ARSENIC SPECIATION ANALYSIS IN ENVIRONMENTAL AND FOOD SAMPLES AND INVESTIGATION OF SULFIDE IN GROUNDWATER

By

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A dissertation submitted to the Graduate Faculty in Chemistry in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York

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ABSTRACT

ARSENIC SPECIATION ANALYSIS IN ENVIRONMENTAL AND FOOD SAMPLES AND INVESTIGATION OF SULFIDE IN GROUNDWATER

by

YI HE

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Professor David C. Locke

This work consists of three parts: (1) analytical methods development for arsenic speciation in natural water samples, (2) speciation analysis and investigation of bioaccessibility of arsenic in food, specifically in rice samples, and (3) determination of trace level sulfide, a crucial compound participating in metal mobility in the environment, and investigating its interaction with Fe(II) in natural water systems.

Two arsenic speciation methods based on cathodic stripping voltammetry (CSV) were developed for arsenic speciation in water samples. These methods are simple, fast, sensitive, inexpensive and applicable both in the laboratory and in the field. The first method employs a hanging mercury drop electrode (HMDE), on which As(III) is deposited in the presence of Cu and Se in HCl medium. As(III) is determined by direct

measurement. Determination of total As was performed by reducing As(V) to As(III) using sodium meta-bisulfite/sodium thiosulfate reagent stabilized with ascorbic acid. As(V) is quantified by difference. The second method further improved the reducing efficiency by using L-Cysteine as reducing reagent and had the capability to differentiate organic arsenic and inorganic arsenic through $Na_2S_2O_8$-assisted UV photooxidation. Concentration of organic arsenic is the difference between total inorganic and total arsenic concentration.

The risk of exposure to arsenic from ingestion of cooked rice was evaluated. Total arsenic of a dozen commercial rice samples was determined by high resolution inductively coupled plasma-mass spectrometry (HR-ICP/MS). The bioaccessibility of arsenic was evaluated by *in vitro* gastric and small intestinal fluid sequential incubation. The effect of arsenic in cooking water was also investigated. Speciation of As using ion chromatography (IC) coupled with ICP/MS showed inorganic arsenic to be the major arsenic species in rice.

A simple approach for determining trace level sulfide was developed using differential pulse CSV (DPCSV). The method was applied on-site to analyze Fe-rich reducing ground water samples collected at landfill sites in Winthrop, Maine, and Vineland, New Jersey. Additionally, based on this method, interactions between Fe(II) and sulfide were studied in both environmental and laboratory solutions and three situations were established according to different Fe(II) concentrations.

Dedicated to

My beloved family

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LIST OF ABBREVIATIONS

AAS	atomic absorption spectrometry
AFS	atomic fluorescence spectrometry
AsB	arsenobetaine
AsC	arsenocholine
CE	capillary electrophoresis
CSV	cathodic stripping voltammetry
DMA	dimethylarsenic acid
DMAE	dimethylarsinylethanol
DPCSV	differential pulse cathodic stripping voltammetry
EPA	Environmental Protection Agency
GC	gas chromatography
GFAAS	graphite furnace atomic absorption spectrometry
GFSI	gastric fluid and small intestinal
HGAAS	hydride generation atomic absorption spectrometry
HMDE	hanging mercury drop electrode
HPLC	High performance liquid chromatography
IC	ion chromatography
ICP-MS	inductively coupled plasma-mass spectrometry

LC	liquid chromatography
LDEO	Lamont-Doherty Earth Observatory
LED	light-emitting diode
MCL	maximum contaminant level
MMA	monomethylarsenic acid
MS	mass spectrometry
ORP	oxidation-reduction potential
RSD	Relative standard deviation
SAOB	Sulfide Anti-Oxidation Buffer
SBRP	Superfund Basic Research Program
SDWA	Safe Drinking Water Act
SFMS	sector field mass spectrometry
TMAO	trimethylarsine oxide
WHO	World Health Organization

Chapter I Introduction

1.1 Project background and research objectives

My Ph.D. research work involves analytical method development for a Superfund Basic Research Program (SBRP) on Health Effects and Geochemistry of Arsenic and Lead, which is an interdisciplinary program including studies in the areas of public health and environmental science and engineering. The investigation sites for earth science component of the program include Bangladesh and US EPA Superfund Sites in Vineland, New Jersey and Winthrop, Maine.

In order to facilitate the success of the program, I focused on two areas. First was the development of analytical methods for arsenic speciation at the μg/L level and their application to samples of environmental and health interest. The second was solving the problem of monitoring nM levels of sulfide in reducing groundwater and investigating the interaction between dissolved sulfide and Fe(II) to provide information for better understanding of arsenic remediation through sulfide precipitation. As a result, the objectives of my research projects were to provide analytical approaches based on the requirement of environmental scientists in the research group and apply these techniques to solve real world problems.

1.2 Arsenic and its environmental and health effects

Arsenic is a common trace element with toxic properties that have been known for centuries. Arsenic is ubiquitous in the environment. The natural abundance of arsenic in the earth crust is 1.8 mg/kg [1]. In regions with abundant volcanic rocks or sulfidic ores, arsenic concentrations are elevated [1]. Arsenic can be released from crust and sediments and enter into aqueous environment depending on geochemical conditions. In modern

industrial society, anthropogenic activities, such as the use of arsenic-containing herbicides, pesticides and wood preservatives, contribute another important source for arsenic contamination [2, 3]. Even though many of these chemicals have been banned from use in recent years, the accumulated arsenic in soil and water continue to have environmental impact [4]. Most recently, there is increasing concern over the use of arsenic as an officially approved chicken feed additive to prevent certain diseases. Most of the ingested arsenic in the additive ends up in the environment through poultry litter and it is highly mobile [5]. Additionally, arsenic in feed may also cause elevated arsenic levels in chicken meat, which is a favorite food in many countries including the U.S.

The arsenic problem has commanded worldwide attention in recent years because drinking water with elevated arsenic has been found in many parts of the world, such as Bangladesh, India, Argentina and Taiwan and has led to devastating public health effects. The World Health Organization (WHO) called the case in Bangladesh as "the largest mass poisoning of population in history" [6]. In Bangladesh and India alone, millions of people drink water containing high levels of arsenic, which is naturally released from aquifer sediment into groundwater, resulting in concentrations as high as several hundred micrograms, or in some cases, milligrams, per liter [7, 8]. In the USA, many areas have been identified as having a groundwater arsenic problem; the most affected areas are southwestern states and the less affected are eastern and central states [9, 10]. Currently about half of the drinking water supply in the US comes from groundwater; 2.5 million people are supplied with water containing more than 25 μg/L arsenic [9, 11].

Arsenic has significant deleterious health effects. Even at low concentration,

diseases resulting from chronic As exposure include skin lesions, cancers of the skin, bladder, and lung, and neurological and cardiovascular diseases [12].

Because of increasing concern over the health impacts of arsenic at low levels, the US Environmental Protection Agency (EPA) recently proposed a much more stringent arsenic standard for drinking water under the Safe Drinking Water Act (SDWA), reducing the maximum contaminant level (MCL) from 50 μg/L to 10 μg/L [9]. Drinking water systems must comply the new standard by January, 2006 [13]. The ultimate MCL goal is 0 μg/L [13].

1.3 Arsenic species and toxicity

Over 20 arsenic compounds of varying degrees of toxicity have been identified in natural environmental and biological systems [14]. In general, inorganic arsenic compounds are much more hazardous than organic arsenic compounds [15]. Inorganic As(III) and As(V) are the major species in natural water samples. Organic arsenic compounds including monomethylarsenic acid (MMA) and dimethylarsenic acid (DMA) are also found in seawater [16] and some fresh water samples [4] as a result of biological activity [4, 17] and anthropogenic contamination [2, 3]. Harmless organic arsenic compounds, such as arsenobetaine (AsB), arsenocholine (AsC) and arsenosugars are mainly found in marine biological samples. Arsenic compounds commonly found in environmental and biological system are summarized in Table 1.1 and relative toxicities of selected arsenic compounds is presented in Table 1.2.

Table 1.1: Common arsenic species in environmental and biological samples

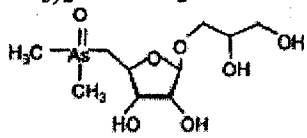
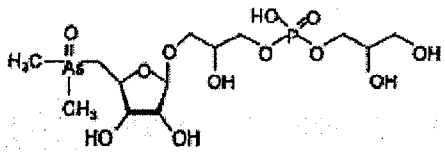
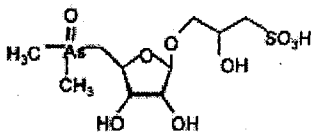
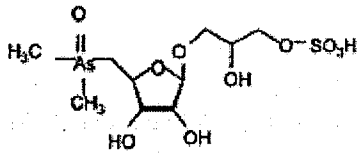
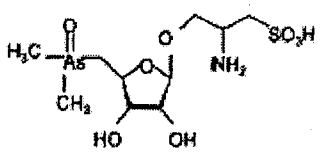
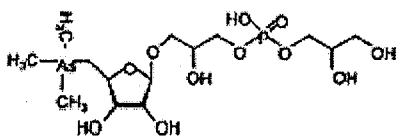
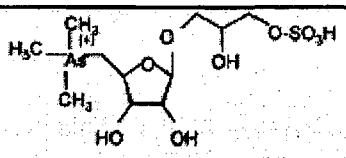
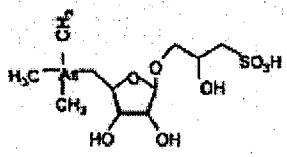
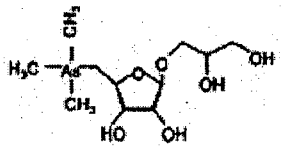
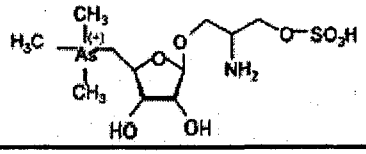
#	Compound	Formula	Ref
1	Arsenous acid (arsenite, As(III))	$OH\text{-}As(OH)_2$	[18]
2	Arsenic acid (arsenate, As(V))	$O{=}As(OH)_3$	[18]
3	MMA	$CH_3AsO(OH)_2$	[18]
4	MMA(III)	$CH_3As(OH)_2$	[19]
5	DMA	$(CH3)_2AsO(OH)$	[18]
6	DMA(III)	$(CH3)_2AsOH$	[19]
7	TMAO	$(CH3)_3AsO$	[18]
8	TMAs+	$(CH3)_4As^+$	[18]
9	AsB	$(CH_3)_3AsCH_2COOH$	[18]
10	AsB-2	$(CH_3)_3AsCH_2CH_2COOH$	[19]
11	AsC	$(CH_3)_3AsCH_2CH_2OH$	[18]
12	DMAE	$(CH_3)_2AsO(CH_2)_2OH$	[18]
13	DMAsAc	$(CH_3)_2AsOCH_2COOH$	[18]
14	Arsenosugar A		[18]
15	Arsenosugar B		[18]
16	Arsenosugar C		[18]
17	Arsenosugar D		[18]
18	Arsenosugar E		[18]
19	Arsenosugar F		[18]

Table 1.1 Continued

20	Arsenosugar G		[18]
21	Arsenosugar H		[20]
22	Arsenosugar I		[20]
23	Arsenosugar J		[20]

Note: Arsenosugars A-E are dimethyl sugars and arsenosugars F-J are trimethyl sugars.

Table 1.2 Toxicity of selected arsenic compounds to mice and rats[21].

Arsenic Compound	**LD_{50} (mg/kg) (species)**
As(III)	34.5 (mouse); 4.5 (rat)
As(V)	14-18 (rat)
MMA	1,800 (mouse)
DMA	1,200 (mouse)
TMA	8,000 (mouse)
TMAO	10,600 (mouse)
AsB	10,000 (mouse)

1.4 Significance of arsenic speciation

Rapid, reliable and accurate differentiation between toxic inorganic arsenic species and the usually less toxic organic arsenic species in water samples is necessary to assist the implementation of the new drinking water MCL for As, which is concerned only with total dissolved inorganic As. Speciation of inorganic As(III) and As(V) also provides important information for environmental engineers for successful design of water treatment processes, because most As remedial technologies are based on sorption processes that are sensitive to the As oxidation state, with As(V) generally removed much more efficiently than As(III) [22]. Moreover, speciation techniques identifying complicated arsenicals in food and biological samples are required with the further study of dietary arsenic bioaccessibility and toxicology, metabolism of arsenic [23] and disease–causing mechanisms at the molecular level.

1.5 Analytical methods for arsenic speciation

Analytical methods for the speciation of arsenic compounds include chromatographic [18, 24-30] and electrophoretic separation [31] and electrochemical, colorimetric [32] and bioanalytical techniques [33].

1.5.1 Chromatographic methods:

Gas chromatography (GC):

GC is not widely used in arsenic speciation because most arsenic compounds are non-volatile. However, methods have been developed based on GC separation after hydride generation or sample derivatization [1, 34]. Volatile arsines, e.g. AsH_3 (b.p. –55

°C), $MeAsH_2$ (b.p. 2 °C), Me_2AsH (b.p. 36 °C), and Me_3As (b.p. 70 °C) [1], are generated from non-volatile inorganic and organic arsenic compounds. MMA and DMA in urine had been derivatized with thioglycol methylate and isolated using a solid phase microextraction (SPME) fiber followed with determination by GC-MS [34].

Liquid chromatography (LC):

Methods based on LC separation currently are the most established, widely used and powerful techniques for arsenic speciation. They have been applied in various sample matrixes such as environmental water and soil and biological samples [18, 24-30]. Because arsenic compounds may be present as anionic, cationic, zwitterionic or neutral species, depending on the pH, different chromatographic separation strategies need to be employed for resolving these compounds [19]. As a result, anion exchange, cation exchange and ion pair chromatographic methods have been developed for the separation [35, 36]. As(III), As(V), MMA, DMA, AsB and AsC can be separated on anion exchange columns, such as Hamilton PRP X-100. Some As-metabolites, such as DMAE, TMAO, TetraMA, etc., are neutral or cationic and thus can be best separated by cation exchange IC procedure [19, 37]. Ion pair separation strategies have been developed for arsenic speciation [38, 39]. Because most arsenic compounds are not retained on reversed-phase columns, the addition of an anion-pairing reagent, such as a tetraethylammonium salt [38], or a cation-pairing reagent, such as hexsanesulfonate[39], is required.

Coupled with LC, detection is usually achieved using an element-specific detector such as inductively coupled plasma-mass spectrometry (ICP-MS), hydride generation

atomic absorption spectrometry (HGAAS), and atomic fluorescence spectrometry (AFS) [40-43]. However, with continuous discovery of new arsenic compounds, identification of unknown arsenic compounds, especially arsenic metabolites, is challenging. For example, at least 12 species have been found in human urine after ingestion of an arsenosugar, only three of which could be positively identified [44]. Le, *et. al.* also reported some unknown metabolites found in urine [45]. Only two of them, dimethylarsinylethanol (DMAE) and trimethylarsine oxide (TMAO), were later identified [46]. Therefore, techniques capable molecular structure interpretation, such as electrospray ionization mass spectrometry, is especially required for unknown determination [47].

1.5.2. Capillary electrophoresis (CE):

The ionic character of most arsenic compounds allows the convenient application of CE, which offers high resolution and fast analysis time, to arsenic speciation. The largest challenge, however, lies in sample detection because of the small volume (nanoliters) of sample injected. Conventional UV detection is not an ideal detector for arsenic analysis because arsenic compounds are transparent above 200 nm, which usually is the lower edge wavelength for a UV detector. In order to increase method sensitivity, sample stacking was employed for As(III), As(V) and DMA separation by CE coupled with UV detection [48]. Conductivity detection was found to be superior to UV detection and was employed for As(III), As(V) and DMA in water samples from tin mine tailings [49]. Highly sensitive element specific detection techniques have been developed in recent years. They include AFS [50], ICP-MS [31], HG-ICP-MS [51],

ICP-atomic-emission spectrometry (AES), and ICP- sector field mass spectrometry (SFMS) [52], *etc.* These methods usually offer sub μg/L detection limits.

1.5.3 Electrochemical methods:

Polarography, cyclic voltammetry, potentiometric titration, and anodic and cathodic stripping voltammetry have long been applied to the determination of arsenic [53-62]. Stripping analysis using hanging mercury drop electrodes (HMDE) utilizes a pre-electrolysis step to concentrate arsenic from solution onto the mercury electrode in the presence of Cu(II) and/or Se(IV), followed by a rest period to allow a more uniform distribution of the arsenic intermetallic compound formed within the amalgam. The compound is then stripped from the electrode upon application of a sufficient potential [55, 63, 64]. This method is especially suitable for trace analysis due to the enrichment of analyte by the pre-concentration, resulting in a detection limit usually at the sub-μg/L level. HMDE effectively avoids memory problems because a new mercury drop is generated for each determination [53-55, 61, 65]. Alternatively, ligands such as pyrrolidine dithiocarbamate can be used to complex arsenite, which is adsorptively deposited on the HMDE [53].

1.5.4 Colorimetric Methods:

Colorimetric arsenic determination is simple and fast. Production of colorful arsenic compounds by reaction with molybdate or mercuric bromide has long been employed as basic principle in colorimetric arsenic determination [66-69].

Molybdenum blue method:

This method is based on the chemistry of formation of blue arsenomolybdate from As(V) and ammonium molybdate [68, 69] . A UV-visible spectrometer is usually used for color detection. A light-emitting diode (LED)-based photometric detector has also been developed [32]. The Johnson and Pilson method [69] has been improved to lower the detection limit for As to 2 μg/L in groundwater containing up to 30 μM phosphate, as well as for As(III) and As(V) speciation [70].

Mercuric bromide method ("Gutzeit" method)

This method takes advantage of the formation of volatile arsine (AsH_3) gas to react with mercuric bromide to form colored product for detection and separate the arsenic from other possible interferences in the sample matrix. The arsine gas replaces bromide attached to the mercuric bromide with an -AsH_2 group in a stepwise manner. Mercuric bromide is colorless but the reaction product gives a yellow color when one arsine group is bound and then becomes progressively browner as more bromide is replaced. Most available field-test kits for arsenic are presently based on this method [67]. However, the operator has to handle the toxic arsine and mercuric bromide.

1.5.5 Biosensor:

Inorganic arsenic detection using a biosensor is an emerging and interesting research field. Test of groundwater and potable water for arsenite was achieved with a bacterial system that contained β-galactosidase, a nonpathogenic laboratory strain of *Escherichia coli*. The natural resistance mechanism of *E. coli* against arsenite and

arsenate produces a visible blue color at arsenite concentrations above 8 μg/L [33].

1.6 Significance of on-site arsenic speciation

There is increasing demand for development of sensitive on-site speciation methods for environmental water samples, especially reducing groundwater, because exposure of samples to the atmosphere during transport and storage allows oxidation of As(III) to As(V). Attempts to prevent this change have met with limited success [71, 72]. The oxidation has pH-dependent kinetics [73]. Also, As(III) oxidation in stream water is accelerated microbiologically [74]. Currently these two inorganic As species are separated in the field using an anion exchange resin with subsequent laboratory determination [75, 76]; however, immediate results cannot be obtained by these methods. The well-established chromatographic methods are not suitable for use in the field. Moreover, on-site arsenic detection techniques generally provide only total inorganic arsenic data. The results using commercially-available arsenic testing kits are far from satisfactory. For example, referring to the widely used Merck test kit, Erickson[66] stated "most experts agree that the kit works well for samples containing arsenic levels < 10 μg/L or > 100 μg/L, but not in between." So far, electrochemical [77, 78], colorimetric [66-68] and biosensors [33] have been reported to be applied on-site.

1.7 Summary of the research projects

In this research, we mainly focused on methods development for on-site arsenic speciation and their application. We chose an electrochemical method, specifically differential pulse cathodic stripping voltammetry (DPCSV), because it is relatively

simple and inexpensive with superb sensitivity and portable instrumentation. Two arsenic speciation methods, applicable both in the laboratory and in the field, have been developed. The methods employ a hanging mercury drop electrode (HMDE), on which As(III) is deposited in the presence of Cu(II) and Se(IV) in HCl medium. As(III) is determined by direct measurement. In the first method, determination of total As is performed by reducing As(V) to As(III) using sodium meta-bisulfite/sodium thiosulfate reagent stabilized with ascorbic acid. As(V) is quantified by difference. This method has been successfully applied for on-site analysis of groundwater at a Superfund site in Vineland, New Jersey and in Bangladesh. The second method further improved the reduction procedure by using L-cysteine and also has the capability to differentiate organic arsenic and inorganic arsenic. By using L-cysteine reduction of As(V) in a batch mode, which is difficult to perform by using sodium meta-bisulfite/sodium thiosulfate reagent, sample analysis throughput can be greatly improved. Organic arsenic compounds are UV photooxidized to inorganic As(V) and total arsenic (inorganic plus organic) subsequently determined. The concentration of organic arsenic is the difference between total inorganic and total arsenic concentrations.

Evaluation of dietary arsenic toxicity and bioaccessibility is essential for accurate arsenic risk assessment. A pilot study of arsenic bioaccessibility in rice has been accomplished in this work through *in vitro* gastric and small intestinal fluid incubation, which provides preliminary information for further investigation of the health impact of dietary arsenic.

The study of dissolution and precipitation of arsenic in natural environmental systems is of great significance for understanding arsenic mobility and providing for

potential remediation approaches to solve the problem of arsenic contamination. Sulfur is an important element exhibiting a range of oxidation states from -2 to +6 and as a result of this redox capability enters into a variety of biogeochemical processes [79]. Because sulfide can form highly insoluble sulfide precipitates with many elements, such as As, Cd, Co, Cu, Fe, Mn, Ni, Pb, Zn, in natural water systems, study of sulfide, especially dissolved sulfide, will undoubtedly improve our knowledge of removal pathways of those elements, which are important in assessing water quality. Conventional batch mode analysis of dissolved sulfide by cathodic stripping voltammetry (CSV) is known to suffer from loss of sulfide in the cell to the waste mercury pool, compromising quantification of sulfide [80]. The development of trace level sulfide analysis by DPCSV effectively solved this problem through a simple approach to keep sample solution fresh.

