic.[2] Arsenobetaine and arsenocholine are considered to be non-toxic. The extent of arsenic poisoning depends on various factors such as dose, individual susceptibility to arsenic and the age of the affected individuals. People who are exposed to toxic arsenic species, can suffer diseases, such as skin cancer, lung cancer, high blood pressure and diabetes. Thus, some arsenic species are classified as genotoxic and carcinogenic by International Agency on Research Cancer (IARC), and relevant compounds impose significant risks to the health of people of many different countries. As many as 60-100 million people globally may be at risk of exposure to relatively high levels of arsenic.[3]

People are exposed to arsenic from many sources. Arsenic contained in water, soil or food, that is ingested arsenic, may quickly enter the human body. When air containing arsenic dust is breathed in, the majority of the dust particles settle onto the lining of the lungs.[4] Very little internal exposure to arsenic occurs via the material passing through the skin into the body, and so there is little risk of arsenic poisoning posed by this route. People are usually exposed to arsenic via diet, with exposure to the inorganic species often occurring through consumption of contaminated drinking water. Consumption of foodstuffs, seafood in particular, most frequently results in exposure to organic arsenic species. Certain agricultural practices, such as the use of arsenic-based herbicides and pesticides, may introduce inorganic arsenic species into the food supply, thereby facilitating human exposure via foodstuffs. For example, the treatment of US poultry with arsenicals in an effort to prevent *Coccidiodes spp.* infection, and enhance growth, introduces an average of 8.07 μg As day^{-1} into the US diet, 65% of which is inorganic arsenic.[5] Use of inorganic arsenic contaminated water for cooking also results in hume exposures. Lots of researches are focused on arsenic contamination in groundwater. A literature search was conducted employing the Web of Science and 992 papers were found with the words "arsenic contamination" and "groundwater: in the titles. Arsenic can be introduced to a groundwater system through many means, including incursion by surface and precipitation as well as from anthropogenic and naturally occurring sources.[6] Human activity can also aggravate and accelerate the release of naturally occurring arsenic.[7] In groundwater, arsenic is predominantly as inorganic As(III) and inorganic As(V), together with a minor amount of methyl and dimethyl arsenic compounds. Arsenic is often found in association with sulfide-bearing minerals, such as among the ores of copper, lead, and zinc, facilitating liberation during mining, smelting and manufacturing operations.[8] Dissolution of inorganic arsenic into drinking groundwater sources from minerals and ores, black or carbonaceous shales in particular, occurs in semi-arid and arid inland regions where groundwater is highly alkaline, as well as in alluvial aquifers where conditions are strongly reducing.[9] The WHO, the European Union (EU) and the U.S. Environmental Protection Agency (EPA) have recommended that the maximum arsenic concentration in groundwater is 10 μg L^{-1}, although Bangladesh, India and several other nations maintain a 50 μg L^{-1} maximum level.[10] However, many countries, such as India, China, Vietnam and Bangladesh, have reported the concentrations of arsenic in groundwater are higher than 10 μg L^{-1}, as shown in Figure 1.1. Yadav et al. reported that the groundwater in South Asia is the worst affected with arsenic contamination in the world.[11] Every day, more then 100 million people in Bangladesh, Cambodia, China, India, Myanmar, Nepal, Pakistan, and Vietnam are exposed to drinking water contamination with arsenic. In 1999, the Department of Water Supply and Sewerage in Nepal was the first organization to conduct a comprehensive study on groundwater arsenic contamination. Of the samples they analyzed, over 20% exceeded the WHO arsenic limit. In Bangladesh, within the area of 38,865 km^2, 42.7 million of the population was exposed to the arsenic-contaminated groundwater.[12] In Vietnam, several million habitants are at considerable risk of chronic arsenic poisoning, with contamination levels reach up to 3,050 μg L^{-1} in groundwater samples.[13] In US, prior to 2000, 18,850 portable water sampling locations were analyzed and 14% of public US groundwater supplies exceeded 10 μg L^{-1}, whereas 22% were contaminated by 1-10 μg L^{-1} inorganic arsenic. Table 1.1 summarizes the concentrations of arsenic in human tissues and urine collected from residents form the arsenic-contaminated areas.

To accurately determine arsenic concentrations in water samples, many techniques have been used, such as AFS,[14] ICP-MS,[15] ICP-AES,[16] HG-AAS.[17] All these techniques can provide high sensitivity and low detection limit, as shown in Table 1.2.[1] Notably, simple flame AA, the most affordable of the atomic spectrometric instruments, is incapable of determining arsenic at the requisite levels without hydride generation. In addition, with atomic spectrometric detection, some form of front-end separation technique is needed if speciation is desired. Moreover, these instruments are large, expensive, and need well-trained operators, stable power supplies, gases, and are usually involved in methods with relatively long analysis times. As a result, for field studies, cheap, portable and easy-operate techniques are needed, such as colorimetric test kits or electrochemical techniques.

1.2 Determination of Arsenic in Environmental Samples by Voltammetry

Voltammetric techniques have been used for the detection of environmentally important metal species detection in natural water since the early 1970's. This electrochemical technique is characterized by its unique selectivity for arsenite, minimum sample preparation, extremely high sensitivity and very low detection limit, which is comparable to that of atomic absorption. Unlike some techniques, electrochemistry does not require arsine hydride generation. For some field kits, arsine was generated during the reaction. With voltammetry, this is a clear advantage for field application and method development. Most hydride generation techniques measure total arsenic unless a chromatographic separation is implemented while arsenite can be measured selectively by electrochemical technique without separation and the total arsenic can be measured after chemical or electrochemical reduction of arsenate to arsenite. The stripping voltammetric techniques for trace metal analysis have been known to chemists for more than 50 years. The basic process involves electrochemical deposition of trace metals onto a suitable electrode for few minutes and then the oxidation of the metals back into solution by a reverse potential scan, which can be either linear or pulsesd. The oxidation current (stripping current) is recorded as a function of scan potential and the resulting peakshaped voltammograms is the analytical signal. The techniques mainly include anodic stripping voltammetry (ASV) and cathodic stripping voltammetry (CSV). The first one is becoming popular because of its low detection limit and easier operation procedure.[18] Moreover, ASV has been demonstrated to be able to detect arsenic in liquid samples in the field. The US EPA has approved a method, SW-846-7063, for arsenic determination by ASV that is able to detect 0.1 $\mu g\ L^{-1}$ arsenic in water samples. This detection limit is much lower than the maximum concentration recommended by the WHO.

Analysis by ASV consists of two steps: deposition and stripping. For the deposition step, samples are acidified with hydrochloride acid, the working electrode is placed in the solution and then arsenate or arsenite is reduced to As(0) at a sufficiently negative potential. The changes in oxidation state are summarized below:

$As(III) + 3e^- \rightarrow As(0)$

$As(V) + 5e^- \rightarrow As(0)$

The arsenic is deposited as a thin layer on the surface of the working electrode. Next, a linearly increasing potential is applied and the arsenic is oxidized, causing an anodic current that is recorded. The resulting plot of current as a function of voltage is known as a voltammogram. Peak area is the quantitative measurement that is related to the amount of arsenic that is removed. This is the stripping step, for which the changes in oxidation state may be summarized as:

$As(0) \rightarrow As(III) + 3e^-$

In CSV, two steps are also involved: accumulation and redissolution. In the accumulation step, an adsorption or deposition inducer is needed to immobilize the analyte on the working electrode. For example, if the working electrode is a hanging mercury drop electrode, copper or selenium ions are often added to react with the arsenic species in solution.[19] For example, arsenite can form the insoluble salt (Cu_3As_2) with Cu^0 on the mercury surface.[20] The reaction may be summarized as

$2As(III) + 3Cu(Hg) + 6e^- \rightarrow Cu_3As_2 + 3Hg$

In the following step, which is carried out under a more negative potential, arsine is formed in a reaction that may be summarized as follows:

$Cu_3As_2 + 2H^+ + 3Hg + 12e^- \rightarrow 2AsH_3 + 3H_2 + 3Cu(Hg)$

Differential pulse (DP) and square wave (SW) voltage wave-forms are commonly used in stripping steps in order to increase the sensitivity.[19,21-23] In differential pulse voltammetry, a series of regular voltage pulses are superimposed on the potential linear sweep, whereas in square wave voltammetry, a special form of differential pulse voltammetry, equal time is spent at the potential of the baseline and the potential of the superimposed pulse. Calibration by standard additions is usually improves the accuracy because the stripping process is largely affected by other solutes. In addition, the electrode needs to be re-plated, re-activated and rinsed with the sample solution before each determination.[24,25]

1.2.1 Anodic Stripping Voltammetry

As reviewed in multiple references, anodic stripping voltammetry has been established as a leading technique in the determination of trace amounts of metals.[26-29] In 1977, Branica and co-workers introduced the concept of ASV by using this technique to determine the mercury traces in seawater.[30] In the following years, ASV has been used for trace metal determinations, such as that of zinc and cadmium.[31,32]

Kaplin and coworkers first reported the determination of trace arsenic concentrations by ASV in 1970[33,34] Since then, many papers have been published in this area.[25,35,36] By employing Web of Science, 151 papers were found has the words "arsenic" and "ASV" in the titles. ASV should be able to detect both arsenite and arsenate. Without adding any reducing agent, ASV determines arsenite. For arsenate determination, the arsenate in the samples need to be chemically reduced to arsenite or can be directly electroreduced at a gold or other working electrode at a sufficiently negative potential.[37] Huiliang et al. reported that arsenate can be directly reduced to As(0) by applying – 1.6 V vs Ag/AgCl electrolysis potential in 4 M HCl using a gold plated platinum-fibre electrode.[38] Most recent studies have been concerned with the detection of arsenic in environmental samples. Because in environmental samples, most arsenic is in presence as inorganic arsenate, reducing agents are always added during the experiment.

A variety of electrode materials can be applied in the determination of arsenic. The working electrode used in the early days was the hanging mercury drop electrode, for which it is reported that the arsenic oxidation peak was a shoulder on the mercury peak, which caused an interference during the arsenic determination.[28] When platinum electrodes are used, hydrogen ions can be easily oxidized on the electrode surface and the hydrogen evolution can interfere with the determination.[28] To overcome this problem, a gold electrode can be applied, because gold has a higher hydrogen overvoltage. However, the response of a gold electrode depends critically on its past history, pretreatment, and the formation of oxide films. Thus, gold-plated glassy carbon electrodes,[39] electrodes modified by gold nanoparticles,[40] gold-modified boron doped diamond electrodes[41] have been used instead of solid gold electrodes. Sun et al. reported a method for arsenic detection in seawater by using a rotating gold-film glassy-carbon electrode.[39] Because arsenate was found to be electrochemically inactive, it was first reduced to arsenite by SO_2 gas. The detection limit was 0.2 $\mu g\ L^{-1}$ when the deposition time was 4 min. Compton and co-workers extensively investigated the determination of arsenic by ASV at gold nano particle modified electrodes. [26,42-45] They developed a gold nano-particle modified glassy carbon electrode to detect arsenous acid in 1 M HCl, and the detection limit was 0.0096 $\mu g\ L^{-1}$.[43] They also found that with increasing deposition time, the peak height increased rapidly. Thus, in order to achieve a low detection limit, a long deposition time of 180 s, was applied. Linear sweep voltammetry (LSV) and square wave voltammetry (SWV) were both investigated. Under the selected parameters, LSV gave a low detection limit (0.0075 $\mu g\ L^{-1}$) whereas SWV gave a high sensitivity (0.96 $\mu A/\mu g\ L^{-1}$). Although ASV can give a low detection limit for arsenic, it suffers interference from interferences from many metals, such as copper, lead, mercury, silver and zinc. Among these metals, copper is the most common interference metal.[26] Dai and Compton investigated gold nanoparticle modified electrodes to decrease the copper interference during arsenic detection by ASV.[26] The gold nanoparticles were deposited on glassy carbon or a basal plane pyrolytic graphite (BPPG) electrode. For the glassy carbon electrode, the sensitivity of arsenic was 0.014 $\mu A/\mu g\ L^{-1}$, which is much higher than that for copper (0.67 $mA/\mu g\ L^{-1}$); for the BPPG electrode, the sensitivity for arsenic was 0.047 $\mu A/\mu g\ L^{-1}$, whereas the sensitivity of copper was not detectable. The detection limits of the two electrodes were 0.5 $\mu g\ L^{-1}$ (glassy carbon) and 20 $\mu g\ L^{-1}$ (BPPG). The stripping peak of arsenic was narrower and more symmetric and the peak of copper was smaller, which indicated that the electrodes could decrease the interference caused by copper. In 2008, the Compton group prepared gold nanoparticle modified carbon nanotubes, which were immobilized onto the surface of a glassy carbon electrode.[45] The electrode showed a long life and gave precise measurements over 10 months. Although the sensitivity was as high as 1985 µA/µM with square wave voltammetry, the detection limit was only 0.1 µg L^{-1}.

Compared to conventional carbon electrodes, highly boron-doped diamond (BBD) electrodes have a wide potential window in aqueous solutions, low background current and weak adsorption of polar molecules. Yamada et al. used a gold-modified BBD electrode to determine arsenic in water.[41] They were able to measure arsenite and arsenate in a mixture when the deposition time was 60 s. A linear calibration was also obtained for a series of concentrations covering the range 100 – 1000 µg L^{-1} arsenate in a mixture solution with 100 µg L^{-1} arsenite and for a series of concentrations of 5 – 30 µg L^{-1} arsenite in a mixture solution with 100 µg L^{-1} arsenate. Detection limits of 5 and 100 µg L^{-1} were achieved for arsenite and arsenate in a mixture, respectively. The reproducibility was, for stripping voltammetry of arsenite and arsenate, RSD (n = 8) of 7.5% and 8.4%, respectively.

1.2.2 Cathodic Stripping Voltammetry

Cathodic stripping voltammetry is often carrying out at a hanging mercury drop electrode (HMDE).[20] Li and Smart developed a method for arsenite detection in natural waters using square wave cathodic stripping voltammetry(SWCSV) at a HMDE.[19] Under the optimized conditions, the detection limit was 0.005 µg L^{-1} when the deposition time was 10 min. Tongesayi and Smart proposed another method to detect arsenic(III) in the presence of dissolved organic matter (DOM) by SWCSV.[23] They reported that arsenic(III) can be accurately quantified in the presence of fulvic acid and natural DOM under the optimum condition of: 0.8 mM Cu(II), 1 M HCl, 40 μM $N_2H_4 \cdot H_2SO_4$, 70 Hz square wave frequency, 60 s deposition time and -0.550 V vs. SCE deposition potential. Eguiarte et al. investigated a method for the determination of total arsenic in the presence of copper in soil after microwave digestion, by DPCSV using a HMDE.[46] They used KI as the reductant and both 3.8 M HCl and 0.24 M $NaHCO_3$ as electrolyte. The detection limit was 0.08 μg L^{-1}. This method showed less interference than hydride generation atomic absorption spectrometry (HGAAS) method, which is usually used for the determination of arsenic in soils, from transition metals such as Ni^{2+} and Co^{2+}.[46] Although CSV can provide a low detection limit, this method still has many problems, such as the toxicity of mercury.

1.2.3 Challenges for Voltammetric Methods

Nowadays, extremely sensitive voltammetry methods for arsenic determination with very low detection limits are available. However, these methods also suffer from interferences and matrix effects, which means that voltammetric methods are rarely used for arsenic determination in complex matrixes, such as food samples. Efforts are still needed in order to overcome those drawbacks. A recent review shows that voltammetry studies have mainly targeted the following goals: developing a battery-powered instrument for field analysis and the combination with flow analysis or other non-electroanalytical instrument.[47] Combining all these improvements, it may be possible to develop small and reliable stripping voltammetry based instrumentation for the determination of inorganic arsenic species in the field.

1.3 The Application of Quartz Crystal Microbalance

1.3.1 The Mechanism of Quartz Crystal Microbalance

The quartz crystal microbalance (QCM) is a mass sensing device based on the piezoelectric effect. Since many important physical and chemical processes are related to mass change, researchers are interested in using microbalances to monitor these processes. The QCM has a wide detection range. Because the QCM is capable of measuring a mass as small as 1 ng on the surface of crystal, it has been applied to many fields of chemistry, including environmental chemistry, clinical chemistry and biochemistry.

The central component of a quartz crystal microbalance, as shown in Figure 1.2, is a thin quartz crystal with electrodes attached to opposite surfaces. When an alternating external electrical potential is applied, an internal mechanical oscillation of characteristic frequency, f, is produced. The QCM is a shear mode device, which means the acoustic wave, produced by the oscillating electric field, propagates perpendicular to the surface of crystal. To ensure this, the crystal has to be cut in a certain orientation. The most commonly used quartz crystals are cut in the AT form (shown in Figure 1.3) because such a crystal has a low temperature coefficient and is very sensitive to mass change.

The oscillation frequency of a crystal will decrease if there is an increase in mass bound to the surface as described by the Sauerbrey equation:

$$\Delta f = - 2\Delta m f^2 / A(\mu\rho_q)^{0.5} = - C_f\Delta m$$

where Δf is the frequency change (Hz), f is the intrinsic crystal frequency, Δm is the mass change (g), A is the electrode area (usually, 0.196 cm^2), ρ_q is the density of quartz (2.65 g/cm^3), and μ is the shear modulus (2.95×10^{11} dyn/cm^2). For a 9 MHz crystal, the sensitivity is 0.903 Hz ng^{-1}. Thus a QCM is able to measure a mass change of about 1 ng, which is 1000 times smaller than that which can be measured by an the electronic mass balance. The Sauerbrey equation is valid when the layer on the crystal surface is thin and rigid. Miller and Bolef were the first to study the effects of the overlayer, a layer of atoms absorbed onto a surface, obtaining a complicated formula for the frequency shift,[48] which was then simplified by Lu and Lewis.[49] This formula contains the density of the overlayer as well as the shear modulus. In addition to the models developed by these researchers, Benes and Schmid,[50] Krimholtz,[51] Nowotny[52] and Crane[53] have all studied various theoretical models of the QCM response.

Many factors can affect the frequency, such as the thickness of crystal, its chemical structure and shape. The diameter of the crystal can affect the stability. For most QCMs, the diameter is usually 0.5 in. On the other hand, the thickness determines the resonant frequency, which is related to mass sensitivity.

When a crystal is immersed in a liquid, the frequency depends on the solvent used. In liquid-phase measurements, the QCM can give information about reactions and conditions at the liquid-solid interface. The Sauerbrey equation does not apply to crystals immersed in liquids. Kanazawa et al. first derived the equation for the frequency change in liquid media to include additional terms for density and viscosity:[54]

$$\Delta f = - f^{3/2}(\rho_L \eta / \pi \mu \rho_q)^{1/2}$$

where ρ_L is the density of the liquid and η is the viscosity.

The liquid-phase measurement led the researchers into a new area. Kelly and Oyama studied the ion and solvent transport in an osmium polymer film using a QCM.[55] Fawcett et al. investigated the QCM response to RNA.[56] Li et al. studied the adsoption behavior of human fibrinogen at different concentrations using a QCM.[57]

1.3.2 Surface Modification of Quartz Crystal Microbalance

Instruments based on QCMs have many advantages: they are 1) simple and inexpensive; 2) sensitive to surface mass binding; 3) can detect viscosity change in solution, and 4) can detect changes in the gold surface area.

Before they were applied to liquid environments, QCMs have been used in vacuum deposition systems: to control the film deposition on crystal surface,[58] to measure air contamination,[59] and to sense the gaseous analytes.[60]

With the development of the QCM technology, researchers found that a QCM can be coated by specific layers or films that can selectively and effectively absorb the analytes. King used a coated piezoelectric crystal as a detector for gas chromatography.[61] Karmarkar et al. made a portable instrument that could monitor air pollutants.[62]

Besides sensing trace mass changes, the QCM can also be applied to detect large molecules, such as DNA and antibodies. For example, in 1972, Shons et al. used a QCM as an immunosensor to detect BSA antibodies.[63] Crystals were coated with antigen and exposed to antisera at different concentrations. They found that only the anisera specific for the antigen could form an additional protein layer on the crystal that caused a frequency change proportional to the concentration of the specific antibody. In 1988, Fawcett et al. were the first to sense DNA with a QCM.[64] This method was based on the large mass increase accompanying hybridization. Nucleic acid strands were attached to the crystal surface, which was modified by polymers, and then nucleic acid strands were melted and incubated with target strands in solution. They indicated that this method also can indentify DNA and RNA.

The detection of heavy metals in water is important because it is related to environmental protection and human health. Although many techniques can effectively and sensitively detect multiple metals in water, such as atomic adsorption spectroscopy, atomic fluorescence spectroscopy and inductively coupled plasma mass spectroscopy, detection with real-time responses is still not possible. Due to the fast-response, low cost and high mass-sensitivity, QCMs have been used in detecting trace metal ions for a few years. Etoeki et al. detected Cd(II) ions in pure solutions by using a QCM sensor that was coated with a self-assembled functionalized thiodisuccinic acid monolayer.[65] Frequency response data were related to the ligand binding to the Au electrode and metal ions binding to the ligand. They pointed out that the functionalized self-assembled monolayer (SAM) had a high binding capacity of $3.8\ (\pm 1.1) \times 10^{6}\ \mathrm{mol^{-1}\ dm^{3}}$. Carrington et al. determined chromium(VI) with a QCM coated with sol-gel films.[66] Nomura et al. demonstrated that a crystal coated with a suitable ligand can absorb metal ions from the solution very effectively.[67] They found that a copper oleate coating was capable of detecting 6.2 -8.2 mg $\mathrm{L^{-1}}$ lead,[68] a poly(vinylpyridine) coating was capable of measuring 0.3 -2.2 mg $\mathrm{L^{-1}}$ copper(II),[69] and a silicone oil coating was capable of detecting 0.3 - 5.58 mg $\mathrm{L^{-1}}$ iron(III).[67]

The QCM also can be used for anion detection. However, compared to the detection of metal ions, less mentioned has been paid to the measurement of anions. Only a few papers describe the detection of anions. Timofeyenko et al. developed a procedure capable of monitoring cyanide in drinking water.[70] The aqueous samples containing cyanide were reacted with the gold electrodes of a QCM. Based on the Elsner reaction, 4

$Au + 8\ CN^{-} + 2\ H_2O + O_2 \leftrightarrow 4\ Au(CN)^{2-} + 4\ OH^{-}$, the loss of gold from the electrode was detected by QCM as the frequency change. The detection limit was 2.7 $\mu g\ L^{-1}$ for an analysis time of 1 h. Also the calibrations showed excellent linearity ranging from low $\mu g\ L^{-1}$ to hundreds of $mg\ L^{-1}$. A QCM coated with a trichloacetic acid-molecularly imprinted polymer (TCAA-MIP) was used for detecting haloacetic acids by Suedee et al.[71] The MIP had a crosslinked structure that could interact with the analytes. The detection limit was 20 - 50 $\mu g\ L^{-1}$, lower than the guidelines for the maximum permissible levels (60 $\mu g\ L^{-1}$). In addition to premade polymers, another viable coating material of choice is the self-assembled monolayer (SAM). Eun et al. investigated a SAM of phosphorylated 11-mercapto-1-undecanol on a QCM gold electrode surface that was used for selenite sensing.[72] Cadmium selenite crystals, which were first formed and then immobilized in the SAM, selectively absorbed selenite ions, which caused the mass change as result. The device responded over the range of concentrations form 12.3 to 114 $mg\ L^{-1}$ at pH 7.4. They also grew barium sulfate crystals on the same SAM surface to detect sulfate ions.[73] The response range was 6.5 - 432 $mg\ L^{-1}$ at pH 7.2. A similar sensing mechanism was developed by Iitaka et al. for the determination of phosphate.[74] The QCM was coated with RTV silicone rubber and insoluble orthophosphate salts ($CePO_4$, $CrPO_4$ and $BiPO_4$) or mixtures were embedded in the coating. The response range was 0.095 - 950 $mg\ L^{-1}$ at pH 7.0. They found few interferences.

1.3.3 The Chemistry of Arsenic and Sulfur Containing Compounds

It is known that arsenic can form As-S bond with thiol groups. Thus, compounds containing thiol groups have been used in arsenic determination in water samples. The compound dithiothreitol (DTT, $HSCH_2CH(OH)CH(OH)CH_2SH$), which contains thiol groups, has been used for inorganic arsenic detection by Kalluri et al. [75] and by Forzani et al.[76] Kalluri and co-workers reported the label-free detection of arsenic by a dynamic light scattering assay based on gold nanoparticles modified with DTT, glutathione (GSH) and cysteine (Cys), for which the detection limit was 10 pg L^{-1}. Forzani and co-workers developed a surface plasmon resonance sensor with DTT as the "recognition element" to detect total arsenic in ground water. They were able to discriminate between arsenic concentrations below and above the US EPA maximum containment level in drinking water. They also pointed out that DTT can reduce arsenate to arsenite, thus, this method can be used for arsenate determination without adding other reducing agents. Recently, Chen et al. reported a ASV method to determine arsenite in environmental and drinking water using a SAMs modified gold working electrode.[77] A good linear relationship was attained between the concentration of the arsenite standard and peak current, in the range of 3 – 100 µg L^{-1}. The limit of detection of this sensing system was determined to be 0.5 µg L^{-1} for a signal-to-noise ratio of 3.

1.4 Gutzeit Method

1.4.1 The Chemistry of the Gutzeit Method

The widely known Gutzeit method has been used for arsenic determination since 1890.[78] In the Gutzeit test, arsenic in the solution is reduced by zinc in acidic media, generating arsine gas which then reacts with mercuric bromide or mercuric chloride on a filter paper forming a yellow to brown color depending on the arsenic concentration. Then, the color spot can be compared with a calibrated color scale, made under the same conditions for a series of arsenic standard solutions. The chemical reactions involved are shown below:[79]

The formation of arsine gas:

(1) $Zn(0) \rightarrow Zn^{2+} + 2e^-$

(2) $2H^+ + 2e^- \rightarrow H_2$

(3) $As(III) + 3e^- \rightarrow As(0)$

(4) $As(0) + 3e^- + 3H^+ \rightarrow AsH_3$

Reactions of arsine gas with detecting reagent

(1)AsH_3 (g) + $2HgBr_2$ (aq) → $AsH(HgBr)_2$ (aq) + 2HBr……………………yellow

(2) AsH_3 (g) + $3HgBr_2$ (aq) → $As(HgBr)_3$ (aq) + 3HBr……………………..brown

Unfortunately, only in one paper do the researchers discus the chemical structure of the colored compound.[80] They passed arsine through an aqueous solution of mercuric chloride. The solution became a light yellow, then red. They propose that the hydrogen of arsine is replaced by the mercurous chloride group.

