DEVELOPMENT OF A GAS-PHASE CHEMILUMINESCENCE DETECTION SYSTEM FOR THE MEASUREMENT OF ARSENIC IN ENVIRONMENTAL AND BIOLOGICAL SAMPLES

by

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DEDICATION

This work is dedicated to my loving mother Tigist Hailemariam.

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My sincere gratitude goes to Dr. Abul Hussam, for giving me the opportunity to work on this important Masters Thesis project. The accomplishment of this project is based on his knowledge, support, encouragement, constructive criticism and directions. I thank Dr. Hussam for his generous financial support through his research grants during my graduate and undergraduate studies. I would like to take this opportunity to congratulate him for his outstanding achievement in developing an award winning arsenic filtration system that is saving the lives of millions throughout Bangladesh.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectroscopy
AC	Activated Carbon
AC	Alternating Current
ACS	American Chemical Society
AD	Auto Dispenser
ADC	Analog to Digital Converter
AFS	Atomic Fluorescence Spectroscopy
AP	Air Pump
APL	Acute Promyelocytic Leukemia
As	Arsenic
As (III)	Arsenite
As (V)	Arsenate
ASV	Anodic Stripping Voltammetery
BDL	Below Detection Limit
CB	Circuit Board
CC	Chemiluminescence Chamber
CL	Chemiluminescence
cm	centimeter
CV	Check Valve
DAC	Digital Analog Converter
DMA	Dimethylarsinic Acid
DC	Direct Current
DC	Data Card
EDTA	Ethylenediaminetetraacetic acid
EHG	Electrochemical Hydride Generation
FEP	Fluorinated Ethylene Propylene
FIS	Flow Injection System
ICP-MS	Inductively Coupled Plasma Mass Spectroscopy
ICP-AES	Inductively Coupled Plasma-Atomic Emission Spectroscopy
ID	Inside Diameter
FC	Flow Controller
GCE	Glassy Carbon Electrode
GFAAS	Graphite Furnace Atomic Absorption Spectroscopy
HG	Hydride Generation
HGAAS	Hydride Generation Atomic Absorption Spectroscopy
HPLC	High Performance Liquid Chromatography

hr	hour
Hz	Hertz
L	Liter
LED	Light Emitting Diode
LOD	Limit of Detection
m	meter
M	Molar
MDL	Minimum Detection Limit
MCL	Maximum Contamination Level
mL	milliliter
mm	millimeter
MMA	Monomethylarsonic Acid
MS	Magnetic Stirrer
mV	millivolt
ng	nanogram
OD	Outside Diameter
OZG	Ozone Generator
PE	Polyethylene
PMT	Photomultiplier Tube
PP	Peristaltic Pump
ppb	parts per billion
ppm	parts per million
ppt	parts per trillion
PS	Power Supply
PTFE	Polytetrafluoroethylene
R	Reactor
rsd	relative standard deviation
S	Syringe
SV	Sample Vial
s	second
SERS	Surface Enhanced Raman Spectroscopy
SRM	Standard Reference Material
TLV	Threshold Limit Value
USEPA	United States Environmental Protection Agency
USGS	United States Geological Survey
V	Volt
WV	Waste Vial
XRF	X-Ray Fluorescence
µg	microgram
Ω	ohm
6-PV	6-Port Valve

ABSTRACT

DEVELOPMENT OF A GAS-PHASE CHEMILUMINESCENCE DETECTION SYSTEM FOR THE MEASUREMENT OF ARSENIC IN ENVIRONMENTAL AND BIOLOGICAL SAMPLES

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Arsenic in groundwater used for drinking is now regarded as one of the most serious health hazards of this decade. Long term exposure to low concentrations of arsenic has been reported to cause cancer of the skin, bladder and other internal organs. Also, various ionic forms of arsenic are known to be very toxic to most microorganisms. Measurement of trace arsenic at parts per billion (ppb) levels in environmental and biological samples is a key component for the mitigation and understanding of this crisis. This M.S. Thesis Project deals with the development of an analytical technique for the measurement of arsenic based on a gas-phase chemiluminescence reaction between ozone (O_3) and arsine (AsH_3) as the detection principle. The approach is capable of analyzing arsenic concentration in a 1.0 mL sample in one minute. The determination of As(III) and As(V) was achieved based on the different pH dependence and the

reducibility of these species to arsine gas by sodium borohydride ($NaBH_4$). The intense chemiluminescence formed in a reflective glass reaction cell from the reaction of $AsH_3 – O_3$ is detected by a sensitive photomultiplier tube (PMT). The signal is further amplified, digitalized and recorded with a complete data acquisition computer controlled system. The limit of detection (LOD) is 0.146 µg/L (ppb or 146 ppt) for total arsenic concentration in 10 determinations.

To validate the performance of the gas-phase chemiluminescence based arsenic analyzer, results were compared with Hydride Generation Atomic Absorption Spectroscopy (HG-AAS) and Atomic Fluorescence Spectroscopy (AFS) techniques. The chemiluminescence detection system was also coupled with a flow injection system to enhance its efficiency. Sequential procedures including direct chemical and data analysis methods and step by step development of the analyzer are described in depth. The system has been effectively tested using standard and unique field water samples from several regions of Ethiopia.

CHAPTER I

ARSENIC

1.1 Introduction

Arsenic (As) is one of the main group elements found in group V-A of the periodic table. It is the 20th most abundant element in the earth's crust, 14th in the seawater and 12th in the human body.[1] Arsenic was first discovered as a metal by a German scientist and philosopher Albertus Magnus around 1250 A.D. The origin of the word *Arsenic* came from the Persian word *Zarnikh* meaning "yellow orpiment" which was later renamed by the Greek as "arsenikon". Since the ancient times, Arsenic has been known and used in Persia and elsewhere, mostly by the ruling class to murder one another and has been called *the poison of kings and the king of poisons.*[2]

Detailed studies confirmed that deaths by arsenic poisoning include Napoleon Bonaparte and King George III of Great Britain. The strong binding affinity of arsenic with sulfur groups causes malfunctioning of important enzymes and proteins which lead to serious implications and death.[3]