Interaction between sulfide and Fe(II) ions, which actively participate in arsenic precipitation, has been qualitatively investigated on site in reducing groundwater and laboratory solutions by applying above-developed sulfide analysis technique. The result provides an engineering consideration for arsenic removal through sulfide precipitation in groundwater, especially in iron-rich groundwater system.

Chapter II

Differential Pulse Cathodic Stripping Voltammetric Speciation of Trace Level Inorganic Arsenic Compounds in Natural Water Samples

The objective of this project was to develop a simple, fast and sensitive DPCSV method that is applicable to on-site analysis. To improve the peak shape and the method sensitivity, Cu(II) and Se(IV) were used together to form an intermetallic arsenic compound, $Cu_xSe_yAs_z$, on the HMDE during the deposition procedure. A reducing reagent system for As(V) determination was also investigated and improved, featuring good stability which is essential in field work. The DPCSV deposition potential and time, amount of Cu(II) and Se(IV) added, and the amount of reducing reagent used and reduction time were optimized. Furthermore, the effect of organic arsenic (monomethylarsonic acid (MMA) and dimethylarsonic acid (DMA)) on the electrode response, and the potential interference of ions (phosphate, iron, and manganese) commonly found in groundwater containing arsenic, were investigated. The accuracy of the method was validated by analyzing the NIST natural water standard, spiked drinking water, and groundwater samples. The on-site analysis capability was demonstrated by analyses of groundwater performed at an EPA superfund site at Vineland, New Jersey. The results compare favorably with those obtained by High Resolution ICP-MS, Graphite Furnace AAS and IC-AFS.

2.1 Experimental Section

2.1.1 Reagents and materials

2.1.1.1 As(III) and As(V) standard solutions

As(V) stock solution containing 1000 mg/L As was prepared by dissolving sodium arsenate (Na_2HAsO_4) (Sigma, St. Louis, MO, ACS reagent) in 1% (v/v) HCl

solution, diluted from 12M HCl (Fisher, Pittsburgh, PA, Optima). As(III) stock solution containing 1000 mg/L As was prepared by dissolving sodium m-arsenite ($NaAsO_2$) (Sigma, 96.7% purity) in 1% (v/v) HCl solution containing 1 mg/mL ascorbic acid [81]. Ascorbic acid served as an anti-oxidant to prevent As(III) from being oxidized to As(V) when refrigerated at 4°C, for up to three months. Working solutions of As(III) and As(V) were freshly prepared daily by dilution with 18 MΩ-cm water (Barnstead Infinity Nanopure system, referred to below as nanopure water).

2.1.1.2 Reducing agent

The reducing agent was adopted from Johnson and Pilson [69] with an important modification: ascorbic acid was added to stabilize the reagent for up to 100 hrs, compared to 6 hrs in the absence of ascorbic acid. 1.4 g Sodium meta-bisulfite (Fisher, Certified ACS) and 0.14g of sodium thiosulfate (Fisher, Certified) were each dissolved in 10 ml of nanopure water. The reducing agent was prepared by slowly adding 5 ml of 10% (v/v) H_2SO_4 to the sodium meta-bisulfite solution, with vigorous shaking, followed by the addition of the 10 ml sodium thiosulfate solution and 0.2 g solid L-ascorbic acid (Fisher, Certified ACS).

2.1.1.3 Auxiliary solutions

Cu(II) and Se(IV) solutions used were the 1000 mg/L AAS reference standard solutions (Fisher). The working solution of Se(IV) (1 mg/L) was prepared by dilution of the standard solution. Monosodium acid methane arsonate (MMA) (Chem Service, West Chester, PA, 99.0% purity) and dimethylarsinic acid (DMA) (Sigma, 98% purity)

stock solutions containing 1000 mg/L As were prepared in nanopure water. A 0.01M Fe(II) stock solution was freshly prepared before use by dissolving $FeCl_2 \bullet 4H_2O$ (Fisher) in nanopure water. The Mn solution was a 1000 mg/L AAS reference standard (Fisher). 0.1M. Phosphate stock solution was prepared by dissolving KH_2PO_4 (Fisher) in nanopure water. Samples with different levels of Fe(II), Mn and phosphate were prepared by spiking the desired amount of stock solution.

2.1.2 Instrumentation for DPCSV

An Eco Chemie μAutolab voltammetric apparatus (Brinkmann Instruments, Westbury, NY) equipped with a Metrohm 663VA electrode stand and controlled by a notebook computer running Eco Chemie GPES 4.9 software was used. A HMDE working electrode, a Pt auxiliary electrode, and a Ag/AgCl/3M KCl double-junction reference electrode were used. The electrode cell was equipped with a nitrogen purge tube to remove oxygen prior to sample analysis, as well as to remove gaseous sulfur compounds produced during the reduction step in the total arsenic measurement. A built-in motor-driven stirrer drives a PTFE stirring rod to stir the sample during purge and deposition. The unit is small and light enough to be portable, and sufficiently rugged to take on-site. An automobile battery (12V, D. C.) was linked to a commercial UPS system (Back-Ups CS 350, APC, Kingston, RI) to provide 110 V A.C. power to the unit. The battery allowed a few days of analysis time before recharging was required.

2.1.3 Analytical procedure for DPCSV

An appropriate amount of standard or sample was added to the analysis vessel containing nanopure water, making the total volume 10.0 mL, followed by addition of 12 M HCl (Fisher Optima) to provide a 1 M HCl supporting electrolyte. Optimized volumes of the 1000 mg/L Cu(II) and 1 mg/L Se(IV) solutions, 50μl and 40μl, respectively, were also added to produce concentrations of 4.6 mg/L and 3.7 μg/L, respectively. Sample was purged for 300 sec with N_2. DPCSV was performed using a deposition potential of –0.44 V versus Ag/AgCl reference, applied for 60 sec with stirring, during which time As(III) was deposited as $Cu_xSe_yAs_z$ intermetallic compound on the Hg electrode. Stirring and purge were stopped. After a 15 sec equilibration time, the stripping potential was scanned from –0.4 V to –0.9 V vs. Ag/AgCl reference electrode with a 10 mV step potential, 50 mV modulation amplitude, 33.3 msec pulse width, 16.7 msec measurement time, and 25mV/s scan rate. The As(III) peak appeared at ~ –0.66 V. The procedure is illustrated in Fig. 2.1. Since only As(III) is electroactive during the DPCSV procedure, optimization procedures for all parameters were carried out using As(III) solution.

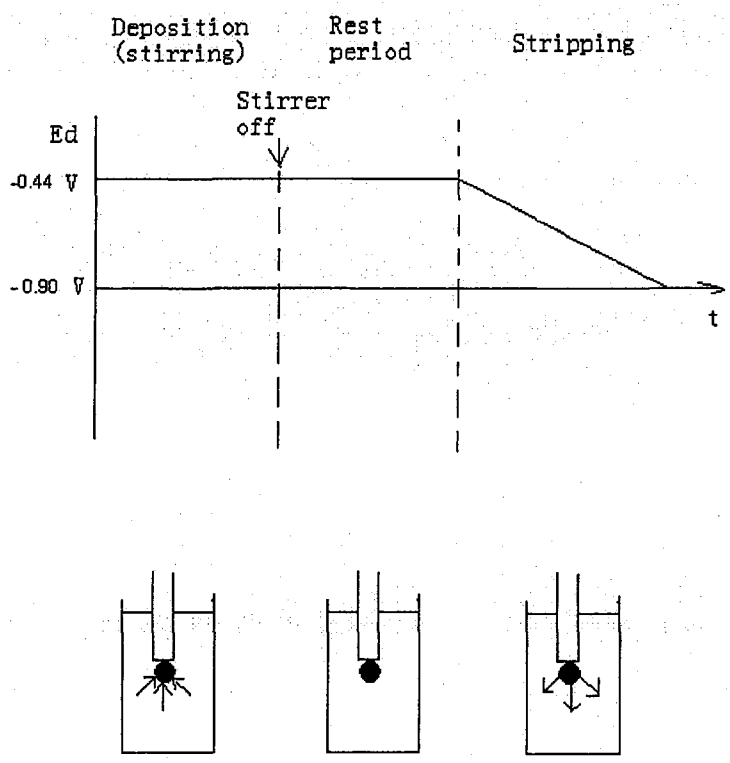


Fig. 2.1 The DPCSV procedure for As(III) analysis

2.1.4 Quantification by standard addition

For samples containing both As(III) and As(V), two 10 mL sub-samples were used. As(III) in the presence of HCl, Cu(II) and Se(IV) was quantified by standard addition in first sub-sample. Total arsenic was determined in the second sub-sample by spiking with concentrations of 2 mg/ml of sodium meta-bisulfite and 0.2 mg/ml of sodium thiosulfate, and allowing reduction to proceed for 420 seconds with N_2 purge before DPCSV analysis. Total arsenic was then quantified by following the standard addition procedure for As(III). The As(V) concentration is the difference between total arsenic and As(III) concentrations. Groundwater samples or samples with high arsenic concentration were diluted with nanopure water to reduce matrix effects or to reduce arsenic concentrations to within the linear range.

2.1.5 Quantification by HR ICP-MS, GFAAS and IC-AFS

Total dissolved arsenic, including both inorganic and organic arsenic in the samples was quantified by standard addition. An Axiom single collector High Resolution (HR) ICP-MS (Thermo Elemental, Germany) at the Lamont-Doherty Earth Observatory (LDEO) of Columbia University was used. Arsenic was quantified by standard addition calibration after drift correction using Ge as an internal standard at a high resolving power of 12,000 to eliminate ArCl interference. The detection limit for As is 0.1 μg/l. A Perkin-Elmer AAnalyst 800 was used for graphite furnace AAS, following a standard GFAAS protocol using $Pd(NO_3)_2$ as matrix modifier. We also speciated the inorganic arsenic in our samples by IC-AFS using a Varian 9012 HPLC

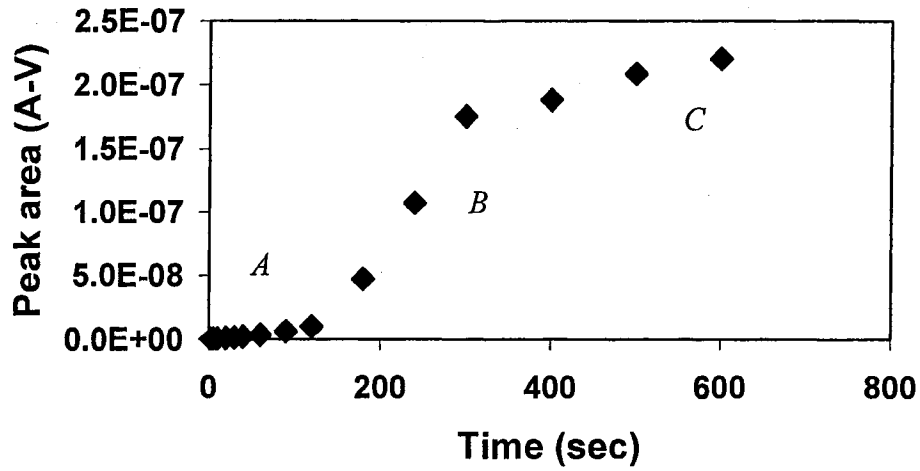
system with a Varian 9100 autosampler with a 250 μL injection loop . The separation column was PRP X-100 (Hamilton, 250mm x 4.1 mm i.d., 10 μm particle size). A guard column with same packing material was connected between the injector and the separation column. The mobile phase was 10mM K_2HPO_4/KH_2PO_4 adjusted to pH 6.25, at a flow rate of 0.8ml/min. Arsenic species were detected by an atomic fluorescence spectrometer (PS Analytical, Kent, UK) after hydride generation. The carrier acid was 12.5% (v/v) HCl and the reductant was 1.4% (m/v) $NaBH_4$ (Fisher) in 0.1M NaOH. Flow of HCl and reductant was set at 50% full pump rate.

2.2 Results and Discussion

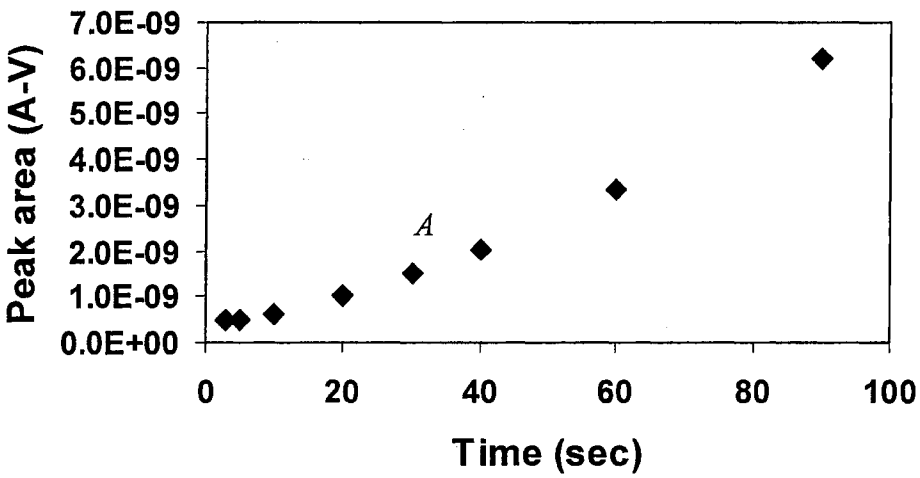
2.2.1 Optimization of deposition potential and time

DPCSV deposition potential and time were investigated. For 10 ml samples containing 45 μg/L As(III), 4.6 mg/L Cu(II) and 3.7 μg/L Se(IV), the deposition potential was varied from –0.35 to –0.70V versus reference electrode. Peak response increased initially and then decreased, maximizing at –0.44 V, the same potential found by Barra and Correia dos Santos [65]. The same peak maximum potential was observed for As(III) formed by reduction of As(V) in the analysis vessel. As(III) peak area increased with longer deposition time (Figure 2.2 a); however, two types of peaks were observed with the increasing deposition time. For deposition times up to 60 sec (range A in Fig. 2.2 a), the peak was sharp and symmetrical and the peak area increased linearly with time (Fig. 2.2 b). For longer deposition times (>60 sec, Fig. 2.2 a), the peak became broader and lower, and the peak position gradually shifted from ~-0.66V to ~0.80 V. The peak area

increased with deposition time between 60 sec and 300 sec (range B, Fig. 2.2 a) but leveled off when deposition time was > 300 sec (range C, Fig. 2.2 a). Both the peak shape change and peak potential shift with deposition time suggest formation of a succession of intermetallic compounds, $Cu_xSe_yAs_z$, with different stoichimetric ratios. Based on peak shape and sensitivity, 60 sec was chosen as the optimized deposition time. For extremely low concentrations, increased deposition time can be used to improve sensitivity, but requires a separate calibration.



(a)



(b)

Fig. 2.2 : Effect of deposition time on As(III) peak area. (a) deposition time up to 700 sec, which includes range A (<60 sec), range B (from 60 sec to 300 sec) and range C (> 300sec) and (b) deposition time up to 100 sec. The scales of y-axis are different for panels (a) and (b). The experiments were performed by varying deposition time at deposition potential of –0.44V using a solution containing 45 μg/L As(III), 4.6 mg/L Cu(II) and 3.7 μg/L Se(IV).

2.2.2 Effect of Cu(II)

Arsenic cannot be electrolytically deposited directly onto a Hg electrode. As(III) reacts with Cu(II) to form an intermetallic compound Cu_xAs_y [54, 64] that is deposited onto the HMDE and is subsequently stripped cathodically. The amount of Cu(II) affects peak area and peak shape as shown in Figure 2.2. For 5 μg/L to 45 μg/L As(III), the peak area increased for up to 4.6 mg/L Cu(II) added; however, Cu(II) concentrations between 4.6 mg/L and 10.1 mg/L produced peak splitting . Further increase in Cu(II) to 18.4 mg/L produced a broad and large single peak. Still higher Cu(II) concentrations led to decreasing peak area, and ultimately the peak disappeared. Increasing Cu(II) concentration also shifted the peak position from ~ –0.65V to ~ -0.77V. This behaviour demonstrated the formation of intermetallic compounds with different Cu:As ratios. On the basis of sufficient sensitivity and good peak shape over the range from 4.5 to 180 μg/L As(III), the entire linear range (see below), we chose the optimum Cu(II) concentration to be 4.6 mg/L. However, under these conditions, the Cu_xAs_y peak was found to exhibit a shoulder, leading to some uncertainty in quantification. A similar peak with a shoulder was also observed by Barra and Correia dos Santos [65].

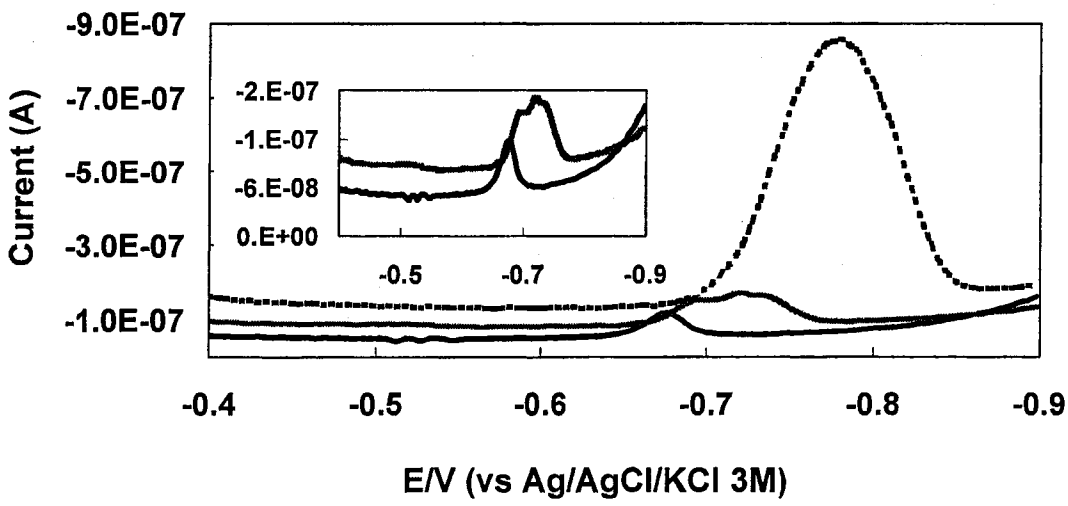


Fig. 2. 3: Effect of Cu(II) concentration on As peak shape. The solid line, shaded line and dashed line refer to Cu(II) concentrations of 4.6 mg/L, 10.1mg/L and 18.4mg/L, respectively. The insert enlarges the voltammograms of the two lower concentrations of Cu(II) addition at 4.6 mg/L and 10.1 mg/L, respectively. The unit of the axis in insert voltammogram is same as the large one. As(III) concentration was 20 μg/L.

2.2.3 Effect of Se(IV)

Addition of 50 μg/L Se(IV) has been used for arsenic measurement in a H_2SO_4-acidified sample [55]. Se(IV) presumably forms an intermetallic compound with arsenic, such as As_2Se_3, during deposition [55]. In the presence of Cu(II), Se(IV) was reported [61] to interfere with the arsenic signal. However, we found a major improvement in As peak shape was achieved by addition of both Cu(II) and trace level Se(IV), as shown in Figure 2.3. The peak shoulder gradually disappeared with increasing trace levels of Se(IV). At a Se(IV) concentration of 3.7 μg/L with 45 μg/L Cu, a sharp and symmetric As peak was obtained, and sensitivity was improved over the linear As(III) range of 4.5 to 180 μg/L. Se(IV) and Cu(II) have not been applied previously together in arsenic measurement. Although further study is needed, we assume a different intermetallic compound, $Cu_xAs_ySe_z$, forms during deposition, which leads to the improvement in peak response.

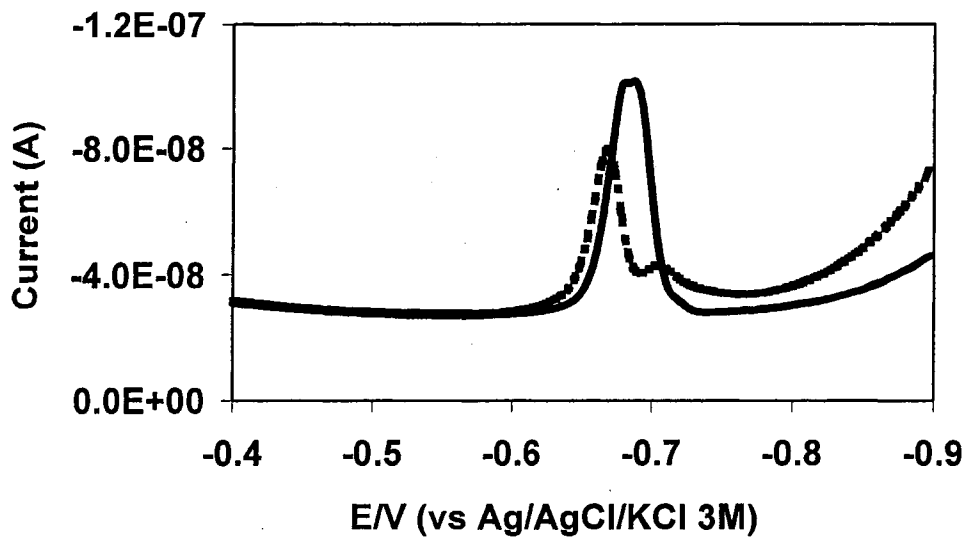


Fig. 2.4: Effect of Se(IV) on As peak shape. The dashed line and solid line represent the voltammograms without and with 3.7 μg/L Se(IV), respectively, in a solution containing 45 μg/L As(III) and 7.4 mg/L Cu(II).