Since the test is cheap, simple and fast, test kits based on the Gutzeit method are sold by many companies, such as Hach, Merck, BVC, ECO, La Motte, and Industrial Test Systems, Inc. (Quick II) and they are commonly used in field analysis procedure.

The tests kits, according to several studies, suffer from a number of drawbacks, such as low sensitivity, poor reproducibility and accuracy. In 2006, Spear et al. reported an evaluation of the test kits by comparing the results with those obtained from graphite furnace atomic absorption spectroscopy (GFAAS).[81] Their results are shown in Figure

1.4. From this figure, it can be seen that 4 out of 6 test kits gave low recoveries for both arsenite and arsenate. Percent recoveries on test kits ranged from 33.3 to 105% for arsenite spiked into water at level of 31.4 $\mu g\ L^{-1}$. Percent recoveries ranged from 33.3 to 97.3% for arsenate spiked at a level of 30.7 $\mu g\ L^{-1}$. All test kits had a standard deviation of < 10 $\mu g\ L^{-1}$. Spear et al. suggest that to obtain the most accurate results, samples should be determined in triplicate.

1.4.2 Modifications of Gutzeit Method

Although the Gutzeit method has been used since 1891, the need to improve the accuracy and sensitivity and avoid some interference have caught researchers' attention. H_2S, which is formed during the Gutzeit test if sulfide is present, has a similar way of action as AsH_3.[82] In 1901, Dowzard reported that H_2S could be separated from AsH_3 by passing the mixed gases over lead acetate solution.[82] He also mentioned, besides eliminating the interference with sulfide, when this modification of Gutzeit test is used, arsenic can be detected in presence of about 2500 times its weight of antimony. Selenium and Tellurium compounds do not interference with this method either. In 1974, Crawford et al. modified the sulfide trap media. They replaced a plug of glass wool that has been impregnated with lead acetate with a glass fiber disk impregnated with lead acetate. They said, "The glass wool plug is ineffective in the complete removal of hydrogen sulfide and the actual preparation of the generators is quite troublesome just from the aspect of handling glass wool." By using a glass fiber disk, their results are closer to the known concentration of the standards than the results obtained using the glass wool. Instead of using glass wool or glass fiber disk, Hach company used scrubbers made of cotton saturated with lead acetate to remove sulfide interferences in their EZ arsenic test kits.

Compounds that react with arsine gas can also be modified. Besides using HgBr or HgCl, several other reagents can detect the evolved arsine. One direction for this involves developing arsenic chemistry that will react with a dye in a way that can be measured and quantified. For example, arsine formed by borohydride reduction reacts with methylene blue in anionic micellar medium, causing the color to bleach. The detection limit was 0.03 mg L^{-1}.[83]

Another approach is to develop arsenic chemistry that directly forms a high colored product. These reagents include silver diethyldithiocarbarnate (AgDEDTC), potassium iodate with carbon tetrachloride and ammonium molybdate. Arsine gas reacts with AsDEDTC in pyridine to form an intense red solution, which can be measured by visible absorption spectrometry at a wavelength of 535 nm.[84] Arsenites can also react quantitatively with potassium iodate in the presence of sulfuric acid and releases an equivalent amount of iodine, which imparts a pink color to a carbon tetrachloride extract. The color can be measured at 520 nm, providing a detection limit of arsenite at 2 µg L^{-1}.[85] To improve the sensitivity of the determination, arsine gas can also be absorbed and oxidized to arsenate in a sodium hypobromite solution. Then, arsenate reacts with ammonium molybdate in the presence of hydrazine sulfate, forming a blue complex. This can also be measured by visible absorption spectrometry at a wavelength of 840 nm and the detection limit was reported as 0.1 µg L^{-1}.[86] However, this reaction has not been applied to environmental measurements in the field because iron, phosphate and silicate in groundwater and soil interfere with this method.

Lawson et al. modified the way of generating arsine gas.[87] Because the zinc used in the experiment for the formation of hydrogen must be purified, or a slight stain on the mercuric bromide paper will be observed for the blank. Instead of using zinc and acid, an electrolytic method was applied. They used lead as the cathode and platinum as the anode and used a porous cup to separate the two electrodes as shown in Figure 1.5. Arsine was generated in the presence of sulfuric acid when a current of 0.9 A at 5 V was applied. They obtained almost 100% recoveries. In 1927, Fink modified Lawson's apparatus by replacing the porous cup with a U-tube, because he found that some of the arsenic was retained within the apparatus.[88] The two arms serve as cathode and anode chambers as shown in Figure 1.6. They reported a sensitivity of 0.00002 mg of arsenious acid, and 0.001 mg of arsenic acid.

1.4.3 Evaluations of Gutzeit Method

As early as in 1901, Bird points out that unless the state of oxidation of the arsenical compound is known, the Gutzeit method fails as an approximately quantitative method, because arsenates yield a much paler stain in a given time than arsenates.[89] He also points out the degree of humidity influences the intensity of the stain, because the water can dissolve the stain completely, and that sulfur, selenium, and tellurium compounds have the potential of interfering with this method. Organoarsenic species, such as monomethylarsonate and dimethylarsinate, cannot be directly detected using this method.[90]

Although there are a few drawbacks of the Gutzeit method, it is still widely applied in field test kits. Since those test kits are used to determine arsenic concentration in many countries, such as Bangladesh, India and China, they should be accurate and sensitive enough to assess the level of arsenic contamination. Much effort has been put into the evaluation of the test kits. Besides Spear's work mentioned above, Ballinger reported in

1962, the minimum detectable limit by using Gutzeit method for arsenic was 10 μg L^{-1} and the accuracy is ± 5 μg L^{-1}.[91] Rahman compared three commercial field kits whose results showed significant variations (up to 50% false negative and false positive) when compared with accepted laboratory methods.[92] Similar research also found that in a comparison of laboratory and field test results, the values for 99 out of a total of 799 randomly selected wells were not in an agreement.[93] The researchers also pointed out that increasing the reaction time to 40 min is a modification of the procedure that could greatly increase measurement accuracy. Table 1.3 summaries most of the published evaluation work.[94] From the table, it is clear that only two test kits are able to determine arsenic concentration around 10 μg L^{-1}, which is the WHO guideline. In addition, the average error is about 20%. To improve the accuracy, digital image analysis was applied to determine the color intensity. Kearns and Tyson successfully applied digital image analysis for data processing of the results Hach kit test.[95] By adopting the Colors program, which analyzes the color intensity in a digital image of the spot on the strip, fewer false positive and or false negative values (standard deviation equals to 6.3 and 4, respectively) are generated at the critical decision values of 10 and 50 μg L^{-1}. More background research on the application of digital image analysis to colorimetric analysis will be included in Chapter 4 in this dissertation.

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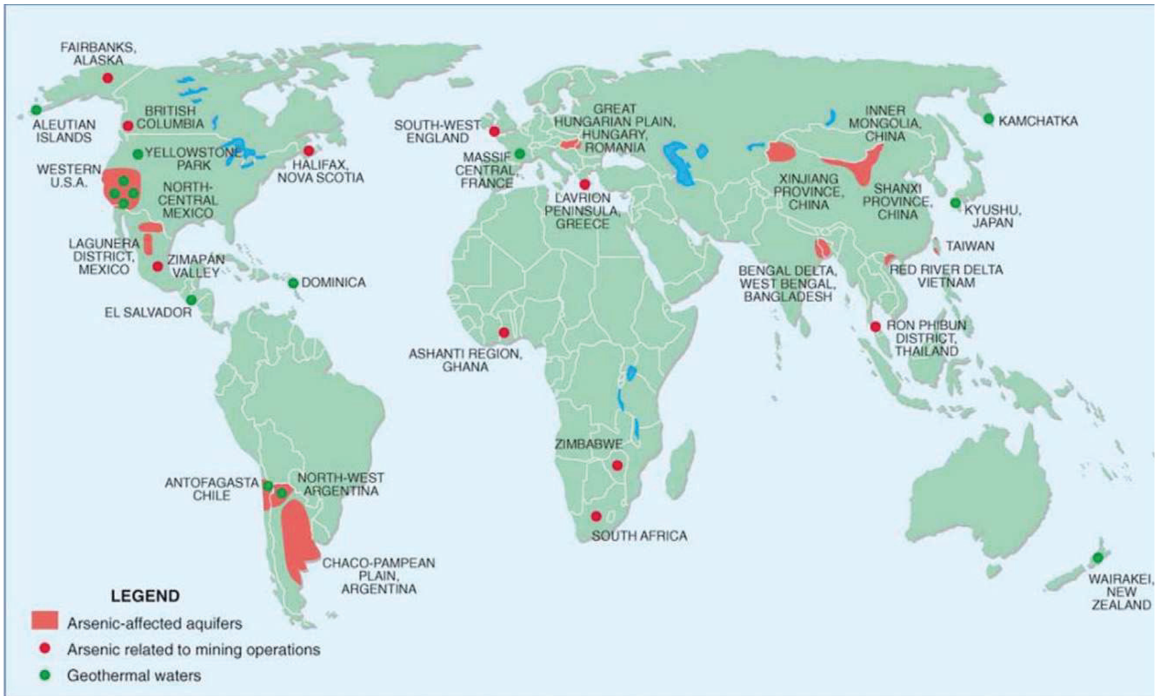

Figure 1.1 Distribution of documented world problems with arsenic in groundwater in major aquifers. Areas in blue are lakes.[96]

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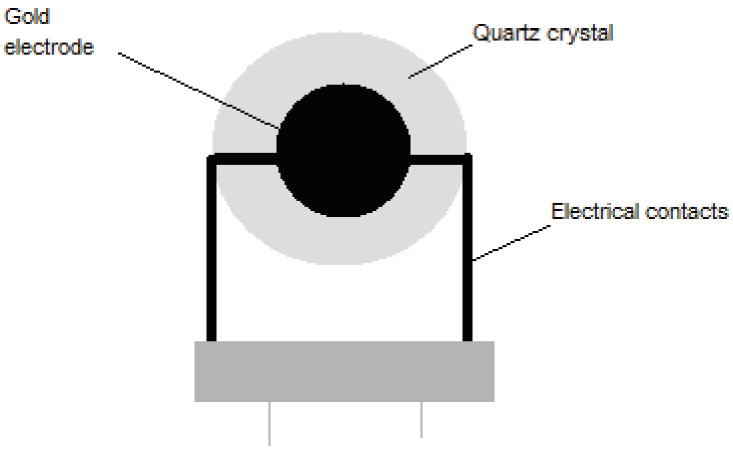


Figure 1.2 Schematic diagram of a quartz crystal and electrical connections in a QCM[97]

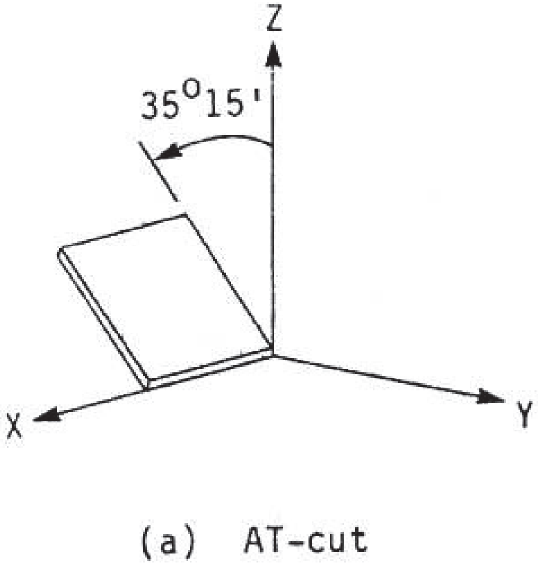


Figure 1.3 AT-cut crystal[97]

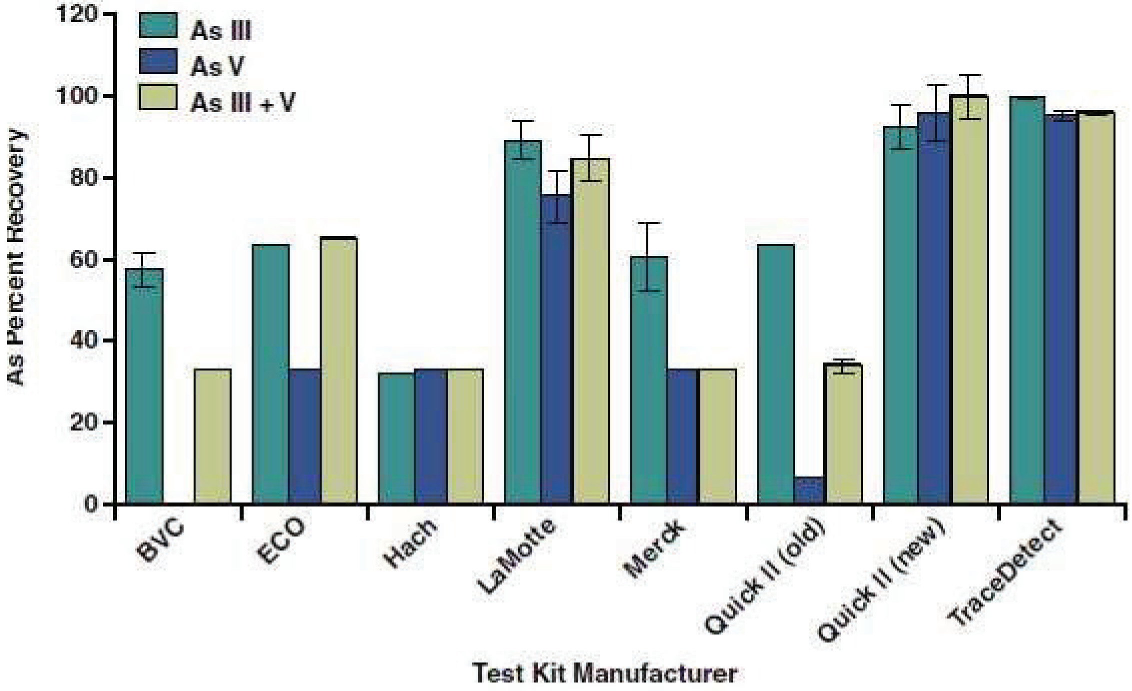


Figure 1.4 Recoveries of arsenite, arsenate and total arsenic from deionized water using six commercial test kits and an ASV method[81]

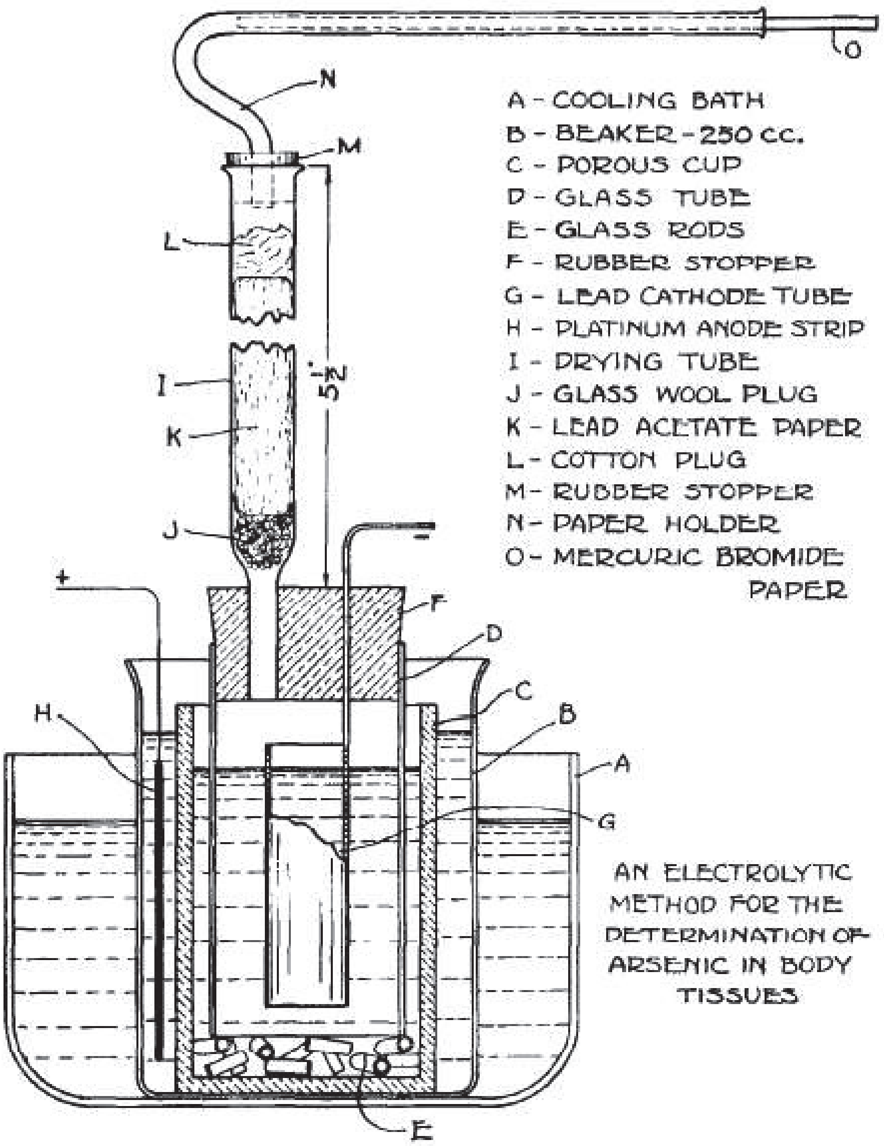


Figure 1.5 Apparatus for electrolytic Gutzeit Method[87]

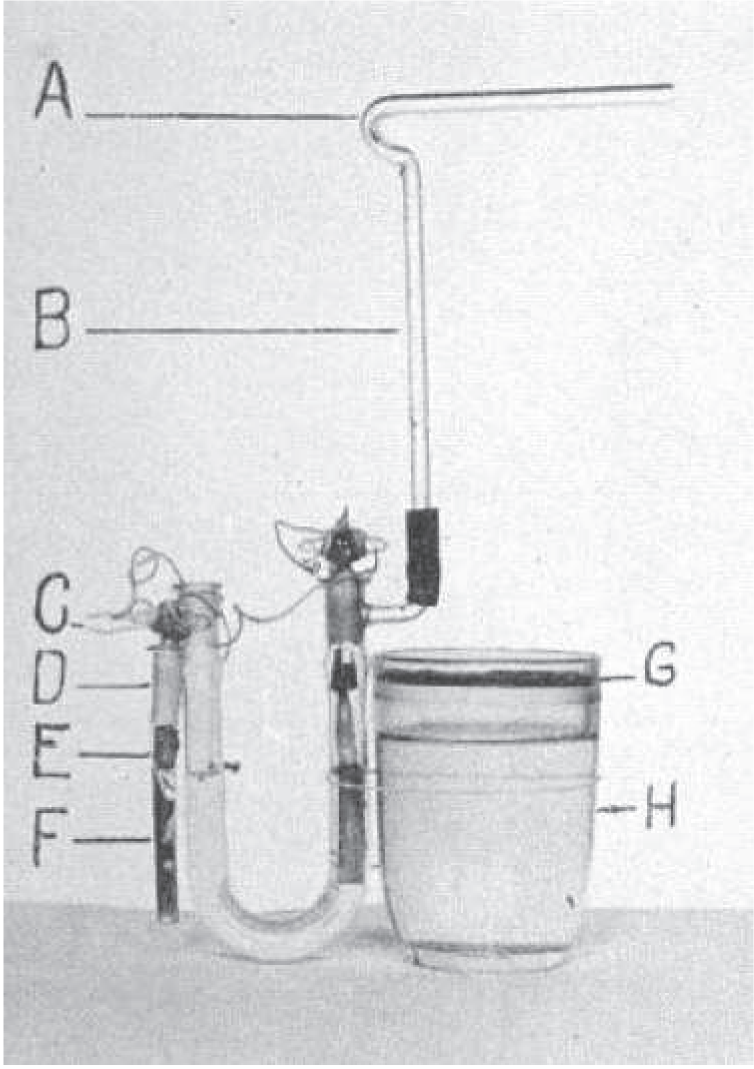
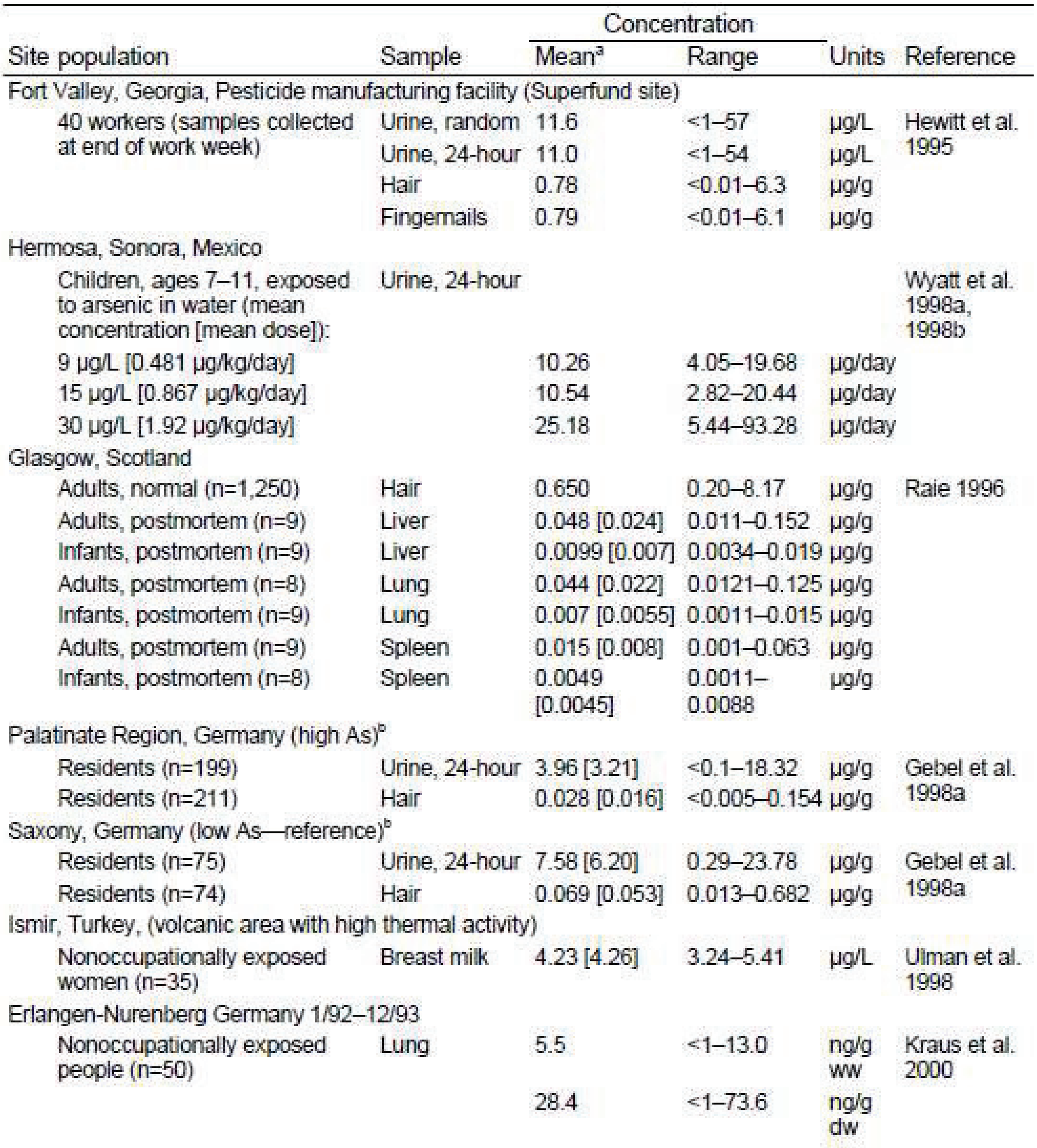


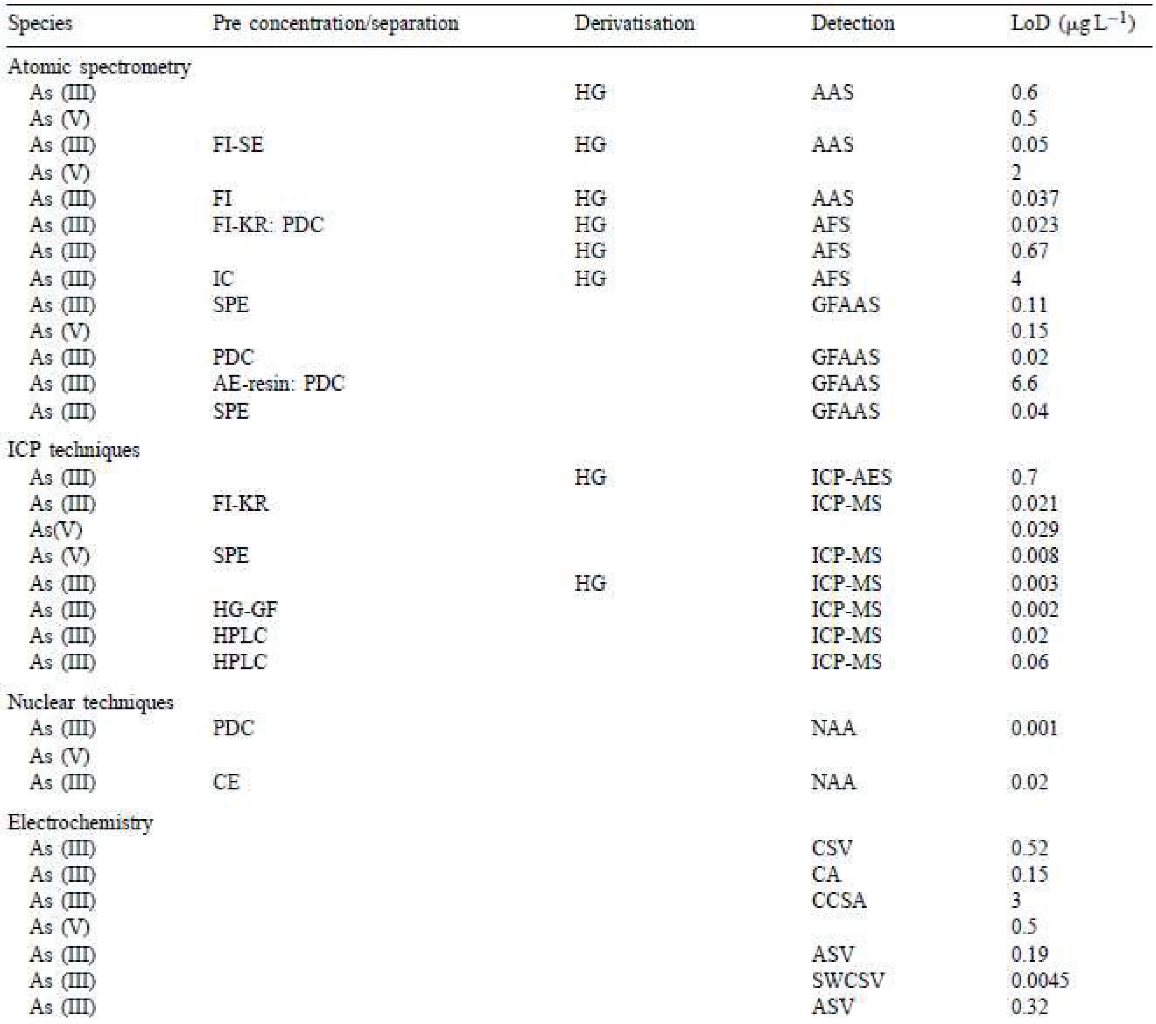
Figure 1.6 The U-shaped apparatus. A: the holder for the strips; B: the portion of tube containing loosely plugged cotton previously soaked in lead acetate and dried; C: the outlet from the anode chamber; D: the ground-glass stopper through which a platinum wire leads to connection with the copper clip; E: the copper clip soldered to the wire; F: a strip of rolled platinum foil held by the clip which serves as an anode electrode; G: the Bakelite for holding the apparatus when immersed in the water bath of the tumbler; H: the tumbler used as a water bath.[88]