Arsenic is odorless and tasteless with grey and yellow allotropes. The yellow or γ-As form is soft, waxy, unstable and non-metallic with density of 1.97 g/cm^3 at 25^{o}C. It is made of the tetrahedral As_4 molecules, similar to the

structure of phosphorus in which each atom is covalently bound to the other three. In the presence of light the yellow form rearranges itself to structures of higher density and darker colors, such as β-As with density of 4.7 g/cm^3 and brown to black in color. The metallic α-As is gray in color, more stable at room temperature and it has a layered rhombohedral (hexagonal) crystal structure. The density of the gray form is 5.72 g/cm^3 at 25^{o}C.[4] α-As is definitely a metal but is brittle and does not melt, rather it sublimes directly into As_4 at 615^{o}C. Elemental arsenic is practically insoluble, where as arsenic compounds may readily dissolve. The following are a few examples of the aqueous solubility of common arsenic compounds. As(III) hydride 700 mg/L, As(III) oxide 20 g/L, As acid (H_3AsO_4 + ½ H_2O) 170 g/L and As(III) sulfide 0.5 mg/L.[5] Table 1.1 shows the list of physical and chemical properties of elemental arsenic.[6]

Table 1.1. Physical and chemical properties of arsenic

Atomic Number	33
Atomic Mass	74.9216 u
Atomic Radius	1.33 Å
Density	5.72 g/cm^3
Melting Point	808 ^{o}C
Boiling Point	603 ^{o}C
Heat of Vaporization	34.76 kJ/mol
Heat of Fusion	369.9 kJ/mol
Specific Heat	0.33 J/g K
Heat of Atomization	301.42 kJ/mol
Thermal Conductivity	0.50 w/cm K
Oxidation States	+/- 3,5
Stable Isotopes	1 with mass number 75
Electronegativity	2.18
First Ionization	9.81 eV
Resistivity	35 μΩ-cm
Coefficient of Expansion	6.95 x 10^{-4} per ^{o}C

The electron configuration of As, [Ar]^{18} 4s^2 3d^{10} 4p^3, indicates oxidation states of +3 or +5. In the periodic table, arsenic shares the same outer shell s^2 and p^3 with N, P, Sb and Bi. N and P are evident nonmetals while Sb and Bi are metals with electric conductivity. Arsenic falls in the middle of these four elements and can not be classified simply as metal or nonmetal. Its classification

as a metal or a nonmetal depends on how it aggregates based on the balance between lowest energy and highest entropy.[7]

The two fundamental oxidation states, trivalent (arsenite, AsO_3^{3-} with As (III)) and the more oxidized form, pentavalent (arsenate, AsO_4^{3-} with As (V)) are rapidly formed in an oxygenated environment. While either can be found in the environment, depending on redox potential and pH, the highly toxic trivalent arsenic is somewhat more common in groundwater while pentavalent arsenic is more common in surface water and less toxic. Organic arsenic compounds like monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) are usually formed as a result of oxygen atom substitution with the methyl group as metabolic products in biological systems. Figure 1.1 shows the list of common inorganic and organic arsenic compounds and their chemical structures.[8] It should be noted that arsenocholines and arsenosugars are naturally occurring arsenic products and are not toxic.[9]

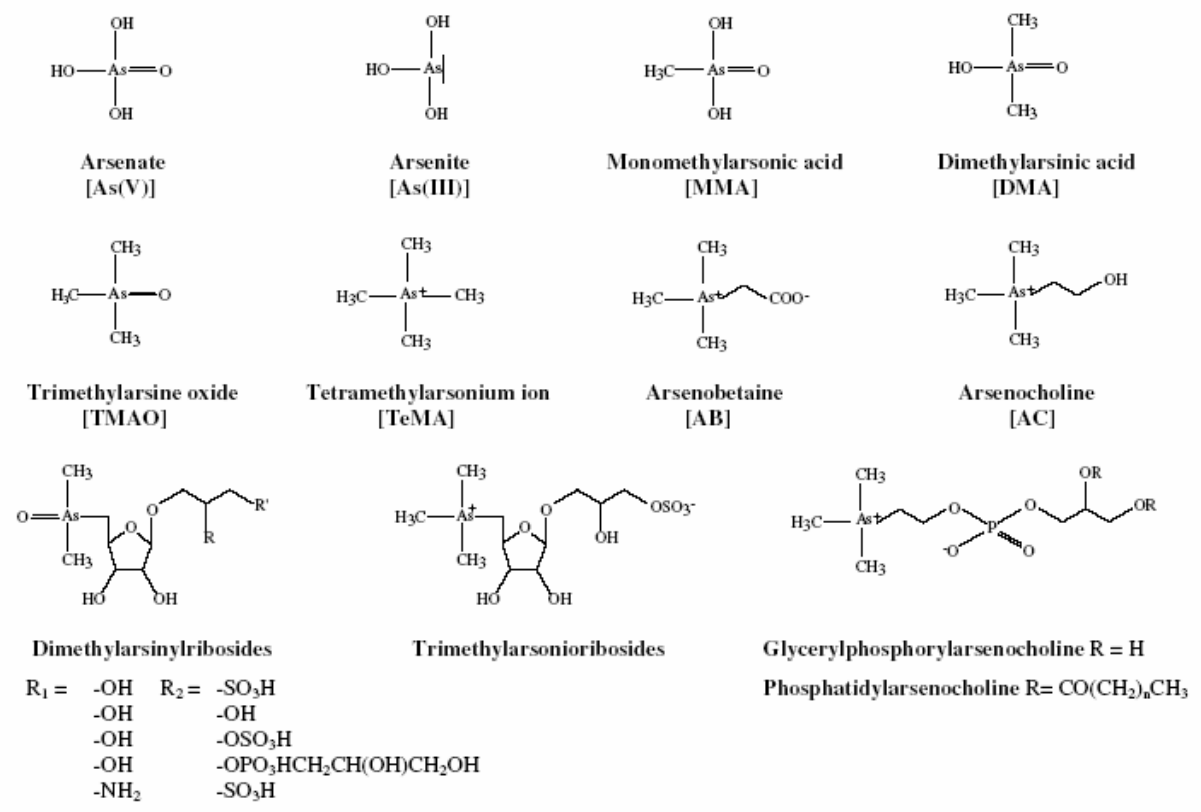

Figure 1.1. Common inorganic and organic arsenic species

1.2 Occurrence

Arsenic is not often found in its elemental state but in mineral ores. It is more common in sulfides and sulfosalts such as arsenopyrite, orpiment, realgar, lollingite and tennantite. Usually elemental arsenic is produced from these ores at high temperature in the absence of air.[10]

Arsenopyrite (FeAsS) is the major ore of arsenic that may contain a small amount of gold as an impurity. A typical arsenopyrite is a brassy white to gray

crystal. It has a diamond like shape with sharp acute angles which makes it different from other sulfides that generally have only obtuse angles.