2.2.4 Effect of reducing agent

Because As(V) is generally electrochemically inactive, total inorganic arsenic determination requires chemical reduction of As(V) to As(III). The reduction procedures employed in reported electrochemical methods are time consuming and not straightforward. For example, Kotoucek reported that As(V) was reduced by hydrobromic acid and solid $N_2H_4 \cdot 2HCl$ with heating for 45min on a steam bath at 95-100°C [59]. Sulfur dioxide was also used as a reducing reagent by passing the gas through a sample solution at 80°C [53, 58]. Manipulations such as sample cooling and volume corrections due to evaporation had to be performed before determination of As(III). A less aggressive reduction method using potassium iodide and ascorbic acid solution, often applied in atomic absorption spectroscopic determinations with the hydride generation technique, was also applied to the voltammetric method [65], but Ferreira and Barros [61] and our own experience found these reagents ineffective in DPCSV. Instead, a reducing reagent with thiosulfate alone [30], or combined with meta-bisulfite were much more effective (see below). Direct As(V) deposition on a HMDE with mannitol serving as an activator was reported, but the determination of total arsenic was restricted because response to As(III) was higher than that to As(V). Thus chemical conversion to a single arsenic species by oxidation of As(III) to As(V) was necessary for accurate assessment [82].

We adopted as a reducing reagent the sodium meta-bisulfite /sodium thiosulfate in sulfuric acid reagent that was previously employed in a colorimetric method [68, 69]. However, a major drawback of this reducing agent is that it was not stable in colorimetric

applications [68] and deteriorates even faster in voltammetric applications (< 6hrs) because of the lower concentrations of reagents used. To overcome this limitation, which would otherwise make this method impossible to use for fieldwork, ascorbic acid was added as an anti-oxidant. The modified reducing agent is stable up to 100 hrs at room temperature.

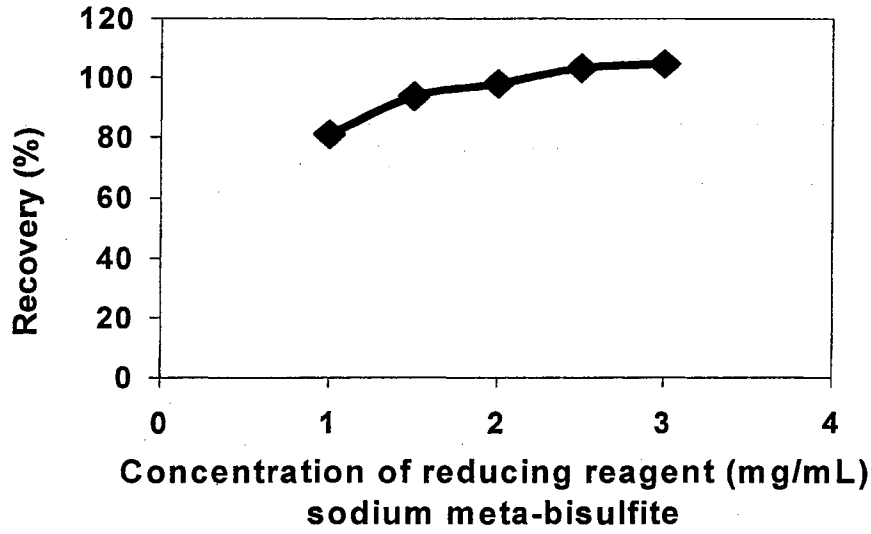
2.2.4.1 Amount of reducing agent

To study the effect of the concentration of reducing agent on the DPCSV response, amounts of reducing agent ranging from 1 mg/ml sodium meta-bisulfite/0.1 mg/ml sodium thiosulfate to 3 mg/ml sodium meta-bisulfite/0.3 mg/ml sodium thiosulfate, were added to 10 mL of solution containing 180 μg/L As(V). The solution was purged for 600 sec during reduction. Purging was important because gaseous sulfur compounds formed from the reducing agent caused a high background and distorted the voltammogram. Generally, the longer the purging time, the better the voltammogram. It was found that for a 180 μg/L As(V) solution, 100% recovery (defined as the percentage of a signal obtained by As(III) reduced from As(V) compared with that where the same concentration of standard As(III) was used) was achieved if at least 2 mg/ml sodium meta-bisulfite/0.2 mg/ml sodium thiosulfate of reducing reagent was added, as shown in Figure 2.4 a.

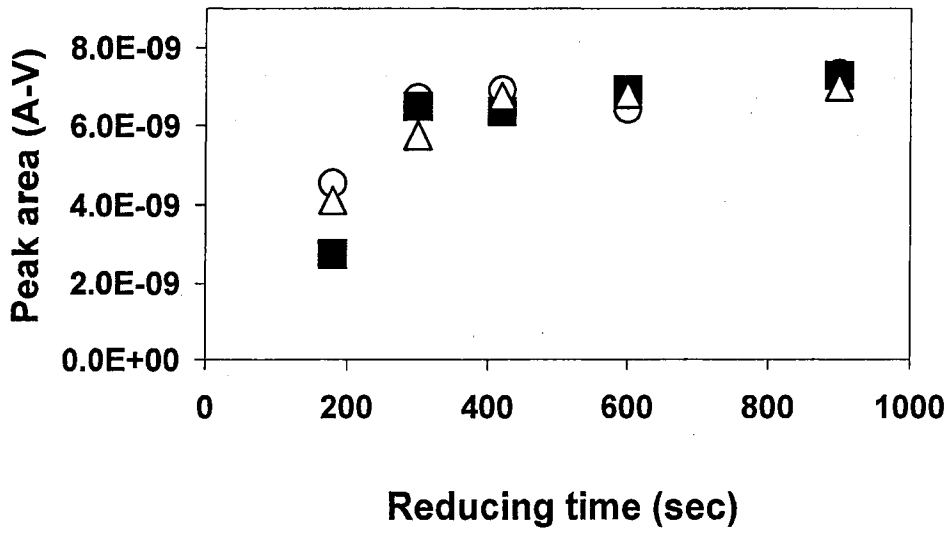
2.2.4.2 Reduction time

To determine the time required to quantitatively reduce As(V) to As(III), the reduction time was varied from 180 sec to 900 sec with addition of 2mg/ml /0.2 mg/ml;

2.5 mg/ml/0.25 mg/ml; and 3mg/ml/0.3 mg/ml sodium meta-bisulfite/sodium thiosulfate, respectively, to a sample containing 180 μg/L As(V). The minimum time required to eliminate background and obtain a reasonable voltammogram with 3mg/ml /0.3 mg/ml sodium meta-bisulfite/sodium thiosulfate was 180 sec. For all three levels of added reducing agent, arsenic response was stable for reduction times greater than 420 sec, as shown in Figure 2.4 b. Thus we chose addition of 2mg/ml /0.2 mg/ml sodium meta-bisulfite/sodium thiosulfate to minimize background current, and 420 sec reduction time to allow quantitative reduction of As(V) to As(III) in the 10 mL sample solution.



(a)



(b)

Fig. 2.5. Optimization of reducing conditions. (a) Effect of the amount of reducing agent on recovery with a reducing time of 600sec. The concentration of sodium thiosulfate is always 10 times less than the concentration of sodium meta-bisulfite in the reducing agent, which is indicated by the X-axis. (b) Effect of reduction time for conversion of As(V) to As(III) with addition of 2 mg/ml / 0.2 mg/ml (open circles), 2.5 mg/ml / 0.25 mg/ml (solid squares) and 3 mg/ml / 0.3 mg/ml (open triangles) sodium meta-bisulfite/ sodium thiosulfate. As(V) concentration in all cases was 180 μg/L.

2.2.5 Effect of organic arsenic compounds

Low levels (<100 μg/l) of organoarsenic compounds such as MMA and DMA may be found in natural waters. Spiking MMA and DMA at levels up to 100 μg/L into a water sample containing inorganic arsenic produced no DPCSV response to either compound, and no obvious peak change for As(III) or As(V). We conclude organic arsenic compounds provide no interference.

2.2.6 Quantitative analysis

As(III) and As(V) were analyzed using the optimized conditions, i.e., in a 10 mL water sample, with addition of 4.6 mg/L of Cu (II) and 3.7 μg/L Se(IV). For the As(V) determination, 2mg/ml /0.2 mg/ml sodium meta-bisulfite/sodium thiosulfate was also added and the reduction time with purging was 420 sec. The range of linearity for As(III) was from 4.5 to 180 μg/l with slope of 2.71 $x10^{-11}$ μg/L /A-V and linear regression correlation coefficient (r^2) of 0.9963. For As(V), the linear range was the same, the slope of calibration curve was $3.66x10^{-11}$ μg/L/A-V with r^2 = 0.9961. The precision was evaluated from six replicate measurements of an arsenic standard containing 45 μg/L, 10 μg/L, and 5 μg/L each of As(III) and As(V), yielding peak area RSDs of 2.4%, 2.5%, 4.2% for As(III) and 8.0%, 6.8%, 9.0% for As(V), respectively. The method detection limit, S/N >3, was determined to be 0.5 μg/L for both As(III) and As(V). Noise is taken to be the fluctuation of the baseline current for a blank solution.

2.2.7 Interferences

Interference caused by substances commonly present in groundwater, Fe(II), Mn(II) and phosphate, was investigated. The DPCSV response to an aqueous solution containing 45 μg/L As (III or V) and different levels of Fe(II) up to 300 μM, and Mn(II) up to 100 μM, was investigated. The presence of these ions was found to have negligible interference. Phosphate concentrations ranging from 5 μM to 100 μM had no effect on the analysis of a 45 μg/L As(III) solution. The response to As(V) in the presence of phosphate, however, was found to be slightly depressed, showing an average peak area decrease of 10%.

2.2.8 Analysis of environmental samples and comparison with other techniques.

Tap water from a laboratory at Queens College was collected in a polyethylene bottle and analyzed immediately. No arsenic species was detected in the sample. As(III) and As(V) standard solution were spiked into this tap water sample at a concentration of 20 μg/L for each analyte. Three replicate determinations using the standard addition method gave average results for As(III) of 20.4 ± 0.7 μg/L and for As(V), 21.7 ± 2.5 μg/L. The optimized method was applied to various other water samples, including a NIST SRM 1640 natural water standard, spiked commercial bottled drinking water, and LDEO groundwater. These samples were chosen because they had been analyzed previously as internal laboratory standards repeatedly by HR ICP-MS at LDEO and GFAAS at Queens College. Because these samples had been stored for months, only As(V) was found (Table 2.1).

On-site analysis of inorganic arsenic by DPCSV was conducted at an EPA

Superfund site in Vineland, New Jersey, in July, 2003 to speciate groundwater extracted from 5 wells (Table 2.1). Because MMA and DMA were known to be present in the Vineland groundwater [4] , the inorganic As speciation data obtained on site by DPCSV was later confirmed using IC-AFS analysis, and the total arsenic determination was confirmed using GFAAS in the laboratory. Because of the conversion of As(III) to As(V), the total inorganic arsenic concentration obtained by IC-AFS was used for comparison with the on-site DPCSV results. Due to time constraints in performing analysis in the field and the rapid change of oxidation state of reducing groundwater upon exposure to air which results in arsenic removal from the sample, each sample was analyzed only twice. Therefore, the concentration error for As(III) and As(III)+As(V) (Table 2.1) was estimated by error propagation from linear regression errors obtained by a least-square fit from both the slope and the intercept of the standard addition curve of each sample. As(V) concentration was based on the difference of As(III) and As(III)+As(V), the error was propagated from errors estimated for As(III) and As(III)+As(V) [83]. Results obtained by the different techniques agree well, demonstrating that DPCSV is not only a technique useful in the laboratory, but is also applicable to on-site inorganic arsenic determination and speciation.

Table 2.1: Summary of results from analysis of arsenic in water samples

In-laboratory sample analysis					
Sample name	DPCSV(μg/l)			HR ICP-MS(μg/l)[a]	GFAAS(μg/l)
	As(III)	As(V)	As (III) + As (V)	Total As	Total As
NIST 1640 [As]=26.7 μg/l	N.D.	26.5 ± 3.4	26.5 ± 3.4	26.3± 0.5	24± 6
Spiked Drinking water	N.D.	40.4±3.2	40.4±3.2	44± 1	45± 4
Spiked LDEO Groundwater	N.D.	323± 23	323± 23	320± 2	311± 9

On-site DPCSV sample analysis (July, 2003)				In-laboratory Analysis			
Sample name	DPCSV(μg/l)			IC-AFS(μg/l)[b]			GFAAS(μg/l)
	As(III)	As(V)	As (III) + As (V)	As (III) + As (V)	MMA	DMA	Total As
GW-1	2.8 ±0.4	904 ± 45	907 ±45	923	<100	<100	1193± 23
GW-2	5.0 ±1.2	1445 ± 136	1450 ±135	1445	<100	<100	1563 ±31
GW-3	N.D.	2.5 ±0.9	2.5± 0.9	3.7	<5	<5	<5
GW-4	1189 ±94	130 ± 102	1319 ±41	1440	216	<100	1872± 24
GW-5	97 ± 11	29± 14	127±10	106	8.2	9.1	124 ±3

Note: Triplicate analysis was performed for all in laboratory samples except when noted. All DPCSV on-site analyses were the average of two measurements.

[a] values obtained base on average of 26 measurements over approx. 1 year

[b] single analysis was performed. Detection limits of IC-AFS method used were 5 μg/L for MMA and DMA. However, GW-1, GW-2, and GW-4 were subjected to 20 times dilution and therefore had a higher detection limit of 100 μg/L for MMA and DMA.

N.D.: non-detectable.

Chapter III

Speciation of Submicrogram per Liter Levels of Arsenite, Arsenate and Organo-arsenic Compounds in Water Samples by Cathodic Stripping Voltammetry

In this study, we report a major improvement in the speciation method by using L-cysteine, which has several qualities making it a more useful reductant. It is itself a stable, fast-acting reagent; produces an As(III) product solution that remains stable for one week; and causes no interference with the CSV determination. L-cysteine ($HOOC\text{-}CHNH_2\text{-}CH_2\text{-}SH$, abbreviated as HSR) is the reducing agent used in hydride generation-spectrometric techniques for determination of analytes such as As, Sb, Se, Sn, Bi, Te and Pb [84]. However, there is no report in the literature applying L-cysteine to As(III) and As(V) speciation using CSV. L-cysteine efficiently reduces As(V) to its trivalent state as a As(III)-thiolate complex. L-cysteine is oxidized to the disulfide form, L-cystine ($HOOC\text{-}CHNH_2\text{-}CH_2\text{-}S\text{-}S\text{-}CH_2\text{-}CHNH_2\text{-}COOH$, abbreviated as RSSR) [84]. The reaction is

$$H_3AsO_4 + 5HSR \rightarrow As(SR)_3 + RSSR + 4H_2O$$

We further demonstrate that the CSV method can be applied to determine non-electroactive organic arsenic compounds following UV photooxidation with peroxydisulfate to convert MMA and DMA to inorganic form.

3.1 Experimental Section

3.1.1 Test chemicals.

As(V) stock solution containing 1000 mg/L As was prepared by dissolving sodium arsenate (Na_2HAsO_4) (Sigma, St. Louis, MO, ACS reagent) in 1% (v/v) HCl

solution diluted from 12M HCl (Fisher Scientific, Pittsburgh, PA, Optima grade). As(III) stock solution containing 1000 mg/L As was prepared by dissolving sodium m-arsenite ($NaAsO_2$) (Sigma, 96.7% purity) in 1% (v/v) HCl solution containing 1mg/mL ascorbic acid (Sigma) [81]. Monosodium acid methane arsonate (MMA) (Chem Service, West Chester, PA, 99.0% purity) and dimethylarsinic acid (DMA) (Sigma, 98% purity) stock solutions containing 1000 mg/L As were prepared in 18 MΩ-cm water (Barnstead Infinity Nanopure system, referred to below as nanopure water). Working solutions of As(III) and As(V) were freshly prepared daily by dilution with nanopure water.

L-cysteine reagent (1.0 M) was freshly prepared before use by dissolving 1.212 g in 10ml nanopure water. $Na_2S_2O_8$ solution (0.25 M) was also freshly prepared before use by dissolving 0.60g $Na_2S_2O_8$ (J.T. Baker, Phillipsburg, NJ) in 10ml 0.2M NaOH solution. Cu(II) and Se(IV) were used in the CSV determination to form an electrochemically better-behaved intermetallic compound with arsenic, $Cu_xSe_yAs_z$, on the HMDE surface [61, 78]. Cu (II) was the 1000 mg/L AAS reference standard solution (Fisher) and 1 mg/L Se(IV) solution was diluted from 1000 mg/L AAS reference standard solutions (Fisher). For the investigation of interferences, Fe(II), Mn(II) and phosphate solutions were used. A 0.01M Fe(II) stock solution was freshly prepared before use by dissolving $FeCl_2 \bullet 4H_2O$ (Fisher) in nanopure water. A 0.1M Mn(II) solution was freshly prepared by dissolving $MnSO4 \bullet H_2O$ (Fisher) in nanopure water. A 0.1M Phosphate stock solution was prepared by dissolving KH_2PO_4 (Fisher) in nanopure water. Samples with different levels of Fe(II), Mn(II) and phosphate were prepared by spiking the desired amount of stock solution.

3.1.2 Instrumentation and As(III) analysis.

An Eco Chemie μAutolab voltammetric apparatus (Brinkmann Instruments, Westbury, NY) equipped with a Metrohm 663VA electrode stand and controlled by a notebook computer running Eco Chemie GPES 4.9 software was used. A HMDE working electrode, a Pt auxiliary electrode, and a Ag/AgCl/3M KCl double-junction reference electrode were used. The procedure was similar to that used in our previous study [78]. Briefly, sample solution containing 1M HCl, 4.6 mg/L Cu(II) and 3.7 μg/L Se(IV) for AsIII or 7.4 μg/L Se(IV) for As(V) and organic As was purged with N_2 for 180 sec before a new HMDE was generated. Analyte was then deposited on the mercury electrode surface for 60 sec at a deposition potential at –0.44V vs. Ag/AgCl reference electrode. After 15 sec equilibrium time, stripping was performed from –0.4 V to –0.9 V. The arsenic peak was observed at about –0.68V.

3.1.3 As(V) reduction

A batch of 8 working standards or samples, the number limited by the available sample rack, was prepared in 30ml HDPE bottles or quartz tubes (2.2 cm i.d., 15 cm length). Optima HCl and 1.0M L-cysteine were added to 10 ml of samples to make concentrations of 0.03 M and 20mM, respectively. The bottles were capped and placed into a water bath (IsoTemp 205, Fisher) at 70 °C for 6 minutes, or held at room temperature for 1.5 hrs. Samples were cooled to room temperature before CSV determination.

3.1.4 Organic arsenic oxidation

Samples with organic arsenic were prepared in quartz tubes in batches of 4, the capacity of the UV irradiator. The concentration of $Na_2S_2O_8$ in 10ml of sample in the quartz tubes was 3 mM or up to 30 mM if Fe(II) and/or Mn(II) were present. The tubes were then capped with Teflon caps and inserted into a pre-warmed 500W UV irradiator (manufactured by Dr. C.M.G. van den Berg, University of Liverpool, UK) for 6 minutes. The samples then were treated by the reduction procedure above before CSV determination of total arsenic. This UV irradiator is portable and amenable to field operation.

3.1.5 Protocol for arsenic speciation

The protocol is illustrated in Figure 3.1. The method of standard additions was used for quantitative analysis. For determination of As(III), three increments of a small volume (< 100 μl) of a As(III) working standard was spiked directly into the electrolysis vessel containing 10 ml of water sample and analyzed, with the highest addition not exceeding the 190 μg/L linear range (see below). For As(V) and organic arsenic analysis, four 10ml sample aliquots were used which included 3 samples spiked with either As(V) or organic As standards. The amount of As addition was based on an estimation of As(V) and organic arsenic from a preliminary determination prior to addition of the increments to ensure the concentration fell within the linear range. The spiked samples were treated following the same protocol for As(V) reduction alone or combined with UV oxidation before determination by DPCSV.