Table 1.1 Levels of arsenic in human urine and tissues of the residents from various arsenic-polluted areas[98]



		Concentration			
Site population	Sample	Mean[a]	Range	Units	Reference
Fort Valley, Georgia, Pesticide manufacturing facility (Superfund site)					
40 workers (samples collected at end of work week)	Urine, random	11.6	<1–57	µg/L	Hewitt et al. 1995
	Urine, 24-hour	11.0	<1–54	µg/L	
	Hair	0.78	<0.01–6.3	µg/g	
	Fingernails	0.79	<0.01–6.1	µg/g	
Hermosa, Sonora, Mexico					
Children, ages 7–11, exposed to arsenic in water (mean concentration [mean dose]):	Urine, 24-hour				Wyatt et al. 1998a, 1998b
9 µg/L [0.481 µg/kg/day]		10.26	4.05–19.68	µg/day	
15 µg/L [0.867 µg/kg/day]		10.54	2.82–20.44	µg/day	
30 µg/L [1.92 µg/kg/day]		25.18	5.44–93.28	µg/day	
Glasgow, Scotland					
Adults, normal (n=1,250)	Hair	0.650	0.20–8.17	µg/g	Raie 1996
Adults, postmortem (n=9)	Liver	0.048 [0.024]	0.011–0.152	µg/g	
Infants, postmortem (n=9)	Liver	0.0099 [0.007]	0.0034–0.019	µg/g	
Adults, postmortem (n=8)	Lung	0.044 [0.022]	0.0121–0.125	µg/g	
Infants, postmortem (n=9)	Lung	0.007 [0.0055]	0.0011–0.015	µg/g	
Adults, postmortem (n=9)	Spleen	0.015 [0.008]	0.001–0.063	µg/g	
Infants, postmortem (n=8)	Spleen	0.0049 [0.0045]	0.0011–0.0088	µg/g	
Palatinate Region, Germany (high As)[b]					
Residents (n=199)	Urine, 24-hour	3.96 [3.21]	<0.1–18.32	µg/g	Gebel et al. 1998a
Residents (n=211)	Hair	0.028 [0.016]	<0.005–0.154	µg/g	
Saxony, Germany (low As—reference)[b]					
Residents (n=75)	Urine, 24-hour	7.58 [6.20]	0.29–23.78	µg/g	Gebel et al. 1998a
Residents (n=74)	Hair	0.069 [0.053]	0.013–0.682	µg/g	
Ismir, Turkey, (volcanic area with high thermal activity)					
Nonoccupationally exposed women (n=35)	Breast milk	4.23 [4.26]	3.24–5.41	µg/L	Ulman et al. 1998
Erlangen-Nurenberg Germany 1/92–12/93					
Nonoccupationally exposed people (n=50)	Lung	5.5	<1–13.0	ng/g ww	Kraus et al. 2000
		28.4	<1–73.6	ng/g dw	

Table 1.2 Different analytical technique and their detection limits[1]



Species	Pre concentration/separation	Derivatisation	Detection	LoD ($\mu g\,L^{-1}$)
Atomic spectrometry				
As (III)		HG	AAS	0.6
As (V)				0.5
As (III)	FI-SE	HG	AAS	0.05
As (V)				2
As (III)	FI	HG	AAS	0.037
As (III)	FI-KR: PDC	HG	AFS	0.023
As (III)		HG	AFS	0.67
As (III)	IC	HG	AFS	4
As (III)	SPE		GFAAS	0.11
As (V)				0.15
As (III)	PDC		GFAAS	0.02
As (III)	AE-resin: PDC		GFAAS	6.6
As (III)	SPE		GFAAS	0.04
ICP techniques				
As (III)		HG	ICP-AES	0.7
As (III)	FI-KR		ICP-MS	0.021
As(V)				0.029
As (V)	SPE		ICP-MS	0.008
As (III)		HG	ICP-MS	0.003
As (III)	HG-GF		ICP-MS	0.002
As (III)	HPLC		ICP-MS	0.02
As (III)	HPLC		ICP-MS	0.06
Nuclear techniques				
As (III)	PDC		NAA	0.001
As (V)				
As (III)	CE		NAA	0.02
Electrochemistry				
As (III)			CSV	0.52
As (III)			CA	0.15
As (III)			CCSA	3
As (V)				0.5
As (III)			ASV	0.19
As (III)			SWCSV	0.0045
As (III)			ASV	0.32

Table 1.3 Evaluation of some test kits for analyzing arsenic in water[94]

Field test kits	Kit capability	Minimum detection limit of arsenic	Detection range	Rate of false positive/false negative	Effects of interferences (sodium chloride, iron, sulfate, acidity)	Occupational hazard potential (OH)	Time required per test	Evaluation reference
Quick™ (industrial test kit, Rock Hill USA)	Semi-quantitative	– 5–20 µg/L	5, 10, 20, 40, 60, 100, 200, … 500 µg/L	0–4%/5–16%	ND	Safe	< 15 min	Environmental Protection Agency-Battelle (2002a)
AS75 (PeCo test kit) (Peters Engineering, Graz, Austria)	Semi-quantitative	– 15–50 µg/L	10, 20, 30, … 100 µg/L 2.5, 5, 10, 20, … 60 µg/L	0–3%/0%	None	Safe	ND	Environmental Protection Agency-Battelle (2002b)
AAN (Asia Arsenic Network, Japan)	Semi-quantitative	– 20 µg/L	20, 50, 100, 200,… 700 µg/L	19%/71%	Some with sulfide	Accidental escape of arsine gas may cause OH.	15 min	Pande *et al.* (2001); Rahman *et al.* (2002)
E. Merck (Germany)	Qualitative for arsenic concentration > 50 µg/L	– 50–100 µg/L	100, 500, 1000, 1700, 3000 µg/L	21%/60%	Some with sulfide	Accidental spillage of acid and escape of arsine gas may cause OH.	30 min	Pande *et al.* (2001); Rahman *et al.* (2002)
NIPSOM (National Institute of Preventive and Social Medicine, Bangladesh)	Qualitative for arsenic concentration > 50 µg/L	– 10–20 µg/L	10, 20, 50, 100, 200, 300 … 700 µg/L	21%/33%	Some with sulfide	Accidental spillage of acid and escape of arsine gas may cause OH.	5 min	Pande *et al.* (2001); Rahman *et al.* (2002)
AIIH-PH (All India Institute of Hygiene and Public Health, India)	Semi-quantitative	– 50 µg/L	> 50 µg/L	25%/1%	Sulfide interference eliminated	Accidental spillage of acid and escape of arsine gas may cause OH.	30 min	Pande *et al.* (2001); Rahman *et al.* (2002)
GPL (General Pharmaceuticals Ltd, USA)	Semi-quantative	– 10 µg/L	10, 50, 100, 200, 400, 500 … 1500 µg/L	10%/32%	ND	Accidental spillage of acid and escape of arsine gas may cause OH.	20 min	Rahman *et al.* (2002)
Aqua (Aqua Consortium, Calcatta, India)	Semi-quantitative	– 100 µg/L	> 50 µg/L	ND	Sulfide interference eliminated	Accidental spillage of acid and escape of arsine gas may cause OH. Contact with HgBr2 paper affects fingers of the user.	15 min	Pande *et al.* (2001)

CHAPTER 2

DETERMINATION OF ARSENIC IN WATER SAMPLES USING ANODIC STRIPPING VOLTAMMETRY AND CATHODIC STRIPPING VOLTAMMETRY

2.1 Anodic Stripping Voltammetry

2.1.1 Introduction

In groundwater, arsenic is present as arsenites, arsenite, arsenates, arsenate and with a minor concentraions of methyl and dimethyl arsenic compounds. The WHO has recommended a maximum arsenic level in drinking water, which is 10 μg L^{-1}. Many lab instrumental techniques, such as ICPMS, ICPOES, GFAAS, and AFS, are able to achieve this low level, however, they are large, expensive and can not be used in field analysis. As a portable instrument, electrochemistry instrumentation, which can perform anodic stripping voltammetry (ASV) and cathodic stripping voltammetry (CSV) has more advantages over the various “naked-eye” arsenic test kits. These electrochemical techniques are able to determine arsenic at trace levels within few minutes. In addition, these techniques can also distinguish between different oxidation states. Besides, they are easy to operate and are comparatively cheap.

ASV has been used for arsenic determination for a few decades. Arsenic in water is reduced and collected on the working electrode and then electrochemically stripped off and measured. The detailed mechanism is explained in Chapter 1 of this dissertation. Under optimum conditions, the detection limits are as low as sub μg L^{-1} level. ASV can be performed with different electrode materials, such as gold, silver, and platinum. The most commonly used working electrodes are gold electrodes, because gold provides a more sensitive response towards arsenic oxidation than other electrode materials and has a higher hydrogen overvoltage. A variety of gold electrodes have been investigated including gold film[1], nanogold particle,[2] gold disk[3] and rotating gold disk electrodes.[4] Some highly cited work is summarized in Table 2.1.[2]

Although ASV has many advantages, it suffers from interferences, especially from copper as Cu^{2+}. Copper co-deposits with arsenic during the deposition step before stripping and can form an intermetallic compound Cu_3As_2 as well as metallic Cu. The corresponding stripping peaks will mask and overlap the arsenic peak. This copper interference can be eliminated by using a uniformed gold film electrode or a gold nanoparticle modified glass carbon electrode, or a platinum electrode.[5]

The TraceDetect Nano-band Explorer is a portable rapid and suitable device designed for analysis in both the lab and filed. The Environmental Protection Agency has verified the performance of this device with respect to the following parameters: accuracy, precision, linearity, method detection limit, matrix interference effects, operator bias, and the rate of false positive/negatives.[6] The Nano-band Explorer working electrode is an array of 100 sub-electrodes made of carbon, each less than 0.5 μm thick. The increased mass transport rate afforded by this array allows measurements of concentrations in single-digit $\mu g\ L^{-1}$ in seconds. The electrode is coated with gold when used for arsenic determination. The auxiliary electrode is platinum and the reference electrode is Ag/AgCl in 3 M KCl. The EPA reported the detection limit for arsenic as 4 $\mu g\ L^{-1}$, however, on TraceDetect's website, the detection limit is 1 $\mu g\ L^{-1}$ for arsenic and mercury. The accuracy based on the verification report is 85%. The relative standard deviation (RSD) is 4 to 11%. The determination was not affected by matrix interferences added to the samples. Another study shows that this device has a detection limit for arsenic at 2.1 μg L^{-1} and the determination was not affected by the presences of 5 mg L^{-1} Sb(V) and 10 mg L^{-1} S as sulfide.[7] In addition to the evaluation work of the performance of TraceDetect Nano-band Explorer against the criteria listed above, the research whose results are described here mainly focused on investigating and optimizing the performance of a TraceDetect Nano-band Explorer device and a comparison of the figures of merit with those obtained by other researches. The possibilities for the determination of inorganic arsenic speciation were also evaluated.

2.1.2 Experimental

2.1.2.1 Apparatus and Reagents

Electrochemical measurements were recorded using a TraceDetect Nano-band Explorer (TraceDetect, Seattle, WA) with a standard three-electrode system. A gold film glassy carbon (GC) electrode (sensing area of 1.5×10^{-4} mm^2) was used as a working electrode. The counter electrode was a platinum plate electrode. The reference electrode was Ag/AgCl (in 3 M KCl). Data was recorded and analyzed with the Nano-Band Explorer software developed by TraceDetect.

All chemical reagents were purchased from Fisher Scientific (Waltham, MA). Solutions were prepared and diluted by using deionized distilled water with a resistivity of not less than 18 MΩ cm. Gold solution (200 mg L^{-1}) used for gold film plating was prepared by diluting a 1000 mg L^{-1} Au standard solution. arsenite and arsenate stock solutions (10,000 mg L^{-1}) were prepared by dissolving 0.1734 g of sodium arsenite and 0.3870 g of sodium arsenate into 10 mL of deionized water respectively. Filter paper was purchased from Whatman (Albany, NY).

2.1.2.2 Procedures

The first step was to buff the electrode. The electrode was buffed on a Buehler buffing pad with 0.05 µm Al_2O_3 for 60 s, and then rinsed with deionized water. After that, it was buffed on another Buehler buffing pad saturated with deionized water without Al_2O_3 for another 60 s. Only working and counter electrodes needed to be buffed. All three electrodes were mounted in an electrode holder after cleaning.

2.1.2.2.1 Gold Film Plating

The three electrodes were put into a beaker containing the 200 mg L^{-1} gold solution. The time-plating method was used for thin film plating. The electrode potential was setting at -50 mV vs Ag/AgCl for 60 s, which deposits 2 ng of gold.[8] Then the electrode was rinsed with deionzied water.

2.1.2.2.2 Analysis of Arsenite and Arsenate Standard Solutions

A series of arsenic standard solutions were prepared freshly by dilution from the arsenic stock solution. Concentrated HCl was added as electrolyte to each standard and sample solution, including blank, to make a final concentration of 0.145 M. For arsenate determination, reducing agent, sodium thiosulfate or 0.25 mL potassium iodide (30%) was added 4 min before each measurement. The final concentration of sodium thiosulfate was 0.4 mM. Other parameters are listed in Table 2.2. The electrodes were rinsed with deionized water between each measurement. Each solution was measured three times and peak area was recorded. A calibration curve was made based on the peak area versus the corresponding arsenic concentration.

2.1.2.2.3 Sample Analysis

For real samples, water could be analyzed directly. No further sample pretreatment was needed, unless the water samples were cloudy. In that case, the water sample was filtered through Whatman filter paper #1. Add a certain amount of HCl to make a final concentration of 0.145 M. For water samples, the arsenite concentration was first determined and then total arsenic concentration was determined following the addition of the reducing agent. The difference between the two concentrations was considered to be the arsenate concentration. Arsenic concentration was calculated based on the calibration curve obtained from above.

2.1.2.2.4 Optimization of ASV conditions

Deposition times of 10, 60, 120 and 180 s were investigated. A 50 mL of 10 $\mu g\ L^{-1}$ of arsenie solution was used with 0.145 M of HCl. Current was recorded as a function of stripping voltage vs Ag/AgCl.

Three acids HCl, H_2SO_4, and HNO_3 were evaluated as the supporting electrolyte. The concentration of acids was 0.145 M. Arsenite concentration was 10 $\mu g\ L^{-1}$ and the deposition time was 120 s. Current was recorded as a function of stripping voltage vs Ag/AgCl.

2.1.3 Results and Discussion

2.1.3.1 Optimization of ASV conditions

Figure 2.1 shows that although arsenic can be detected under all deposition time, the peak height and peak area increased with the increasing deposition time. By plotting with a larger peak area, a larger slope of arsenite or arsenate calibration curve could be obtained. As a result, the sensitivity was improved. However, there is only a small difference in the areas of the peak area for 120 and that for180 s. To save analysis time, 120 s was selected.

Three acids HCl, H_2SO_4, and HNO_3 were evaluated as the supporting electrolyte. Among those, only HCl provided a well-formed Gaussian peak, so it was selected. The peak shape is also a function of HCl concentration, because chloride can form a gold-chloride complex which would destroy the gold film surface.[9] The optimum concentration was 0.145 M, which is also recommended by the TraceDetect company.

2.1.3.2 Reducing Agent

Since arsenate is electroinactive, it needs to be reduced to arsenite and then determined by ASV. This allows ASV to be able to perform arsenic speciation analysis: arsenite is determined first, and then following the addition of a reducing agent, total arsenic is determined. The difference between the two concentraions is the arsenate concentration.

Sodium thiosulfate,[10] potassium iodide,[11] hydrazine,[12] cysteine,[13] calcium chloride,[14] mannitol[15] and ascorbic acid[11] are commonly used reducing agent in ASV. However, among those reducing agents, only sodium thiosulfate gave good results, which was agreed with the results of research by Ferreira and Barros' research results.[16] They found that the best one was thiosulfate, because it yields a rapid and complete reduction at room temperature. Different concentrations of thiosulfate were studied. The results are shown in Figure 2.2 and the optimum concentration was 3.16 g L^{-1}.

2.1.3.3 Calibration curve and sample analysis

Calibration plots were made under optimum conditions. As shown in Figure 2.3, this method is more sensitive for arsenate. However, the reason remains unknown. The detection limits, calculated as the concentration giving a signal equal to 3 times the standard deviation of the blank (n = 3), for arsenite and arsenate were 2.2 and 0.13 µg L^{-1}, respectively.

This method was applied to determine arsenic concentration in real water samples. The water sample was obtained from a well in Amherst. The water was filtered before measurement. 2.1 µg L^{-1} of arsenic was found in the sample, and then the sample was spiked with 10 µg L^{-1} arsenite standard solution. The recovery was 119%. No significant interferences were found. All figures of merit were listed in Table 2.4.

2.2 Cathodic Stripping Voltammetry

2.2.1 Introduction

Polarography, cyclic voltammetry, anodic and cathodic stripping voltammetry have been applied for the determination of arsenic. Cathodic stripping voltammetry (CSV), which was first introduced for arsenic determination by Henze et al[17], avoids the interferences of copper that are problematical for ASV. In fact, CSV is performed in the presence of Cu or Se with a hanging mercury drop electrode (HMDE). Their method has a linear range between 0.2 and 20 µg L^{-1}. In 1997, their group further developed this method by adding mannitol-sulphuric acid as the supporting electrolyte, which can help reducing the usage of Cu.[15] They also found that As(V) becomes electroactive when mannitol was used. Thus, by varying the composition of the supporting electrolyte, it was possible to differentiate between As(III) and As(V). The detection limit was 0.9 µg L^{-1}. Besides Cu and Se, ligands such as pyrrolidine dithiocarbamate (PDC) can also be used to complex arsenic.[18] Under the optimum condition (PDC = 0.5 µM, pH = 0.8), the detection limit of the method is 0.2 µg L^{-1} As. The procedure with a HMDE utilizes a pre-electrolysis step to concentrate arsenic from solution onto the mercury surface by forming an compound. Then, the compound is stripping from the electrode at a certain potential. The mechanism is discussed in Chapter 1 in this dissertation. CSV is a sensitive method. Usually, the detection limit is at the sub- μg L^{-1} level. In addition, the use of a HMDE avoids memory problems because a new mercury drop is formed for each measurement. However, although the HMDE is able to produce fresh surface, it has a few drawbacks. For example, it is difficult to reproduce the exact same size of drop each time. More importantly, the use of mercury creates hazardous waste, which has to be deal with appropriately. Besides, the HMDE is mechanically unstable so that it is not suitable for certain types of on-site analysis (e.g. those involving an uneven surface, or operating on a board ship).[19] To address some of these limitations, a mercury film electrode (MFE) has been used in some studies.[13] Glassy carbon is the preferred substrate material in most cases due to its chemical inertness, broad useful potential range, low residual current, low cost, wide availability and ease of polishing.[19] Adeloju et al. determined arsenite by cathodic stripping potentiometric (CSP) on a glassy carbon mercury film electrode in the presence of Cu(II) ions.[13] As little as 2 μg L^{-1} of arsenic was determined at an electrolysis potential of – 600 mV vs Ag/AgCl (1 M HCl) and an electrolysis time of 30 s. Interferences from copper interference can be overcome by co-deposition with arsenic onto a mercury electrode and organic interference can be overcome by applying UV irradiation of the sample prior to the analysis. The result for the analysis of arsenic in boeine liver (0.65 ± 0.28 mg kg^{-1}) was close to the results (0.61 ± 0.04 mg kg^{-1}) obtained by inductively coupled plasma atomic emission spectrometry (ICP-AES). In a MFE review paper, Economou pointed out that the MFE is used in many other trace metal determinations except arsenic.[19] There is no paper that described the determination of arsenic by CSV using a TraceDetect Nano-Band Explorer. Since the determination of arsenic by CSV is affected by the following factors: the Cu concentration, the concentration of supporting electrolyte, and time and the deposition potential. In order to achieve low detection limit, those factors were optimized in this study to achieve a low detection limit, and the method was applied to the analysis of a real water sample. In CSV determination, Li et al. found that arsenic peak decreased and eventually disappeared over time after multiple determinations at the same electrode without regeneration.[20] The addition of hydrazine can reduce or combine with mercuric ions to prevent them from reacting with arsenite. As a result, the peak current of arsenic was constant during repeated scans. So, hydrazine was also applied in the study reported in this dissertation.

2.2.2 Experimental

2.2.2.1 Apparatus and Reagents

Electrochemical measurements were recorded using TraceDetect Nano-Band Explorer (TraceDetect, Seattle, WA) with a standard three electrodes system. An mercury film glassy carbon (GC) electrode (sensing area 1.5×10^{-4} mm^2) was used as a working electrode. A platinum electrode was used as a counter electrode. An Ag/AgCl (in 3 M KCl) was used as a reference electrode.

All chemical reagents were purchased from Fisher Scientific (Walthem, MA). Solutions were prepared and diluted by using deionized water with a resistivity of not less than 18 MΩ cm. Mercury(II) chloride in 2 M HCl solution (200 mg L^{-1}) used for mercury film plating was prepared by dissolving 0.541 g mercuric chloride in 2 L of 2 M HCl solution. Arsenite and arsenate stock solutions (10,000 mg L^{-1}) were prepared by dissolving 0.1734 g of sodium arsenite and 0.3870 g of sodium arsenate in 10 ml of deionized water respectively. Copper stock solution (10,000 mg L^{-1}) was prepared by dissolving 1.058 g of copper(II) chloride (anhydrous) in 50 mL of deionized water. N_2H_4 stock solution (2600 mg L^{-1}) was prepared by dissolving 0.105 g of hydrazine sulfate in 10 mL of deionized water.

2.2.2.2 Procedures

The electrodes were cleaned the same way as described for ASV in section 2.1.2.2.

2.2.2.2.1 Mercury Film Plating

The three electrodes were put into a beaker containing 200 mg L^{-1} mercuric(II) chloride solution, which was purged with nitrogen gas for 4 min. The time-plating method was used for thin film plating. The electrode potential was setting at -600 mV vs Ag/AgCl for 240 s.[21] Then the electrodes were rinsed with deionzied water.

2.2.2.2.2 Arsenite and Arsenate determination

A series of arsenic standard solutions were prepared freshly by diluting from the arsenic stock solution. The appropriate volume of concentrated HCl was added as electrolyte to each standard and sample solution, including the blank, to make a final concentration of 0.0242 M. The sample volume was 50 mL. 2.15 mL of $CuCl_2$ and 0.0247 mL of N_2H_4 solution were added to make a final concentration of 430 mg L^{-1} and 1.28 mg L^{-1}, respectively. For arsenate determination, sodium thiosulfate reducing agent was added 4 min before each measurement. The final concentration of sodium thiosulfate was 0.02 M. The operating parameters are listed in Table 2.3. The electrodes were rinsed with deionized water between each measurement. Peak area was recorded and the calibration curve was based on the peak area as a function of the corresponding arsenic concentration. Each solution was analyzed three times.

2.2.2.2.3 Optimization of HCl, $CuCl_2$ and N_2H_4 Concentrations

In order to achieve a low detection limit, the concentration is HCl, $CuCl_2$ and N_2H_4 were optimized. To get started, the optimum condition in Li's research was used as a reference (2 M HCl, 0.8 mM $CuCl_2$, 40 μM N_2H_4)[20]. The HCl concentration was optimized in the range from 0 – 0.72 M with 0.8 mM $CuCl_2$ and 40 μM N_2H_4. The $CuCl_2$ concentration was optimized in the range from 0.8 – 8 mM with the found optimum HCl concentration and 40 μM N_2H_4. The N_2H_4 concentration was optimized in the range from 0 – 100 μM with the found optimum HCl and $CuCl_2$ concentration. After got the optimum concentrations by using Li's condition, the HCl, $CuCl_2$ and N_2H_4concentrations were optimized again with the other two reagents under the optimum concentration. Peak area was recorded as a function of HCl, $CuCl_2$ or N_2H_4 concentration.

2.2.3 Results and Discussion

2.2.3.1 Optimization of HCl, $CuCl_2$ and N_2H_4 Concentrations

To achieve a higher sensitivity, the concentrations of HCl, $CuCl_2$ and N_2H_4 were optimized to obtain a larger arsenic peak area. Chloride ions can greatly enhance the arsenic signal, because the high concentration of chloride at the electrode surface can increase the amount of As-Cu compound deposited through stabilization of Cu(I).[22] To obtain the optimum concentration of HCl, the concentration was varied from 0 to 0.72 M and the results are shown in Figure 2.4. The arsenic signal increased with the increasing of HCl concentration, which demonstrates that HCl helps the reduction of As(III) and

Cu(II) as indicated in the reaction mechanism.[20] The 24.2 mM was chosen as the optimum HCl concentration.