Orpiment (As_2S_3) is yellow in color and usually found with realgar (As_4S_4). Realgar is one of the only few sulfides that are not metallic or opaque. It resembles sulfur in most respects except its color is orange to red. Realgar's structure alternates between sulfur atoms and arsenic atoms producing rings of As_4S_4 while sulfur has a structure composed of 8 sulfur atoms linked in a ring. It is usually found in hot spring deposits and volcanoes.

Lollingite is silver to gray opaque crystal often associated with FeAsS, and it usually contains cobalt and nickel. Tennantite is very similar with a much more common mineral tetrahedrite but richer in arsenic concentration.[11]

1.3 Applications

Arsenic has long been used in healing practices, agrochemicals, semiconductor industries and other industrial applications. Arsenic has an extended history of use in Chinese and western medicine before and after it was purified as arsenic trioxide (As_2O_3) by the most famous Arabic alchemist Jabir Ibn Haiyan in the 700's A.D.[12] As_2O_3 was used for the first time on controlled studies in patients with acute promyelocytic leukemia (APL) in China during the 1970's. The therapeutic effect of As_2O_3 in the treatment of APL is well documented but still an arguable matter.[13] The mechanism of action of As_2O_3 also suggests that

it may be applicable for the treatment of multiple myeloma and chronic myelogenous leukemia.[14]

Arsenic was the main ingredient of agrochemicals such as pesticides, fungicides, insecticides and herbicides before synthetic organic agrochemicals were available. Wood sold for outdoor uses is often treated with an arsenic compound called chromated copper arsenate ($CuHAsO_3$) to prevent decaying. Chicken litter, which is commonly spread on fields as fertilizers, contains high levels of arsenic because the chickens are fed organic arsenic compounds to control infection and increase weight gain.[15]

The use of arsenic has increased in the semiconductors industry. Indium arsenide (InAs) is used in infrared detectors and alloys of gallium arsenide (GaAs) are used for the production of light emitting diodes (LEDs) in watches, calculators, lasers, photodetectors and numerous other instrument displays and transistors. For example, most cellular phones utilize a gallium arsenide chip because of its excellent property to transfer electrons in microwave oscillations.[16]

Arsenic has other industrial uses as an additive alloying element. For instance, it is used in alloys to harden lead, increase the roundness of lead shot and in the production of lead-acid battery plates. Approximately, 0.5% arsenic is added to glass melts to remove the unwanted green tint produced by iron impurities. Addition of 0.15% – 0.5% arsenic to copper raises the annealing temperature of copper without destroying its conductivity, so that copper does not

become soft when exposed to heat.[17] Table 1.2 shows common inorganic As compounds and their industrial uses.[18]

Table 1.2. Common arsenic compounds and their uses

Compound	**Chemical Formula**	**Uses**
Arsenic acid	H_3AsO_4, H_2O	Arsenate manufacturing, glass making, defoliant, desiccators for cotton
Arsenic disulfide	As_2S_2	Shot manufacture, pest control, pyrotechnics
Arsenic pentafluoride	AsF_5	Doping agent in electroconductive polymers
Arsenic pentasulfide	AsS_5	Light filters
Arsenic pentaoxide	As_2O_5	Arsenates, weed killer, colored glass, metal adhesives
Arsenic thioarsenate	$As(AsS_4)$	Scavenger for certain oxidation catalysts and thermal protection for metal-bonded adhesives and coating resins
Arsenic tribromide	$AsBr_3$	Analytical chemistry
Arsenic trichloride	$AsCl_3$	Intermediate for organic arsenicals, ceramics
Arsenic trifluoride	AsF_3	Catalyst, ion implantation source, dopant
Arsenic trioxide	As_2O_3	Ceramic enamels, decolorizing agent in glass, insecticide, rodenticide, herbicide
Arsenic trisulfide	As_2S_3	Reducing agent, pyrotechnics, glass used for infrared lenses, semiconductors
Arsenic hydride	AsH_3	Organic synthesis, doping agent for solid-state electronic compounds

1.4 Environmental impacts

Arsenic is naturally present in the environment in different chemical species. The toxic species (H_3AsO_3, $H_2AsO_4^-$ and $H_2AsO_4^{2-}$) are found in rocks and soil, but they do not pollute the surface water because they bind to iron hydroxide ($Fe(OH)_3$), a compound abundant in soil. The Fe^{3+} (ferric ion) is reduced to Fe^{2+} (ferrous ion) by different types of iron reducing bacteria in the underground. The resulting ferrous ions are more soluble than ferric ions and break apart from the arsenic, releasing it into the groundwater.[19] Volcanoes, minerals, forest fires and geothermal water (hot springs) have very high arsenic concentrations.[20]

Generally normal atmospheric levels of arsenic in areas away from human releases range from 1 - 3 ng/m^3, while in urban locations it may range from 20 - 100 ng/m^3. In most soil types the natural arsenic level is about 5 mg/kg. Typical concentrations of arsenic in fresh water range from 1 - 80 µg/L with As(III) and As(V) being the major species.[21] An average of 2 µg/L of arsenic is found in the drinking water of the United States although most of the water supplies are filtered from sources containing more than 20 µg/L arsenic concentration. Due to natural and anthropogenic activities, arsenic is unevenly distributed in the environment. Anthropogenic activities are manmade causes including mining and smelting, irrigation, agrochemicals, coal and wood combustion, and industrial waste releases. As a result of these activities, arsenic may end up retained in the solid phase of the soil, volatilized into the atmosphere, taken up by organisms

and/or leached into bodies of water. According to the recent groundwater survey conducted by the United States Geological Survey (USGS), high levels of arsenic are documented in many areas across the U.S.[22] Figure 1.2 shows arsenic contaminated sites throughout the U.S.