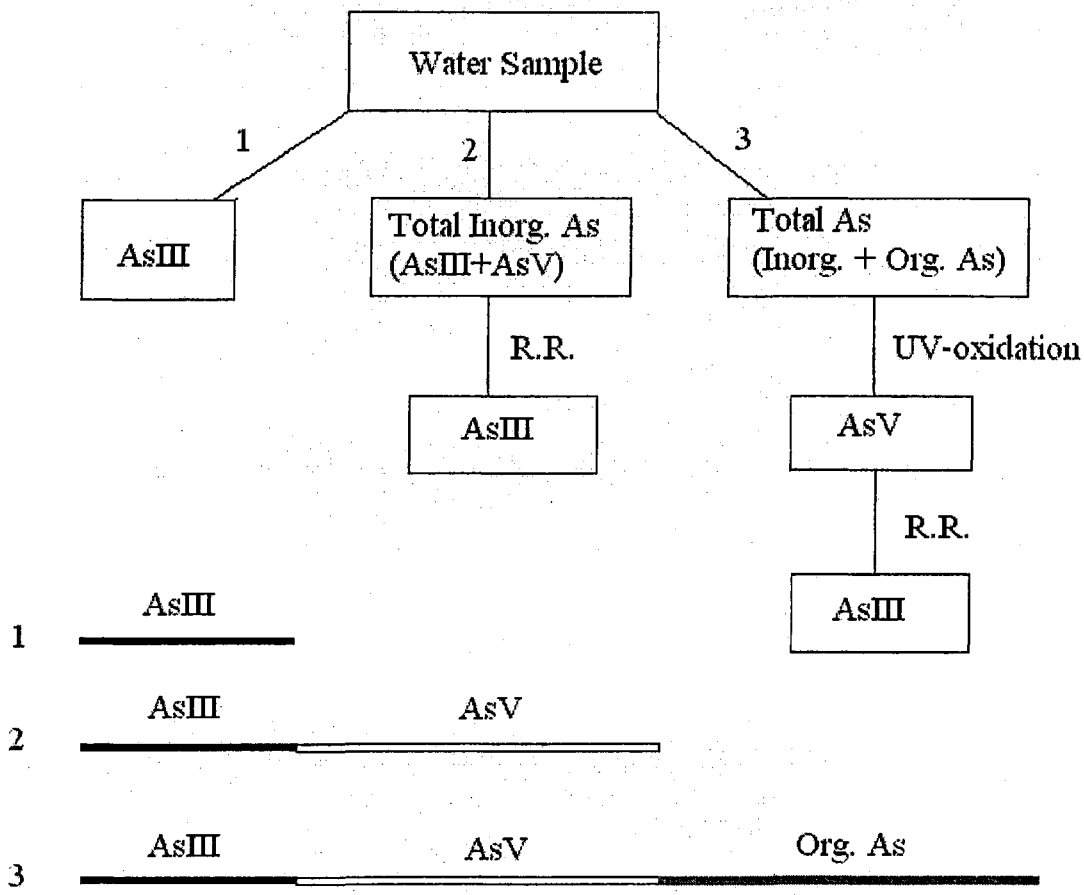


Figure 3.1: Analytical protocol for arsenic speciation. Three procedures are used: (1) direct CSV analysis of As(III), (2) total inorganic arsenic determination through L-cysteine reduction of As(V) to As(III), and (3) total inorganic and organic arsenic measurement through UV-photooxidation using $Na_2S_2O_8$ and L-cysteine reduction. The As(V) concentration is the difference between the total inorganic and As(III) concentrations. Organic arsenic concentration is the difference between total arsenic and total inorganic arsenic. RR means reducing agent.

3.2 Results and Discussion

Optimization of the determination of As(III) was studied previously [78]. Optimization of conditions used for As(V) speciation by L-cysteine reduction and organic arsenic determination though UV photooxidation with $Na_2S_2O_8$ is described here[84][78][61][58, 59].

3.2.1 L-cysteine concentration

Optimization of the L-cysteine concentration was conducted in solutions containing 190 μg/L As(V), the upper limit of linearity of the CSV method (see below). The As(V) solutions were acidified to the optimal acidity of 0.03 M HCl before reduction at the optimal temperature of 70 °C for 6 minutes L-cysteine concentrations were varied from 5 mM to 80 mM. About 80 to 95% of As(V) was reduced when L-cysteine concentrations were between 5 and 15 mM, and 102 ± 1 % reduction of As(V) was observed for L-cysteine concentrations 20mM or higher. Thus 20mM L-cysteine was determined to be the optimum concentration for samples containing or diluted to contain less than 190 μg/L As(V) (Fig. 3. 2 a).

3.2.2 Temperature

The rate of reduction is greatly accelerated by increasing temperature. Initial optimization experiments were conducted with a 50 μg/L As(V) solution containing

20mM L-cysteine but without HCl. Quantitative conversion of As(V) to As(III) requires less than 4 min at 90 °C, about 10 min at 85 °C, and about 20 min at 70 °C. However, to avoid evaporative sample loss and due to sample handling difficulties at higher temperatures, 70°C was chosen as the optimal reduction temperature for laboratory work. In the field, if no thermostat is available, 80 min. is required at ambient temperature (Figure 3.2 c). Samples containing 190 μg/L As(V) treated with L-cysteine were found to be stable for at least one week at room temperature.

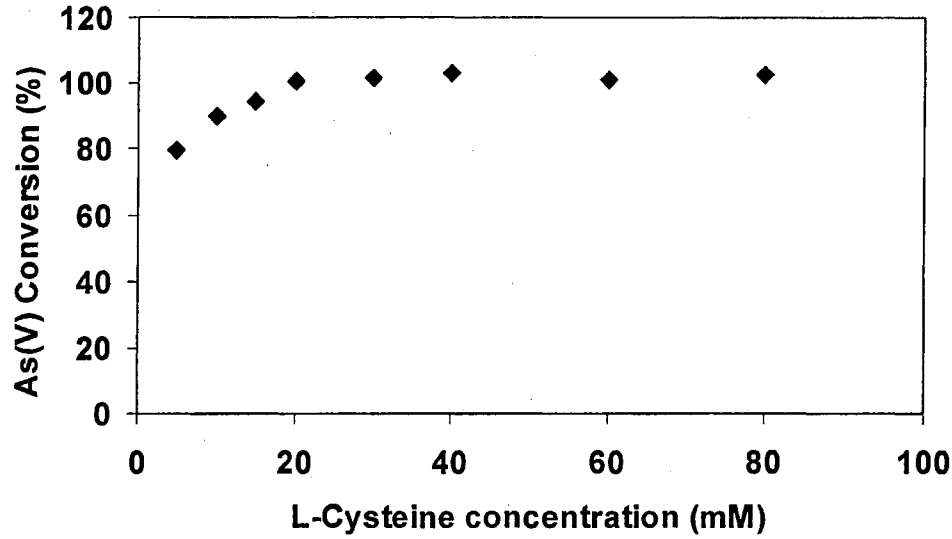
3.2.3 Acidity

The rate of reduction can be further accelerated by adding HCl. A 50 μg/L As(V) solution containing 20 mM L-cysteine and 0.03M HCl was quantitatively converted to As(III) at 70°C in 6 min, vs. 20 minutes without HCl. The optimal amount of HCl was determined to be 0.03 M by varying the concentration of HCl from 0 M to 1M in a 50 μg/L As(V) solution containing 20 mM L-cysteine (Fig. 3.2 b).

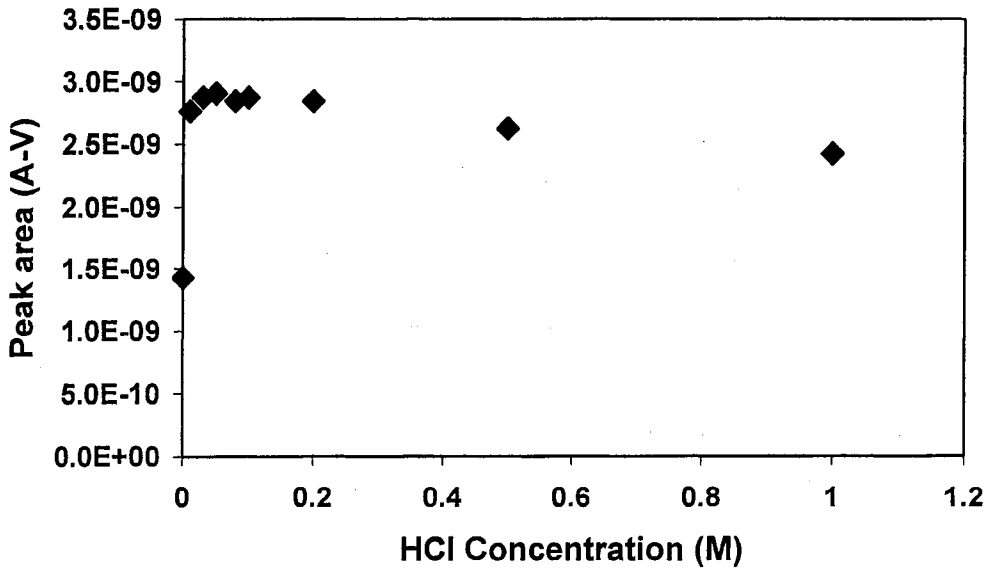
3.2.4 Electrode response to the As(III)-thiolate complex

Our previous work [78] showed that deposition of As(III) on Hg in the presence of Cu and Se resulted in the formation of an intermetallic compound, $Cu_xSe_yAs_z$, on the HMDE. Addition of 4.6 mg/L Cu(II) and 3.7 μg/L Se(IV) are necessary to ensure a useful peak shape for the As peak at -0.66 V. The product of the L-cysteine reduction of As(V) is As(III)-thiolate, for which, however, 7.4 μg/L Se(IV) is needed to eliminate a

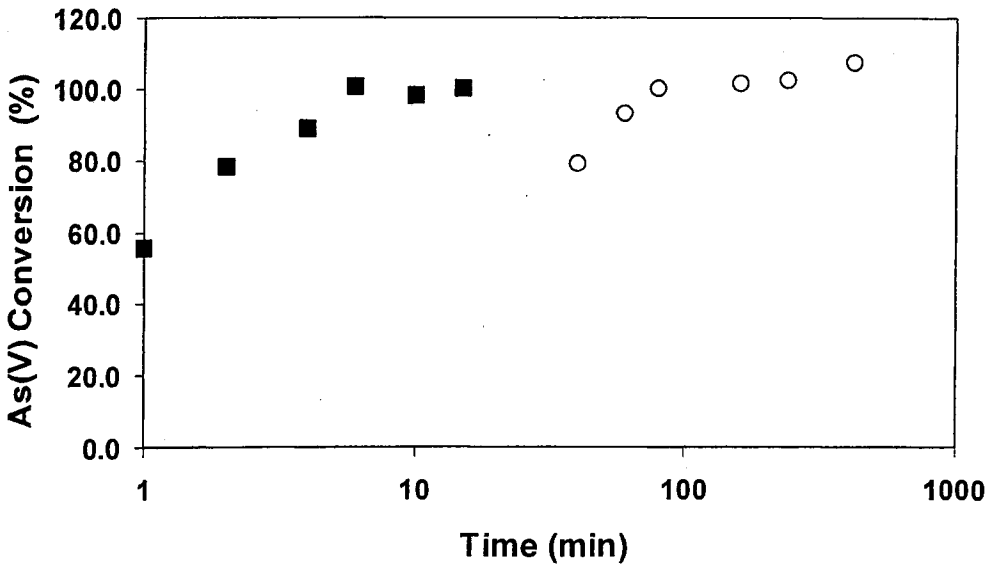
shoulder on the As peak. The peak is shifted to -0.68 V (Fig. 3. 3). We also found the CSV peak area for 190 μg/L As(III)-thiolate produced using the optimized conditions was always about 10 ~ 20% larger than the peak for 190 μg/L As(III). Although cystine is known to react with Hg through sulfur-Hg interaction [63], how this might enhance peak area is not clear. Because of these differences in electrode response, quantification of As(V) and organic arsenic (MMA and DMA) using standard additions requires addition of As(V), or organic arsenic standard followed by same L-cysteine or UV photooxidation combined with L-cysteine treatment.



(a)



(b)



(c)

Figure 3.2 Parameter optimization for As(V) reduction by L-cysteine. (a) Effect of L-cysteine concentration. 190 μg/L As(V) solution containing 0.03 M HCl was reduced with L-cysteine at 70 °C for 6 min. (b) Effect of acid concentration. 50 μg/L As(V) solution was reduced at 70 °C for 6 min. with 20mM L-cysteine at different HCl concentration. (c) Kinetics of As(V) reduction by L-cysteine at 70 °C (solid squares) and room temperature (open circles). The samples were 190 μg/L As(V) solution reduced with 20mM L-cysteine and 0.03M HCl. Reduction time is in logarithm scale.

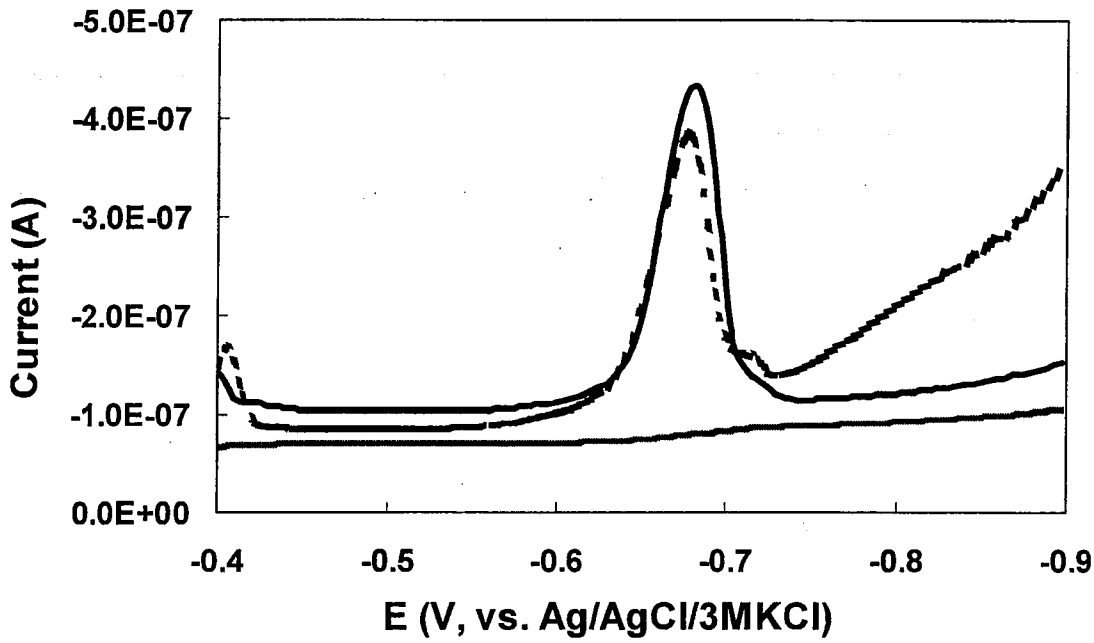


Figure 3.3 Voltammograms for a 190 μg/L As(V) solution treated with 20mM L-cysteine at 70 °C for 6 min. The shaded line is for a 190 μg/L As(V) solution only. The dashed line refers to a 190 μg/L As(V) solution with 4.6 mg/L Cu(II) and 1M HCl only. The solid line is the voltammogram of a 190 μg/L As(V) solution containing 4.6 mg/L Cu(II), 7.4 μg/L Se (IV), and 1M HCl.

3.2.5 Organic arsenic UV photooxidation with peroxydisulfate.

A 10 min UV irradiation alone converted only 60% of 50 μg/L MMA and 40% of 50 μg/L DMA to inorganic As. Rapid, quantitative conversion to As(V) is achieved using UV irradiation in the presence of peroxydisulfate [30, 87].

CSV signals for As were detected for 50 μg/L MMA or DMA solutions after a 10 min UV irradiation in the presence of 6 mM $Na_2S_2O_8$, only after L-cysteine reduction of the oxidation products. Similarly, UV photooxidation of an As(V) solution gave no arsenic response unless L-cysteine reduction was carried out.

Concentrations between 0.6 mM and 25 mM $Na_2S_2O_8$ quantitatively converted solutions containing 190 μg/L MMA and DMA individually, or 95 μg/L MMA and DMA each together, to As(V) in 10 min. A $Na_2S_2O_8$ concentration of 3 mM was used to study the effect of UV irradiation time on a DMA solution. Quantitative conversion of DMA to As(V) required 4 min, but to ensure the completeness of photooxidation, 6 min was taken as the optimum time.

3.2.6 Quantitative analysis

Results of the quantitative analysis of As(III), As(V), MMA and DMA after treatment are summarized in Table 3. 1.

Table 3. 1: Figures of merit for quantitative analysis using UV photooxidation with peroxodisulfate and L-cysteine reduction [a]

	Linear range (μg/L)	Calibration curve [d]	R^2	Detection limit [e] (μg/L)	Relative standard deviation (RSD, %) [f]		
					50 μg/L	10 μg/L	5 μg/L
As(III)	2.5 -- 190	y=3.23e-11 x +5.55e-10	0.9817	0.3	2.6	3.7	2.7
As(V) [b]	2.5 -- 190	y=3.69e-11 x +4.69e-10	0.9891	0.3	1.8	2.6	4.0
MMA [c]	2.5 -- 190	y=3.28e-11 x +6.49e-11	0.9988	0.3	6.2	7.1	8.0
DMA [c]	2.5 -- 190	y=3.28e-11 x +7.42e-11	0.9985	0.3	5.3	7.2	8.0

[a] 10 ml sample was analyzed by CSV with addition of 4.6 mg/L of Cu (II) and 7.4 μg/L Se(IV).

[b] L-cysteine treatment only

[c] UV photooxidation with peroxydisulfate and L-cysteine reduction

[d] The slope (unit is μg/L /A-V) of the plot of peak area, y, vs. concentration, x.

[e] The detection limit was determined by S/N >3 .

[f] Based on eight measuremen

3.2.8 Study of Interferences.

Potential interferences caused by ions commonly found in groundwater, especially Mn(II), Fe(II), and phosphate, were investigated. Interfering ions at various environmentally realistic concentrations were added to water samples followed by (a) L-cysteine reduction or (b) UV photooxidation with peroxydisulfate and L-cysteine treatment. The signals obtained were compared with those for which no interfering ion was added. Up to 120 μM and 200 μM, respectively, of Mn(II) and phosphate, were found to have no effect on the As(V)-L-cysteine reduction procedure. However, the As(III) CSV signal was depressed about 10% by 100 μM Fe(II).

To investigate interferences in the UV photooxidation procedure, a 50 μg/L DMA solution was used. Phosphate at concentrations up to 200 μM showed no interference. However, Fe(II) and Mn(II) are more easily oxidized by the peroxydisulfate than is DMA. These ions interfere at higher concentrations by consuming the oxidant. For a 10 min UV irradiation in the presence of 3 mM $Na_2S_2O_8$, addition of Mn(II) at 20 μM and 120 μM resulted in As(III) CSV signals about 75% and 4%, respectively, of that obtained in the absence of Mn(II). The DMA/Mn solution turned a brown/pink color, indicating formation of MnO_4^{2-}. Increasing the $Na_2S_2O_8$ concentration to 30 mM eliminated the Mn(II) interference up to a concentration of 100 μM. Similar results were obtained for Fe(II). At a $Na_2S_2O_8$ concentration of 30 mM, 100 μM Fe(II) resulted in an As(III) peak area about 90% of that in the absence of Fe(II).

We tried to apply this method in the field to brown-colored porewater samples with high dissolved organic material (DOM). However, peak response to As(III) and As(III)-thiolate was greatly depressed, which might be caused by adsorption of the

organic compound onto the HMDE surface. Natural groundwaters usually have dissolved organic carbon concentrations < 10 mg/L and at such levels the CSV response is not depressed. In field work where high DOM concentrations are found, we recommend sample dilution and standard addition to quantify arsenic concentration.

3.2.8 Arsenic speciation in Standard Reference Material and in spiked water samples.

The method was validated by analyzing (a) the NIST Standard Reference Material 1640 natural water standard, and (b) an As-free Queens College (QC) tap water sample and Lamont-Doherty Earth Observatory (LDEO) groundwater spiked with arsenic. The measurements were performed three times. The analytical results for the NIST sample were no detectable As(III) and 26.9 ±2.0 μg/L As(V) , compared to the certified value of 26.7 μg/L. The results for spiked samples are summarized in Table 3. 2.

Table 3. 2: Spiked tap water and groundwater analysis using L-cysteine reduction and UV-photooxidation with $Na_2S_2O_8$

Sample name	Spiked amount (μg/l)				CSV analysis(μg/l)		
	As(III)	As(V)	MMA	DMA	As(III)	As(V)	Org. As
QC Tap water	20	20	10	10	21.0 ±0.5	21.3±1.6	17.8±2.6
LDEO Groundwater	5	5	5	5	5.2 ± 0.2	5.9 ± 0.6	8.3 ± 0.7
	10	10	10	10	9.5 ± 0.3	11.6 ± 0.6	21.1 ± 1.3
	50	50	50	50	50.0 ± 2.1	50.4 ± 3.7	101.8 ± 4.0

Chapter IV

A pilot study of arsenic bioaccessibility in rice using *in vitro* gastric and small intestinal fluid sequential incubation

Dietary inorganic arsenic intake from food of terrestrial origin is of increasing concern because of its potential impact on public health even at low trace levels. Arsenic in rice, a staple food in many areas in the world, was studied in this project. Total arsenic of a dozen commercial rice samples was evaluated. In order to gain insight into arsenic bioaccessibility, a rice sample with highest arsenic concentration was chosen and further investigated. Bioaccessible arsenic extracted using different extraction methods, effects of arsenic-containing cooking water, and arsenic speciation were investigated in this chapter.

4.1 Introduction:

Dietary arsenic is abundant in seafood such as fish, shrimp, clams, and seaweed, in which it is present at concentrations at the mg/kg level. Fortunately, most As in seafood is in the form of non-toxic organic arsenicals such as arsenobetaine (AsB) and arsenocholine (AsC) [88-90]. There has been less research conducted on arsenic in foods of terrestrial origin, which have lower total arsenic content (at μg/kg level). However, of the total As in each, inorganic arsenic identified by chromatographic separation followed by As detection is present at a higher percentage in terrestrial foods such as rice [28, 91, 92], vegetables [93, 94], and chicken [3] than in seafood. While the identification of inorganic arsenic by available speciation methods may require further improvement, inorganic arsenic intake from foods of terrestrial origin may be a health concern, especially in areas where significant amounts of arsenic are already present in drinking water. Considering the greater toxicity of inorganic arsenic, three aspects of the problem should be addressed in investigating dietary sources of arsenic: (1) total arsenic

determination, (2) arsenic speciation analysis, and (3) study of the bioaccessibility of the toxic arsenic species.