Similarly, the peak current also increased with copper and hydrazine concentration and reaches the maximum at 430 mg L^{-1} (3.2 mM), 1.28 mg L^{-1} (40 µM), respectively. As shown in Figure 2.5 and 2.6, if the copper and hydrazine concentration increased further, the peak current dropped. As a result, 430 mg L^{-1} and 1.28 mg L^{-1} were chose as the optimum Cu(II) and hydrazine concentration. This trend also found by Li et al., however, they didn't explain the reason why the peak current drops when Cu(II) and hydrazine concentrations are higher than the optimum values.[20]

2.2.3.2 Calibration curve and sample analysis

Calibration plots were made under optimum conditions. As shown in Figure 2.7, this method is more sensitive to arsenite determination, which is different from the ASV method. The detection limits, calculated as the concentration giving a signal equal to 3 times the standard deviation of the blank (n = 3), for arsenite and arsenate were 0.58 and 2.7 µg L^{-1}, respectively.

2.3 Conclusion

For both ASV can CSV method, the total analysis time, including the sample handling time and the electrode preparation, of one sample is less than 20 min, which is short than the method by using portable Hach kit. This research demonstrated a fast and sensitive method for arsenic determination in water samples by both ASV and CSV method. A MFE was applied in the CSV method that was performed for the first time being by a TraceDetect Nano-Band Explorer, which is a proven portable instrument for ASV. Speciation can also be performed after reducing arsenate to arsenite. For the ASV method developed, a better arsenic signal (good and smooth peak shape with a larger pear area) was obtained under the modified plating time compared with the signal obtained with the default setting suggested by TraceDetect. The detection limits for both arsenite and arsenate were below 5 $\mu g\ L^{-1}$, which is lower than the critical level (10 $\mu g\ L^{-1}$) recommended by the WHO and the US EPA. Although the detection limits for both arsenite and arsenate by CSV are higher than those reported by other researches, the mercury usage is decreased. This instrument provides more accurate results compared to those obtained from field test kits, however, the cost per test is $2.50, compared with the Hach kit costs $1.06 per test.[23]

2.4 References

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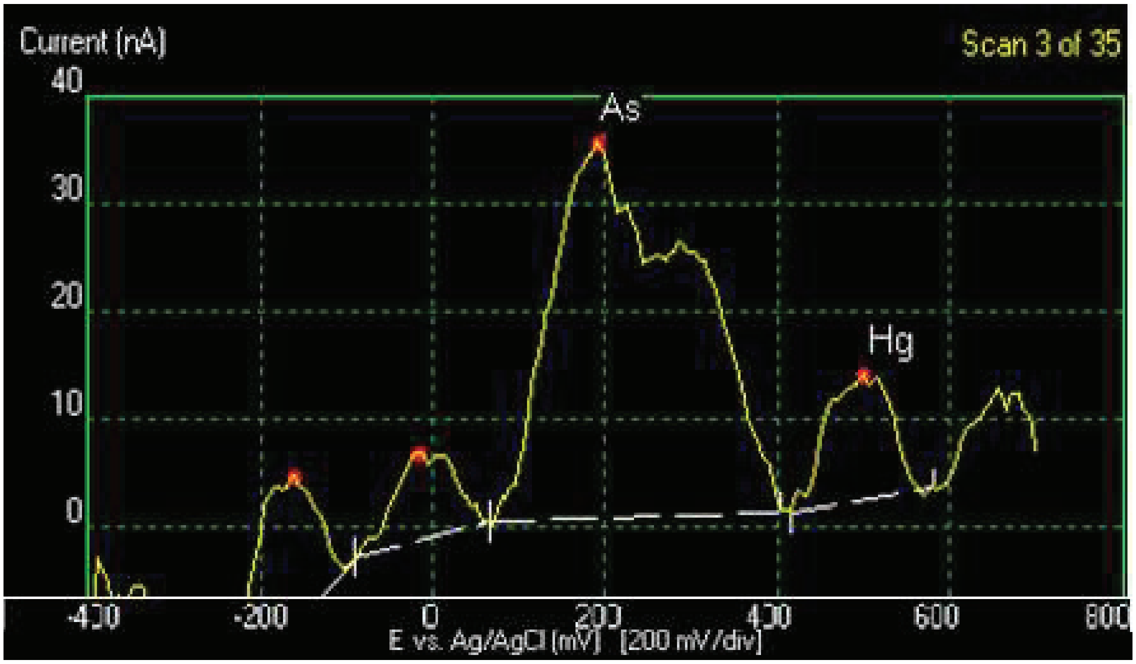
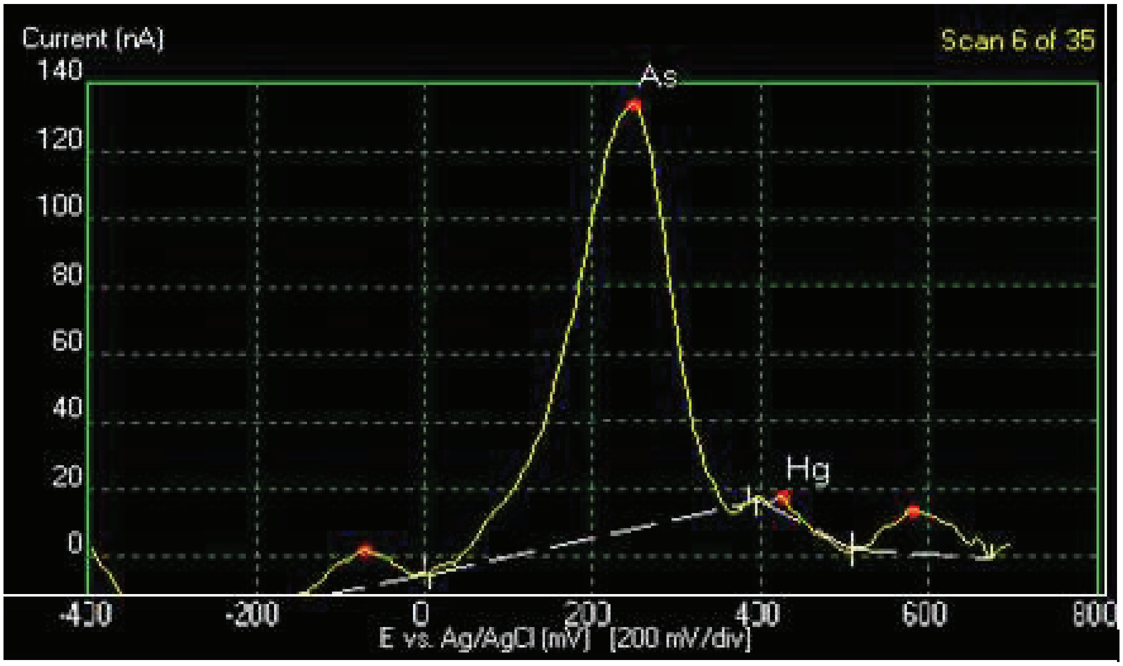
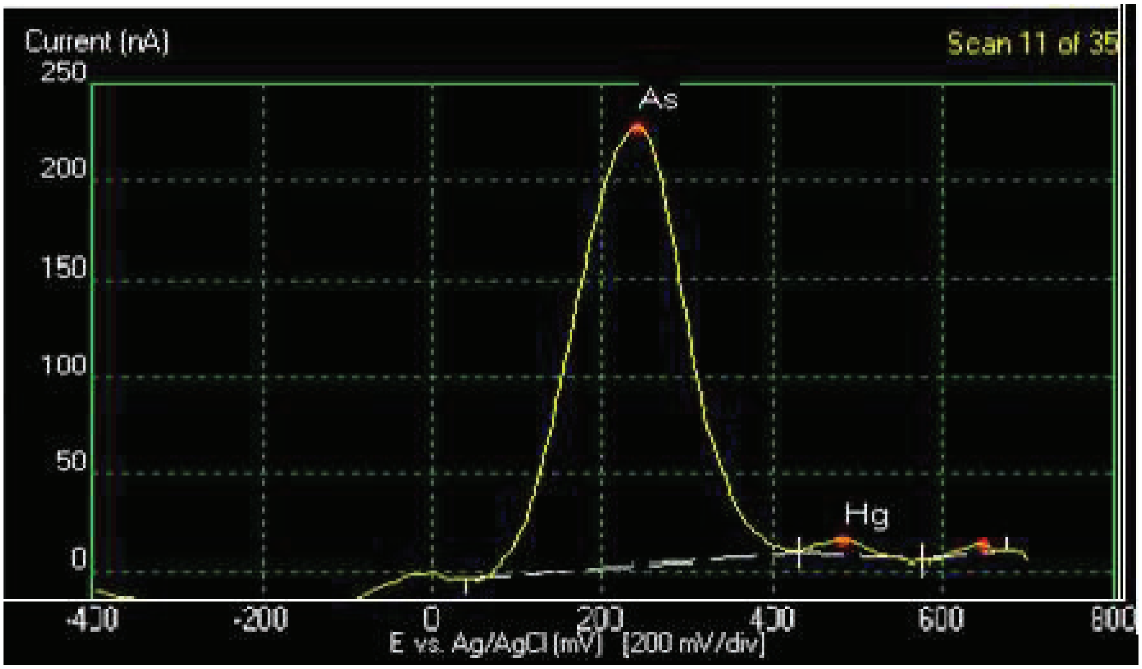
(a)


Current (nA)
Scan 3 of 35
40
30
20
10
0
As
Hg
-400
-200
0
200
400
600
800
E vs. Ag/AgCl (mV) [200 mV/div]

(b)


Current (nA)
Scan 6 of 35
140
120
100
80
60
40
20
0
As
Hg
-400
-200
0
200
400
600
800
E vs. Ag/AgCl (mV) [200 mV/div]

(c)



(d)

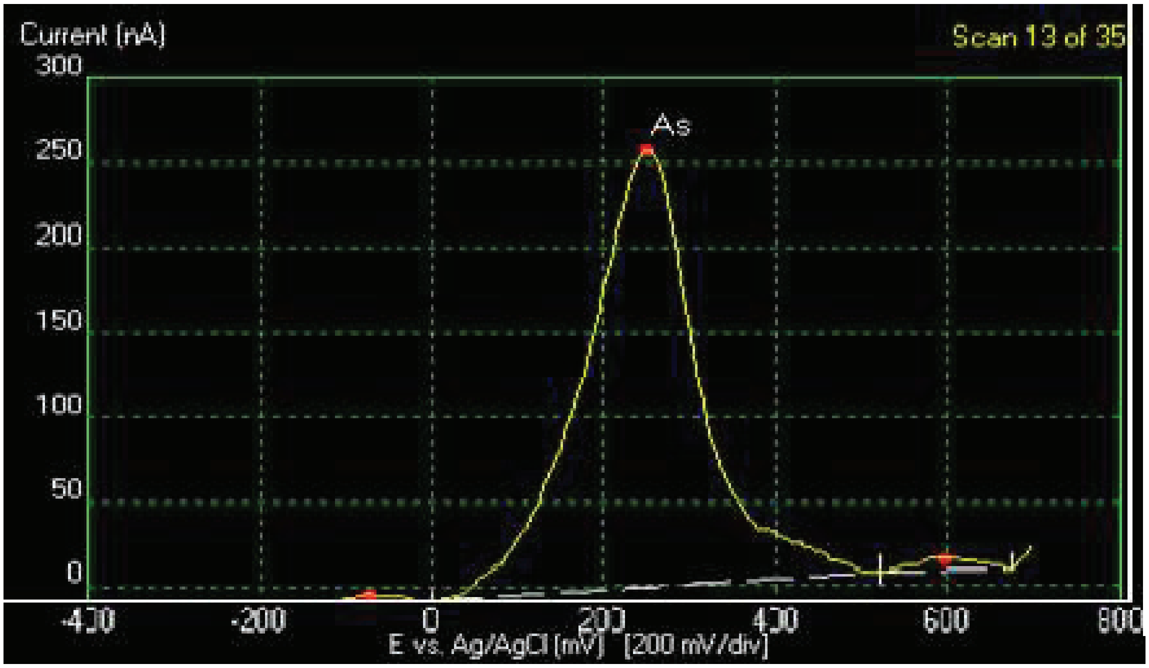


Figure 2.1 Voltammogram of 10 µg L^{-1} arsenite solution at different deposition times: (a) 10 s, (b) 60 s, (c) 120 s, (d) 180 s

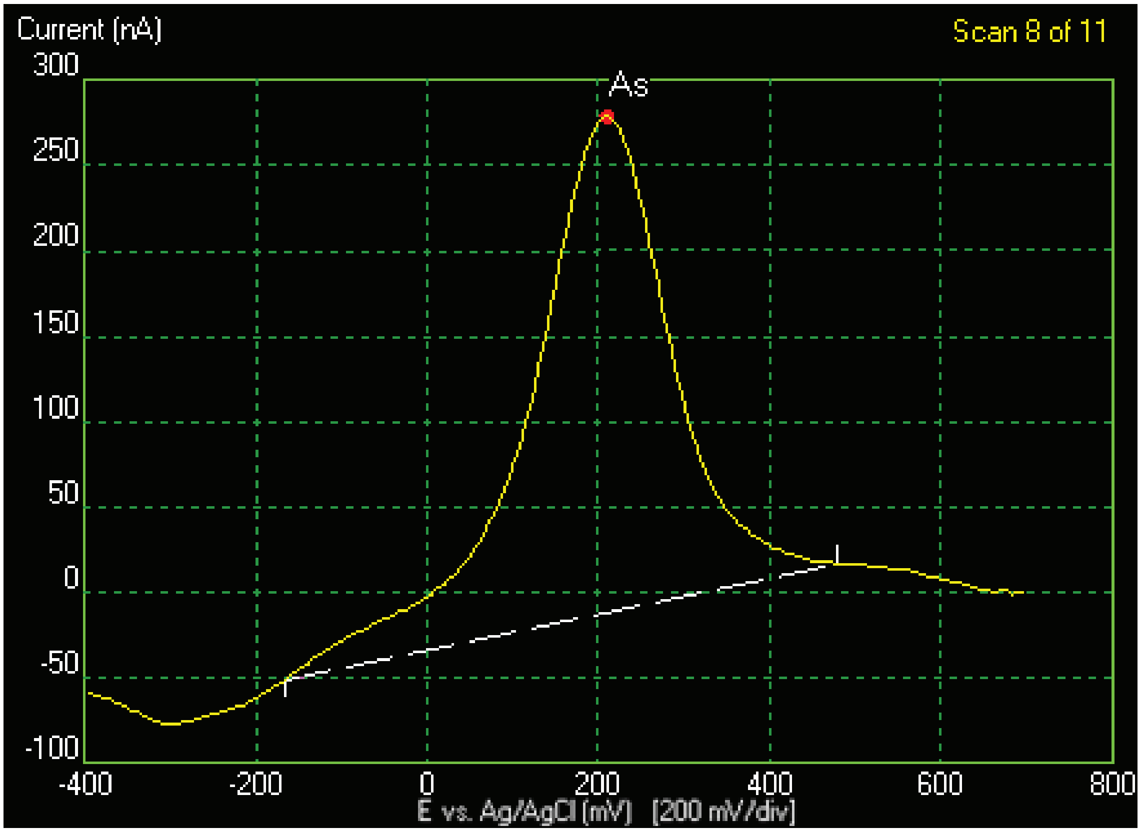
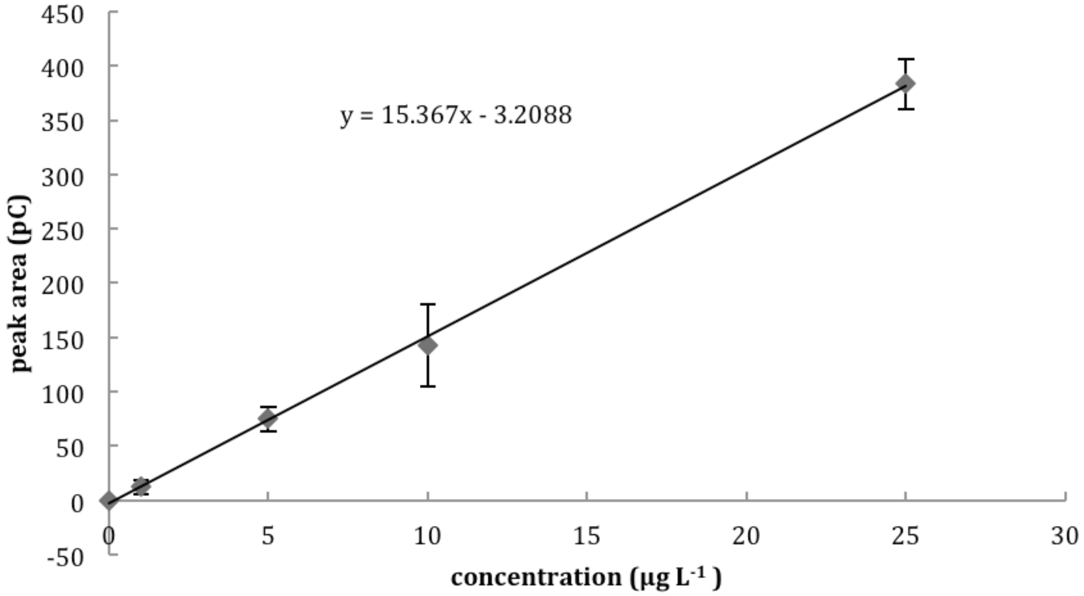


Figure 2.2 Voltammogram of 10 µg L^{-1} arsenate solution in 3.16 g L^{-1} $Na_2S_2O_3$

(a)



(b)

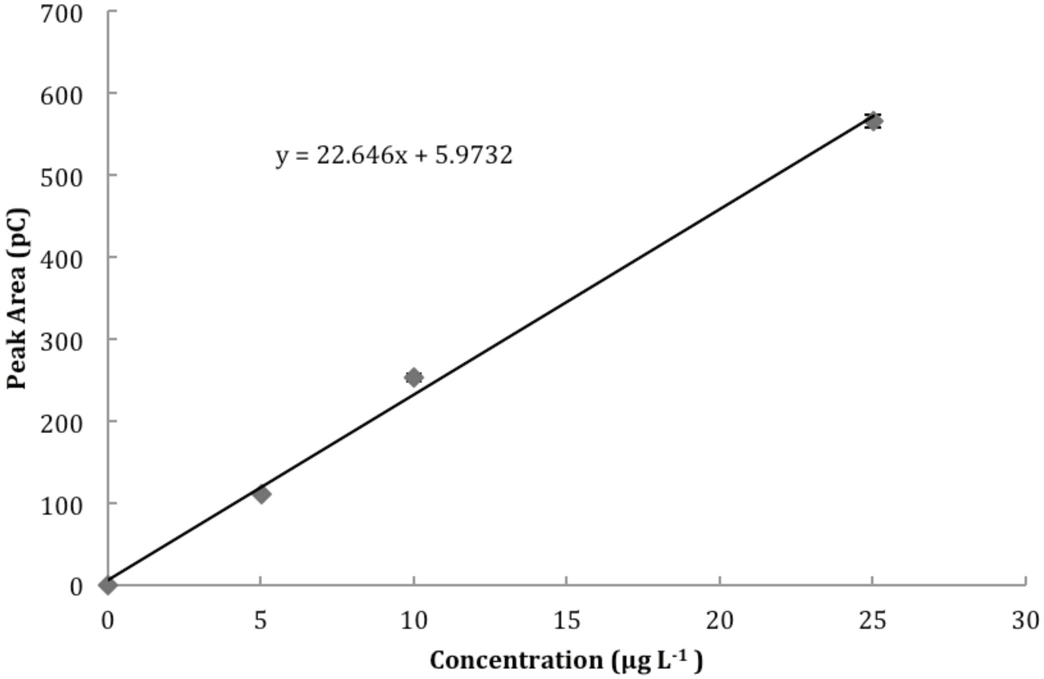


Figure 2.3 Plot of peak area vs. arsenic concentration, the black line is the best fit by an unweigted linear least squares, fitting procedures, the error bars are equal to the ± one standard deviation, (a) arsenite, (b) arsenate

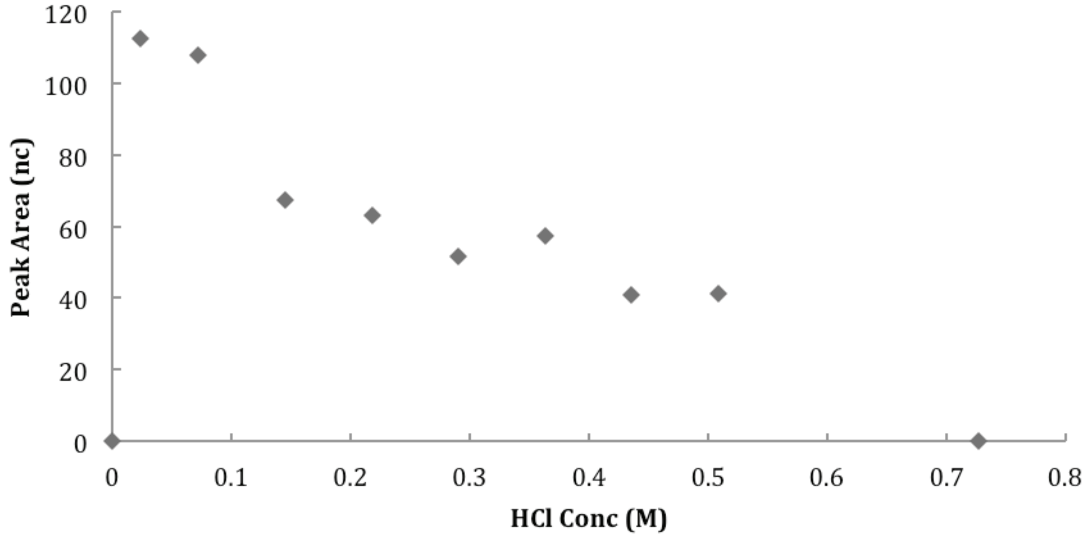


Figure 2.4 Plot of peak area vs. HCl concentration for 10 μg L^{-1} arsenite in the presence of 430 mg L^{-1} Cu(II) and 1.28 mg L^{-1} N_2H_4

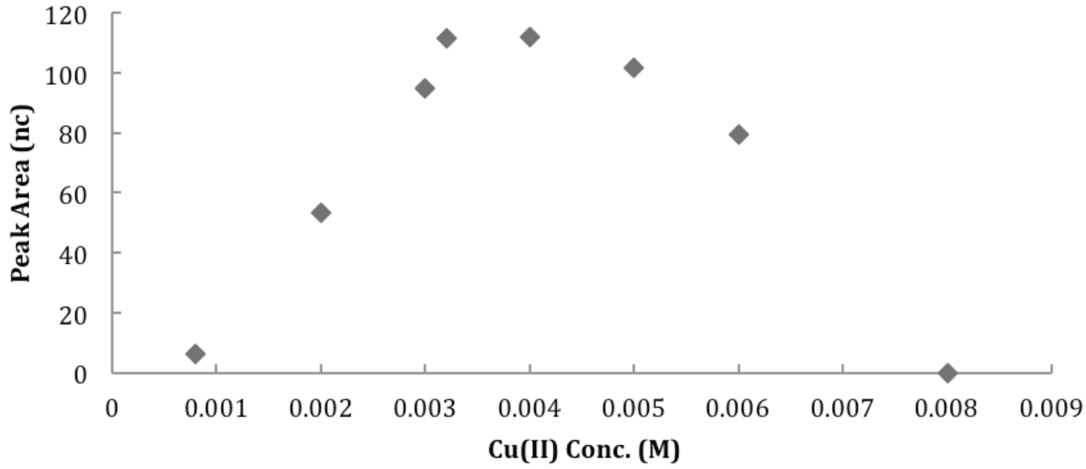


Figure 2.5 Plot of peak area vs. Cu(II) concentration for 10 μg L^{-1} arsenite, in the presence of 24.2 mM HCl and 1.28 mg L^{-1} N_2H_4

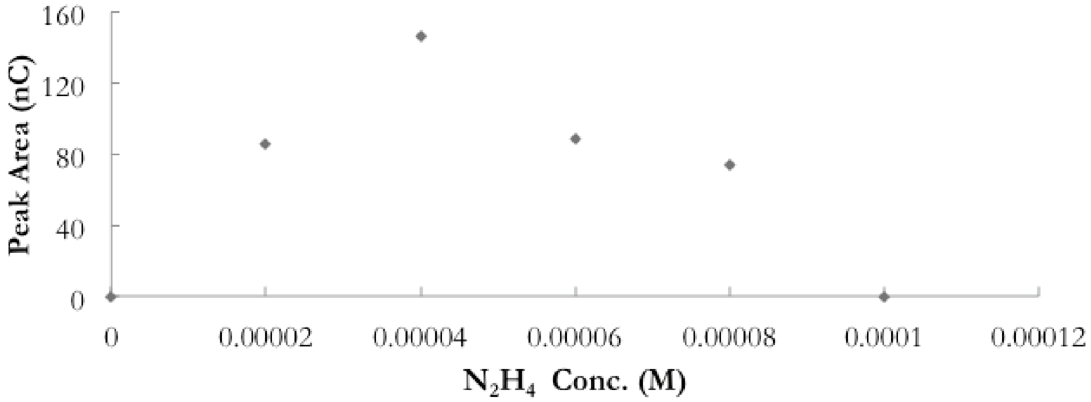
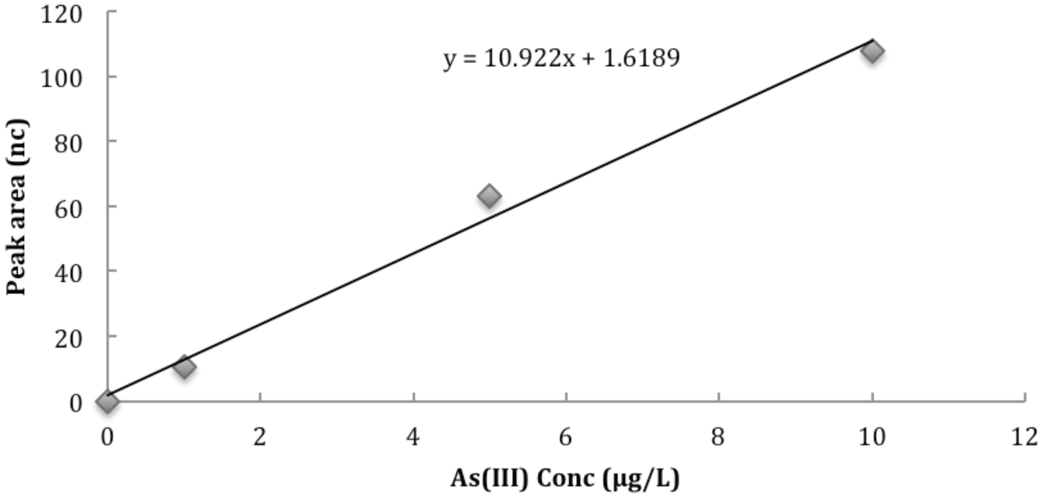