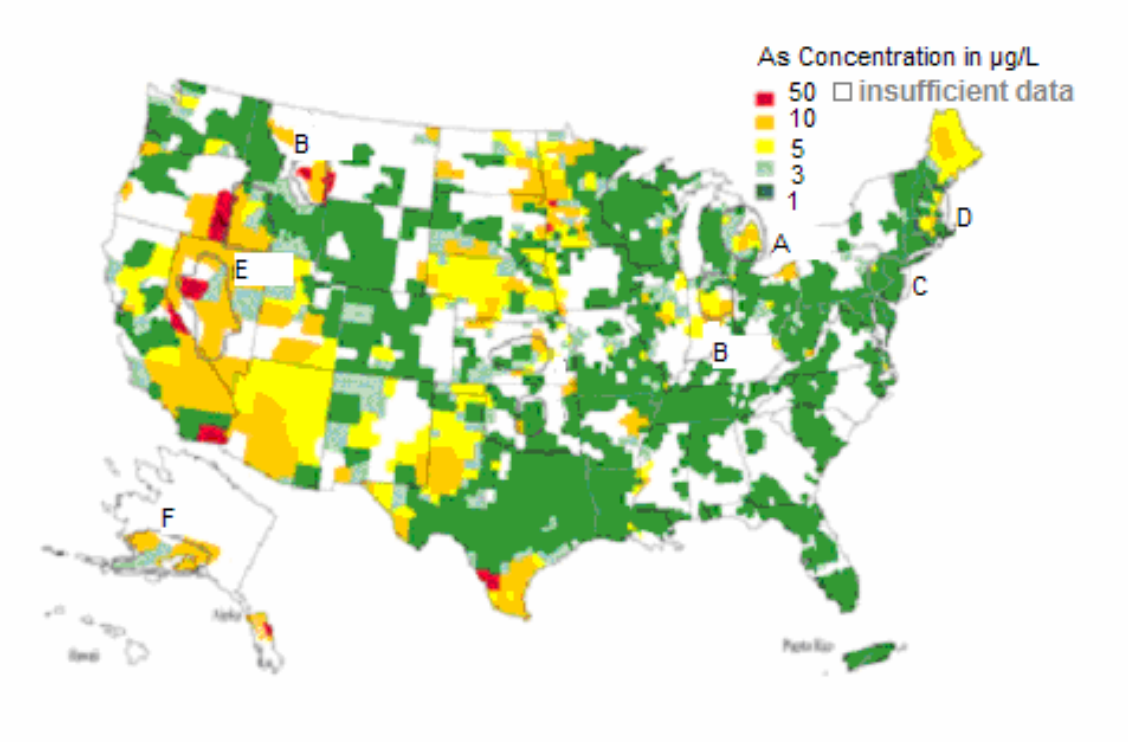

Figure 1.2. Distribution of arsenic in drinking water across the United States on a scale of µg/L. Reprinted from Ryker, S. (2001). Available online at https://www.agiweb.org/geotimes/nov01/feature_Asmap.html

The map represents 75% of arsenic concentration in the U.S., computed from 31,000 samples. Proportions were computed from counties with at least 5 wells where 25% had arsenic concentrations of $\geq$ 10.0 µg/L. Areas indicated by A and B show similar proportions of high values while area D has proportionally higher values than area C. Areas E and F show relatively sparse values.[23]

Arsenic undergoes several oxidation-reduction reactions and precipitations in the environment. The As (V) is often deprotonated as arsenate anion in contrast to the As (III) which remains in its neutral form as arsenite. Sediments usually adsorb most of the arsenic and release it into air mainly in the form of particulate matter. Highly soluble arsenic oxides are generally produced as a result of combustion of coal and oil. Arsenic oxides are also evolved from fossil fuel and other industrial processes. Additionally, other sources of arsenic have been reported in the form of arsenic trichloride from refuse burning, arsenic trisulfide from coal combustion and organic arsines from oil combustion.[24] A variety of arsenic species resulting from manmade emissions are eventually returned to the ground during dry and wet deposition events.

Sandy soils contain the lowest concentration of arsenic while soils with significant organic mater composition, igneous rock and sediment contain the highest arsenic concentration. Arsenite and arsenate are the major species in most soils and groundwater samples. Their distribution is influenced by the oxidation-reduction potential, pH, temperature, salinity, iron concentration and other geological factors. For example, arsenate is more common at pH > 6 while

arsenite is common in reducing conditions.[25] Arsenic speciation in the atmosphere, water source or soil sample is very important because toxicity levels are determined based on the chemical speciation. In general, chemical speciation includes various analytical activities of identifying and quantifying one or more individual chemical forms in a sample. Chemical speciation provides critical information about subsequent distribution, mobility and toxicity of chemical species in the environment.[26]

1.5 Biological Impacts

Some microorganisms can produce arsine from naturally occurring arsenic in soil. Non volatile oxidized arsine gases that are settling back to the ground are often taken in by plants and animals. Arsenic is found in many foods at concentrations that usually range from 20-140 μg/kg with grains being significant sources of inorganic arsenic while fresh vegetables have the lowest concentration. In plants arsenate is preferentially absorbed up to 3-4 times the rate of arsenite. A moderate level of arsenic is required for normal growth and reproduction. Arsenic deficiency has been linked to impaired growth and abnormal reproduction in rats, hamster, chicks, goats and miniature pigs.[27]

Diet is the largest source of exposure to arsenic for most people. Total arsenic in dietary uptake of 50.6 μg/day and 58.5 μg/day has been reported in the U.S.. The average inorganic arsenic uptake among adults is 11 – 14 μg/day.[28] Higher levels of arsenic were observed in cooked food compared with

raw in areas where the potable water has elevated arsenic concentration.[29] Seafood is generally the predominant dietary source of arsenic. Scallop and crab contain 27.0 – 63.8 ppm while crawfish, shrimp and lobster contain up to 54 ppm per dry weight.[30] However about 90% of the arsenic in most seafoods is organic arsenic (eg. arsenobetaine, arsenochloline and dimethylarsenic acid) and approximately 10% is inorganic arsenic. Surveys of inorganic arsenic in seafood samples from markets suggests a level of 0.002 μg/g.[31] Food and water are the major contributors of arsenic intake compared to that of air and soil.

In areas where biological activities are high, arseno-organic species such as MMA and DMA predominantly exist. Although, inorganic arsenic is considered responsible for most carcinogenic effects, As (III) and As (V) may also be metabolized by the body to toxic organic forms of arsenic. Metallic arsenic (zero oxidation state) is not absorbed from the stomach and it is rapidly eliminated with other organic arsenic compounds in the urine.[32] The normal concentration of arsenic in human urine is approximately 3.6 μg/L depending on arsenic exposure in food and water. The urine As level is independent of age, sex or various diseases including hepatic injury or diabetes.[33] DMA is the major species detected in human urine. However MMA, arsenobetaine, As(III) and As(V) may also exist. DMA is more common, because a variety of the ingested inorganic arsenic species are often methylated to DMA.