In this project, rice was chosen for investigation because rice is a staple food in many areas of the world, including the US. In developing countries such as arsenic-affected Bangladesh and India, the normal daily adult rice consumption level is measured in kilograms. To investigate arsenic bioaccessibility resulting from the digestion process, we used *in vitro* gastric and small intestinal fluid (GSIF) sequential incubation, which mimics the digestion process occurring inside the human body. We realize GSIF sequential incubation is a simplified model system using only the major enzymes and controlling the main variables. The real digestion process is far more complex. But this method provides insight into the bioaccessibility of arsenic released from rice during digestion. This project is to be considered a pilot study, and clearly more work will be necessary to provide more information and better understanding of this topic.

4.2. Experimental:

4.2.1 Reagents:

Arsenic standards, As(III), As(V), MMA, and DMA, were prepared as described in section 2.1.1. Concentrated HNO_3 (Optima) and 30% H_2O_2 solution were purchased from Fisher Scientific (Pittsburgh, PA).

4.2.2 Gastric fluid

Artificial gastric fluid was prepared based on the US Pharmacopeia [95] and

modified as suggested by Ruby [96] by adding organic acid[96]. 1.0g NaCl (Fisher, Certified ACS) was dissolved in nanopure water and transferred to a 500 ml volumetric flask; 3.5 ml concentrated HCl (Fisher, trace metal) was added and the solution was diluted with nanopure water almost to the mark and shaken very well. Then 1.6g purified pepsin (1100 units/mg prot, from Porcine stomach mucosa, Sigma), 0.25g citric acid (monohydrate, Sigma), 0.25g DL-malic acid (98%, Avocado Research Chemical Ltd.), 210 μl lactic acid (Racemic, Sigma), and 250 μl acetic acid (Fisher) were added and the volume was finalized for immediate use.

4.2.3 Sample collection

Twelve husk-free US rice samples of different brands and types were bought at random from supermarkets and ethnic food stores in New York City in February 2004. Only two of these samples were labeled indicating the US state of origin. All the samples were dried in 55°C oven for at least 24 hrs. The moisture content of these samples was consistently about 8%.

4.2.4 Sample digestion for total arsenic measurement

About 10 to 20 grains of dried rice (~ 0.3 g) were transferred to a Teflon beaker. Three ml concentrated Optima HNO_3 was added and the rice soaked at room temperature for about 10 min. The rice samples were then digested on an adjustable temperature hot plate (Corning PC-35) in an acid-safe fume hood. The beaker was gently heated on the hot plate with temperature control set at "3" until evolution of the brown NO_2 fumes ceased and the solid grains were totally dissolved. Then 2ml of 30% H_2O_2 was added and

the beaker continuously heated until the solution was reduced to a small drop. The drop was diluted with 1% HNO_3 and quantitatively transferred into a 25 ml volumetric flask.

4.2.5 Pre-treatment of samples for speciation analysis and GSIF incubation

Dried raw rice was ground to powder using a coffee grinder. Cooked rice was prepared in a commercial rice cooker (Salton). About 2 grams raw rice were placed in a Teflon beaker, and 6 ml of nanopure or arsenic-containing cooking water was added. A batch of approximately 12 rice samples in their Teflon beakers was put onto the rack of the rice cooker and steam-cooked for 1hr. Rice was well-done. The weight of the cooked rice in each beaker was about 7.5 g. The rice samples were then cooled and squashed with a plastic spoon before further treatment.

4.2.6 In vitro gastric and small intestinal fluid sequential incubation

Aromatic American Basmati (#12 rice, see below) was chosen for GSIF sequential incubation because of its highest total arsenic concentration in all samples. Samples of ground raw rice or cooked rice were placed into 60 ml HDPE bottles and gastric fluid was added. Extractable arsenic was investigated at different ratios of gastric fluid (ml) / rice (g) , ranging from 6 to ~140, because incomplete extraction of arsenic from various solid matrices, including rice, with methanol/water has been reported previously [91, 97, 98]. The typical adult human physiological condition corresponds to a ratio of 10 mL/g, which was the ratio used for investigation of the bioaccessibility of arsenic in the cooking water in this study. A batch of rice samples (~2.5g each) with gastric fluid (25ml) was placed in an incubator shaker (New Brunswick Scientific Co.

Inc., series 25) for 2hr at 37 °C. This procedure simulates stomach digestion. After gastric fluid digestion, samples were either analyzed or subjected to small intestinal incubation. For the latter, samples were neutralized by adding 2.6 ml 1M $NaHCO_3$ solution, followed with addition of 0.42g bile extract salt (porcine, Sigma) and 0.12g pancreatine (from porcine pancreas, activity at least equivalent to U.S.P. specifications, Sigma). Samples were then incubated in the shaker at 37 °C for another 3 hr.

When GSIF sequential incubation was finished, a 10 ml sample from each HDPE bottle was transferred to a 15 ml centrifuge tube. Samples were centrifuged at least 3000 rpm for 20 min. Sample supernatant was filtered through a 0.2 μm syringe filter before analysis.

4.2.7 Instrumental analysis

Total arsenic was determined either by HR-ICP/MS (Thermo Elemental, Germany) at the Lamont-Doherty Earth Observatory (LDEO) of Columbia University or by GFAAS (Perkin-Elmer AAnalyst 800) at Queens College. The analytical procedures were same as those described in section 2.1.5.

Arsenic speciation was performed by ion chromatography. The system was comprised of a Dionex HPLC pump, a PRP X-100 (Hamilton, 250mm x 4.1 mm i.d., 10 μm particle size) separation column, and a guard column with same packing material connected between the injector and the separation column. The mobile phase at a flow rate of 0.8 ml/min was 10 mM $NH_4H_2PO_4$ adjusted to pH 8.2. Arsenic species were detected by on-line ICP/MS (VG-Plasma Quard, or Thermo Elemental, Germany) or off-line HR-ICP/MS (Thermo Elemental, Germany). For off-line detection, eluent

fractions were collected in 1 ml sample vials at the end of the separation column every 30 seconds.

4.3 Results and Discussion:

4.3.1 Total arsenic analysis

Total arsenic levels in the dozen commercial rice samples obtained in New York City was determined by HR-ICP/MS. The results are summarized in Table 4.1. The As concentration in these samples ranges from less than 100 μg/kg to nearly 600 μg/kg. Rice with the lowest arsenic concentrations was bought in Asian food stores. For two samples labeled "grown in U.S.", sample #9, from Texas, contained 136 μg/kg, and #12, from Arkansas, a gourmet Basmati rice, was surprisingly high at 574 μg/kg. Because of its high total arsenic concentration, rice sample #12 was chosen for the investigation of extractable arsenic using the different treatment methods and for evaluation of the bioaccessibility of arsenic in the cooking water as discussed below in sections 4.3.2 and 4.3.3.

Table 4.1: Total arsenic in market available rice samples bought in New York City:

Sample I.D.	Rice type	As concentration in dry rice (μg/kg)
1	Enriched long grain	193
2	Asian medium grain	64
3	Asian medium grain	73
4	Enriched long grain	185
5	Enriched medium	262
6	Whole grain brown	137
7	Extra long grain	111
8	Jasmine	96
9	Natural long brown	136
10	Enriched long, pre-boiled	154
11	Aromatic rice	91
12	Aromatic American Basmati	574

4.3.2 Evaluation of extractable arsenic by different treatment methods

Aromatic American Basamati (#12) raw rice powder was subject to nanopure water extraction, gastric fluid incubation, and gastric and small intestinal fluid sequential incubation. Total arsenic in the three extracts was measured by AAS to evaluate extraction efficiency. The percentages of extractable arsenic at the different liquid/ solid ratios (ml/g) are summarized in Fig. 4.1. For all three methods, extractable arsenic generally increases with increase in the liquid/solid ratio. GSIF sequential incubation has

higher extractable arsenic than both water extraction and gastric fluid incubation, with ~ 90% soluble arsenic at liquid/solid ratio of 120 mL/g. The highest extractable arsenic by water extraction is about 50% at liquid/solid ratio of 130 mL/g. [91, 97, 98] For low liquid/solid ratios (~6 mL/g to ~12 mL/g), there is not much difference in extractable arsenic between gastric fluid incubation and water extraction, both of which extracted less than 20%. At high liquid/solid ratios, extractable arsenic by gastric fluid incubation greatly increased, for example more than 80% at a ratio of 130 mL/g.

However, we should note that such a high ratio is not achieved in the real digestion process after a meal. The common physiological liquid/solid ratio is about 10 mL/g [96]. At this ratio, extractable, i.e. bioaccessible defined here, arsenic by GSIF incubation is about 35%, which is higher than that obtained by water extraction and gastric fluid incubation only, which are less than 20%. Enzymes digesting starch and protein in gastric fluid and small intestinal incubation solution might contribute to arsenic release from rice samples during incubation. More enzymes and longer sample treatment time by GSIF sequential incubation may result in release of more arsenic. After ingestion, extractable forms of inorganic arsenic are absorbed extensively from the gastrointestinal tract of humans and most laboratory animals [99].

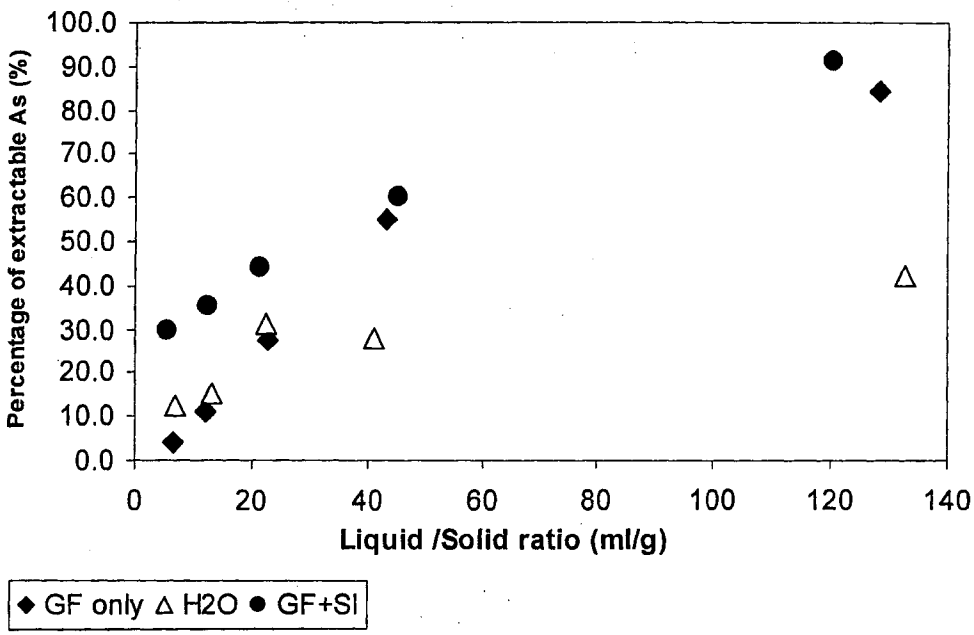


Figure 4.1. Extractable arsenic in raw rice by water extraction, gastric fluid, and gastric and small intestinal fluid sequential incubation at different liquid/solid ratios

4.3.3 Bioaccessibility of As in As-containing cooking water

Cooking water is another important factor that should be considered for arsenic risk assessment, especially in areas with high levels of arsenic in drinking water. Bioaccessibility of As in cooking water was investigated by cooking rice with arsenic-spiked nanopure water followed by GSIF incubation. Cooking water with different levels of As(III), As(V) and DMA, ranging from 0 to 4000 μg/L, the lowest and highest arsenic concentrations found in Bangladesh groundwater, were used. The liquid/solid ratio of 10 mL/g was used in GSIF incubation to simulate the physiological digestion process. The % recovery of total arsenic from the spiked cooking water was measured by AAS, and it was found as shown in Figure 4.2 that, surprisingly, around 40% of the arsenic from the cooking water was recovered, i.e. about 40% was bioaccessible, contrary to the conventionally held notion in health risk assessment [100] that arsenic in cooking water is 100% available[100]. The reason for low extractable arsenic in rice cooked by arsenic laced cooking water is not clear. We assumed that arsenic is absorbed by rice during cooking, which resulted in the difficulty of release in the digestion process.

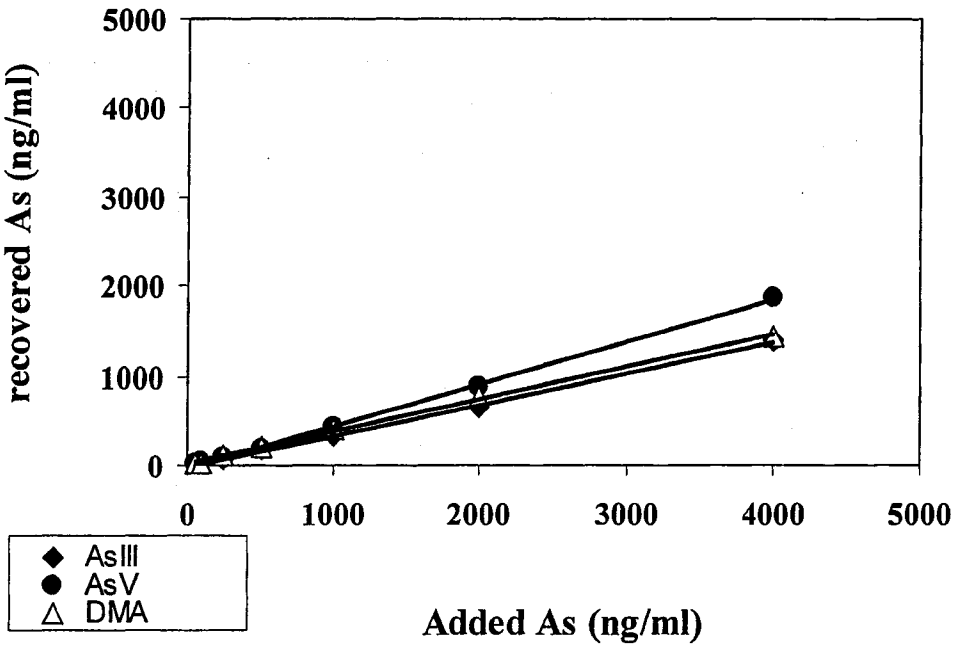


Figure 4.2 Evaluation of bioaccessibility of arsenic in cooking water by mass recovery.

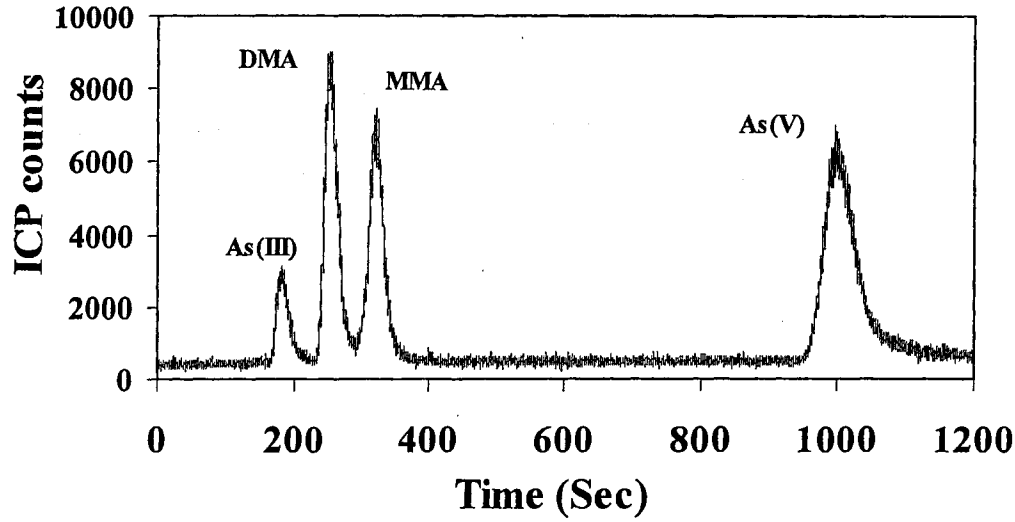
4.3.4 Arsenic speciation

Arsenic speciation analysis was achieved by IC separation coupled with ICP-MS detection. Figure 4.3(a) shows a typical chromatogram obtained for a arsenic standard solution containing 100 μg/l each As(III), As(V), MMA and DMA. The detection limits (S/N > 3) using the VG-Plasma Quard on-line detection for a 10 μl sample injection were 7μg/l , 10μg/l , 5μg/l and 5μg/l respectively.

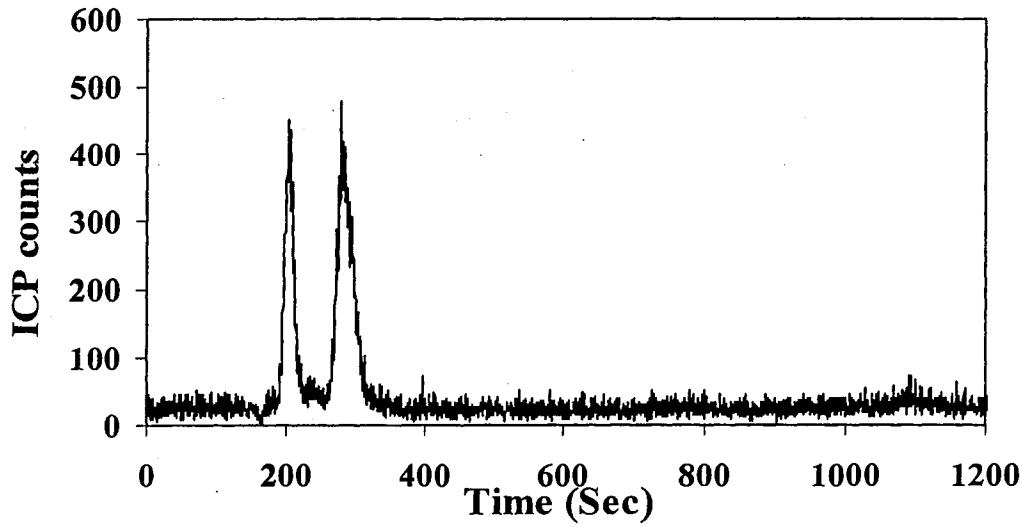
The speciation separation of GSIF extractant of aromatic American Basamati (#12) raw rice powder by IC followed by HR-ICP-MS determination showed that major arsenic species in this rice sample were inorganic As(III) and organic DMA (Figure 4.3 (b)), with extractable concentration of 106 μg/kg and 158 μg/kg, respectively. Extractable MMA and As(V) were not observed in this sample. The total extractable arsenic by this measurement accounted for 35% of total arsenic.

Another preliminary speciation test of a rice sample obtained in November, 2003 and treated by gastric fluid incubation followed by IC separation and off-line HR-ICP-MS detection indicated that major arsenic species (over 50%) in that sample were inorganic As(III) and As(V). Results obtained from two samples showed that different types of rice have different arsenic species. That might be true that arsenic species varies between rice with different growth origin, type, etc. Another possibility is species conversion might happen during sample storage. So far, we don't have any knowledge on the stability of the inorganic As(III) extracted by GSIF. As(III) is well known to be unstable and easily oxidized to As(V) by oxygen in air in natural water samples due to its reducing status, but we don't know if it is also true for inorganic As(III) in biological samples. Stability of As(III) in GSIF samples during sample storage,

therefore, is worth investigating. In order to have more comprehensive knowledge of arsenic species in rice as well as their health impact, more detailed work is surely necessary in future work.



(a)



(b)

Figure 4.3 (a) IC-ICP/MS chromatogram of arsenic standard mixture containing 50 μg/l As(III), 100 μg/l each DMA, MMA, and As(V). Mobile phase was 10 mM $NH_4H_2PO_4$ at pH 8.2. Flow rate was 0.8 ml/min. (b) IC-HR-ICP/MS chromatogram of #12 rice sample treated by GSIF incubation. The elution order of the two peaks is As(III) and DMA.

4.4 Summary and future work

The major findings of this project are: (1) hundreds of μg/kg levels of arsenic were found in rice samples bought from local US markets; (2) about 35% As is bioaccessible at *in vitro* physiological condition; and (3) around 40 % As in cooking water was bioaccessible; (4) inorganic arsenic were found to be major species of bioaccessible arsenic. For better understanding of arsenic components in different rice samples and arsenic dietary toxicology, more speciation work is of great necessity in future research.

Appendix:

Total arsenic concentrations in the seven rice samples collected in Bangladesh are summarized in Table 4.2. Arsenic levels in the rice husks is several times higher than that in grain, a result consistent with that of Abedin, *et al.* [101].

Table 4.2 Total arsenic in Bangladesh rice samples:

Rice sample	μg/kg
RA-1A grain	269
RA-1 A grain +husk	338
RA1-A husk	700
RA-1B grain	239
RA-1C grain	153
RA-1 Amon grain	148
RA-1 Amon grain+husk	165
RA-1 Amon husk	250
RA-2A grain	357
RA-2A grain+husk	512
RA2-A husk	1470
RA-2B grain	278
RA-2C grain	91

Note:

(1) All rice samples were collected from 2 different sites in Bangladesh in October 2003. Three retained the husk on the grains.

(2) ~0.3 g rice or ~ 20 mg rice husk removed from grains were used for analysis.

(3) Amon means wet season.

Chapter V

Differential Pulse Cathodic Stripping Voltammetric Determination of Nanomolar Levels of Dissolved Sulfide Applicable to Field Analysis of Groundwater

5.1 Introduction

Various methods are available for sulfide analysis. Dissolved sulfide in the concentration range of μM to mM can be determined by electrochemical titration using Hg(II) or Pb(II) as titrant with a silver/silver sulfide indicator electrode [102, 103]. The standard colorimetric method is based on methylene blue generated from the reaction of sulfide with N,N-dimethyl-p-phenylenediamine under acidic conditions [Cline, 1969 #897; Lindsay, 1988 #1144]. Nanomolar levels of dissolved sulfide in oxic surface water were measured by solid phase extraction of methylene blue followed by a high performance liquid chromatographic (HPLC) separation and spectrophotometric detection [104]. Nanomolar levels of dissolved sulfide in pore water, and sub-□M levels of sulfide in serum can be separated and detected by using ion chromatography (IC) with electrochemical detection [105, 106]. Sub-pM detection limits were obtained by helium stripping of H_2S from large quantities of acidified water samples followed by cryogenic trapping and gas chromatography with flame photometric detection [107]. Although chromatographic methods have low detection limits, they generally require complex and time-consuming sample preparation procedures, and are not easily adapted to field analysis. It is important to note that reliable measurement of dissolved sulfide in environmental samples requires determination immediately on sample collection because of the rapid oxidation rate of sulfide by dissolved oxygen [108].