Figure 2.6 Arsenic peak area vs. hydrazine concentration for 10 µg L^{-1} arsenite, in the presence of 24.2 mM HCl and 430 mg L^{-1} Cu(II)

(a)



(b)

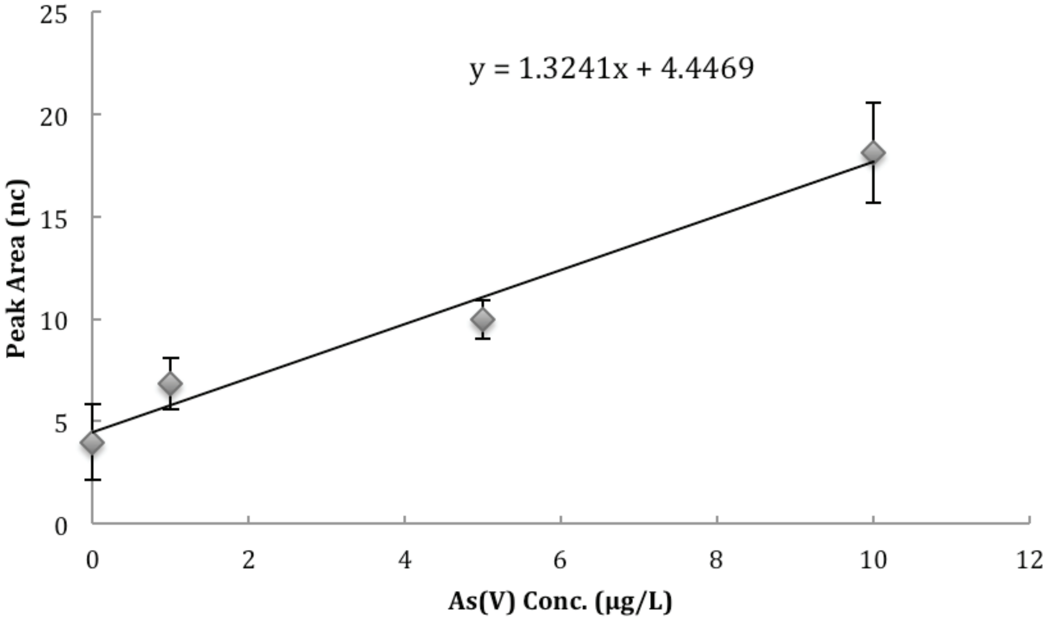


Figure 2.7 Plot of peak area vs. arsenic concentration, the black line is the best fit by an unweigted linear least squares, fitting procedures, the error bars are equal to the ± one standard deviation, (a) arsenite, (b) arsenate

Table 2.1 A summary of some different electrochemical methods developed for arsenic determination[2]

Authors	Electrode material	Method	Deposition time (s)	LOD (μg L^{-1})
Forsberg et al.[3]	gold disk	DPASV	600	0.02
Hua et al.[24]	gold ultramicroelectrode	CCSV	240	0.20
Kopanica and Novotny[25]	gold disk	DPASV	300	0.15
Hamilton and Ellis[26]	gold film	ASV	200	0.56
Bodewing et al.[27]	rotating gold disk	DPASV	240	0.20
Sun et al.[28]	rotating gold disk	DPASV	240	0.19
Feeney and Kounaves[9]	gold ultromicroelectrode array	SWV	300	0.013

Table 2.2 Anodic stripping voltammetry operating parameters

Clean time	0.1 s	Plate time	120 s
Clean voltage	600 mV	**Plate voltage**	- 400 mV
Start voltage	- 400 mV	**Stop voltage**	700 mV
Step voltage	5 mV	**Frequency**	2000 Hz
Range	1.25 µA	**Wave form**	staircase

Table 2.3 Cathodic stripping voltammetry operating parameters

Clean time	1 s	Plate time	60 s
Clean voltage	-800 mV	**Plate voltage**	- 400 mV
Start voltage	- 400 mV	**Stop voltage**	-800 mV
Step voltage	2 mV	**Frequency**	70 Hz
Range	12.5 µA	**Wave form**	Square wave

Table 2.4 Detection limits of ASV and CSV

ASV		CSV	
arsenite	**arsenate**	**arsenite**	**arsenate**
2.2 µg L^{-1}	0.13 µg L^{-1}	0.58 µg L^{-1}	2.7 µg L^{-1}

CHAPTER 3

DETERMINATION OF ARSENIC IN WATER SAMPLES WITH A QUARTZ CRYSTAL MICROBALANCE

3.1 Introduction:

Arsenic contamination in groundwater is a serious problem all over the world. The World Health Organization recommends that the maximum concentration of arsenic in drinking water is 10 μg L^{-1}.[1] To accurately determine the arsenic concentration in water samples, many techniques have been used, such as AFS,[2] ICP-MS,[3] ICP-AES,[4] or HG-AAS.[5] All these techniques can provide high sensitivity and low detection limit. However, the instruments are, for the most part, (a) large, heavy, and expensive, (b) need stable power and gas supplies, and (c) require a long analysis time. For measurement "in the field", colorimetric test kits have been used for a long time, but they are inaccurate and time consuming.[6] These fields test kits are based on the "Gutzeit" method, which was developed over 100 years ago.[7] In this procedure, the inorganic arsenic in a water sample reacts with zinc in acid solution to form arsine gas, which reacts with mercuric bromide on a test strip to give a colored product or products. However, sulfur, selenium and tellurium compounds can cause interferences.[8] Rahman et al. investigated three field test kits (NIPSOM, GPL and Merck). They analyzed 2,866 field samples containing arsenic concentration from <3 to 600μg L^{-1} using the field test kits and compared the results with those obtained by flow injection hydride generation atomic absorption spectrometry (FI-HG-AAS). They reported that when the arsenic concentration was less than 50 μg L^{-1}, the false detection of the field test kits was up to 80.91%. They comment that these field test kits are not at all quantitative and at best qualitative. Other investigations of field test kits are discussed in Chapter 4 of this dissertation.

As a result, accurate, fast measurement of arsenic in the field remains a technical challenge.[9] As was discussed in Chapter 2, portable electrochemistry instruments with three-electrode systems can be applied for field studies. However, portable instruments are not limited to voltammetric techniques. In addition to electrochemical instrumentation, another portable instrument with high sensitivity, which can also be potentially used in field studies is the quartz crystal micro-balance (QCM), a mass sensing instrument based on the piezoelectric effect.[10] Measurement by QCM may be rapid, depending on the surface chemistry employed to capture the analyte species. Due to the high sensitivity and low cost of the QCM, it has become popular in recent years. In the Web of Science database, there are 3,368 papers with QCM (or quartz crystal microbalance in the title) dating from 1911 to the present (August 2012). In most of these studies, the QCM has been used in investigations of the kinetics of small bio-molecule interaction processes (interaction of small biomolecules, such as melittin with a membrane)[11] or as an immunosensor.[12-14] In addition to the application of the QCM to biochemical studies, procedures have been developed for the determination of anions, such as phosphate, sulfate and selenite, in which the surface of the crystal was coated with the appropriate sensing materials.[15-17] More details of these analytical procedures can be found in Chapter 1.

Hydrogels are three-dimensional networks that are always hydrophilic, flexible and able to adhering to many different surfaces due to the gel property. Barakat et al. found that the arsenic ions migrated into the 3D network of hydrogels by electrostatic interactions.[18] Some hydrogels are proved to have the ability to absorb arsenic from water with removal efficiencies of more than 50%. The detailed background to this research is discussed in Chapter 5. Hydrogels could be used to modify the surface of QCM to detect arsenic from water.

The compound dithiothreitol (DTT, $HSCH_2CH(OH)CH(OH)CH_2SH$), which contains thiol groups, has been used for inorganic arsenic detection by Kalluri et al. [19] and by Forzani et al.[20] Kalluri and co-workers reported the label-free detection of arsenic by a dynamic light scattering assay based on gold nanoparticles modified with DTT, glutathione (GSH) and cysteine (Cys), for which the detection limit was 10 pg L^{-1}. Forzani and co-workers developed a surface plasmon resonance sensor with DTT as the "recognition element" to detect total arsenic in ground water. Recently, Chen et al. reported a ASV method to determine arsenite in environmental and drinking water using a gold working electrode modified with SAMs of glutathione, dithiothreitol and N-acetyl-L-cysteine.[21] Since DTT has two sulfur-containing groups, one can form a sulfur-gold bond with the surface of the sensing device and the other can form a sulfur-arsenic bond with the arsenic species in water. DTT can also reduce arsenate to arsenite, thus, this method can be used for arsenate determination without adding other reducing agents.[13]

A real water sample contains potential interfering ions derived from metals such as iron and copper, which can bind with DTT. To investigate the elimination of possible interferences, ethylenediaminetetraacetic acid (EDTA) was added to the sample. EDTA is a well known chelating reagent whose first major industrial use was the removal of trace metal impurities before the dying of fabrics in the textile industry.[22] The logarithms of the stability constants for the reaction of EDTA with copper(II) and Fe(III) are 18.8 and

25.7, respectively. As the values for the reaction of DTT with the metals are 15.3, this means EDTA will preferentially form complexes with the ions and keep them from binding to DTT.[23] The goal of the work reported in this chapter is the development of a potential portable arsenic sensor based on a QCM whose oscillating crystal electrodes are modified with DTT. The desired performance is determination of inorganic arsenic concentrations from 1 to 50 μg L^{-1} within 5 min.

3.2 Experimental:

3.2.1 Apparatus and Reagents:

This study was carried out with the QCM200 Quartz Crystal Microbalance Digital Controller equipped with the QCM25 5 MHz Crystal Oscillator (Stanford Research System, Sunnyvale, CA, USA). The electrode on the crystal surface is chromium/gold electrode, the diameter of the crystal is 2.54 cm and the surface area of the crystal is 0.4 cm^2.

Analytical reagent grade chemicals were used. Methylenebisacrylamide (MBA), 75% (3-acrylamidopropyl)trimethylammonium chloride (APTMACl), tetramethylethylenediamine (TEMED), ammonium persulfate (APS), 1, 6 – hexanedithiol, dithiothreitol ($C_4H_{10}O_2S_2$), sodium arsenate, sodium arsenite, copper(II) chloride, iron(III) chloride, hydrogen peroxide, ethanol and sulfuric acid were all purchased from Fisher Scientific (Pittsburgh, PA, USA). Ethylenediaminetetraacetic acid disodium salt was purchased from Mallinckrodt Chemical Inc. (St. Louis, MO, USA). All working solutions were prepared in deionized water (18.0 MΩ cm) obtained from a Barnstead E-Pure water system (Dubuque, IA, USA).

3.2.2 Arsenite and Arsenate Determination:

To make 10.00 g L^{-1} arsenite and arsenate stock solutions, 0.1734 g of sodium arsenite and 0.4165 g of sodium arsenate were weighted and separately dissolved into 10 mL of deionized water. The 15.43 g L^{-1} (0.1000 M) DTT stock solution was made by dissolving 0.1543 g of DTT solids into 10 mL of deionized water. The lower concentration solutions, 1.498 mg L^{-1} (20.00 μM) arsenite, 1.498 mg L^{-1} (20 μM) arsenate, 3.085 mg L^{-1} (20.00 μM) DTT and 1.543 mg L^{-1} (10.00 μM) DTT, were prepared by serial dilution from the stock solutions. The low DTT concentration solutions were freshly prepared before each experiment and purged with nitrogen gas for 4 min.

Before each experiment, the crystal was immersed into 10 mL "piranha" solution (3 + 1 mixture of concentrated sulfuric acid and 30% hydrogen peroxide) for 3 minutes followed by rinsing with deionized water. Finally, the crystal was dried with nitrogen gas.

The initial frequency was recorded in air before each measurement series. For arsenite measurements, the crystal holder was first immersed into a 50 mL 20.00 μM or 10.00 μM DTT solution. Care was taken to ensure that the whole crystal was completely under the water level. Measurements took 5 min until the signal was stable. The frequency was recorded. 50 μL 1.498 mg L^{-1} arsenite solution was added repeatedly to the solution, until the final arsenite concentration was 50.00 μg L^{-1} (0.6700 μM). Each addition 50 μL produced an increase of approximately 1.5 μg L^{-1} in the concentration. The pH of the solution was not controlled and was about 5.3 throughout the experiments. After each addition, the stable frequency was recorded.

For arsenate solutions, an additional 5 min was allowed before the frequency was noted. Calibration curves were constructed by plotting frequency change as a function of arsenic concentrations in Microsoft Excel and the equations of the unweighted, least-squares, best-fit lines calculated.

1,6 – Hexanedithiol (HDT) was used as an alternative way of modifying the QCM surface. 0.1182 g of HDT solids were dissolved in 10 mL of 5% ethanol in order to make an 11.82 g L^{-1} (0.1000 M) stock solution. The lower concentration solutions, 2.364 mg L^{-1} (20.00 μM) HDT were diluted prepared from the stock solutions by serial dilution. The crystal was immersed in 20.00 μM HDT solution for 1 h before measurements were made.

3.2.3 Hydrogel Preparation

Poly(3-acrylamidopropyl)trimethylammonium chloride, p(APTMACl) hydrogels were synthesized following the method Barakat.[18] Basically, 5 mg of methylenebisacrylamide (MBA), 10 g of 75% (3-acrylamidopropyl)trimethylammonium chloride (APTMACl), 1 mL of tetramethylethylenediamine (TEMED) and 0.57 g of ammonium persulfate (APS) were added to and mixed in 10 mL of deionized water and reacted for 24 h at room temperature. After hydrogels were formed, the hydrogel was immersed in distilled water for 2 days. Then it was dried in an oven at 50 °C until constant weight was reached.

The investigation of the arsenic capacity of the hydrogel was carried out by using ASV. To a 50 mL of 100 μg L^{-1} arsenite solution, a 75 mg of p(APTMACl) hydrogel was added, and the arsenic concentration in the solution was determined every 10 min by using the optimum ASV method, which was described in Chapter 2.

3.2.4 Interference Study

To study possible interferences, a water sample was prepared as Bangladesh groundwater.[24] In order to overcome the interferences, EDTA was used as the chelating agent. In this experiment, a certain amount EDTA was added to the samples, to make the final concentration at 1.86 g L^{-1}, which were then boiled on a hotplate for an hour. After cooling to room temperature before measurements were made.[25]

3.2.5 Real Water Sample Analysis

A rainwater sample was collected in a clean bucket after 5 min from the start of the rain. The bucket was rinsed with rainwater a couple of times. All the samples were filtered through 0.45 μm filter paper and spiked with a known amount of arsenic concentration. EDTA was added to the samples following the procedure described in the inference study in order to get rid of the inferences. For determination, the crystal was first immersed into 50 mL 20.00 μM DTT solution and the frequency was recorded after 5 min when the signal was stable. This frequency was used as the baseline. Then, after EDTA treatment, 10 μL 15.43 g L^{-1} (0.1000 M) DTT was added to 50 mL of sample. The final concentration of DTT was 3.085 mg L^{-1} (20.00 μM). In order to determine the arsenic concentration in water samples, standard addition was applied. For each addition, 100 μL 1.498 mg L^{-1} arsenite solution was added and the frequency was recorded after each addition when the signal was stable.

3.3 Results and Discussions:

3.3.1 Analytical Performance for Determination of Arsenic

A typical response of the QCM sensor immersed in DTT solution after adding 50 μL of a solution containing 1.50 mg L^{-1} of arsenic(III) solution is shown in Figure 3.1, from which it can be seen that the frequency decreased relatively rapidly in the first minute and then more slowly to reach a stable value after 5 min.

The calibration curves are shown in Figures 3.2 and 3.3 for arsenic(III) and arsenic(V), respectively. It can be seen that a usable calibration range of up to 50 μg L^{-1} (0.67 μM) was achieved in the case of arsenic(III), and of up to 15 μg L^{-1} (0.20 μM) in the case of arsenic(V). The equations for the best fit lines are $\Delta F = 1.81\ C + 714$ for arsenic(III) and $\Delta F = 2.44\ C + 699$ for arsenic(III), where ΔF is the frequency change in Hz and C is the concentration in solution in μg L^{-1}.

It is assumed that when the crystal is first immersed in the DTT solution, DDT binds to the surface and that this is responsible for the decrease in frequency seen in Figure 3.1. As the response levels off, it is concluded that all available binding sites on the surface are occupied by a sulfur atom of a DTT molecule. When arsenic(III) is then added, it is considered that there are two possible reactions: (1) arsenite reacts with DDT in solution, exchanging up to three OH groups for the S of DTT, and (2) arsenite reacts with the exposed thiol groups of the DTT bound to the crystal surface. If reaction 1 predominates, then it is considered that a second reaction occurs in which the arsenic-DTT species are exchanged for surface-bound DTT molecules. As the system responds to arsenate, it is deduced that reaction between solution arsenate species and DTT occurs to reduce arsenate to arsenite and that this reaction is followed by whatever processes are responsible for the binding of an arsenite species to the electrode surface. It is noted that the slope of the calibration for arsenic(V) is higher than that for arsenic(III), but that the linear range is shorter. It is proposed that the arsenic-containing species bound to the electrode surface responsible for the change in vibrational frequency is heavier in the case of arsenic(V) than that bound in the case of arsenic(III), and this suggests that the reduction of arsenic(V) by DTT does not lead to exactly the same species as are formed when arsenic(III) and DTT are present in the solution. As DTT contains a sulfur atom at each end, it is possible that dendrimer-like molecules can form in solution; however, no evidence of such formation was detected. It might be expected that the product of the redox reaction with arsenic(V) would be a dithiane ring (cyclic disulfide),[26] which in turn could react with the arsenic(III) in solution, but would not be available to assist with the binding of an arsenic-species to the surface. It is noted that the response to arsenite continues into a region in which there are more arsenite molecules than DTT molecules, suggesting that a more complex mechanism is responsible for the frequency than simple binding via DTT to the surface.

For arsenite, the working linear range can be improved by increasing the concentration of DTT solution. The same experimental procedure was also applied to a lower DTT concentration. As shown in Figure 3.4, the linear range was only up to 10.00 μg L^{-1} (0.1300 μM) of arsenite when using the 1.543 mg L^{-1} (10.00 μM) DTT solution. A possible explanation is that when solution contains excess DTT, the DTT can form a multilayer structure with arsenite, that has more arsenite binding sites, instead of forming a single layer on the QCM surface. The detection limit of arsenite is 0.6 μg L^{-1}, which is calculated based on the standard deviation of the frequency changes over 5 min when measuring a blank.

Arsenite can also be detected using a HDT-modified QCM. However, the crystal pretreatment time was around 1 h in order to obtain a linear response. Moreover, HDT can not be completely dissolved in water. Thus, ethanol was added to the solution (at least 5%). Besides, HDT has an unpleasant smell. Considering the analysis time and the simplicity of the procedure, HDT was not used in this study.

The p(APTMACl)) hydrogels showed the ability of absorbing arsenite (67 μg g^{-1}) from water. However, the absorbing process took 1 h. When the crystal modified with hydrogel was put in the sample solution, it also took a long time (around 30 min) to equilibrate. Thus, hydrogel was not used in these studies either.

3.3.2 Interference Study

To demonstrate the selectivity of the QCM method, some common interference ions were investigated, such as Na^+, K^+, Ca^{2+}, Mg^{2+}, Pb^{2+}, Cu^{2+}, Fe^{3+}, Cl^-, No^{3-}, and SO_4^{2-}. Among those ions, only Cu^{2+} and Fe^{3+} interfered with arsenic when the Cu^{2+} concentration was higher than 90 μg L^{-1} and the Fe^{3+} concentration was higher than 0.4 mg L^{-1}. By boiling the solution, arsenite is oxidized to arsenate and additional time is needed before the QCM measurement in order to make sure arsenate is reduced to arsenite by DTT. An arsenite response curve is shown in Figure 3.5 with the presence of 90 μg L^{-1} of Cu^{2+}, 0.4 mg L^{-1} of Fe^{3+} and 1.86 g L^{-1} of EDTA. It is clear that after adding EDTA, Fe(III) and Cu(II) interferences are successfully eliminated.

3.3.3 Determination of Total Arsenic in Real Water Samples

The real water sample analysis was conducted by Aydan Elçi Başaran, who was a visiting graduate student from Aegean University in Turkey. Real water samples included rainwater, water collected from Puffers Pond (Amherst, MA), the Connecticut River (Northampton, MA), and bottle water were purchased from local supermarket. Results are listed in Table 3.1. The ± term is the standard deviation ($n = 3$ or more, when $n > 3$, three best results were chose based on Q-test). All recoveries were from 84.8% to 128.6%, indicating that this method is suitable for arsenic determination in real water samples. It should be noted that, since each time the DTT layer formed on the gold electrode surface is different, standard addition is strongly recommend for real water analysis.

3.4 Conclusions

It is concluded that a viable analytical procedure for the determination of inorganic arsenic species in solution can be developed in which arsenite is sensed by binding to the surface of the gold electrode of the oscillating quartz crystal with DTT causing a decrease in the oscillation frequency. From the behavior of the calibration function at the low concentration end, it is deduced that the detection limit is single-digit $\mu g\ L^{-1}$. The method was successfully applied to real water analysis without interferences. Compared with performance characteristics of field test kits, the QCM method is more sensitive, accurate and faster. It has the potential to be used in field studies in future.

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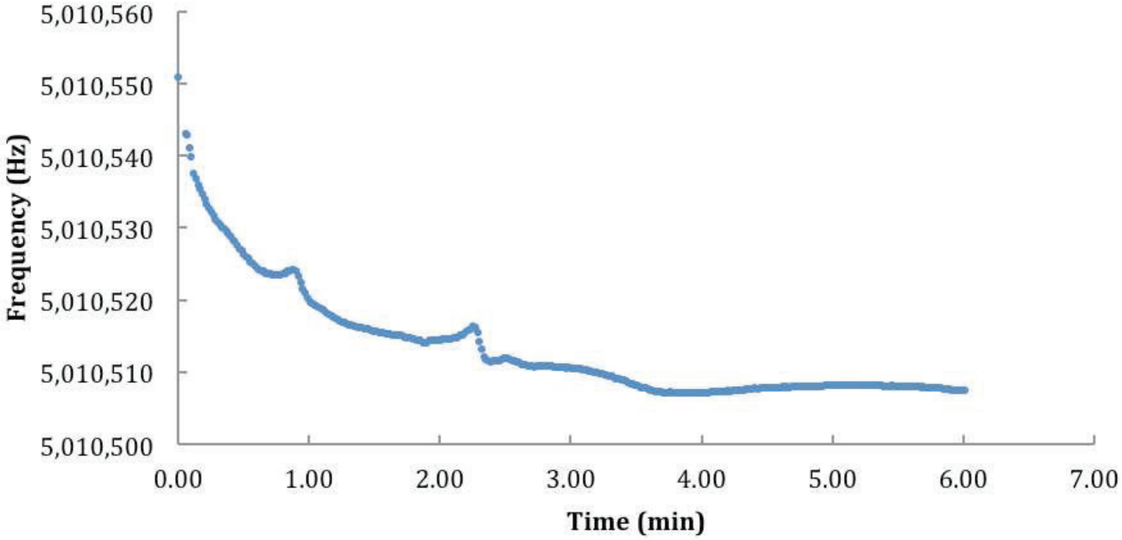

Figure 3.1 Frequency response of QCM sensor

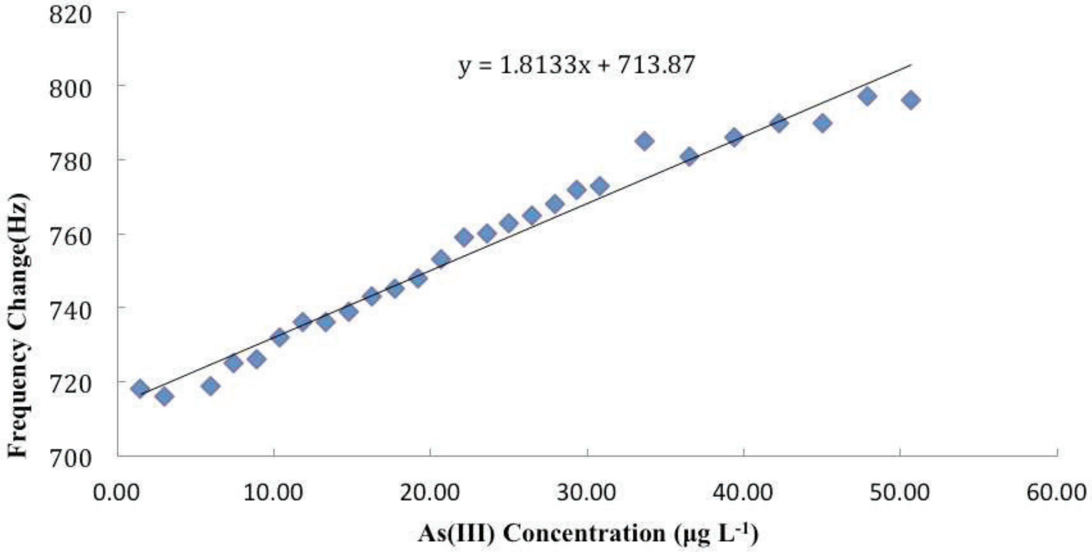

Figure 3.2 Arsenite concentration dependence of the frequency change (the concentration of DTT was 3.085 mg L^{-1}. The straight line represents the best linear fit to the data)

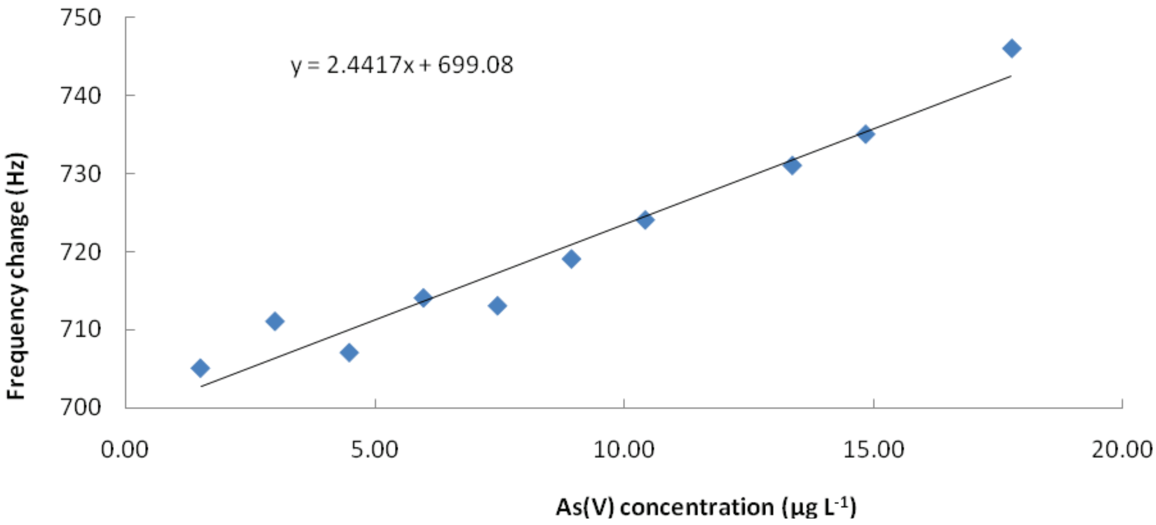

Figure 3.3 Arsenate concentration dependence of the frequency change (the concentration of DTT was 3.085 mg L^{-1} The straight line represents the best linear fit to the data)