Hair and nails are interesting because the sulfur atom in the protein known as keratin binds to the ingested arsenic and the sample of hair or nail can last for

a relatively long time. Since hair and nails are constantly growing, they can be used to determine the level of arsenic in someone's body as it changes over time.[34] Under aerobic conditions organic arsenics are often transformed into inorganic species, most of the time to arsenates. Bacterial oxidation of arsenic from arsenite to arsenate is proposed to occur via a detoxification mechanism. In general, bacteria are more resistant to methylated arsenic compounds than inorganic arsenic species.[35]

1.6 Toxicity and Exposure Hazards

Arsenic is classified as a known human carcinogen by the International Agency for Research on Cancer and the World Health Organization. Arsenic poisoning is characterized by serious illnesses due to hyperkeratosis on palms and feet, fatigue symptoms of arsenicosis, cancer of the bladder, skin, lung, and other organs. Chronic exposure has also been linked to respiratory, reproductive, developmental, neurological and immunological defects.[36] Studies show that arsenic toxicity is related to its ability to inactivate several enzymes and it weakens the integrity of structural proteins that are involved in DNA repair and cellular metabolism.[37] Prolonged consumption of water from arsenic contaminated aquifers lead to chronic arsenic poisoning in many parts of the world. The worst and most publicized situations exist in Bangladesh, India, Nepal, China, Argentina, New Mexico and Chile.[38] Toxic level concentration in groundwater has been documented in 26 other countries around the globe.[39]

In the 1970's about 10 million tubewells were drilled throughout Bangladesh in an effort to eliminate waterborne diseases originating from polluted surface water. It was later discovered that the waters from these tubewells were contaminated with high levels of arsenic. It is estimated that 77-95 million people out of a total population of 140 million in Bangladesh are drinking groundwater containing more than the maximum contamination level (MCL) of 50 µg/L.[40] According to the United States Geological Survey, 32 million people in the US drink water containing 2-50 µg/L arsenic. In 2006, the U.S.EPA reduced the maximum permissible level of arsenic in drinking water from 50 to 10 µg/L due to the increasing health hazard related to arsenic exposure.[41] Clearly there is an urgent need to monitor and document drinking water sources around the globe. However, the lack of a rapid, reliable and field deployable method for the measurement of arsenic has been the challenge for decades. The primary motivation of this M.S. Thesis work is to develop innovative technology based on chemiluminescence of arsine with ozone in a simple instrumental setup amenable to remote laboratories in Bangladesh and elsewhere.

1.7 Removing Arsenic

Arsenic removal methods are often dependent on adsorption or ionic rejection. The most common raw materials used for arsenic removal contain anions such as silica, fluoride, phosphate or sulfate, and cations like iron or manganese. The presence of natural iron in water is beneficial because iron

precipitation can adsorb As (V) if left in open air for a few minutes and retained for removal by filtration.[42]

Arsenic can be removed from water using different processes including iron adsorption, activated alumina adsorption, ion exchange, reverse osmosis, nanofiltration, lime softening, iron and alum coagulation and coagulation assisted micro-filtration. In the normal pH range of drinking water, As (V) is easier to remove than As (III) because most of these methods rely on ionic charge. Therefore if arsenite is present, it is necessary to oxidize it to arsenate in the first step. The arsenate and other As (V) compounds such as iron oxides are immobilized on geological surfaces.[43] However, other environmental activities and bacteria may convert them back to more toxic and mobile forms of arsenic.[44]

Arsenic speciation must be performed in order to determine the presence of As (III). Water containing only As (V) does not require further oxidation for filtration. Some of the chemicals that have proven to oxidize arsenic are free chlorine, hypochlorite, potassium permanganate and ozone. As long as the contact time is sufficient, solid oxidizing media such as those used for iron, manganese or sulfide can also be applied.[45]

All arsenic removal technologies can be classified into two main categories called adsorbents and membrane separation. Adsorbents include any technique that binds arsenic to a material that can keep it in a complex form for removal and disposal. Reverse osmosis and nanofiltration are membrane

separations in which both are cross-flow processes that do not rely on chemical processes but only on size exclusion.

Dr. Abul Hussam, professor of chemistry at George Mason University, Fairfax, VA (the director of this M.S. Thesis project) has been engaged in developing a filtration system for the removal of arsenic in the most affected areas of Bangladesh. He has developed a sustainable, affordable and environmentally friendly household arsenic filtration unit that is allowing millions of people in Bangladesh to drink arsenic-free water. Dr. Abul Hussam was awarded the 2007 Grainger Challenge gold medal award and $1 million for his SONO® filter by the National Academy of Engineering. More description of the challenge is found in Appendix A. One of the purposes of this work is to develop an inexpensive but reliable instrumental method for the measurement of trace arsenic in the field. This development has the potential to be very useful for arsenic contaminated water surveillance and arsenic filter monitoring in addition to its regular use in laboratory measurements.

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CHAPTER II

ANALYTICAL METHODS FOR TRACE ARSENIC DETECTION

2.1 Introduction

Effective measurement and monitoring of arsenic in groundwater and soil has become very important because of its high toxicity and widespread occurrences. Most of the techniques used for arsenic detection measure the total arsenic present in a sample without speciation. To determine the potential transformation and toxicity risk assessment of arsenic in the environment and biological samples, other specific separation techniques such as High Performance Liquid Chromatography (HPLC) interfaced to an Atomic Absorption Spectrometer (AAS) or Atomic Fluorescence Spectrometer (AFS) are usually applied for speciation.[46] Other techniques for measuring the bioavailability of arsenic that can be absorbed by living organisms are the subject of ongoing research.[47] Some of the standard analytical methods for the measurement and speciation of arsenic are described below.

2.2 Spectrophotometers

Spectrophotometers in the UV-VIS range are currently the preferred laboratory methods to measure arsenic in parts per billion (ppb) or µg/L levels.