Among the highly sensitive (sub-nM detection limit) sulfide analytical techniques, cathodic stripping voltammetry (CSV) is the most suitable method for field analysis because of its relatively simple and portable instrumentation, lack of required sample preparation, rapid analysis time (< 10 minutes per sample), small sample volume,

and ease of quantification. Oceanographic applications using CSV techniques include the determination of sulfide in sea water and pore water from marine sediments [109-111]. To the best of our knowledge, however, no one has applied CSV to the determination of dissolved sulfide in groundwater. The sulfate-reducing groundwater that is of interest here is a more complicated matrix because of the presence of dissolved Fe with trace levels of dissolved sulfide [Bottrell, 20000 #1019].

Recently, it has been shown that conventional batch-mode CSV suffers from the loss of dissolved sulfide by reaction with the waste mercury pool in the voltammetric cell during the analysis [80]. As shown in Figure 5.1, we have confirmed this observation. To overcome the loss of sulfide, Al-Farawati and van den Berg [80] constructed a flow-cell system with in-line purging. Here, we show this problem can be solved by simply using a fresh aliquot of sample for each voltammetric scan. This minimizes the loss of sulfide to mercury by limiting the time for sulfide-mercury interaction as well as reducing the quantity of waste mercury in the cell. A standard CSV apparatus is used, with no need to manufacture a special voltammetric cell or complex sample delivery and purging systems. We studied factors that influence sulfide stability in voltammetric cell, as well as the effect of the electrolyte medium on the determination of sulfide in reducing groundwaters containing dissolved Fe. We applied the method in the field to groundwater samples obtained at a landfill site in Winthrop, Maine.

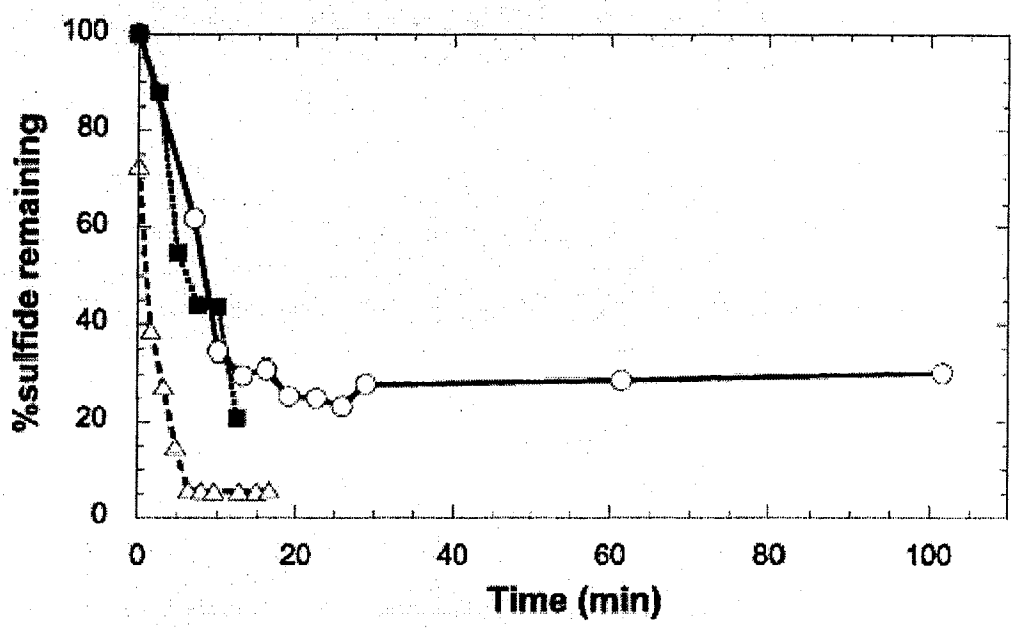


Fig. 5.1 Percentage of sulfide remaining in solution in contact with free Hg as a function of contact time. Open circles represent 1M NaOH + 0.09M Na_2EDTA spiked with 1mM sulfide. Na_2EDTA was added to the NaOH buffer with the intention to form a complex at the surface of the waste Hg but sulfide loss was not prevented. Solid squares represent reducing groundwater in $Na_2CO_3/NaHCO_3$ supporting electrolyte. Voltammetric conditions used in this study are described in Section 5.2.5. Open triangles represent seawater spiked with 100nM sulfide [80].

5.2 Experimental

5.2.1 Instrumentation for DPCSV

The Eco Chemie μAutolab voltammetric apparatus was the same as that used for arsenic speciation, except that the Metrohm 663VA electrode stand was controlled by a personal computer running Eco Chemie GPES 4.4 software. The electrodes include a hanging mercury drop electrode (HMDE) as the working electrode, a Pt auxiliary electrode, and a Ag/AgCl/3M KCl reference electrode. A built-in motor-driven stirrer drives a PTFE stirring rod to stir the sample by manual or automatic control.

5.2.2. Reagents and solutions

5.2.2.1 Supporting Electrolyte Solutions:

(a) NaOH (0.005M) supporting electrolyte (pH 12) was prepared by dissolving 0.4130 g NaOH (Sigma Ultra-pure grade) in 2000 ml of 18 MΩ-cm nanopure water (Barnstead Infinity Nanopure system).

(b) $Na_2CO_3/NaHCO_3$ supporting electrolyte (pH 8.3) was prepared by dissolving 0.0094 g of Na_2CO_3 anhydrous (ACS grade, Fisher Scientific) and 0.3205 g of $NaHCO_3$ (ACS grade, Fisher Scientific) in 2000 ml of 18 MΩ-cm nanopure water.

(c) Supporting electrolyte (b) diluted with an equal volume of aged and filtered oxic groundwater. This groundwater was produced by letting a covered sample of anoxic groundwater from the Winthrop site stand for several weeks until diffusive uptake of atmospheric oxygen oxidized all the dissolved Fe(II) to Fe(III), which precipitates.

5.2.2.2 Sulfide Standard Solutions:

A primary sulfide standard solution (0.1M) was prepared by dissolving 2.4018 g $Na_2S•9H_2O$ (ACS grade, Aldrich) in 100 mL of nitrogen-purged NaOH electrolyte medium. The secondary sulfide standard solution (50 μM) was freshly prepared daily by diluting the primary sulfide standard solution with the nitrogen-purged NaOH supporting electrolyte. Sulfide solutions are more stable at high pH.

5.2.2.3 Pb(II) titrant:

A primary Pb(II) titrant (0.1M) was prepared by dissolving 4.6014g $Pb(ClO_4)•3H_2O$ (ACS grade, Aldrich) in 100ml nanopure water. A secondary Pb(II) titrant solution (0.5 mM) was prepared by diluting the primary Pb(II) titrant with nanopure water.

5.2.2.4 Sulfide Anti-Oxidation Buffer (SAOB) solution:

SAOB was prepared by dissolving 18g NaOH (Sigma Ultra grade), 72g sodium salicylate (ACS grade, Fisher Scientific) and 16.2g ascorbic acid (ACS grade, Fisher Scientific) in 1 liter of 18 MΩ-cm nanopure water. The solution was stored in a refrigerator (4 °C) and could be used for ~ 1 week after which the solution turned dark brown and was discarded.

5.2.3. Electrochemical titration of sulfide standard

The secondary sulfide standard was standardized immediately after preparation by electrochemical titration using Pb(II) as titrant and a silver/silver sulfide indicator

electrode (Orion) connected to an Orion model 250 pH/mV meter. Ten ml of the secondary sulfide standard solution and 10 ml of the SAOB solution were pipetted into a 25 ml Teflon beaker containing a magnetic stirrer bar and titrated with the 0.5mM standard $Pb(ClO_4)_2$ solution using a 2ml micro-burette. The end-point of the titration is indicated by a rapid decline in the potential reading. The sulfide concentration is calculated from the moles of Pb titrant used divided by the volume of sulfide standard titrated.

5.2.4. Groundwater sampling

A 20 ml polypropylene syringe (Aldrich) with a three-way luer lock valve (Cole-Parmer) was employed for groundwater sampling from wells installed on the site. The syringe was flushed with groundwater at least three times, and studious care taken to exclude air bubbles. The sample was placed in an ice-chilled cooler during the ten minute transport between the groundwater well and the DPCSV instrument and was analyzed immediately.

5.2.5. Analytical procedure for DPCSV

Between analysis of each sample and standard, the voltammetric cell was rinsed with nanopure water at least three times and dried using Kimwipes. The cell was acid-cleaned daily to prevent Fe oxyhydroxide and Fe sulfide precipitates from accumulating in the cell. The supporting electrolyte background was monitored daily and found to have a negligible amount of sulfide. Groundwater samples were analyzed as follows: 5.0 ml of the $Na_2CO_3/NaHCO_3$ electrolyte medium (b) was pipetted into the

voltammetric cell. The solution was then purged with purified nitrogen to remove oxygen, with the purge time set for 300 s. In order to override the instrument's automatic introduction of mercury drops at the end of the set purge time (the instrument quickly releases four Hg drops, knocking each off, and then a fifth drop, which is the working HMDE), purging was stopped after 297 s by aborting the run. This prevents Hg from being present in the cell while the sample is introduced and stirred. At this point, 5.0 ml of the groundwater sample was injected through a luer-lock sample injection port installed in the Teflon lid of the cell, without opening the cell to the atmosphere. The solution was then mixed by stirring for 30 s without purging. The purge time was then re-set to zero seconds and the program re-started to perform DPCSV by starting with deposition directly and followed by equilibration and scan. Such a protocol minimizes the contact time between the waste Hg drop with sample. DPCSV analysis was performed using a deposition potential of -0.45V applied for 60 s with stirring, during which time the sulfide undergoes an adsorptive interaction with mercury on the surface of the HMDE to deposit insoluble HgS on the electrode [112, 113]. After a 10 sec equilibration time, the cathodic stripping potential ramp from –0.3 to –1.2V vs. Ag/AgCl reference electrode was applied with a 10mV step potential, 50 mV modulation amplitude, 33.3 msec pulse width, and 16.7 msec measurement time. The cathodic peak corresponding to the reduction of deposited HgS from the mercury surface appeared at around –0.65 V, as shown in Figure 5.2 in three different electrolyte mediums. Quantitation was based on the peak area calculated by the instrument' s integration protocol.

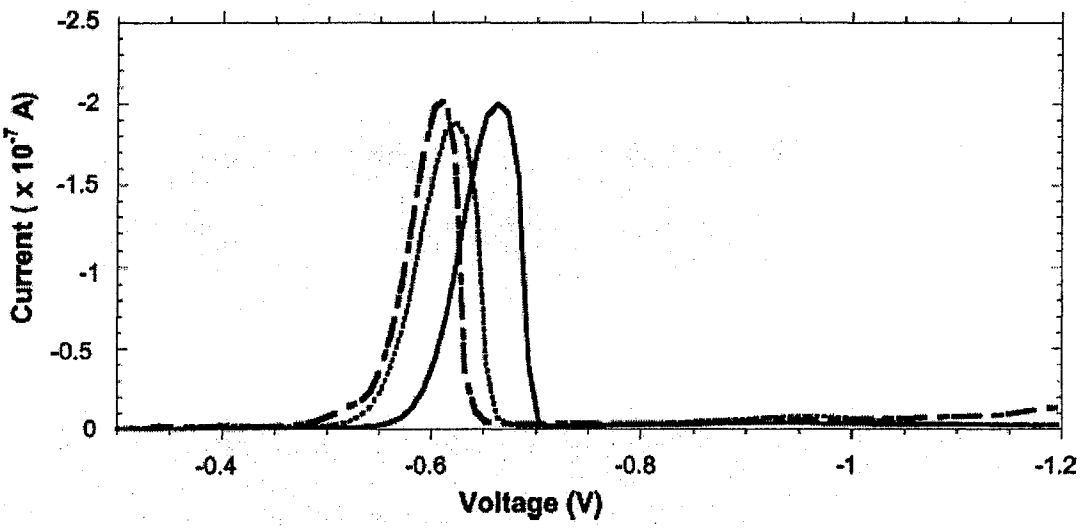


Fig. 5.2. DPCSV voltammograms of 300nM sulfide in different supporting electrolytes. Solid line represents NaOH at pH 12; dotted line represents $Na_2CO_3/NaHCO_3$ at pH 8.3; dash dotted line is $Na_2CO_3/NaHCO_3$ at pH 8.3 mixed in the ratio of 1:1 with oxic groundwater.

The procedure for analyzing sulfide standards was similar except that 10 ml of medium was used, and the secondary sulfide standard solution was added to the cell by quickly removing the plug from the port on the cell lid, pipetting in the sample using a micropipetter, and replacing the plug.

5.2.6. Determination of ORP

The ORP was determined using a platinum combination electrode with Ag/AgCl as reference electrode (Orion 9678) connected to a pH/mV meter (Orion model 250A+) following a procedure described by Walton-Day et al. [Walton-Day, 1990 #1143]. All values were reported as ORP relative to Ag/AgCl reference electrode, not to standard hydrogen potential.

5.3 Results and Discussion

5.3.1 Effect of supporting electrolyte

Three supporting electrolytes were evaluated. The NaOH medium (a) was used previously for CSV analyses of sulfide standards[112]. A potential advantage of using a high pH medium is that sulfide exists as S^{2-} which is less subject to loss by volatilization than the sulfur species that exist at lower pH values, HS^- or H_2S. However, since groundwaters often contain dissolved Fe(II) at concentrations up to the mM range, we were concerned about the potential interference in the determination by precipitation of $Fe(OH)_2$. The $Na_2CO_3/NaHCO_3$ supporting electrolyte (b) has a pH of 8.3, at which $Fe(OH)_2$ will not form [114] and >95% of the sulfide is present as HS^- [115]. The third medium, carbonate plus aged oxic groundwater (c), provided a medium similar to that of

the groundwater samples, although not chemically reducing. The aged oxic groundwater sample was acidic, which resulted in the pH of the 1:1 mixture to drop to 7.0. Stripping voltammograms of a 300 nM sulfide standard using these supporting electrolytes are shown in Figure 2. There is a small cathodic pH-induced shift in the peak potential and a small increase in peak height for the NaOH medium [116].

Calibration plots in the three media produced by standard additions of the secondary sulfide standard to the supporting electrolyte in the cell had quite similar slopes, 4.24×10^{-11} for medium (a), 3.46×10^{-11} for (b), and 3.70×10^{-11} for (c), with linear regression correlation coefficients of 0.9926, 0.9948, and 0.9939, respectively. However, linearity was observed to 800 nM for (a), 1.2 mM for (b), and 1.6 mM for (c). Linearity in the NaOH medium is probably higher, but for concentrations of sulfide higher than 800 nM, the instrument gave an current overload message. The wider linear range of supporting electrolyte (c) makes it the medium of choice for the analysis of groundwater samples. The limits of detection in the three media calculated based on 3σ are 1.3 nM, 5.5 nM and 4.0 nM respectively .

To compare the stability of the sulfide solutions at pH 12 and pH 8.3, two supporting electrolytes were spiked with 20 nM sulfide and purged with nitrogen for periods up to 120 sec. As shown in Figure 5.3, about 60% of the sulfide remained in the cell containing the $Na_2CO_3/NaHCO_3$ medium after 120s of purging, whereas about 85% remained in the NaOH supporting electrolyte. However, in our procedure, no purging is performed in the presence of sulfide so we expect all the sulfide in groundwater samples will be retained. To estimate the method precision, seven replicate analyses of an 80 nM sulfide standard in supporting electrolyte (a) yielded a peak area RSD of 2.5%.

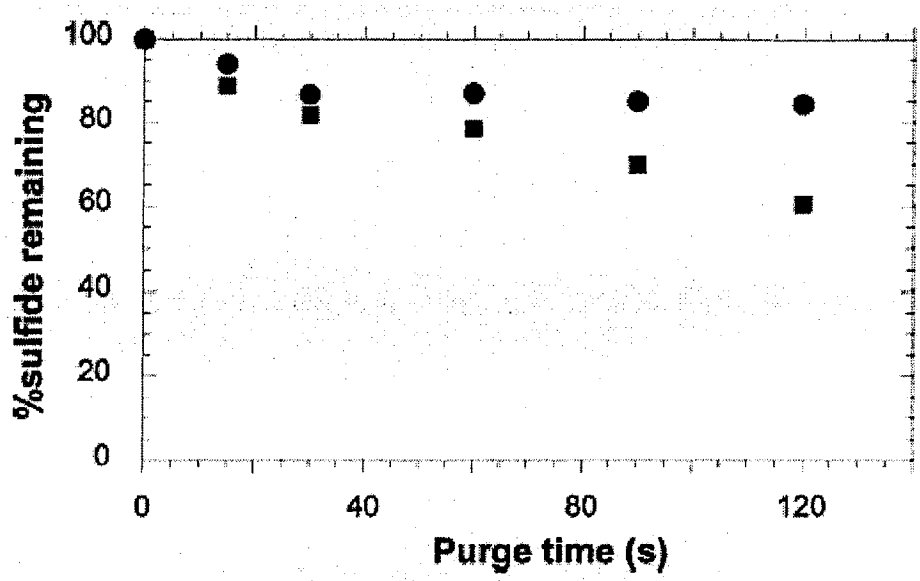


Fig. 5.3. Percentage sulfide remaining after nitrogen purge as a function of purge time. Solid circles are NaOH at pH 12 supporting electrolyte, while solid squares are $Na_2CO_3/NaHCO_3$, pH 8.3 matrix, with both spiked initially with 80nM sulfide.

5. 3.2 Factors contributing to sulfide loss in the voltammetric cell

In conventional batch mode analysis, the amount of waste mercury in the cell and the contact time of sulfide in sample and this waste mercury are usually not controlled. Al-Farawati and van den Berg [80] postulated that the main pathway for loss of sulfide to the waste mercury in the voltammetric cell during batch-mode analysis is precipitation of HgS due to contact of sulfide and mercury. The source of the Hg^{2+} ions is presumably the oxidation of metallic Hg by traces of oxygen in the cell. However, it was not clear whether the important factor in suppressing the sulfide signal is the amount of waste mercury in the cell or the contact time of sulfide and mercury.

To investigate the effect of mercury on the loss of sulfide, two experiments were performed to simulate a conventional batch-mode standard addition analysis. First, different amounts of Hg (0, 5, 15, 20 and 25 drops) were introduced into the cell containing 20nM sulfide solution as the first, second, and subsequent runs of conventional analysis. Multiples of 5 drops were used because the instrument automatically introduces 5 drops of mercury in each run. During each run, there is always a presence of 4 drops of waste Hg as described above (section 5.2.5) when the working HMDE is introduced. Sulfide was determined for each after contact times of 30 sec and 150 sec. As shown in Figure 5.4 A, the sulfide peak area remained fairly constant at both contact times, although the amount of sulfide remaining is in general less after 150 sec. Secondly, with 20 drops of waste mercury in cell, the contact time of sulfide and mercury was varied from 30 sec to 1230 sec. The sulfide peak area decreased rapidly with the increase of contact time, as shown in Figure 5.4 B. Presumably the slow diffusion of oxygen into the cell causing oxidation of the Hg is

more significant than the increased surface area of free Hg, i.e. the increase in the amount of mercury. We conclude that if the contact time of sample sulfide and waste mercury is minimized, batch-mode DPCSV can be applied to the quantitative analysis of groundwater samples even in the presence of small amounts (4 drops) of waste mercury.