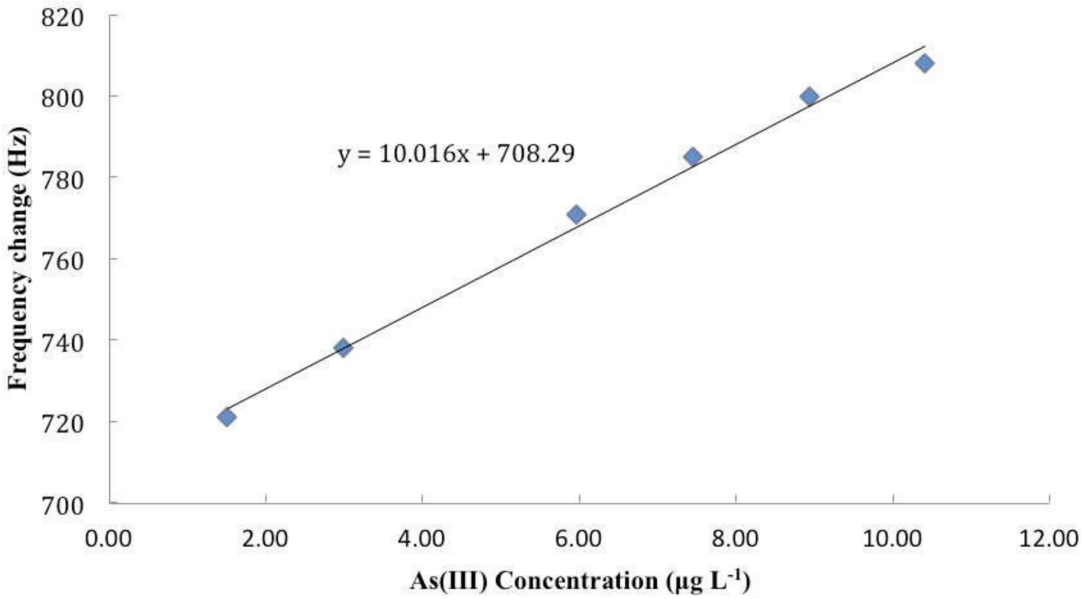

Figure 3.4 Arsenite concentration dependence of the frequency change (the concentration of DTT was 1.543 mg L^{-1} The straight line represents the best linear fit to the data)

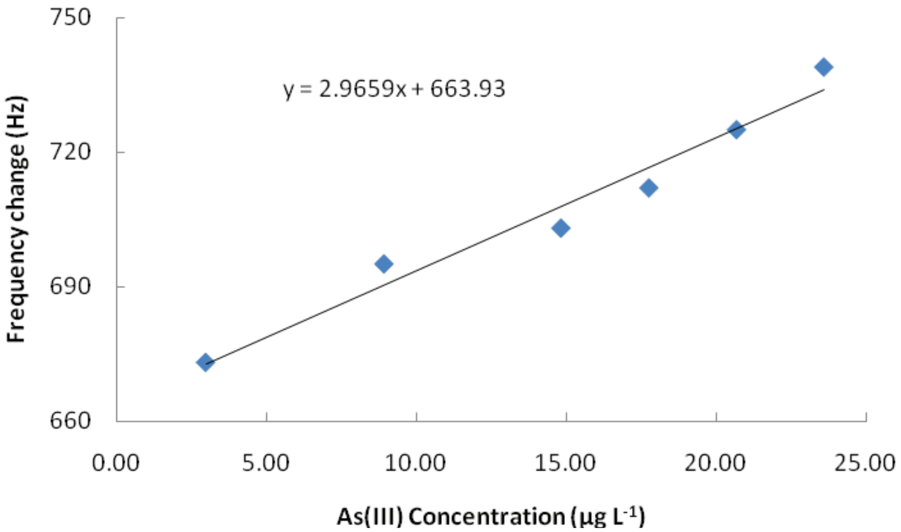

Figure 3.5 Arsenite concentration dependence of the frequency change with the presence of EDTA as the masking reagent (the concentration of DTT was 3.085 mg L^{-1}, Cu^{2+} was 90 µg L^{-1} , Fe^{3+} was 0.4 mg L^{-1} and EDTA was 1.86 g L^{-1}. The straight line represents the best linear fit to the data)

Table 3.1 Recoveries of determination of arsenic in water samples

Sample	Spike level (μg L^{-1})	Found (μg L^{-1})	Recovery (%)	Mean of Recoveries (%)
Puffer Pond	0	Not detected		
	6.00	5.53	91.7	95.1 ± 9.8
	7.00	7.65	110	n = 4
	8.00	7.04	88.0	
	9.00	8.19	91.0	
Connecticut River	0	Not detected		
	6.00	6.27	105	105 ± 6
	7.00	7.46	107	n = 5
	8.00	9.10	114	
	9.00	8.96	99.5	
	10.0	10.4	104	
Rain Water	0	Not detected		
	5.00	5.05	101	101 ± 10
	6.00	5.71	95.2	n = 5
	7.00	6.09	87.1	
	6.00	6.51	109	
	7.00	7.89	113	
Fuji Water	0	7.63 ± 1.43 (n=3)	-	104 ± 3 n = 10
	2.00	9.32 ± 0.62	84.8	
	3.00	10.7 ± 0.4	103	
	4.00	12.8	129	
	5.00	13.4 ± 0.5	116	
	6.00	15.8	136	
	7.00	15.3 ± 0.3	111	
	8.00	15.0 ± 1.3	92.4	
	9.00	15.6 ± 0.3	88.4	
	10.0	16.1 ± 0.6	85.2	
	11.0	17.6 ± 0.6	90.6	

CHAPTER 4

INVESTIGATION OF THE KINETICS OF THE GUTZEIT REACTION IN THE HACH KIT

4.1 Introduction

Arsenic contamination in groundwater is a serious problem all over the world. Exposure to arsenic may cause skin and lung cancers, high blood pressure and diabetes. The World Health Organization recommends that the maximum concentration of arsenic in drinking water is 10 $\mu g\ L^{-1}$, due to its toxicity.[1] However, in some parts of Asia, especially in Bangladesh and India, the arsenic concentration in groundwater is even higher than 50 $\mu g\ L^{-1}$.[2] To accurately determine arsenic concentrations in water samples, many techniques have been used, such as AFS,[3] ICP-MS,[4] ICP-AES,[5] HG-AAS.[6] All these techniques can provide high sensitivity and low detection limit. However, these instruments are large, expensive, and need stable power supply, gases, and a long analysis time. According to Melamed, “Accurate, fast measurement of arsenic in the field remains a technical challenge…the central goal of developing field assays that reliably and reproducibly quantify arsenic has not been achieved.” [7]

There are many field test kit for arsenic determination can be found on the market, such as Quick Arsenic (Industrial Test Systems Inc), EZ arsenic test kit (Hach Company), BVC-100 (Fukuoka), ECO-W100(Fukuoka), LaMotte Arsenic test kit(LaMotte), and Merck(Merck and Company Inc). One of the advantages of using the field test kit is arsenic concentrations in water sources can be immediately determined without costing much money. In US, traditional laboratory analyses typically cost around \$25 – 50 per sample.[8] However, the cost of using the field test kit is only around \$1 – 2 per sample.[8]

In addition, training and other personnel costs are not including in the calculation. Moreover, the water samples need to be shipped to the specific laboratory for analysis, which might be hundreds miles away. In comparison, field test kit can be taken out and analyze water sample onsite. This will ensure that the water sample is fresh and not contaminated during the transportation. The field arsenic test kits have been used in many of the field studies. Most of the test kits are based the Gutzeit method.[9] In the original version of the procedure, arsine gas (AsH_3), generated by the reduction of arsenite on a Zn surface in the presence of H_2SO_4, reacted with $AgNO_3$ forming black compounds. The following reactions are given by[10]

(1) $Zn(0) \rightarrow Zn^{2+} + 2e^-$

(2) $2H^+ + 2e^- \rightarrow H_2$

(3) $As(III) + 3e^- \rightarrow As(0)$

(4) $As(0) + 3e^- + 3H^+ \rightarrow AsH_3$

(5) $AsH_3\ (g) + 3AgNO_3\ (s) \rightarrow AsAg_3\ (s) + 3HNO_3$

The advantages of the Gutzeit method are the simplicity and ease of AsH_3 generation, and the ability to separate AsH_3 from interferences. Similarly, silver diethyldithiocarbomate (AgDDC) can react with arsine and form color complex.[11] Lakso et al. reported a determination method of arsenite, arsenate, monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) by using AgDDC. Four arsenic species were reduced to their corresponding arsines after reduction by sodium borohydride at certain pHs. The arsine reacted with AgDDC to give a chromophore which absorbs maximally at 540 nm. The methyl- and dimethylarsines produced a chromophore, which absorbs maximally at 440 nm. The qutitative limit is 1.0 μg of elemental arsenic for four arsenic species. As alternatives to AgDDC and $AgNO_3$, researchers have used $HgBr_2$ or $HgCl_2$ to react with AsH_3.[12,13] Bird made a careful study of the conditions when using $HgCl_2$ paper, with special reference to the interference of the hydrides of sulfur, phosphorus, and antimony.[14] His work was considered to be quantitative, but he reported that the stain obtained from a given amount of substance might be shown to be greater or less than the stain representing a fixed limit of arsenic for that amount. Rahman developed a colorimetric method for the determination of arsenic by using a test paper impregnated with $HgBr_2$ and rosaniline chloride ($C_{20}H_{19}N_3 \cdot HCl$).[12] Under the optimum condition, this method was able to measure up to 30 $\mu g\ L^{-1}$ of arsenite and arsenate in 15 ml of the sample solution with a detection limit of 5 $\mu g\ L^{-1}$. Many efforts have been put into modifying the Gutzeit method. More work can be found in Chapter 1 in this dissertation.

In the Gutzeit reaction, arsenite in solution forms AsH_3 in the presence of acid on Zn surface, and then reacted with $HgBr_2$ on the test strip, forming a yellow or brown product depending on the arsenic concentration. The higher concentrations produce darker colors. The identities of the yellow and brown compounds are not well understood. However, according to Sanger, the yellow compound is $AsHHg_2Cl_2$:[15]

$$2\ HgCl_2 + AsH_3 = AsHHg_2Cl_2 + 2HCl$$

and the brown compound is $AsHg_3Cl_3$:

$$3\ HgCl_2 + AsH_3 = AsHg_3Cl_3 + 3HCl$$ [15]

This test kit is portable and cheap, but it has a few drawbacks as well. Besides generating toxic arsine gas during the reaction, the reproducibility and accuracy are not good.[16,17] Steinmaus et al. evaluated two field test kits (Quick Arsenic and Hach EZ arsenic test kit) by comparing the results with those obtained form atomic fluorescence spectroscopy (AFS).[8] The results show that when arsenic concentration is higher than 20 μg L^{-1}, 99% (Quick Arsenic) and 97% (Hach EZ arsenic test kit) of the results are agreed with those obtained by using AFS. When arsenic concentration is below 15 μg L^{-1}, the percentages drop down to 71% (Quick Arsenic) and 62% (Hach EZ arsenic test kit). In 1930, Barnes and Murray investigated the accuracy of the Gutzeit method. They applied a formula to calculate the probable error, which is

$$PE = \sqrt{(PE_1)^2 + (PE_2)^2},$$

where PE is the protable error of method as a whole, PE_1 is the probable error of reading referred to the standard, and PE_2 is the probable error of standard. They pointed out, if the experiment was performed under ordinary commercial conditions, the probable error of the mean of duplicate strips in the Gutzeit method was ± 0.0039 mg.[17] Thus, for quantities less than 35 μg, the error ranges from 11 to 100%. Based on the study of Van Geen et al., when the As concentration was in the range of 50 – 100 μg L^{-1}, 44% of the results obtained by Hach Kits were not in agreement with those obtained by ICP-MS.[16] They also suggest if using Hach Kits, a longer reaction time would give a better result. A detailed comparison study was carried out by Spear et al. He and his colleagues investigated the performance of six commercial arsenic test kits (BVC, ECO, Hach, Merck, Quick, and LaMotte) and compared the results by using graphite furnace atomic absorption spectroscopy (GFAAS). Based on their investigation, BVC kit costs least ($0.30 per test) and requires the shortest test time (13 min) and Hach costs most ($1.06 per test) and requires the longest test time (40 min). Quick II provides the best recoveries for both arsenite and arsenate, however, Hach provides the worst. LaMotte works best with Sb and Sulfide, however, Hach and BVC are effected badly. By comparing the results with those obtained by GFAAS, LaMotte results are the closest to the GFAAS results and BVC results are far off.

Lots of factors can cause the poor performance of the field test kit, such as interferences, incomplete reaction, contamination and a part of the error is cause by naked eye determination. It leads to poor repeatability and a considerable determination error of 20 -35 %.[13] As a result, nowadays, digital image scanning can be used to determine the color intensity on the test strip.[13,18] Muhammad et al. proposed a method for the estimation of inorganic arsenic at low levels (µg L^{-1}). The arsine gas was passed through a mercuric bromide filter paper giving a yellowish brown color. The color intensity of the spot was calculated by scanning the spotted paper and analyzing the image using specially designed software (Visual Basic). The detection limit was reposted as 1 µg L^{-1} with a linear range from 2 – 20 µg L^{-1}.[13] Similarly, Sharma et al. developed a arsenic sensor based on immobilizing reagents, which are sulfanilic acid and N-(1-naphthyl) ethylene diamine dihyrochloride (NEDA), on thin layer chromatography (TLC). Arsenic can react with the reagents and form a purple color, which can be scanned and analyzed. The red, green, and blue (RGB) values were analyzed using MATLAB software. The proposed sensor is linear in the range 1 – 10 and 25 – 100 µg mL^{-1}. The detection limit is 0.018 µg mL^{-1}.[19] In most cases, a flatbed scanner and appropriate software are needed for digital image scanning. Basically, the color strip was scanned by a flatbed scanner and then the red, green and blue components of each pixel of the spot (RGB) was measured by the software. The RGB values of a pure white color are 255,255,255 and of a pure black color are 0,0,0. Since the colored spot is yellow, the blue value is more suitable as a measure of the extent of the color formation than the red or green values. A darker spot always has a lower blue value.

Since Hach Kits are commonly used in field studies, to improve the accuracy and reproducibility are becoming important.[18] Thus, the goal of the experiments described in the dissertation is to investigate the kinetics of the reactions in Hach Kit, establish a mass balance for the arsenic and suggest modified conditions to improve the sensitivity(i.e. the ability to detect low concentrations), reproducibility and accuracy.

4.2 Experimental

4.2.1 Apparatus and Reagents

A Perkin-Elmer Model ELAN 6100 inductively coupled plasma mass spectrometer (Waltham, MA, USA) was used for monitoring the rate of arsenic gas generation. The operating parameters are listed in Table 4.1. A flatbed scanner Epson 1280 (Long Beach, California, USA) was used for scanning the strips and obtaining the pictures of the strips. The images of the scanning pictures were analyzed by Color.exe program from Redwoodsoft. A CEM MARS Xpress microwave digestion system with PFA vessels (Matthews, NC, USA) was used for digesting the strips and the temperature program is listed in Table 4.2. The solution after digestion was analyzed by the same inductively couple plasma mass spectrometer listed above in order to obtain the mass information of arsenic on the strip. For analyzing the mass of arsenic in the solution, a continuous flow hydride generation atomic florescence spectrometer (Millenium Excalibur, PS Analytical Ltd., Orpington, Kent, England) equipped with a glass gas-liquid separator was used. Arsenic was determined at a wavelength of 193.7 nm. To investigate the possible chemical structures of the color compounds, strips were analyzed using a scanning electron microscopy (SEM) from Zeiss (EV050 XVP) coupled with Bruker AXS EDS system (Oberkochen, Germany). Esprit-Quantax program (Xflash 4010 SDD) was used to quantitatively analysis elements weight percentage of the crystal. Vessels and the salfamic acid and zinc powder reagents (catalogue. Number 2822800) were provided by the Hach Company (Loveland, CO). High purity water (18 MΩ.cm) was obtained from a Barnstead E-pure unit. Standard solutions were prepared by dissolving the appropriate mass of sodium arsenite ($NaAsO_2$) supplied by Fisher Scientific (Pittsburgh, PA). Concentrated HNO_3 used for microwave digestion, L-Cysteine, hydrochloric acid, $NaBH_4$, NaOH, KI and I_2 used in arsenic determination in solution were purchased from Fisher Scientific (Pittsburgh, PA).

4.2.2 The Rate of Hydrogen Generation

A Hach kit vessel and a 50 ml cylinder were set up as shown in figure 1. The vessel was filled to the mark with 50 ml of deionized water and the reagents (Zn and sulfamic acid) from EZ arsenic test kit were added. A tube was inserted into the hole in the cap of the vessel and the other end of the tube was placed into an inverted cylinder, which was filled with water, and then close the cap. The volume was recorded every 5 minutes until no futther bubbles were generated.

4.2.3 The Rate of Arsine Generation

A Hach Kit vessel (headspace volume is about 80 mL) containing a certain concentration of arsenite standard solution (10, 25, 50, and 100 $\mu g\ L^{-1}$) was directly connected to the plasma source mass spectrometer by a short tubing. After adding the two reaction reagents, close the vessel. The arsine gas was pumped by a Minipuls pump at 0.5 $mL\ min^{-1}$. During the experiment time, about 30 mL of gas mixture was pumped into the ICP-MS. The ion intensity at *m/z* 75 (^{75}As) was monitored using the ELAN software. The aim of this experiment is to monitor and compare the rate of arsine gas generation under non-stirring and stirring conditions, not to obtain the concentration information.

To investigate the effect of Zn surface area, two packets of zinc power (about 1.05 g each packet) were added into the solution instead of adding one. Arsine was detected by ICP-MS as described above.

4.2.4 The Speed of Color Formation

A series of the Hach test kit vessels were filled with 50 ml of arsenite standard solutions at 10,25,50,100 μg L^{-1} concentration respectively. A test strip was inserted into each cap before adding reagents. Then the caps were closed. The strips were removed in order after 1, 2, 5, 7, 10, 15, 20, 30 and 60 min and scanned using a flatbed scanner. Blue values were recorded using the method below.

4.2.5 Digital Image Analysis

Images obtained at 400 dpi resolution and a 24-bit color scale were analyzed using the *Colors* program, which provides the red, green and blue values in an image.[20] The images were cropped to 45 by 45 pixels. The blue values were recorded.

4.2.6 The Distribution of Arsenic in Hach Kit

In the Hach kit reaction, arsine gas is generated from arsenous acid in the presence of zinc and acid, which is then absorbed by a strip containing $HgBr_2$. During the process, part of the arsine gas is absorbed by the strip, part of arsine gas is remaining in the headspace, and part of arsine gas is dissolved into the solution, together with the unreacted arsenites. It would be interesting to know the distribution of arsenic inside the

Hach kit vessel after a certain time. To investigate the arsenic distribution, three Hach kit vessels (#1, #2 and #3) containing 100 µg L^{-1} of arsenite standard solution were prepared. After the first hour, all three strips were removed and a new strip was immediately inserted in #2 and #3 vessels, respectively. The solution in #1 vessel was then filtered into a digital tube through a 0.45 µm filter paper. After the second hour, the two strips were removed and a new strip was immediately inserted in #3 vessel. The solution in #2 vessel was filtered as the first one. After the third hour, the strip was removed and the solution was filtered in the same way as well.

All the strips were immediately scanned using a flatbed scanner and then microwave digested. The microwave digested solutions were analyzed by ICP-MS in order to obtain the arsenic mass on the strip. The solutions filtered from the Hach kit vessels were analyzed by AFS in order to obtain the arsenic mass in the solution. The arsenic mass in the headspace was calculated by subtracting the arsenic mass on the strip and the arsenic mass in the solution from the total arsenic mass.

4.2.7 Microwave Digestion of the Strips

The end of the test strip, which contained the colored spot, was cut off and place into a PFA vessel, to which 3 ml of concentrated HNO_3 was added. The operating program of the microwave system was shown in Table 4.2. After cooling, the solution was filtered into a plastic centrifuge tube through a 0.45 µm filter paper and diluted to 15 ml with deionized water. To determine the mass of arsenic on the strips, standards were made from blank strips and arsenic standard solutions that were digested at the same time as the sample strips.

4.2.8 Arsenic Determination in the Solution Using AFS

The sample solution in the Hach kit vessel was filtered into a plastic centrifuge tube through 0.45 μm filter paper after a certain amount of time. $NaBH_4$ solutions were prepared daily by dissolving appropriate amounts of powered $NaBH_4$ in 5% NaOH solution to make a final concentration of 0.5%. The flow rate of $NaBH_4$ is 4.5 mL/min, and sample is 9 mL/min. Calibration solutions were prepared daily by dilution of a stock standard of arsenite to the appropriate concentrations with DI water. In order to improve the signal, L-Cys was added to both calibration and sample solutions, so that the final concentration of L-Cys was 0.5%.[21] HCl was added to standards and samples to make a final concentration of 0.6 M. Arsenite standards were also matched with the Zn concentration. Argon was used as a carrier gas. An external cylinder of hydrogen was needed to support the flame. Instrumental control and data acquisition were preformed with the software provided by PSA company.

4.2.9 The Investigation of Possible Chemical Structures of the Color Compounds

Four Hach kit vessels were filled with arsenite standard solutions, which concentration was at 0, 25, 100 and 500 $\mu g\ L^{-1}$. Strips were removed from the vessels after an hour in order to obtain white, yellow, orange, and brown color. The end of each strip was cut into a 0.5 mm × 0.5 mm square, and stick on an aluminum plate. Those plates were plated with Pt first and then scanned by SEM.

4.3 Results and Discussion

4.3.1 The Rate of Hydrogen and Arsine Generation and Color Formation

A classic approach for generating arsine gas is by reaction with zinc and acid to generate “nascent” hydrogen.[22] At the same time, hydrogen ions are reduced by zinc powder forming hydrogen gas. Figure 4.3 illustrates the rate of arsine and hydrogen gas generation. The blue line refers to hydrogen, which volume was directly measured. The red line refers to arsine, which was generated from a Hach kit vessel and measured by ICP-MS as arsenic intensity. The percentage was calculated by the volume at a certain time over the total volume or the intensity at a certain time over the maximum intensity. In Figure 4.3, the arsine gas was generated from a solution containing 100 μg L^{-1} of arsenic. However, if different arsenic standard solution was applied, the percentage profile was almost similar. As shown in Figure 4.3, arsine gas and hydrogen were generated at the same time and both reached to a maximum at about 50 minutes. This indicates that a reaction time of 20 minutes, which is suggested by the Hach company, is not long enough for arsenic reduction. Only 70% of maximum concentration of arsine gas over the headspace was generated at 20 minutes.

Figure 4.4 shows the color formation process using a series of arsenic standard solutions at 100 μg L^{-1}. Strips removed at 1, 2, 5, 7, 10, 15, 20, 30, and 60 min were scanned by a flatbed scanner. The blue values were obtained by using the color program. Detailed parameters were described in the 4.2.5 section. The blue value reached to a minimum after 30 min, which means the color was fully developed as shown in Figure 4.5. This indicates that the absorption of arsine gas onto the strip takes longer than 30 minutes. A former study in our group also reported the absorption process is extremely slow.[23] To obtain more intense color results, strips can be removed after 24 hours.

4.3.2 The Rate of Arsine Generation Under Stirring Condition

The experiment condition suggested by Hach company is to leave the vessel on a flat surface without continuously stirring, but to swirl twice during the measurement. In that case, since arsine gas is quite soluble in water (solubility of arsine in water is 0.07 g / 100 mL, 25 ºC), some arsine gas may be trapped in the solution, despite the degassing effect of the hydrogen bubbles. We assume that under stirring condition, arsine gas bubbles formed on the zinc surface would be brought to the headspace by hydrogen gas more quickly, so that less arsine gas would be dissolved in the solution. In other words, the stirring condition increases the amount of arsine, which is available to be determined. To investigate this possibility, the solution was stirred continuously with a magnetic stir bar during the reaction. From Figure 4.6, it clearly shows under stirring condition, more arsine gas was determined by ICP-MS at the same concentration. Based on the data listed in Table 4.3, when arsenic concentration is at 5 and 10 μg L^{-1}, the intensity increases by 200 and 100% respectively. When arsenic concentration is above 25 μg L^{-1}, the intensity increases by 80%. Also from the test strip, the spot is much darker under the stirring condition(Figure 4.7). When the stirring condition was applied, a strip prepared under arsenic concentration at 5 μg L^{-1} gave almost the same color intensity as a strip prepared under arsenic concentration at 10 μg L^{-1} without stirring. This illustrates that under the stirring condition, even when arsenic concentration is lower than 10 μg L^{-1}, the color is able to shown on the strip, which broads the range of determination by Hach kit.

4.3.3 The Rate of Arsine Generation Using More Reagent

Since arsine gas is formed on the surface of zinc powder, the surface area is playing an important role. Increasing the surface area can not only speed up the arsine gas generation, but also help bringing more arsine to the headspace. As shown in Figure 4.8, the amount of arsine gas was increased almost by 40%.

4.3.4 The Distribution of Arsenic in Hach Kit

4.3.4.1 The Mass of Arsenic on the Strip

To determine the mass of arsenic on the strip, the strip was digested using a microwave digestion system. The solutions after the digestion were filtered, diluted and then analyzed by ICP-MS as described in 4.2.7 section. Results are listed below. The ± term is equal to the standard deviation. As shown in Table 4.4, as time increased, less arsine was absorbed by the strip. This may be caused by the decreasing of the inside gas pressure.