The standard Spectroscopic analytical methods include Atomic Fluorescence Spectroscopy (AFS), Graphite Furnace Atomic Absorption Spectroscopy (GFAAS), Hydride Generation Atomic Absorption Spectroscopy (HGAAS), Inductively Coupled Plasma-Mass Spectroscopy (ICP-MS) and Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES). These methods involve pretreatment of the sample with acidic oxidation or extraction in order to convert all of the arsenic in the sample into an arsenic acid solution.[48-49] In general, atomic spectroscopic instruments are expensive, bulky and consume large amounts of pure gas. In terms of cost and performance, Atomic Fluorescence Spectrometry is a better technique in this category.[50] In our laboratory an AFS (Millennium Excalibur PSA 10.055 from PS analytical, UK.) was used efficiently to detect parts per trillion (ppt) levels of As concentrations. The technique is based on continuous flow hydride generation. The hydrides and excess hydrogen are swept out of the generation vessel using a stream of argon into a chemically generated hydrogen diffusion flame. The hydrides are atomized and the resulting atoms are detected by atomic fluorescence.

Arsenomolybdate chemistry is utilized in some direct spectrophotometer methods. However, only As (V) is sensitive to this chemistry. Therefore a pre-oxidation is required if As (III) is to be determined.[51] As (V) anions react with molybdenum oxide to form an arsenic polyoxomolybdate complex. When reduced, it becomes arsenomolybdate anion with an intense blue color and a defined absorbance band that can be measured easily. However interference

from phosphate and other cationic species can hinder reliable trace level detection.

Another useful spectrophotometer is the Surface Enhanced Raman Spectroscopy (SERS), a powerful tool for classifying unknown chemicals by their vibrational spectra.[i] Raman spectra of arsenite and arsenate in solutions and soils are identified by the change in wavelength when a laser light is reflected off the adsorbed molecule. The Raman spectrum provides the fingerprint of the desired species out of a large sampling environment with computer assistance. The minimum detection limit (MDL) for arsenate and arsenite by SERS has not been determined yet.[52-53] This technique has low sensitivity, very expensive and may not be a viable field analytical technique.

Laser-Induced Breakdown Spectroscopy (LIBS) provides another alternative approach for the detection of arsenic. A small laser induced breakdown called laser spark is formed by high powered pulsed laser beam focused directly into the targeted sample. The sample molecules are then vaporized, atomized and electronically excited as a result of the high temperature. The electrons within these atoms subsequently emit light at characteristic wavelengths as they gain energy. The resulting emission frequency spectrum can be used for arsenic speciation.[54] The lowest MDL

[i] Raman Effect was named after Chandrasekhara Venkata Raman of India who invented this spectroscopic technique and earned the 1930's Nobel Prize in Physics. His work was published for the first time in the *Indian Journal of Physics*, 1928, *2*, 387 under the title "A New Radiation". He was the chief editor of the *Indian Journal of Physics* of which he founded in 1926.

reported is 400 ppm which is considered poor when compared with other techniques.[55]

2.3 Electrochemical Methods

Detection of arsenic by electrochemical assays has been demonstrated to be promising. Liquid samples are ideal for this method. Solid samples must be extracted and dissolved into liquid form before testing. The EPA has approved an anodic stripping voltammetry (ASV) method for the measurement of trace arsenic. ASV is capable of measuring as low as 0.1 ppb level of arsenic in a liquid sample. Among commercially available ASV instruments, the one from Tracedetect® (Washington, USA) may be readily transported and used in the field.[56] Tracedetect® was tested in Bangladesh by this lab and was found to be unsuitable for routine use. In this laboratory a home made ASV instrument built by Dr. Abul Hussam has been used for arsenic related research and teaching purposes.[57] This instrument and protocol are now used to measure arsenic in thousands of samples by laboratories in Bangladesh.

Both As (III) and As (V) are equally detectable by the ASV method, but the arsenate has to be reduced chemically before measurement. The method uses anodic stripping to quantify free dissolved As (III) ions on a conditioned gold-plated-glassy-carbon electrode. The As oxidation peak appears at a potential of +145 - 200 mV with respect to the saturated Ag/AgCl, KCl reference electrode. There are three major steps involved in the ASV analysis. First, the samples are

made acidic by adding hydrochloric acid, which acts as the supporting acid electrolyte. Then, a glassy carbon electrode (GCE) is prepared by plating a thin film of gold onto the electrode and it is conditioned. The electrode is placed in the sample solution, and the dissolved arsenic is reduced onto the electrode surface ($As^{3+} + 3e^- \rightarrow As^0$ on Au). A layer of arsenic is formed on the gold electrode from the solution and subsequently oxidized off (the reverse reaction). The quantitative measure of arsenic that was removed from the solution can be determined by the amount of electrical current required to strip (or remove) the arsenic oxidatively (anodic process) from the GCE. The electrochemical response could be interfered by dissolved copper at a concentration greater than 100 times that of the arsenic concentration.[58] High concentrations of mercury, lead and zinc may also interfere with the determination of arsenic.[59]

Recently, ASV methods are being coupled with more affordable and readily available microelectrodes. Gold microelectrode arrays can be mass-produced using photolithographic methods, unlike the previous GCE which requires preparation of a new gold electrode for each new set of measurements. Microelectrodes improved the MDL to 0.05 ppb but a common criticism of using microelectrodes or GCE is electrode fragility and irreproducibility.[60] However, some studies suggest that microelectrodes are long lasting. In one study a gold microelectrode array lasted 30 days.[61] Although this is an indication of improvement, more research is required to make ASV a handy long-term technology.

Capillary ion electrophoresis is another technique for the measurement of arsenic which has not been widely applied but it has shown proof of concept in the laboratory. This technique is limited to only extraction and separation of ions from a sample matrix. It requires another sensitive instrument such as ICP-MS to be coupled with it for detection and measurement.[62] Capillary ion electrophoresis has a detection limit of 1.0 ppm for arsenic species, however below 1.0 ppb has also been achieved with indirect absorbance detection. In indirect absorbance detection, a strongly absorbing species is placed in the buffer to change the spectrum for that region.[63, 64] A recent study developed a similar technique called isotachophoresis fitted with a conductivity detector. The MDL for this device ranges from 2.0–5.0 ppm, which is higher than capillary electrophoresis, but the size, durability and ease of use makes it preferable.[65] Both techniques and others on the horizon suffer from poor detection limits and a prohibitively high cost of implementation.

2.4 Field Techniques

It is critical that reliable analytical field techniques are identified and approved for the measurement of arsenic. A field monitoring instrument must be portable, resilient, easy to use, selective, sensitive and accurate. Moreover, it has to be environmentally friendly and affordable. There are a variety of established field techniques for the measurement of arsenic, but there has not been a best technique that meets all the necessary criteria. The shortcomings of

field analytical capabilities have left millions of people at risk. It may be noted that, in Bangladesh alone, there are 10 million samples to be analyzed for at least a couple of times. Without a fast and reliable field technique this task would take many years in some remote villages.