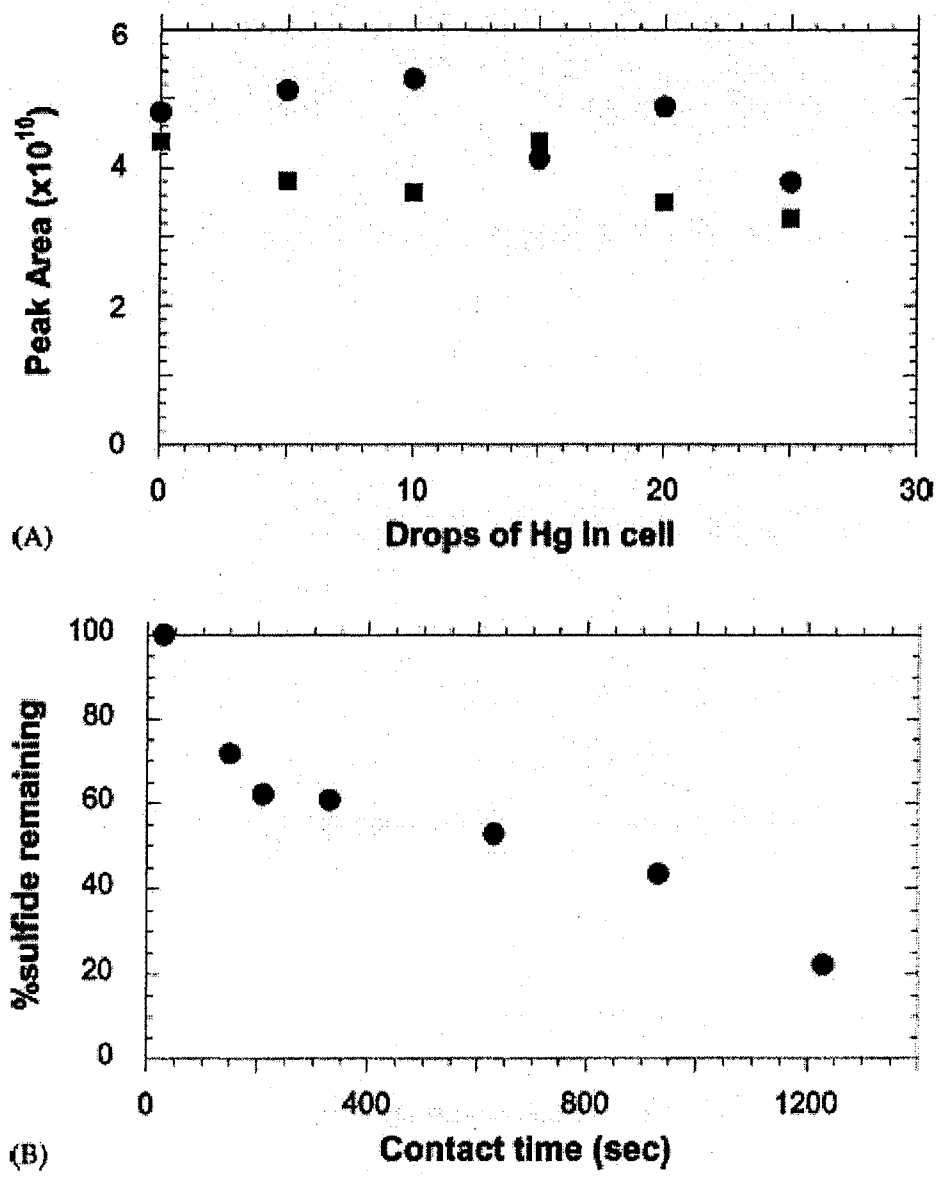


Fig. 5. 4. (A) Effect of the amount of mercury in contact with 20nM sulfide standard in NaOH medium. Solid circles are for 30 s contact time, and solid squares are for 150 s contact time; (B) effect of contact time of sulfide and mercury on percentage sulfide remaining, when 20nM sulfide standard was spiked to NaOH electrolyte plus 20 drops of mercury initially in the cell.

5.3.3 Method application to groundwater analysis

Groundwater samples from multi-level observation wells and extraction wells inside (Table 5.1 a) and in the immediate vicinity (Table 5.1 b) of a closed and capped municipal landfill located about 20 km south west of Augusta, ME in the town of Winthrop, were quantified for sulfide concentrations by DPCSV on site in July of 2001. The subsurface water is strongly reducing and high in dissolved Fe(II). The precision based on six replicate analyses of the extraction well sample (44 mg/L dissolved Fe) yielded a RSD of 4.5%. The extraction well was chosen to evaluate groundwater sulfide analysis precision due to the ease of sampling of this well compared to other observation wells because the extraction well was continuously pumped for the site's pump and treat facility. The poorer precision than that obtained for the sulfide standard (2.5%) reflects the use of a syringe to introduce the sample into the cell vs. the more precise micropipetter for the standards.

A wide range of sulfide concentrations (< 4 nM to 3254 nM), together with ORP values are reported in Table 5. 1. Concentration of sulfide increased with decreasing ORP value, indicating that a reducing environment favors the production of sulfide via sulfate reduction.

Table 5.1a: Characterization of Groundwater from Multilevel Wells Beneath a Landfill in Maine, July 2001

Well ID	level	Date	Depth (m)	pH	Temp (°C)	ORP (mV)	Fe(II)[a] (mg/l)	Sulfide[b] (nM)	Sulfate (μM)
EW2		7/12/2001	16.2	6.5	11.8	-112	44	470	n.a.
OW1		7/11/2001	20.5	6.9	11.7	-131	33	175	n.a.
OW11	1	7/09/2001	18.6	6.3	15.7	-105	45	27	1758
OW11	2	7/09/2001	17.1	6.3	16.7	-96	32	46	1513
OW11	3	7/09/2001	15.5	6.2	21.3	-89	27	59	960
OW11	4	7/09/2001	14.0	6.2	19.6	-51	30	n.d.[d]	1231
OW11	5	7/09/2001	12.5	6.3	16.9	-81	38	n.d.	1444
OW12	1	7/10/2001	20.6	6.5	13.6	-136	49	278	1288
OW12	2	7/10/2001	18.0	6.6	14.2	-129	46	356	970
OW12	3	7/10/2001	16.5	6.7	15.2	-131	35	226	505
OW12	4	7/10/2001	14.9	6.7	14.0	-143	34	486	499
OW12	5	7/10/2001	13.4	6.8	14.9	-144	35	648	519
OW12	6	7/10/2001	11.9	6.8	15.6	-138	36	662	420
OW13	1	7/09/2001	19.5	6.4	15.1	-109	47	29	382
OW13	2	7/09/2001	17.4	6.4	15.7	-112	46	60	1290
OW13	3	7/09/2001	15.9	6.4	15.3	-121	n.a.[e]	160	1079
OW13	4	7/10/2001	14.3	6.6	14.7	-146	27	407	788
OW13	5	7/10/2001	12.8	6.7	15.4	-151	33	657	605
OW13	6	7/10/2001	11.3	6.8	17.4	-147	39	519	501
OW14	1	7/09/2001	18.6	6.5	16.0	-91	38	61	953
OW14	2	7/09/2001	16.2	6.6	18.8	-98	39	141	971
OW14	3	7/09/2001	14.6	6.5	16.5	-102	32	14	915
OW14	4	7/09/2001	13.1	6.7	19.4	-121	30	n.d.	551
OW14	5	7/09/2001	11.6	6.8	17.6	-51	32	n.d.	808
OW15	2	7/10/2001	14.3	6.7	17.6	-134	33	83	1106
OW16	1	7/09/2001	20.4	6.5	14.3	-117	50	380	1084
OW16	2	7/10/2001	18.3	6.5	14.5	-109	50	476	1353
OW16	3	7/10/2001	16.8	6.6	14.4	-130	n.a.	546	1170
OW17	1	7/10/2001	19.2	6.5	14.5	-106	55	621	1118
OW17	2	7/10/2001	17.1	6.9	16.1	-110	46	857	1123
OW6		7/12/2001	10.0	6.7	15.0	-168	41	531	384
OW7		7/10/2001	9.9	6.5	12.7	-115	52	137	331

Table 5.1b, Characterization of Groundwater from Multilevel Wells Outside the Landfill in Maine, July 2001

Well ID	Date	Depth (m)	pH	Temp (°C)	ORP (mV)	Fe(II)[a] (mg/l)	Sulfide (nM)	Sulfate (μM)
MW8A	7/08/2001	34.0	7.4	10.7	n.a.	6	142	1500
MW8B	7/11/2001	29.1	6.8	11.0	-146	60	49	7104
MW8C	7/08/2001	12.2	5.5	14.5	n.a.	0.2	12	5698
MW212A	7/11/2001	38.5	8.1	12.3	-215	n.d.	307	250
MW212B	7/11/2001	22.7	6.4	12.4	-130	37	12	10500
MW212C	7/11/2001	10.7	5.9	11.4	116	0.1	n.d.	800
MW12	7/11/2001	6.1	7.2	7.5	44	n.d.	n.d.	150
MW13A	7/10/2001	10.7	6.4	9.4	118	n.a.	n.d.	n.a.
MW211A	7/11/2001	26.5	10.6	8.5	-321	1	3254	50
MW211B	7/11/2001	9.8	7.6	8.8	-164	2	719	n.d.
MW204A	7/12/2001	29.1	9.0	9.0	-285	1	112	146
MW204B	7/12/2001	13.7	8.1	8.8	-206	1	70	100

Note:

[a] The Fe(II) inside landfill data were measured in April, 2001 and outside landfill data were obtained in July, 2000

[b] Sulfide concentration outside landfill is different from that reported in previous work (17) because the calibration slope used in previous work was based on standard addition calibration to groundwater inside landfill. The slope of groundwater inside groundwater was depressed because of polysulfide, resulting overestimation of sulfide concentration.

[d] n.d. means non-detectable (< 4nM).

[e] n.a. means not available

Chapter VI

Investigation of Fe(II) (Bi) Sulfide interaction by Cathodic Stripping Voltammetry in groundwaters and laboratory solutions

6.1 Introduction:

The formation of insoluble metal sulfides plays a critical role in metal and sulfur cycling. It has long been recognized that precipitation of and/or adsorption onto highly insoluble sulfides are effective pathways to remove many dissolved metal species (e.g. As, Cd, Co, Cu, Fe, Mn, Mo, Ni, Pb, Sb, Zn) that are important to water quality [117, 118]. Studies of metal-sulfide interactions are of interest because they may also affect the bioavailability of trace metals [119, 120]. In addition, recent findings indicate that better understanding of sulfide interactions with metals is much needed because metal (bi)sulfide complexes (a) affect the solubility of metal sulfide precipitates and (b) are the intermediates in the formation of sulfide minerals[120]. Soluble metal (bi)sulfide complexes have been found in various water environments, such as oxic marine waters[116], pore water[121], river waters[122], and anoxic lake waters [123].

Although sulfur species could significantly influence environmental mobility and bioavailability of metals and metalloids in groundwaters by stabilizing both insoluble sulfide minerals and soluble aqueous species [124], no previous studies have investigated dissolved labile reduced sulfur species in groundwater. This probably has been the case to some extent because conventional sulfide analysis methods [125] are not capable of on-site measurement of these species at trace levels (<1 μM). This limitation was recently overcome through application of the differential pulse cathodic stripping voltammetry (DPCSV) method [126].Yet metal–sulfide interactions in groundwater systems have not been investigated by DPCSV, to our knowledge.

We carried out a DPCSV investigation of the dissolved sulfide system in groundwaters obtained from two sites, Winthrop, Maine and Vineland, New Jersey.

Groundwater from both sites containing elevated dissolved As concentrations were reducing and *in situ* remediation through formation of insoluble As-sulfide may be a viable option. Groundwaters from investigated sites displayed a wide range of dissolved Fe(II) concentrations (from non- detectable to ~ 60 mg/L), and resulted in three different types of sulfide voltammograms. Therefore, laboratory experiments were conducted to investigate Fe(II) - (bi)sulfide voltammetric response at different Fe(II) and sulfide levels that were encountered in groundwater systems in the field. This allows a qualitative explanation of different types of sulfide voltammograms observed in the field and identification of different Fe-sulfide species. In addition, it provides the basis to assess saturation state with respect to amorphous FeS minerals and to estimate the stoichiometry of dissolved Fe-sulfide species.

6.2 Experimental:

6.2.1 Reagent:

Preparation of sulfide stock and working standards, and the pH 8.3 $Na_2CO_3/NaHCO_3$ medium was the same as described in section 5.2.2. Fe (II) working solutions were freshly prepared by dissolving $FeCl_2 \cdot 4H_2O$ (Fisher) in nanopure water and spiked into samples to achieve the desired concentrations.

6.2.2. Instrumentation and analysis

Instrumentation and analysis procedures are the same as sulfide determination described in section 5.2.1, except in this study the range of scan was expanded to – 1.7 V in some cases.

6.2.3 Field site descriptions:

6.2.3.1 Winthrop, Maine

Highly reducing groundwaters below the central area of the landfill are pumped into a treatment facility to remove dissolved iron (~50 mg/L) and arsenic (100-400 μg/L) by intensive oxidation, using large quantities of sulfuric acid, and then re-injected at a few wells on the perimeter of the landfill. Groundwater samples were collected from wells installed inside and outside the landfill .

6.2.3.2 Vineland, New Jersey

This site previously was occupied by a chemical company that manufactured arsenic-based herbicides. The improper storage of byproduct arsenic salts resulted in the contamination of groundwater and the nearby Black Water Branch of the Maurice River. Like the Winthrop site, groundwater containing > 10,000 μg/L As is being pumped and treated on site before re-injection or discharge to the Black Water Branch.

6.2.4 Field sampling and analysis of groundwaters

Groundwater samples were collected in July 2001 from various depths of multilevel wells from both below and outside the perimeter of a landfill in Maine, and in July 2003 in Vineland, New Jersey. After continuous pumping until temperature, conductivity, pH, and oxidation-reduction potential (ORP) reached stable values, samples were transferred into a 20 ml polypropylene syringe (Aldrich) with either a three-way luer-lock valve (Cole-Parmer) or via a 60 ml BOD bottle. In most cases, our samples

were collected with syringes because this allowed quantitative transfer of water with minimum exposure to air. Sulfide loss in both type of sampling device were observed over a period of 50 hrs (see section 6.4) despite storage at near 0°C. Therefore all samples were analyzed within 1 hour of collection.

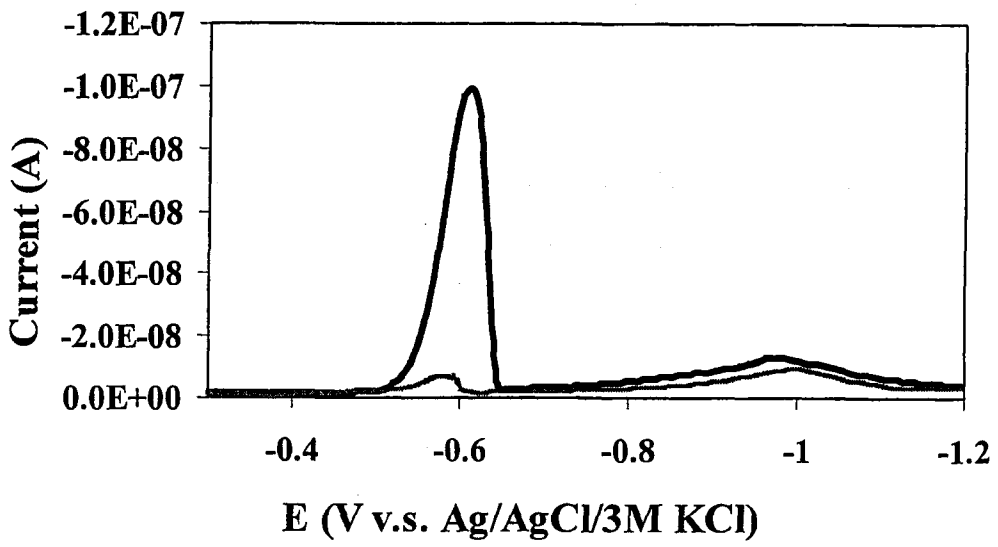
6.3 Results and Discussion:

6.3.1 Fieldwork results:

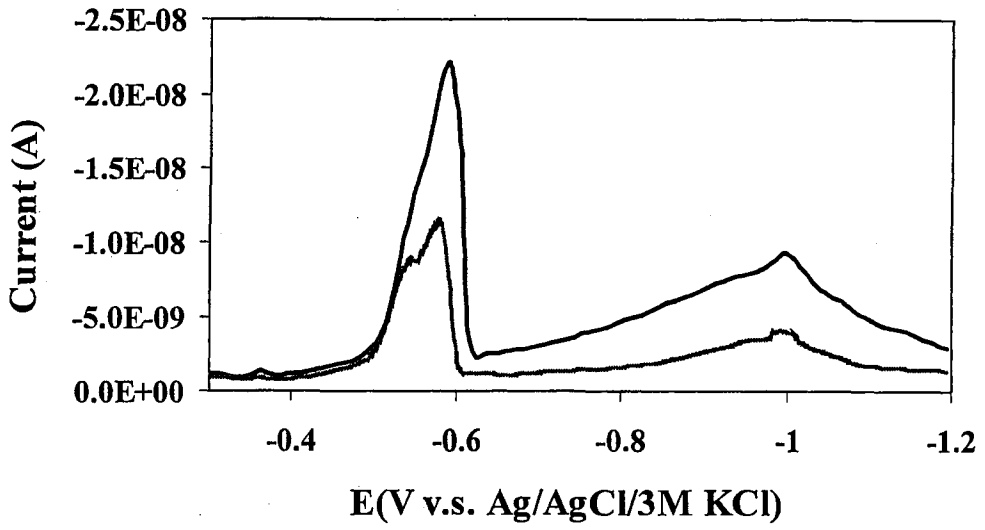
Three different types of voltammogram have been observed in field samples from groundwaters from both sites. The three types are (a) a distinctive peak at ~ -0.6 V (vs Ag/AgCl/3M KCl except otherwise indicated) (Fig. 6. 1 a); (b) a peak with potential ranging from ~ -0.6 V to ~ -0.75 V and a broad profile with a peak potential between ~ -0.9 V to ~ - 1.0 V (Fig. 6. 1 b); and (c) a peak at ~ -0.5V and a peak centering at ~ -0.97V (Fig. 6. 1 c). Both peaks in this case were labile and sulfide-related because they decreased with N_2 purge and increased with the addition of sulfide standard.

Voltammograms similar to types (a), (b) and (c) have been observed by Luther III *et. al.* in marine pore waters [127] and Davison *et. al* in anoxic lake water [123]. The peak in type (a) voltammogram was identified as free sulfide. The peaks have been attributed to Fe-(bi)sulfide complex in types (b) and (c) voltammogram. However, to our knowledge, this is the first such an observation reported in reducing groundwater system. The following section reports laboratory studies that simulated the range of Fe(II) and sulfide concentrations common in groundwater system to explain the three types of voltammograms observed in the field.

(a)



(b)



(c)

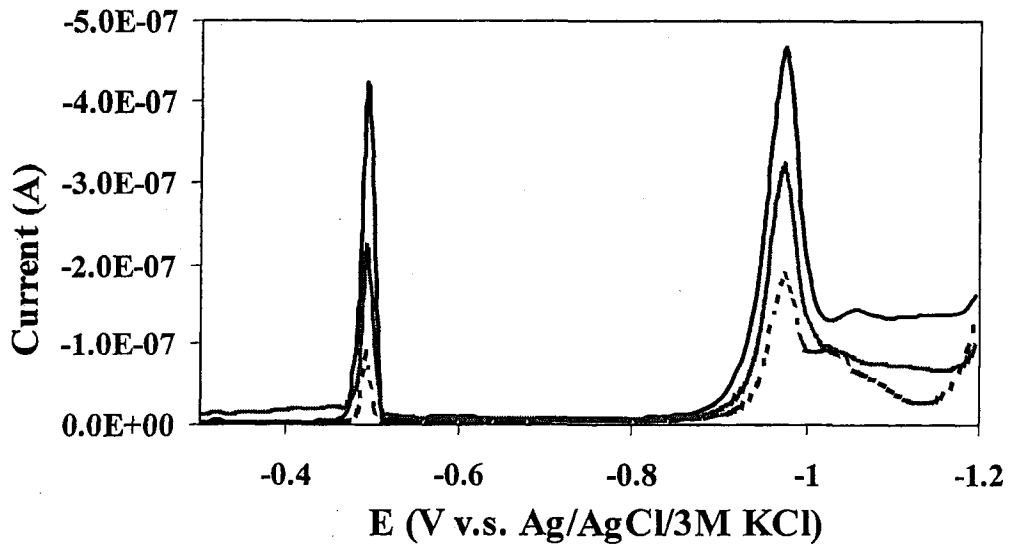


Fig.6.1

Fig. 6.1 : (a) Voltammogram of groundwater from outside the perimeter of the landfill (MW212A) at Winthrop, Maine. The solid line and shaded line represent the sample before and after 90 sec N_2 purge. (b)Voltammogram of groundwater from a monitoring well (WI MW204B) at Winthrop, Maine. The solid line and shaded line represent the sample before and after 90 sec N_2 purge. (c) Voltammogram of groundwater from beneath the landfill (EW2) at Winthrop, Maine. Solid, dotted and shaded lines indicate samples analyzed without purge, with purge using N_2 for 90 sec, followed by addition of 300nM sulfide to a purged sample, respectively. All potentials are v.s. Ag/AgCl/3M KCl reference electrode.

6.3.2 Laboratory study:

Dissolved sulfide levels are generally low (<1000 nM) in the groundwater systems (Table 5.1a and b) compared to marine pore waters (up to 10μM) [121] and anoxic lake waters (up to 6 μM) in previous studies[123] . The concentrations of Fe(II), however, spanned over a much wider range from not detectable (< 0.1 μM) to 1000 μM. Investigation of sulfide systems were carried out for three representative dissolved Fe concentrations: (a) No dissolved Fe(II) , (b) low dissolved Fe(II) up to 0.5 μM, and (c) high dissolved Fe(II) up to 500 μM.

6.3.2.1 No Fe(II) system

A laboratory solution containing 75 nM of dissolved sulfide and no dissolved Fe displayed a voltammogram (Fig. 6.2a) similar to the type (a) voltammogram obtained in the field (Fig 6.1a). The first and only peak showed at potential of –0.61 V. Most sulfide in groundwater from Winthrop well MW212A, containing non-detectable Fe(II) and 307nM sulfide, was present as free sulfide ion, which was further confirmed by sample loss upon N_2 purge (Fig. 6.1a).

6.3.2.2 Low Fe(II) system (<0.5 μM)

In a system containing 0.5 μM of dissolved Fe(II), voltammograms (Fig. 6.2b) for dissolved sulfide concentrations of 0, 37.5, 75, 325, and 575 nM were comparable to type (b) voltammogram obtained in the field (Fig. 6.1b). The potential (E_p) of the first peak shifted negatively from – 0.57 V to – 0.66 V upon addition of free sulfide to a matrix of constant pH and Fe(II) (Fig. 6.2b). Luther III *et.al* [121] found similar shift of E_p when

Fe was complexed with sulfide, suggesting that the 1st peak we observed in both laboratory and field samples indicates Fe(II)-complexed sulfide, not free sulfide.

Another indication that Fe-sulfide complexation has occurred despite the low dissolved Fe(II) concentration is the broad second peak at ~ -0.9 V. This broad peak is likely the initial DPCSV response to yet another Fe-S complex because in system containg much higher Fe(II), a well defined 2nd peak ~ -1.0 V is found (see section 6.3.2.3 below).