As mentioned above, the blue value is related to the amount of arsenic on the strip. A lower blue value means a larger amount of arsenic. Figure 4.9 shows the correlation between the blue value and the mass of arsenic, which demonstrated the statement.

4.3.4.2 The Mass of Arsenic in the solution

The arsenic in the solution came from two sources: dissolved arsine gas and unreacted arsenous acid. In this experiment, duplicates were prepared. After the reaction in Hach kit vessels was done, the arsenic concentration in one vessel was determined by AFS. Nitrogen gas was bubbled through the solution in the other vessel in order to remove the dissolved arsine gas and then the arsenic concentration was determined by AFS. The second value should indicate the concentration of unreacted arsenous acid and the difference between the two results should correspond to the amount of dissolved arsine gas.

The solution contains a very high level of zinc (800 mg L^{-1}), which will affect the hydride generation efficiency.[21] Rigby et al. reported that in a 20 μg mL^{-1} Zn(II) solution there was only a 65% recovery of the arsenic signal from arsenite, whereas from the same solution containing 5 mg mL^{-1} L-cys, a 99% recovery was obtained. In my study, the same situation was found. Without adding L-cys in the solution, less than 100 ng of arsenic was determined by AFS, which is much lower than the estimated mass. However, by adding L-Cys, the interference of zinc can be minimized and more As was determined as a result. The calibration curve was shown in Figure 4.10. Mass of arsenic in the solution is listed in Table 4.5. The ± term is equal to the standard deviation. As shown in Table 4.5, as time increased, less arsenic was left in the solution.

4.3.4.3 The Mass of Arsenic in the Headspace

Based on the mass of arsenic on the strip and in the solution, most of the arsenic was in the form of arsine gas in the headspace. According to the mass balance, at the beginning, in a Hach kit vessel containing 50 mL 100 μg L^{-1} arsenic solution, the total mass of arsenic is 5000 ng. After an hour, the mass of arsenic on the strip is around 1000 ng and in the solution is around 450 ng. Theoretically, 3550 ng of arsenic is remaining in the headspace. To measure the arsenic in the headspace, arsine gas needs to be dissolved back into the solution and then measured by AFS. To point out, this mass obtained by AFS is the sum of the mass of As in the headspace and in the solution (section 4.3.4.2). So the mass of arsenic in the solution needs to be subtracted from the result.

To dissolve arsine gas back into the solution, KI and I_2 were added in a ratio of 2.5 : 1 and the final concentration of I_3^- was 1.97×10^{-3} M.[24] Arsine was oxidized to arsenate by I_3^-, and then determined by AFS. All operating procedure and parameters are the same as described in section 4.2.8.

4.3.5 The Investigation of Possible Chemical Structures of the Color Compounds

Arsine gas was absorbed on a test strip, which contains $HgBr_2$, forming a color compound. However, few studies were found on investigating the color compounds. Among these studies, they believe that during the reaction, H in AsH_3 was replaced by Hg-Br, forming a color compound and HBr. The yellow compound has a formula of $AsHHg_2Br_2$, and the brown compound has a formula of $AsHg_3Br_3$.[15] Figure 4.11 shows the SEM images of strips which were prepared at As(III) concentration 25, 250, and 500 $\mu g\ L^{-1}$, representing yellow, orange, and dark brown respectively. From the images, the crystals have different morphology at different arsenic concentrations. Elements percentages of those crystals were calculated by Burker program and results were listed in Table 4.6. Under each concentration, three different locations on the crystal were chose to obtain the element percentage information. The ratio of each element was also calculated. It is clear that when arsenic concentration is over 250 $\mu g\ L^{-1}$, the ratio between Hg, Br, and As is close to 3:3:1, which is close to sanger's statement.[15]

4.4 Conclusions

The portable Hach kit device is able to measure arsenic concentration in water samples, but the accuracy is limited. Within the suggested reaction time, only 60% of arsenic was determined as arsine gas. Thus, the time is not long enough to produce all arsine gas, which causes the inaccuracy. In addition, when the measurement is done, lots of arsenic is remaining in the headspace as arsine gas. To obtain more accurate results, increasing the amount of arsine that is absorbed by the strip are becoming important. One way to increase the amount of absorbed arsine gas is to apply a longer reaction time. Kearns research shows that a longer reaction time gives more accurate results and my study provides this a theoretical support.[18] To improve the performance, stirring condition can be applied. The intensity was largely improved when arsenic concentration is lower than 10 μg L^{-1}. Thus, the determination range of Hach kit can be increased.

4.5 References

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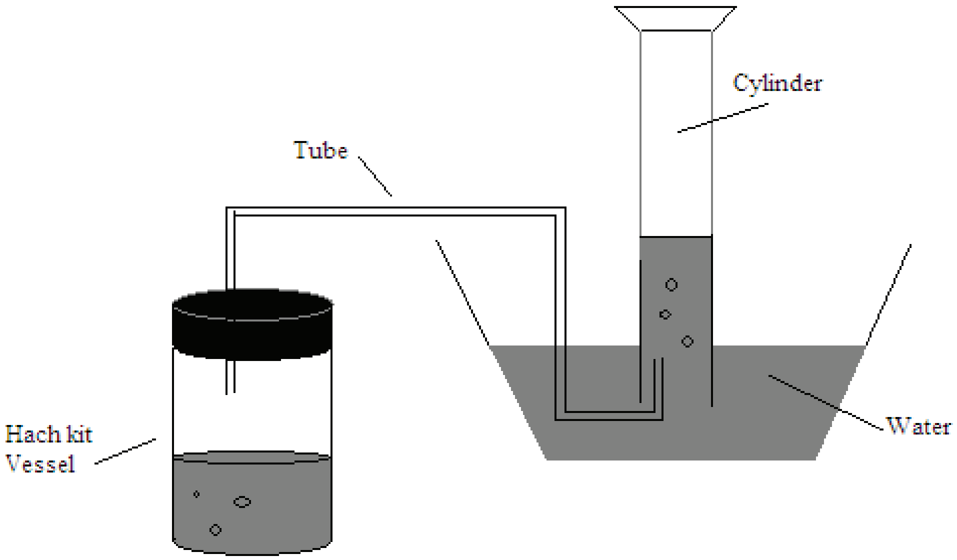


Figure 4.1 Schematic diagram of hydrogen gas collection

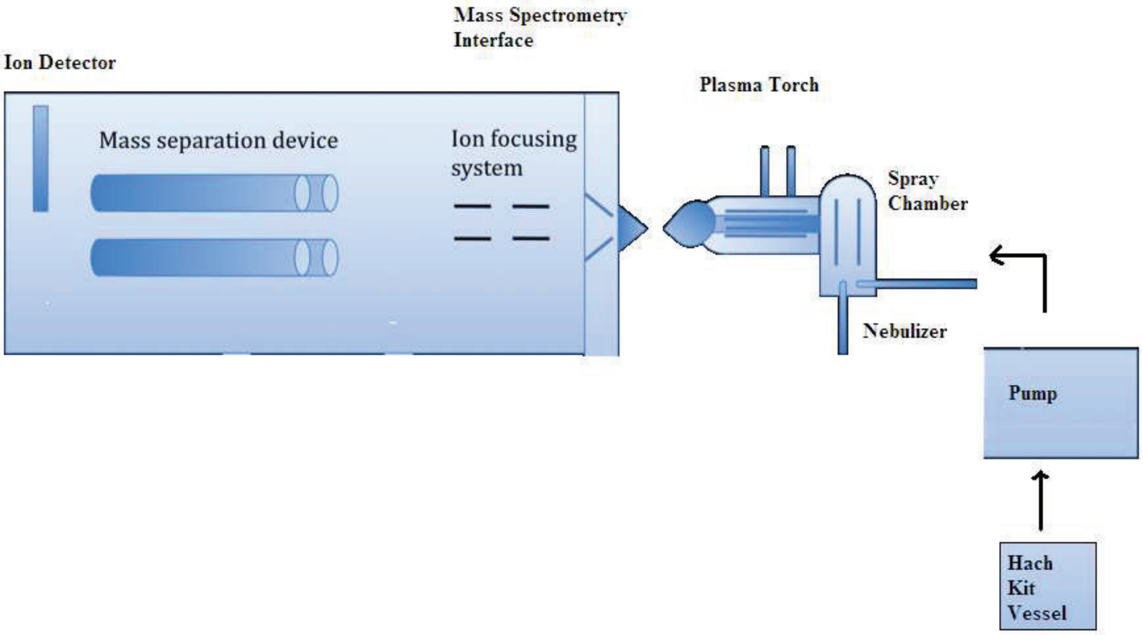


Figure 4.2 The scheme of the set up for arsine gas monitoring

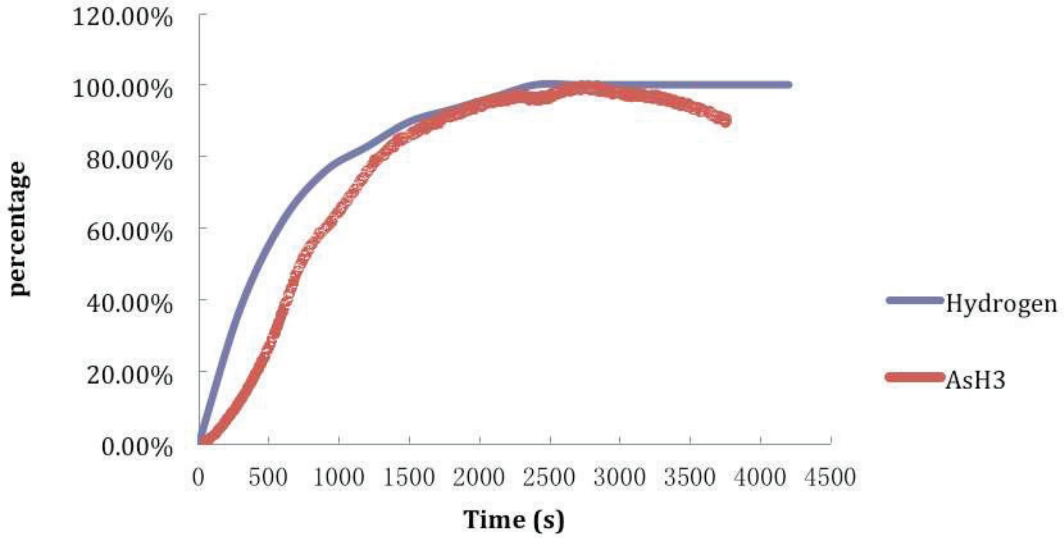


Figure 4.3 Plots of percentage as a function of time for arsine and hydrogen generation

Figure 4.4 Digital images of a sequence of test strips exposed under increasing time for a solution containing 100 µg L^{-1} of arsenic

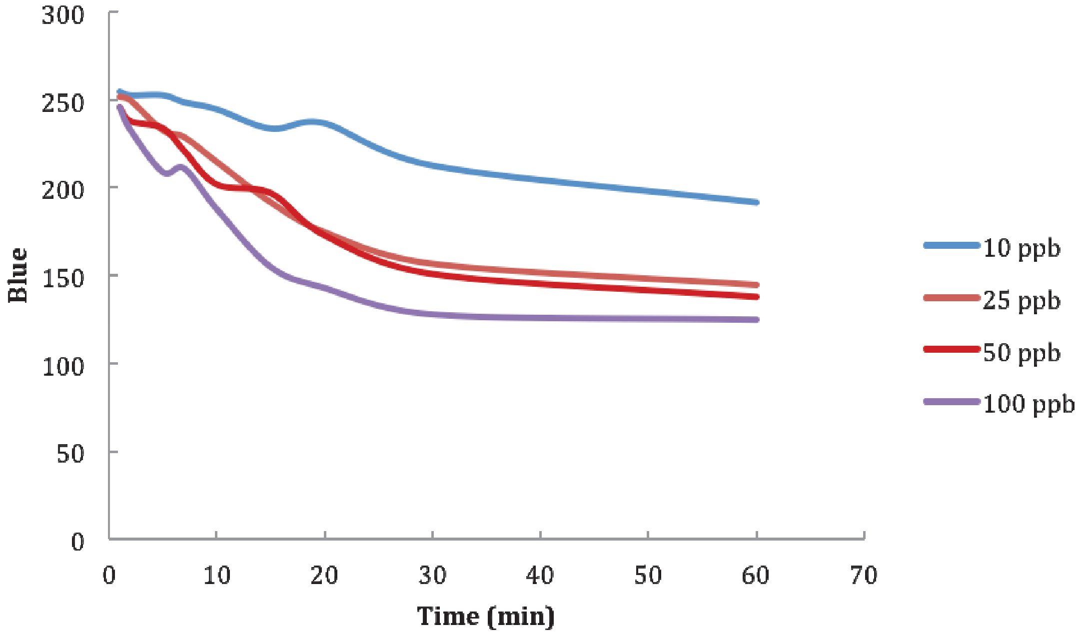


Figure 4.5 Plot of the measured blue values vs. time for different arsenic concentrations

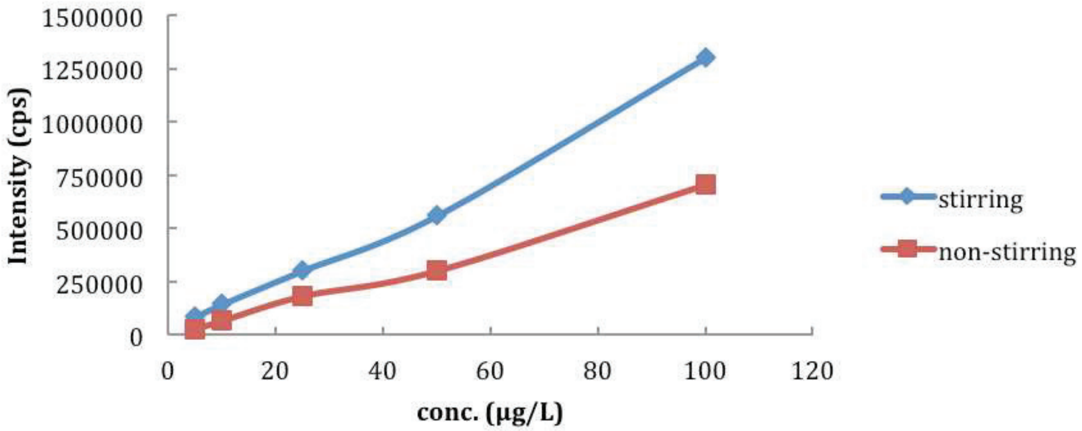


Figure 4.6 Comparison of intensity vs. arsenic concentration under stirring or non-stirring condition

Figure 4.7 Strips prepared at arsenic concentration = 100 μg L^{-1} (left : non-stirring, right : stirring)

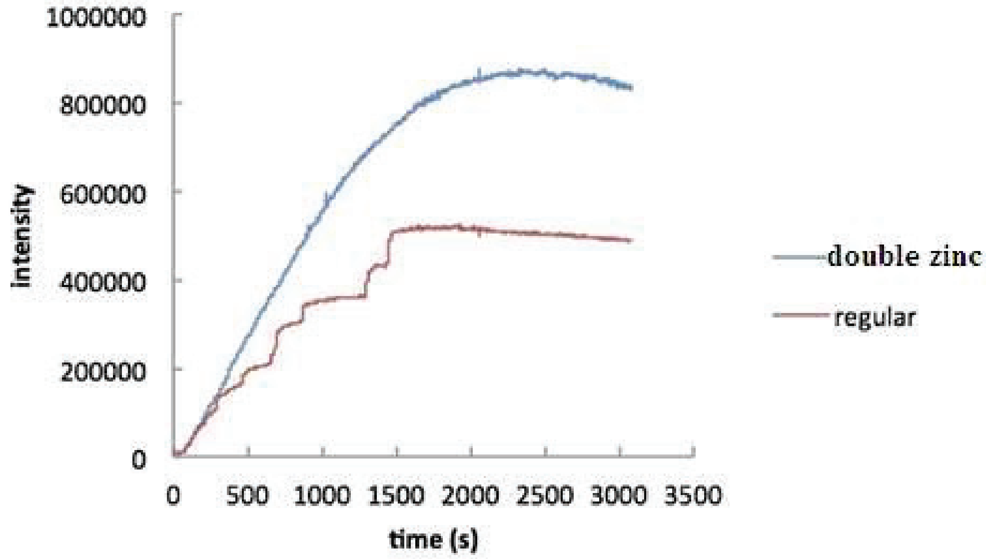


Figure 4.8 Plot of the intensity vs. time at different amount of zinc

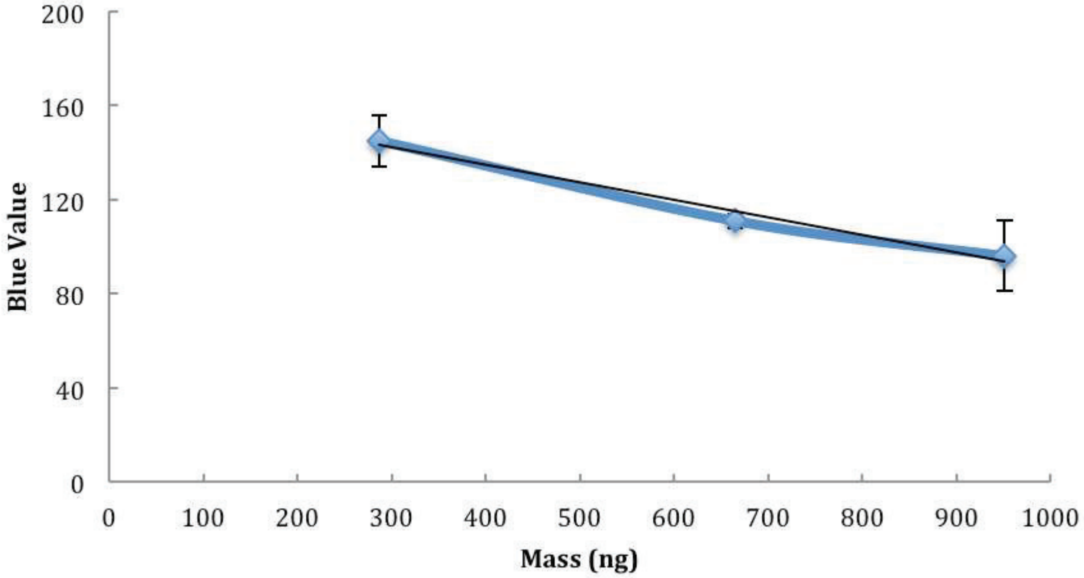


Figure 4.9 Plot of blue value vs. the mass of arsenic

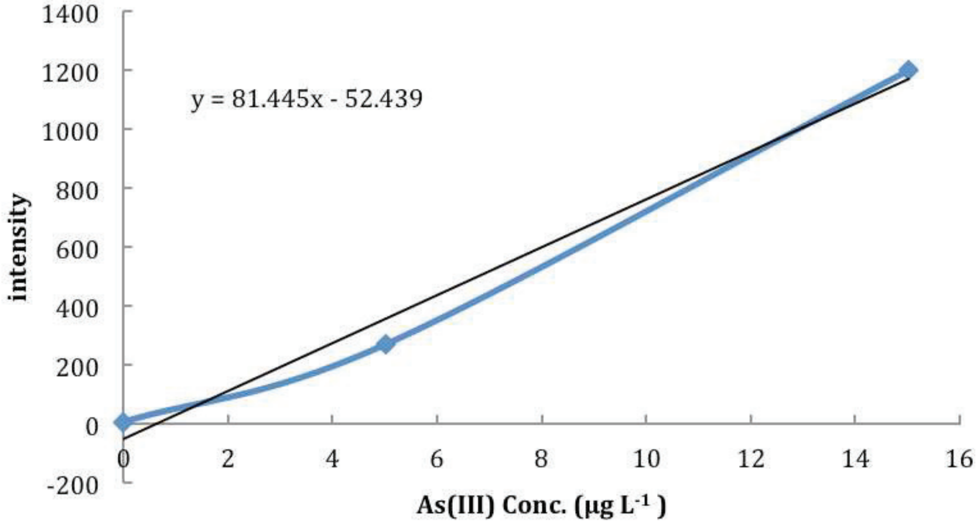
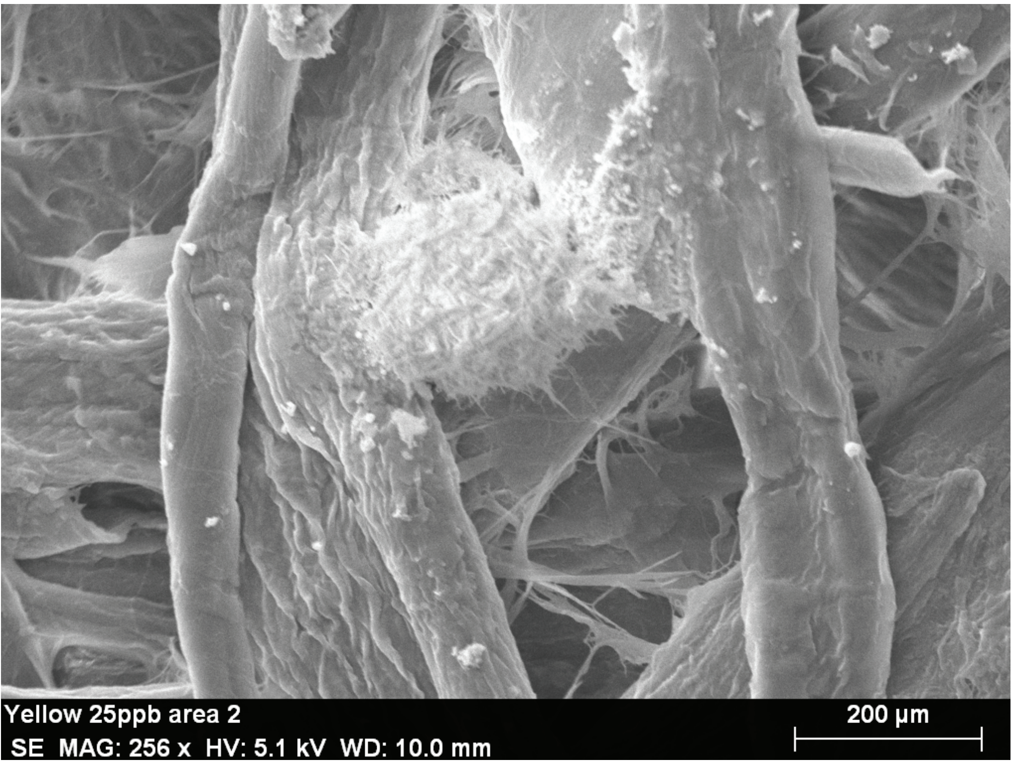
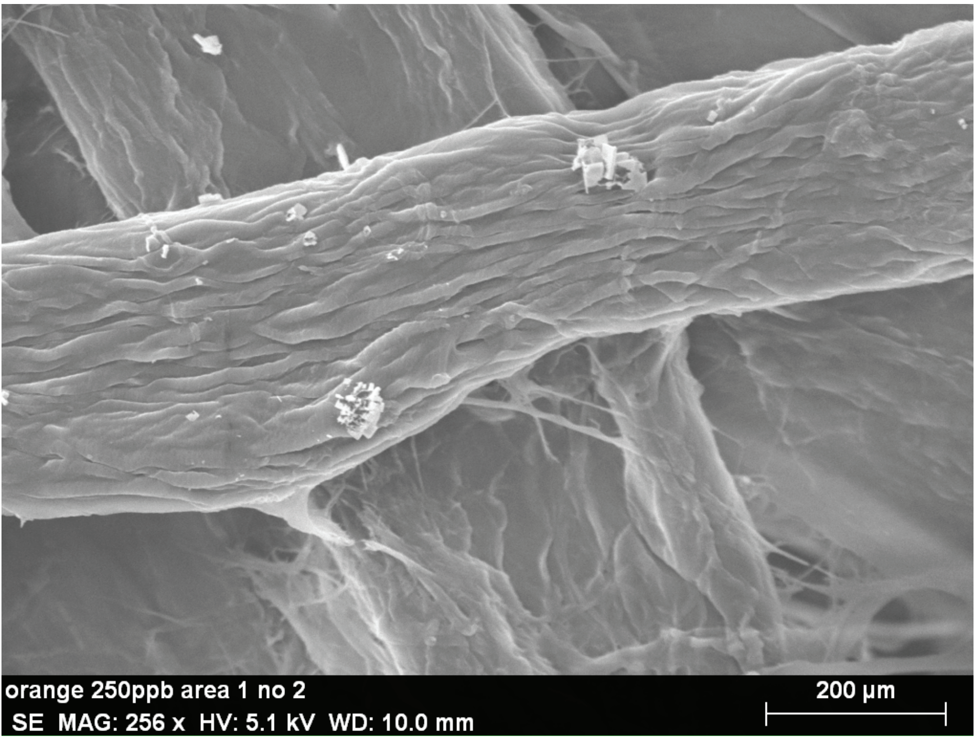


Figure 4.10 Plot of intensity vs. arsenite concentration. The black line represents the best linear fit to the data.