The current baseline field methodology involves the "Gutzeit" method, developed 100 years ago.[66] The Gutzeit derived methods have been almost exclusively used on water samples, although they may be applied to soil or waste samples. These methods involve the addition of a reducing agent that transforms all the arsenic in the water sample into arsenic trihydride (AsH_3, arsine gas) to separate the arsenic from the water. The diffused arsine gas is then exposed to a paper soaked with mercuric bromide ($HgBr_2$). The reaction with the paper produces a colored compound and the arsenic concentration can be visually approximated from a list of calibrated color scale. Compounds of sulfur, selenium and tellurium can interfere with the accuracy of the Gutezeit tests. Additionally, organoarsenic species, such as MMA and DMA can not be directly detected by these methods. In the presence of a strong reducing agent such as sodium borohydride ($NaBH_4$), MMA and DMA can form CH_3AsH_2 and $(CH_3)_2AsH$ respectively, however these compounds do not react with mercuric bromide on the test strip to give the desired color change.[67]

The lack of reproducibility and accuracy are the two main pitfalls of the available field testing kits that rely on the Gutzeit methods. Various studies show significant differences and false negative or positive readings when compared

with other laboratory methods. Besides, the arsine gas produced by the Gutzeit method exceeds the recommended threshold limit value (TLV) of 0.05 ppm and the testing strip produces toxic mercury solid waste.[68]

Several important improvements have been made to advance the performance of the field kit technology. For example, the reducing agent used for arsine gas generation has been changed from zinc powder to $NaBH_4$ because zinc metal decreases reactivity. The sulfide contaminates that are known to interfere with the test are now oxidized to sulfates to avoid the interference.[69] However, the use of toxic chemicals in the field is prohibited except when monitored by authority.

Another promising field technology for detecting arsenic is X-ray fluorescence (XRF). The big advantage of this technique is that it can directly measure arsenic in soil without aqueous extraction. XRF is field-ready, weighs 20 lbs and is powered by a battery with MDL of 50 ppb.[70] The durability of XFR is advantageous but the technique is limited in its sensitivity. Even though it seems like there are a number of instruments to measure arsenic, the search for a suitable field technique is still continuing in the laboratory.

2.5 Biosensors

Biological systems show potential for measuring arsenic. For instance, bacteria have a specific genetic mechanism for detoxifying arsenic that involves activation of proteins regulated by arsenic exposure.[71] An Arsenic biosensor can be created using these proteins as they are creating a visible signal, usually fluorescence bright yellow when the bacterium comes in contact with arsenic compounds.[72] However, it is not clear whether the bacteria are sensitive just for the bio-available arsenic or all of the arsenic in the entire sample. There has not been enough research conducted in this area but it is a very interesting and may be promising notion to study in-depth. Biosensors involving the use of plants to detect arsenic in the environment may also be another concept to study. Biosensors also suffer from degradation of sensing proteins, low sensitivity and high cost.[73]

2.6 Chemiluminescence (CL) Based Method

Chemical reactions using highly oxidized species, such as peroxides are commonly termed as chemiluminescent reactions. These reactions usually involve the fragmentation or cleavage of the O-O bond resulting in a large energy release. Chemiluminescent reactions are very useful in forensic science for the detection of bloodstains. The oxidation of luminol by hydrogen peroxide catalyzed by hemoglobin or the enzyme peroxidase of the bloodstain in an alkaline medium, produces an excited 3-aminophthalate ion detectable at a

wavelength of 425 nm.[74] Light emitting reactions which take place by the use of electrical current are designated as electrochemiluminescent reactions. Those arising from living organisms, such as the firefly or jellyfish are commonly termed as bioluminescent reactions.[75]

Ozone (O_3) is by far the most commonly used CL reagent for gas phase reactions. Gas-phase CL reactions are usually used for the measurement of unsaturated hydrocarbons, sulfur, nitrogen, boron, phosphorus, etc., in atmospheric pollutants. The most popular CL based environmental analysis for monitoring nitrogen monoxide was first proposed by Fontijin *et al*. in 1970.[76] This CL was generated from the reaction of $NO + O_3 \rightarrow NO_2 + O_2$.

CL based methods for the measurement of ppb level arsenic are rather simple and do not necessarily require the use of a spectrophotometer or automated tools.[77] The measurement is based on detecting the CL emission from mixing arsine and ozone. Arsine gas (AsH_3) is initially generated in acidic media by a strong reducing agent or electrochemical reduction. The AsH_3-O_3 reaction in a closed glass cell generates intense CL that can be detected by a photomultiplier detector. The resultant glow from this reaction is a bluish band within the wavelength range of 400 nm to 520 nm in the visible region.[78] The analog output from the photodetector can be recorded on a strip-chart recorder or a data acquisition system and the peak height or peak area is measured for quantitative determination of arsenic. Some CL based analyzers for arsenic measurement utilize a flow injection system for faster analysis. In one particular

study, a gas-liquid separator based on membrane separation in a flow cell was discussed. However, the study does not present a diagram of signal response. In another study, a peristaltic pump was used to move the solutions. A carrier gas and a drier gas were also supplied, usually argon and helium. [79] The biggest advantage of CL based method over previously discussed arsenic detection and measurement techniques is that it is capable of differentiating between As (III) and As (V). Acidic potassium permanganate ($KMnO_4$) is the common reagent used in this method to detect only As (III) from total arsenic concentration in the sample.[80] The effort to make a chemiluminescence based arsenic analyzer a suitable field technique is still continuing behind laboratory doors. We have been a part of this effort for the last three years and our results appear to be promising.