6.3.2.3 High Fe(II) system(5 - 250 μM)

In a system containing 500 nM dissolved sulfide, dissolved Fe(II) concentrations were increased from 5, 50, 75, 100, to 250 μM (Fig. 6.2c). Upon addition of Fe(II), the single first peak was observed to shift positively from a potential of – 0.61 V to – 0.49 V, confirming that Fe(II) began to complex sulfide in the solution. The peak shape at ~ -0.5V turned from sharp peak to a broad peak and became indistinguishable from the baseline at 250 μM Fe(II) addition. All peaks with potentials at ~ -1.0 V and ~ -1.2V increased as more Fe(II) was added to the solution, suggesting that they are all related to Fe(II). The last peak at ~ -1.2V responded quantitatively ($y = 1.24e\text{-}10\ x + 1.20e\text{-}9$, $R^2 = 0.9924$; where x is the Fe(II) concentration and y is the peak area.) to addition of Fe(II) and was absent when Fe(II) was not in solution, suggesting that it is an Fe(II) peak. A similar slope was observed in standard addition of Fe(II) in Vineland groundwater (Well RW2 VI44). This 2nd peak at ~ -1.0 V is similar to that obtained by Luther et al. (1993)[121] and Davison et al. (1998)[123], suggesting that it is an Fe-S complex. Due to the peak shift observed in 1st peak upon addition of Fe(II), we attribute the 1st peak as a

Fe-S complex as in the type (b) voltammogram. The response observed at high Fe(II) conditions is similar to the type (c) voltammogram observed in the field (Fig. 6.1c).

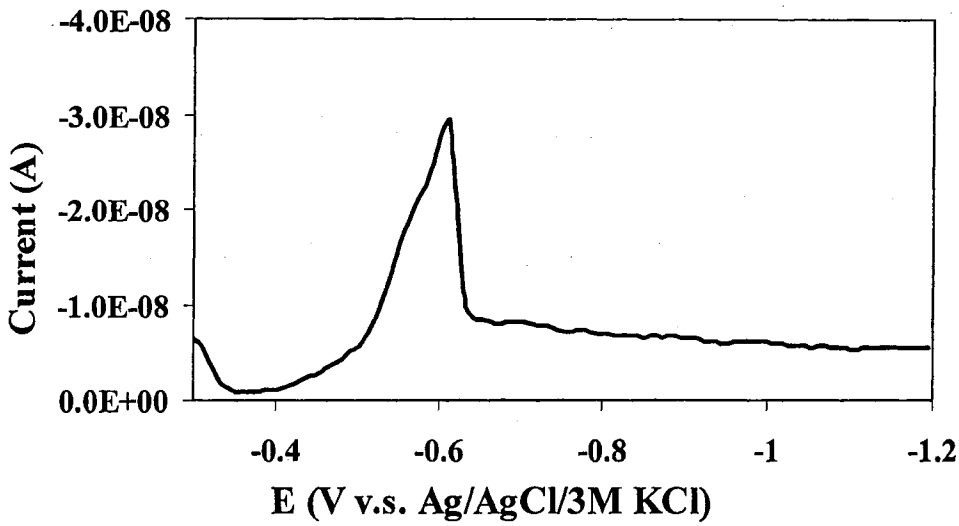


Fig. 6.2a Voltammogram of Fe-sulfide species in laboratory solutions containing no dissolved Fe(II) and 75 nM of dissolved bisulfide.

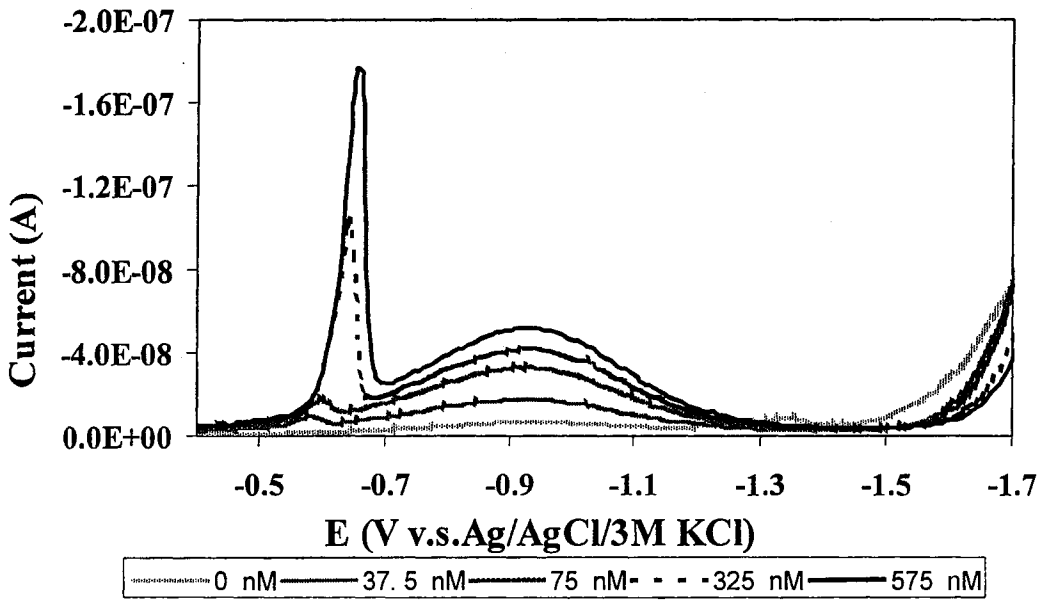


Fig. 6.2b Voltammograms of Fe-sulfide species in laboratory solutions with low level of Fe(II) (< 1 μM). The Fe(II) concentration was constant at 0.5 μM and sulfide

concentrations were increased from 0, 37.5, 75, 325, to 575 nM respectively.

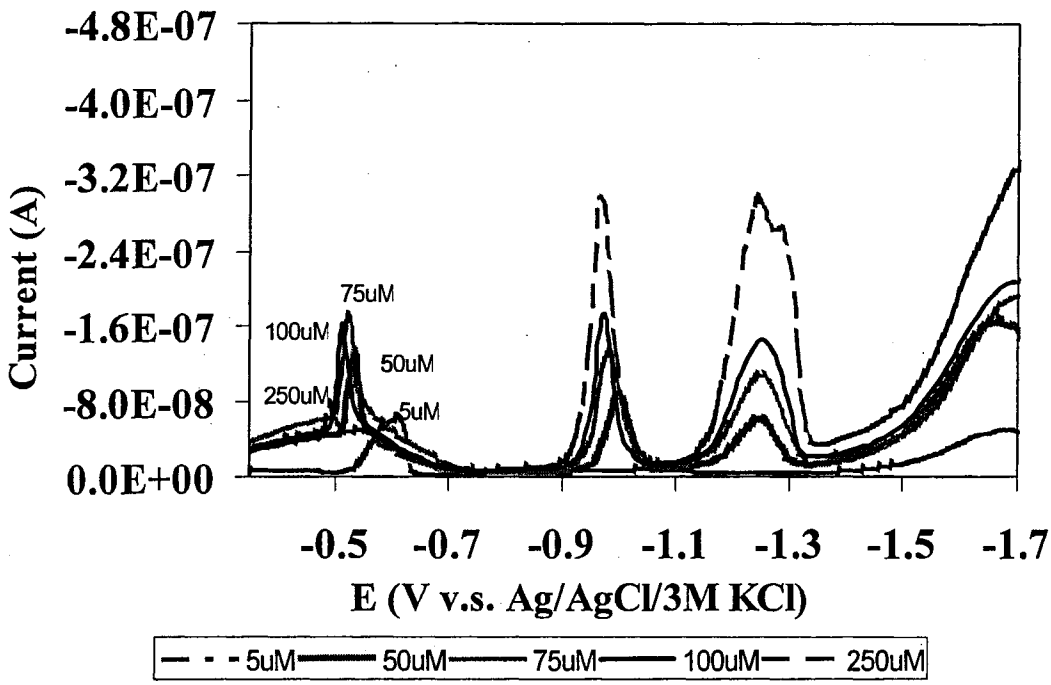


Fig. 6.2c. Voltammogram of Fe-sulfide species in laboratory solutions with high level Fe(II) (> 5 μM). The Fe(II) concentration changed from 5, 50, 75, 100 to 250 μM while the sulfide concentration remained constant at 500 nM. Both the Fe(II) and sulfide concentration level were similar to groundwater in Winthrop, Maine.

6.3.3 Stoichiometry of Fe-S complexes

The stoichiometry of Fe-S complexes found at ~ -0.5 V and ~ -1.0V can be estimated based on the potential shift upon Fe(II) addition [128-130] and the relationship between peak area at –1.0V and the ionic activity product (IAP) [127].

6.3.3.1 Fe-S complex at –0.5V

The stoichiometry of the Fe-S complex at –0.5V can be determined by metal titration by using the Lingane expression at constant pH [121].

$$(E_p)_c - (E_p)_s = (RT/nF)\ \{\ln[D/D^*]^{1/2} - \ln K_p - \ln [X]^p\} \qquad (1)$$

Where c refers to complexed and s to free. E_p is the peak potential, D and D* are the diffusion constants, and K_p is the conditional stability constant. The ligand ([X]) for sulfide is Fe(II). A plot (Fig. 6.3) of ln[FeII] versus the peak potential $[(E_p)_c]$ for sulfide at constant sulfide concentration of 500 nM and varying Fe(II) concentrations from 5 to 250 μM was linear with a slope of 0.0317. This slope is equal to the stoichiometry of the complex, p, times the constant of RT/nF, where n=2. The stochiometry of Fe-S complex in laboratory solution was determined as p=1.1. Field data obtained similarly by spiking a purged Vineland, New Jersey groundwater with 32 μM Fe are also plotted, and yield a p value of 0.7 (Figure 6.3). This groundwater cannot give a reasonably good sulfide peak without purge due to presence of trace level oxygen as a result of re-injection of pump-and-treated groundwater.

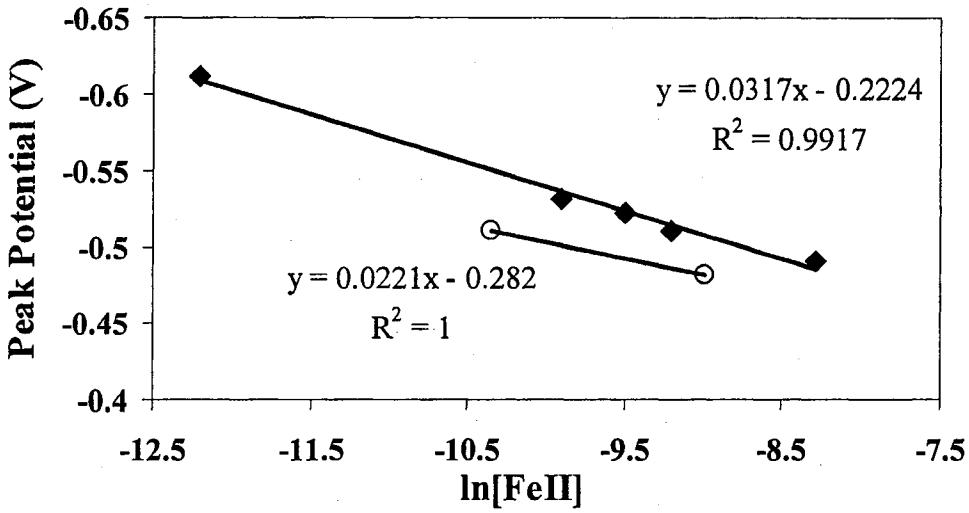


Figure 6.3 Plot of E_p versus ln [FeII] for titration of Fe(II) with constant sulfide. The line with solid diamond was laboratory solution with sulfide concentration of 500 nM. The line with open circle was purged groundwater obtained from well RW2 VI44 in Vineland, New Jersey spiked with Fe(II) standard.

6.3.4 Fe-S complex at –1.0V:

Theberge *et.al* [127] conducted a laboratory study in a sea water matrix that investigated Fe-S complex formation at –1.0V and found that the response at –1.0V was negligible until the ionic activity product (IAP) of Fe and dissolved sulfide exceeded the solubility product of amorphous FeS. The IAP is calculated using the following equation:

$$IAP = \gamma_{Fe}^{2+} [Fe^{2+}] * \gamma_{HS}^{-} [HS^{-}] / \gamma_{H}^{+} [H^{+}] \qquad (2)$$

Where [ ] represent concentration and γ is the activity coefficient. Peak areas of Fe-S complex peaks at –1.0V obtained on groundwaters from Winthrop, Maine vs. IAP was plotted (Fig. 6.4). In our calculation of IAP, activity coefficients were assumed to be 1 for both Fe and sulfide although in seawater matrix they are 0.43 and 0.11, respectively [127] because seawater has higher ionic strength than groundwater due to its high salinity. Nonetheless, we observed that only after the IAP exceeded ~ 10^{-3}, peak area of Fe-S complex at –1.0V in groundwater increased rapidly. This turning point corresponded to the value of K_{sp} of amorphous FeS [127] and also is consistent with electrochemically determined values by Davison, $10^{-2.95}$, [131] and Theberge, $10^{-2.94\pm 0.1}$[127]. For samples that displayed IAP > 10^{-3}, a reasonably good correlation (R^2 = 0.71) was obtained between peak area and IAP, suggesting a 1:1 stoichiometry of this Fe-S complex as found in sea water system [127]. Unambiguous assignment of stoichiometry of Fe-S complex in groundwater is, however, difficult because the scatter in the data is large as previously noted in a study of lake water [131].

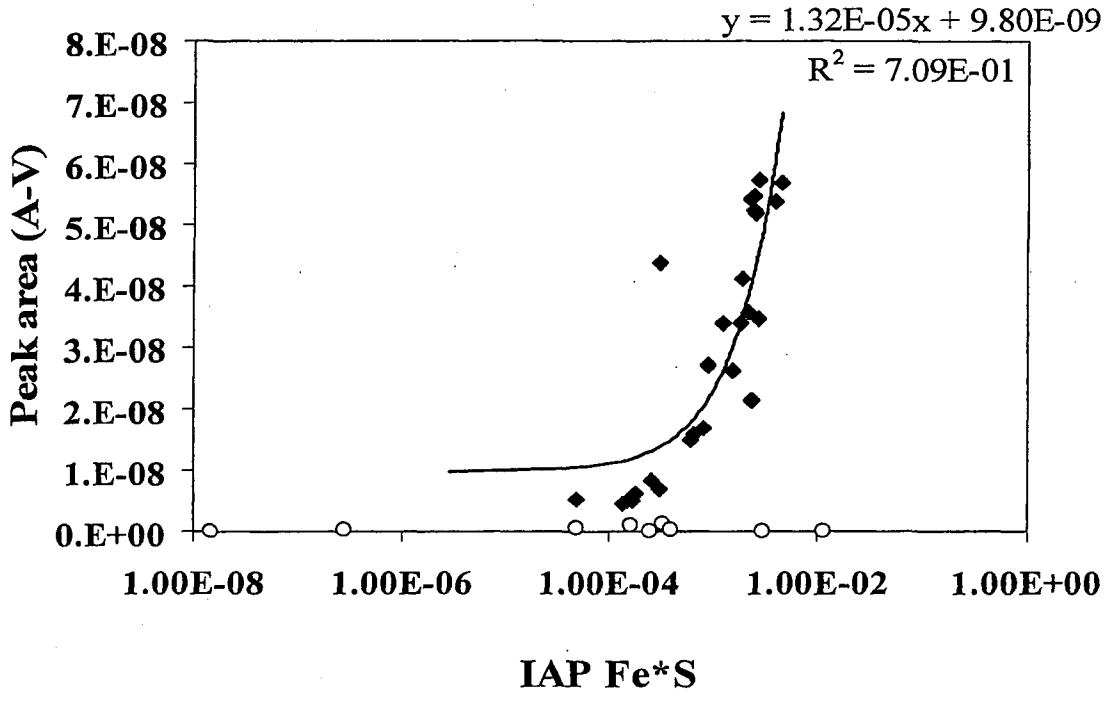


Figure 6.4. Plot of peak area of Fe-S complex at –1.0V vs IAP of $[Fe^{2+}]*[S^{2-}]$ calculated using equation (2) for groundwater samples from Winthrop. The solid squares were data from inside the landfill and open circles were data from outside the landfill.

6.3.5 Saturation state of groundwater with respect to amorphous FeS

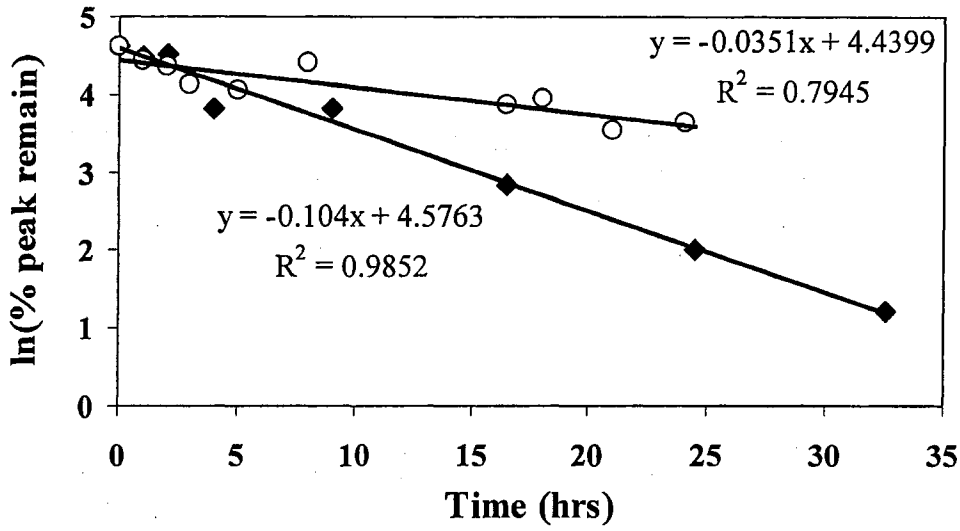
Assessment of saturation state with respect to amorphous FeS in the natural environment remained difficult. This is because computation of the IAP requires accurate representation of activity coefficients, which most often is obtained through an ion interaction model in solution (Eby, 2004). Aqueous geochemical model programs such as MINTEQ or PHREEQC utilizes such model to compute coefficients but rarely is such assessment confirmed by laboratory measurements.

DPCSV investigation of the dissolved sulfide system in groundwater provides an important experimental means to assess the saturation state of groundwater with respect to amorphous FeS. Appearance of a well-defined Fe-S complex peak at –1.0V serves as a good indicator that the system has reached super-saturation with respect to amorphous Fe-S. While the interaction of such a precipitate with in situ As removal in groundwater requires further investigation, this is a first important step towards that goal.

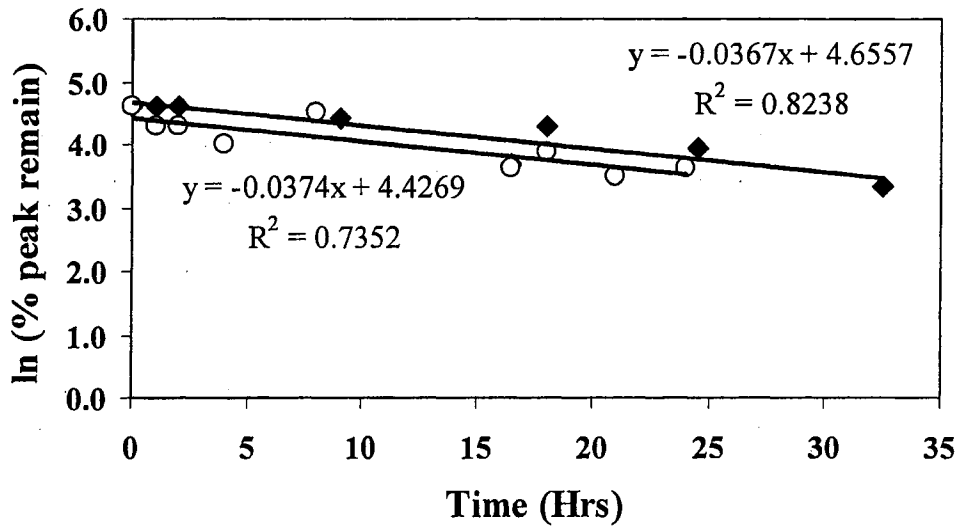
6.3.6 Loss of sulfide upon sample storage

Fe(II) (bi)sulfide complex loss kinetics in reducing groundwater was studied because little is known about the stability of these naturally occurring species. The complex loss was monitored in EW2 water sample (ORP –112 mV) over a 50 hr period for samples stored in closed plastic syringes and BOD bottles. The linear regressions of the two Fe-S complex at –0.5V and –1.0V indicate first-order loss kinetics (Fig. 6.5). Based on the assumption of first-order kinetics, the half-time was calculated as 6.7 hrs and 18.9 hrs respectively for the –0.5V compound and –1.0V compound by syringe storage. The results were similar to those of free sulfide loss half-time in laboratory

solutions [108, 115], but slower than those of metal sulfide complex loss half time obtained in oxic river water, which had a half-time of the order of days [122].



(a)



(b)

Figure 6.5 Loss kinetics of Fe(II) (bi)sulfide species in groundwater collected in EW2 well (Winthrop, Maine). (a) first peak at ~-0.5 V and (b) second peak at ~ -0.97 V. The solid diamonds refer to samples stored in syringes and the open circles to samples stored in BOD bottles. The slope is the observed first-order reaction rate constant k (h^{-1}). The half-time for the first peak is 6.7 hrs (Syringe) and 19.7hrs (BOD) and that for the second peak is 18.9 hrs (Syringe) and 18.5 hrs (BOD).

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