Yellow 25ppb area 2
200 µm
SE MAG: 256 x HV: 5.1 kV WD: 10.0 mm

(a)


orange 250ppb area 1 no 2
200 µm
SE MAG: 256 x HV: 5.1 kV WD: 10.0 mm

(b)

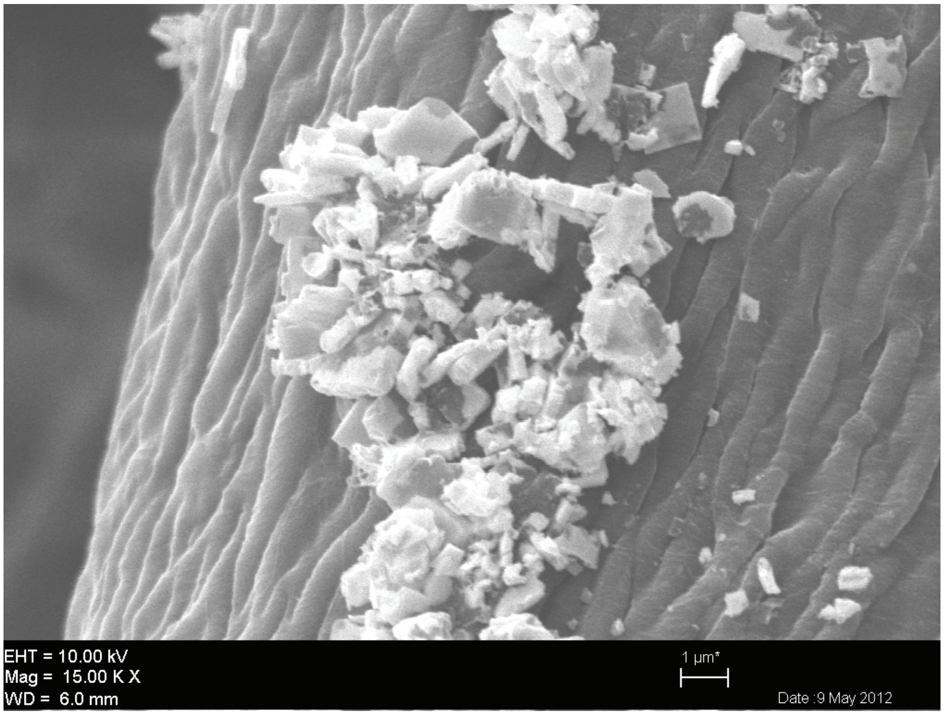
(c) 

Figure 4.11 SEM images of the color test strip prepared under different arsenite concentrations. (a) yellow (arsenite 25 µg L^{-1}), (b) orange (arsenite 250 µg L^{-1}), (c) dark brown (arsenite 500 µg L^{-1})

Table 4.1 ICP-MS operation parameters

Perkin Elmer ELAN 6100	
RF Power	1500 W
Nebulizer Gas Flow	1.01 L min^{-1}
Nebulizer Type	Gem Tip Cross-Flow II
Spray Chamber	Scott
Detector Mode	Dual mode
Sampler/Skimmer Cones	Nickel
Scanning Mode	Peak hopping
Dwell Time	100 ms per point
Number of replicates	10(solution),1000(gas)
Isotope Monitored	^{75}As

Table 4.2 CEM Mars Xpress microwave digestion temperature program

Temperature (°C)	Time (min)
Ramp to 180	20
Hold at 180	60

Table 4.3 Intensity of arsenic under stirring and non-stirring conditions

	Stirring	Non-stirring
As concentration (μg L^{-1})	Intensity (cps)	
5	85088	27534
10	144702	65902
25	302588	181592
50	560676	300475
100	1302559	704297

Table 4.4 The mass of arsenic on the strip

Sample name	Mass of As (ng)	Blue Value
1st hour strip	951±157	96±15
2nd hour strip	665±97	111±3
3rd hour strip	287±32	145±11

Table 4.5 The mass of arsenic in the solution

Sample name	Mass of As (ng) with L-Cys
Solution filtered after 1 h	437±22
Solution filtered after 2 hs	237±6
Solution filtered after 3 hs	137±4

Table 4.6 Element percentages of color compounds

Strip color	Hg (at.%)	Br (at.%)	As (at.%)	Hg:Br:As
Yellow	45.1	27.8	0.6	79:48:1
Orange	2.22	2.38	0.93	2:3:1
Dark brown	3.57	3.46	1.19	3:3:1

CHAPTER 5

SUGGESTIONS FOR FUTURE WORKS

5.1 Introduction

Arsenic contamination is a world-wide problem. Although there are many techniques, which can accurately determine arsenic concentrations in a wide variety of sample materials, few of them can be applied to measurements in the field (i.e. outside the laboratory). The most used portable techniques are colorimetric field kits, portable X-ray fluorescence (XRF), and anodic stripping voltammetry (ASV). As discussed in Chapter 4, colorimetric test kits are suffering from low sensitivity and high detection limit. Research efforts have been made to improve the performance, for example, by using a dye that can be bleached by arsine instead of the test strip, or adding reagents that can form a highly color material with arsenates.[1] XRF is an effective way. An EPA test method has been reported with an interference-free detection limit of 40 mg kg^{-1}. Some XRF research is focused on determining arsenic in aqueous solutions after pre-concentration, for example, using zirconium-loaded activated charcoal to collect arsenic.[1] The detection limit was reported as 50 mg kg^{-1} in liquid. Among the colorimetric test kits, portable XRF and ASV techniques, ASV is the most sensitive. Different kinds of electrodes have been investigated in order to decrease the detection limit or solve the fragility problem.[1] There results of the evaluation of three methods (ASV, cathode stripping voltammetry (CSV) and quartz crystal microbalance (QCM) method) based on portable instruments, which can be used in field study, have been presented. Future work is still needed (a) to improve the performance of the methods, such as increase the linear range of QCM method, or to improve the detection limit of CSV method, and (b) more effort is needed as to understand the nature of the compound formed when arsine reacts with mercuric bromide.

In Bangladesh, where the arsenic contamination in ground water is a serious problem, the portable test kit is a better choice over other techniques. Based on the researches, most parts of Bangladesh have an arsenic concentration over 50 μg L^{-1} in ground water. If the optimum conditions mentioned in Chapter 4 are used, the Hach kit is able to determine arsenic concentration below 50 μg L^{-1}, even 10 μg L^{-1} with little costs. Thus, in most of the cases, Hach kit is good enough. When arsenic concentration is too low to be detected by Hach kit, more advanced techniques, such as ASV, QCM then can be used.

5.2 Improve the Voltammetry Method

5.2.1 Modified the Gold Electrode Used in ASV

Several kinds of gold electrodes, such as gold film[2], gold nano-particle[3], gold disk[4], and boron-doped diamond[5] have been used in ASV to determine arsenic concentrations. Recently, researchers have turned their attention to the possibilities of chemically modified electrodes (CMEs), other than only gold electrodes. These CMEs have been applied to some trace element determinations, such as the determination of Sb, Pb, Hg and Cu.[6,7] CMEs are different from other electrodes because they have a molecular monolayer or film. Nano particles can be also attached to the surface of materials to modify their electronic and structural properties. Those modifications can bring the electrode with special new chemical properties in terns of physical, chemical, optical, transport and other useful properties.[8] Lan et al. modified a glassy carbon electrode with Au-Pd nanoparticles, which have been proven to have a high activity for the remediation of various inorganic and organic groundwater contaminants.[9] To make this CME, a glassy carbon electrode was polished with 0.05 µm Al_2O_3 powder. 5 µL of the prepared Au-Pd nanoparticles was cast on the clean surface and dried at room temperature. The detection limit of arsenite was 0.25 µg L^{-1} with no interference from Pb(II), Cd(II), Mn(II) and Zn(II). Also, a gold electrode surface can be modified with some ligands, which can selectively bind analytes of interest from water.[10] As a result, interference can be eliminated. Chen et al. modified the gold electrode with the mixture of glutathione (GSH), dithiothreitol (DTT) and N-acetyl-L-cysteine (NAC) self-assembled monolayers (SAM) and the detection limited obtained was 0.5 µg L^{-1} with a linear range from 3 – 100 µg L^{-1}. Clearly, these modified electrodes can avoid the interference caused by other metal ions, especially by copper ions, which is very common in ASV determinations. Based on Chen's research, 1,6-hexanedithiol, which contains thiol group can also be used to modify the gold electrode, since this ligand can selectively bind arsenite from water. By modifying the gold working electrode in this way, the interferences can be avoided and the sensitive and accuracy can be improved as a result.

5.2.2 Determination of Arsenate by ASV

Inorganic arsenic found in the groundwater is usually oxidized to arsenate by dissolved oxygen as the water is abstracted for use. Arsenic in surface waters is mostly arsenate. For a long time, arsenate was considered to be electro-inactive,[11] and arsenate is determined as arsenite at the working electrode surface after reduction. However, the directly measurement of arsenate is not impossible. As early as in 1988, Huang et al. reported a flow potentiometric and constant-current stripping method for arsenate determination without prior chemical reduction to arsenite by using a gold-coated platinum-fiber electrode.[12] They found that in the presence of 4 M HCl and 2.5 M $CaCl_2$, arsenate can be reduced at -1.80 V vs. Ag/AgCl after 60 s of electrolysis time. The detection limit was reported as 0.1 μg L^{-1}. In 1997, Viltchinskaia et al. modified the method by using a lower concentration of HCl (0.1 M) and detected arsenate after deposition at – 0.8 V vs. Ag/AgCl with a detection limit of 0.4 μg L^{-1}.[13] The traditional reduction step, which involves adding reducing agents (ascorbic acid, sodium thiosulfate and potassium iodide), has a few drawbacks, such as the additional handling time and possible contamination caused by adding the chemical reagent. Since only a few papers describe the direct measurement of arsenate, efforts can be put into developing possible modified electrodes at which arsenate can be directly determined.

5.2.3 Combine Sample Pretreatment with Electrochemical Detection Techniques

In order to determine trace arsenic in water samples, besides increasing the sensitivity of the technique, sample pretreatment procedures can be applied before the electrochemical detection technique. Traditional preconcentration and separation methods, such as liquid-liquid extraction[14] and solid phase extraction[15], have been used for arsenic removal or determination. These methods are time-consuming and labor intensive, besides requiring relatively large amount of toxic solvents, which have to be disposed off properly.[16] Recently, cloud-point-extraction (CPE) methods have been used to facilitate preconcentration and separation of the analyte from complex matrices.[16] Basically, in CPE, arsenic forms a complex with reagent, such as molybdate or ammonium pyrrolidine dithiocarbamate.[17] Then the complex is extracted by a surfactant, such as Triton X-114. The presence of surfactant not only facilitates extraction of analyte efficiently but also enhances the sensitivity of the method.[18] By using this extraction method, an enrichment factor of 50 can be obtained.[17] If CPE is combined with ASV or CSV method, arsenic can be preconcentrated and removed from the matrix. The possible interferences can be eliminated and a better detection limit and accuracy can be achieved as a result.

5.3 Improve the Quartz Crystal Microbalance Method

The quartz crystal microbalance is a potential portable instrument for arsenic determination in the field. The relatively cheap equipment is smaller and lighter than most laptops and does not require an external gas supply. The QCM method described in this dissertation has demonstrated that with an appropriate coating on the crystal/gold electrode surface, it can sensitively and selectively determine arsenic in water samples within a relatively short time. To make this method practical, the AC adapter can be replaced by a connection to a vehicle power outlet. In addition, the device can be redesigned so that it will have two crystal holders. One crystal will act as the reference. By applying the reference crystal, matrix and background effect can be eliminated.

In this method, mass was increased in order to change the frequency during the process. Ligands, other than dithiolthreitol, that can selectively bind arsenites can be investigated. Although 1,6 hexanedithiol (HDT) has been tried in this research and gave negative results, reaction conditions can be varied in order to achieve better results, such as using the phytochelatin-like peptide.[19] Similarly, to change the frequency, mass can be decreased by replacing light ones with very heavy species that have been bonded to the surface. It is well known that arsenate can react with molybdenum forming arseno-molybdate,[20] which has a very large mass. It may be possible that arsenate react with molybdenum and are absorbed on the crystal surface via some media. Then the arsenomolydbate complex is then displaced from the crystal surface by another stronger binding ligand causing mass change. This mass change will be a lot larger than the mass change caused by binding arsenite from water. As a result, a better detection limit can be achieved.

5.3.1 Explore the Possibility of Using Hydrogels

Hydrogels are three-dimensional networks that are always hydrophilic, flexible and able to adhering to many different surfaces due to the gel property. Barakat et al. found that the arsenic ions migrated into the 3D network of hydrogels by electrostatic interactions.[21] Some hydrogels have the ability to absorb arsenic from water with removal efficiencies of more than 50%. QCM is a mass sensing device that can sense mass change as small as 1 ng.[22] Thus, if a hydrogel with a high capacity (ability to absorb 50% of arsenate) are applied on a QCM surface and put in a 50 mL of 100 μg L^{-1} arsenate solution, then 2500 ng mass change will occur. This is heavy enough for the QCM to detect. Thus, a hydrogel could be used to modify the QCM surface. The capacity is crucial because the more arsenate it absorbs, the heavier mass change will cause. A search of the Web of Science database using “arsenic” and “hydrogel” as keywords reveals that there are 10 papers in which the removal of arsenic from water by hydrogels is reported. For example, Barakat et.al. synthesized and applied the poly (3-acrylamidopropyl)-trimethylammonium chloride (PAPTMACl) hydrogels as adsorbents for removal of arsenate from water. PAPTMACl hydrogels were synthesized in the form of small cylinders, with using N, N’-methylene-bisacrylamide (MBA) as crosslinker. N,N,N’,N’-tetramethylethylenediamine (TEMED) was used as an accelerator, and ammonium persulfate (APS) was used as redox initiator. The hydrogel was regenerated by eluting the adsoded arsenate with 1 M NaOH. The adsoption process was optimized by adding hydrogels to a 50 mg L^{-1} arsenate solution at pH 9 for 360 min. The solution finally had 1.5 mg/L of hydrogels. The liquid was filtered through 0.45 μm syringe filters and analyzed by inductive coupled plasma optical emission spectrometer. The removal efficiency of arsenate was 99.7%.[21] Sahiner et al. synthesized (3-acrylamidopropyl)-trimethylammonium chloride (APTMACl) hydrogel in different sizes (bulk, micro- and nano-sizes). The crosslinker was N,N'- methylenebisacrylamide (MBA), the initiators were ammonium persulfate (APS) and 2,2'-azobisisobutyronitrile (AIBN), the accelerator was N,N,N',N'-tetramethylethylenediamine (TEMED), the surfactants were L-α-phosphatidylcholine (lecithin) and dioctyl sulfosuccinate sodium salt (AOT), and solvents cyclohexane and 2,2,4-trimethylpentane (isooctane) were used. The micrometer-sized cationic hydrogel particles were very effective in the removal of arsenic from an aqueous environment: 96% of the arsenic was removed in less than 10 min from a 55 mg L^{-1} aqueous stock solution.[23] Sahiner et al. synthesized p(4-vinylpyridine)-based (p(4-VP)) hydrogels. The nano-sized hygrogels were able to remove 85% of arsenate from a stock solution (10 mg L^{-1}) in 15 min and could be reused after elution with NaOH.[24] Fe(II)-loaded pectin based hydrogels[25] and poly(acrylonitrile-co-1-vinylimidazole) (p(AN-c-1-VI)) hydrogels[26] have also been shown to have the ability to remove arsenic from water.

If hydrogels can be synthesized in a cylinder with 1-inch diameter, they can be cut into slices and put onto the quartz crystal surface. Hydrogels can be applied to the surface of crystal directly and their 3D network provides more potential adsorption than a monolayer of adsorbent. An Fe(II)-loaded pectin based hydrogel is a possible choice.[25] The capacity is 126 mg g^{-1} when the hydrogel was immersed in arsenic solution for 2 h.

The absorption process takes a little bit more time, but a shorter reaction time can be tried in an experiment. The capacity obtained in 2 h is 4 times higher than those of other hydrogels, so a shorter reaction time could still allow the adsorbtion of enough arsenic from water. Another possibility is poly(3-acrylamidopropyl)trimethylammonium chloride (p(APTMACl)) hydrogel which has a capacity of 33.2 mg g^{-1}.[21] The two hydrogels are both bio-degradable and reusable. No hazardous waste was generated after the experiments. In addition, a flow cell can be used in order to investigate the kinetics of the adsorption. To get a strong signal output and a better detection limit, the mass change should be as big as possible. The ion exchange with heavy counter ions, such as molybdate ions, could be tried. Molybdate ions could be absorbed on the surface of the hydrogel at the first step. When it is immersed into arsenate solution, the arsenate will react with molybdate ions and form $AsMo_{12}O_{40}^{3}$. Compares to the mass change caused by the arsenic adsorbed on hydrogel, the mass change of replacing $AsMo_{12}O_{40}$ is a lot larger.

5.3.2 Improve Arsenate Determination

In Chapter 3, experiments were described in which arsenate was determined directly without adding additional reducing agents, because DTT can reduce arsenate to arsenite. However, without adding additional reducing agents, the linear range for the determination of arsenate was limited to an upper value of 15 $\mu g\ L^{-1}$. The reason is probably because some of the DTT were reacted with arsenate and could not form a uniform and evenly distributed DTT layer to bind arsenite or DTT can not completely reduce arsenate within a short time. Increasing the amount of DTT has been tried, which did not provide a better result. Although this method is good for determination of arsenate below 10 μg L^{-1}, which is the WHO level, it would be better to have a wider linear range in order to avoid the need for further dilution.

Several additional reducing agents, such as sodium thiosulfate, KI, L-Cys, and ascorbic acid (which were discussed in Chapter 2), could be added prior to arsenate determination. These reducing agents are able to completely reduce arsenate to arsenite within a few minutes. In addition, these will not reacts with DTT, so the formation of DTT layer will not be disturbed. So by addition the reducing agent prior to arsenate determination, the process will behave similarly as the determination of arsenite process. As a result, the linear range can be increased.

5.3.3 Modify the Quartz Surface

Instead of modifying the gold electrode surface, the quartz crystal surface can also be modified. Eguchi et al. determined phosphate ion by adhesion of a precipitate of ammonium phosphomolybdate onto a one-electrode-seperated QCM.[27] A similar idea can be used for arsenic determination. Just like a column used in HPLC, C18 can be attached to the quartz crystal surface. However, this method maybe suffered from interference ions. Hydride generation is a good way to eliminate the interferences. Arsine gas can be dissolved in water and then determined by the modified QCM.

5.4 Hach Kit Study

5.4.1 Investigation of the Chemistry of Arsenic Mercuric Bromide Compound

Arsine gas reacts with mercuric bromide, forming one or more colored compounds.[28] However, in searching the literature for information about the nature of these compounds, only one paper was found.[28] As this was published in 1907, it appears as though no instrumental techniques have been applied to the elucidation of the structure and stoichiometry of these compounds. In the work described in this dissertation, the strip was scanned by SEM coupled with energy dispersive X-ray spectroscopy (EDS) and the elemental mole ratios were obtained as well. However, the ratio was probably not calculated only solely for the colored products, but the value also includes a contribution from some mercuric bromide. This means that the number is only an approximation. Thus, there is not enough information to provide the chemical structure of the compound. In addition, SEM only provides surface information about the surface of the strip (maximum depth of about 5 μm), so the element ratio or the structure information can not be obtained when the depth is over 5 μm.

Other instruments, such as total reflection X-ray fluorescence (TRXRF), Auger electron spectroscopy (AES) can be applied in investigating the element ratio or the chemical structure of the compound. TRXRF is a surface elemental analysis thenique that often used for the ultratrace analysis of particles on smooth surfaces. It is an enery dispersive XRF in a special geometry, which can reduce measurement background contributions by elimination of sample acattering and increase the elemental measurement sensitivy. The advantage of using AES is it can give the information about the oxidation state. This will help with the study of the colored compound chemical structure.

The study does not have to be done on the surface of filter paper. Powdered mercuric bromide could be exposed to arsine gas in a reactor of XRF or AES. Once all the mercuric bromide had reacted, the product(s) could be examined.

5.4.2 Investigate Other Possible Arsine Gas Detectors

Although the test strip saturated with mercuric bromide is able to determine arsine gas by forming the color spot, the method is not rigorously quantitative, as was discussed in Chapter 4. Replacing the strip with a more accurate arsine detector will enhance the performance of methods based on arsine generation. For example, Pal et al.[29] designed a vessel with a sidearm, as shown in Figure 5.1. A polymer screen-printed sensor saturated with phosphate buffer solution was kept suspended from the lid. Then the sensor was connected to the terminals of an amperometric electrochemical analyzer that responded to arsine gas. Saturated sodium borohydride solution was added to the sample from the sidearm. After arsine was generated, the response was noted. This method was able to determine 2 $\mu g\ L^{-1}$ of arsenic. If this can be combined with Hach Kits, it will have a lower detection limit than that of the ordinary Hach kits.

5.4.3 Improve the Arsine Gas Reaction Efficiency

As was discussed in Chapter 4, to improve the accuracy of the Hach kit, more arsine gas needs to be absorbed by the strip. In the work described in this dissertation, a longer reaction time and a higher reaction temperature have been studied. In future, a smaller headspace can be tried. The commercial Hach kit vessel has a headspace of 80 mL. If a smaller vessel is used under the same condition, arsine gas concentration in the headspace will be higher, which will increase the possibility of absorption by the strip. Since arsine gas is quite soluble in water, during the reaction process, part of the arsine gas was dissolved in water. The set-up in Figure 5.3 can solve this problem. By heating the vessel, arsine gas is transferred to the empty flask, which prevents from dissolving in water.

5.5 Other Possible Instruments

5.5.1 Ultraviolet-visible Spectrometer

Although it is well known that arsenates can react with molybdenum and form a deep blue color, there are other chemicals that can also form colored compounds with arsenic species. Arsenite reacts quantitatively react with potassium iodate in the presence of sulphuric acid releasing an equivalent amount of iodine.

$$5\ AsO_3^{3-} + 2\ IO^{3-} + 2\ H^+ \rightarrow I_2 + 5\ AsO_4^{3-} + H_2O$$

The iodine needs to be extracted immediately with carbon tetrachloride to form a pink color.[30] This color can be measured at 520 nm giving a detection limit for arsenite of 2 μg L^{-1}. Similarly, another possible colorimetric method is the malachite green method (MG). Arsenite reacts with potassium iodate in HCl medium to liberate iodine quantitatively, which then selectively oxidizes leuco malachite green (LMG) to malachite green (MG), as shown in Figure 5.2. The green color is developed in an acetate buffer medium (pH 4.0 – 4.8) on a heating in a water bath (40 °C) for 5 min. The product has a maximum absorption at 617 nm. The detection limit of arsenate was also 2 μg L^{-1}.[31]

Some sample preconcentration step, such as cloud point extraction, solid phase extraction or liquid-liquid extraction could be tried prior to the detection reaction. Since arsenic is removed from the matrix, the potential interferences can be eliminated. The detection limit and accuracy can be improved as a result.

5.5.2 Surface Plasmon Resonance Sensor

Surface plasmon resonance (SPR) spectroscopy is a sensitive method for detecting analytes adsorbed on a metal surface. Basically, surface plasmons are collective oscillations of free electrons in a metallic film. Under an appropriate condition, the plasmons can resonate with an incident light beam and absorb the energy of the beam. Because the resonance condition is extremely sensitive to the refractive index of the medium adjacent to the metallic film, the presence of molecules on the surface of the metallic film can be accurately detected.[32] This technique has been successfully applied to bio-molecule sensing,[33] and heavy metal detection.[32] There is one paper was found using dithiothreitol modified SPR to detect arsenic in groundwater.[34] The detection limit was 3 ng L^{-1}. Similar ideas can be applied to modify SPR surface, such as using glutathione (GSH) and hexanediol (HDT). The detection limit would be low.

5.5.3 Ion Chromatography Coupled with Electrospray Ionization Mass Spectrometry

An ion exchange column is able to provide retention of arsenic species that includes anions and arsenobetaine. A literatures search shows that the IonPac AS 7 or AS 18 columns from Dionex have been successfully used to do the separation.[35,36] Kutscher et al. separated and determined arsenic in apple juice, for which 20 mmol L^{-1} or 200 mmol L^{-1} of ammonium carbonate (pH = 9) was used as the eluent. Arsenic species were separated within 15 min with a flow rate of 0.3 mL min^{-1}. Gradient elution was applied., in which the ammonium carbonate concentration was increased from 20 to 200 mmol L^{-1} in 15 min. They reported retention times for AsB, DMA, arsenite, AsC, MMA, and arsenate of 185, 205, 215, 270, 400, and 800 s respectively.

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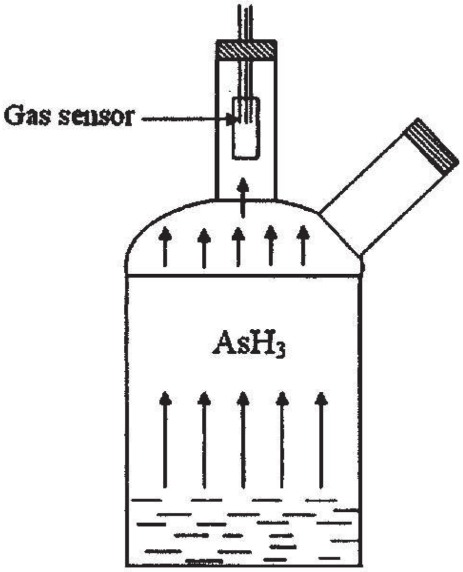

Figure 5.1 Schematic diagram of arsine detection arrangement using polymer gel and screen-printed electrodes[29]

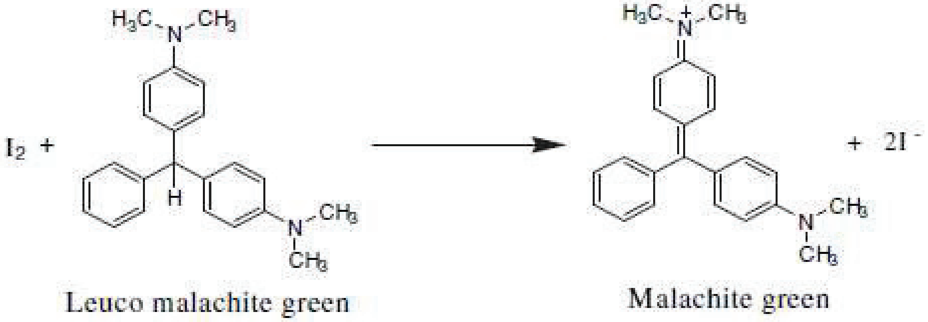

Figure 5.2 Malachite green formation reaction[31]

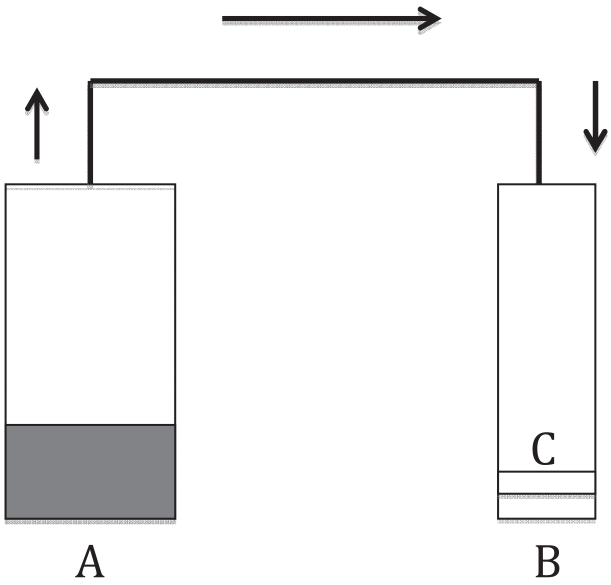

Figure 5.3 Schematic diagram of arsine detection arrangement by separating arsine from the Hach vessel (A: Hach vessel, B: flask, C: strip, arrows indicate the path of the arsine)

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