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CHAPTER III

DEVELOPMENT OF A GAS-PHASE CHEMILUMINESCENCE DETECTION SYSTEM FOR THE MEASUREMENT OF ARSENIC IN ENVIRONMENTAL AND BIOLOGICAL SAMPLES

3.1 Intent of the work

The intent of this work is to develop an easy, fast, environmentally friendly, portable, and computer-controlled field analyzer for the detection and quantification of arsenic without sacrificing the analytical figure of merits i.e., the detection limit, sensitivity and long term reproducibility. The technique is based on the generation of gas-phase chemiluminescence (CL) from the reaction of arsine gas (AsH_3) and ozone (O_3) in a reaction cell attached to a photo-detector.[81] Although the approach was last discussed extensively more than a decade ago, there has been a notable increase of emerging studies.[82] A number of results have been reported on the use of liquid-phase CL coupled with a flow injection analyzer. Most of the techniques described in these previous studies determine organic and inorganic species based on liquid state oxidation of luminol by hydrogen peroxide.[83] There are very few reports on the determination of inorganic arsenic species using gas-phase CL detection.[84] The sensitivity of a gas-phase CL detection method for measuring arsenic in environmental and

biological samples is attracting researchers in the field of analytical chemistry. This is mostly due to continuous reports on widespread arsenic poisoning around the globe and at the same time the lack of a dependable detection system. In this work, we show a promising homemade gas-phase chemiluminescence analyzer coupled with a simple fluidic system.[85] The study involves the development of the system with complete data acquisition and control. The ultimate goal of this development is to put this analytical instrument in the field in a completely portable configuration.

3.2 Experimental Section

3.2.1 Chemicals and Reagents

All chemicals used in this experiment are guaranteed reagents (GR) suitable for use in the chemistry laboratory which meet or exceed American Chemical Society (ACS) requirements where required. Standard stock solutions of As (III) and As (V) were prepared at concentrations of 400 µg/L using sodium arsenite ($NaAsO_2$, Baker & Adams) and sodium arsenate heptahydrate ($Na_2HAsO_4 \cdot 7H_2O$, Fisher). In most cases pure deionized water (18 MΩ-cm at 25^oC) was used for dilution of the stock solution. In some cases 2.0 M H_2SO_4 was used for dilution immediately before use. The reducing agent, 4% $NaBH_4$ solution for hydride generation, was prepared from sodium borohydride (sodium tetrahydroborate, $NaBH_4$, 99% reagent plus, Sigma Aldrich) dissolved in 0.5 M NaOH prepared from solid sodium hydroxide pellets (NaOH, Amresco) and 1.0

mM disodium ethylenediamine tetraacetate (Na_2EDTA, Fisher). The NaOH was used to stabilize the borohydride and Na_2EDTA was added to form a highly stable coordination compound with the trace metals which are known to catalyze the reduction of borohydride. (Note: NaOH absorbs water from accessible air and its weight can be altered instantly if left in open air for more than a minute or more.) The 5.0 M H_2SO_4 solution prepared from concentrated H_2SO_4 (95–98% ACS Reagent, Sigma Aldrich) was used for total As, As (III) and As (V) determinations. Sodium citrate crystals ($Na_3C_6H_5O_7 \cdot H_2O$, Mallinckrodt) were used to prepare 1.0 M citric acid ($C_6H_8O_7$) and adjusted to pH 4.5 with 1M NaOH. The citrate buffer was used for the selective reduction of As (III) only in the presence of As (V) species.

Silver nitrate ($AgNO_3$, Fisher), ammonium hydroxide (NH_4OH, Sigma Aldrich), potassium hydroxide pellets (KOH, Fisher), dextrose sugar ($C_6H_{12}O_6$, J.T. Baker) and concentrated nitric acid (HNO_3, Sigma Aldrich) were used for the silvering of the reaction cell. Potassium iodide (KI, 99% Reagent Plus, Sigma Aldrich) was used for detecting the presence of ozone in the flow system and to absorb excess ozone on the exit line of the system. Granular activated carbon was also used at the air pump inlet to capture biogenic organic contaminates and at the end of the system to destroy ozone. (HAZARD: activated carbon at higher temperature may cause fire upon high levels of ozone flow.)[86-87] Silica gel desiccant (SiO_2, Fisher) and Drierite ($CaSO_4$, Fisher) were used as drying agents. For sample analysis purposes, random unknown sample solutions were

prepared from 1000 µg/L As stock solution. SRM (Standard Reference Material, Fisher) was used for validation of results. Other samples tested included tap water from Fairfax, VA and groundwater and surface water samples from different regions of Ethiopia.

3.2.2 Procedure

3.2.2.1 Materials and General Instrument Setup

For a batch measurement system it is necessary to deliver the reducing agent ($NaBH_4$) into the reaction cell as fast as possible. This was accomplished initially with a syringe pump (SYVA, Cavro Scientific Instruments). Later, a 10mL automated glass dispenser (Metrohm, 655 Dosimate, Brinkmann) was also used as an alternative replacement to deliver the reducing agent into the arsenic containing sample. Experience shows that the strong alkaline borohydride solution is a very strong reducing agent and could destroy plastic materials and make them brittle. A 3 mm cylindrical teflon magnetic stirrer was used to swirl the sample. The sample container was a 7 mL glass vial with screw top and Teflon line septa (Supelco). Three different sizes of fluorinated ethylene propylene tubes (FEP, Upchurch Scientific) were used for various connections on the analyzer. The outside diameters (OD) were 1/16, 1/8 and 1/4 inches and inside diameters (ID) were 1/50, 1/16 and 1/5.3 inches, respectively. Two flow controllers (Micro Metering Valves Assy 1/16 P-446 and 1/8 P-447, Upchurch Scientific) and two unidirectional check valves (02-19CV0012N, 1/8'' ID, Ark-

Plas) were used for fine tuning the flow rate and to prevent backflow on each side of the air pump and O_3 lines. Another inline check valve (CV-300, Upchurch Scientific) was also used between the air pump and the sampling vial for similar purpose. Fittings, nuts and unions (P-200, P-207, P-307, P-630, P-602 and U-665, Upchurch Scientific) were used for connecting tubes based on their sizes. Solenoid pinch valves (Biochem Valve Inc. PIN075T2NC12-62 and PIN075P2NC24-02SQ) powered by 12 V and 24 V DC external power supplies respectively, were used in some cases to explore the control of AsH_3 and O_3 flow as needed. An ozone generator (ozone output capability of 300 mg/hour using a power source of 120 V AC / 60 Hz Model EOZ 300Y, Enaly Trade Co., Ltd. China) was used to generate the necessary ozone for the CL reaction from pure oxygen in ambient air. The ozone generator consumes a total power of 12 W to operate. The air is supplied to the ozone generator and to the sample vial by a small air pump (Altech Thomas ANR: 50020019, SRN 44007047 Germany) powered by 24 V power supply and controlled by a simple switch (I024-2, JUD. CO). A 30.0 mL plastic syringe (Lauer Slip Plastic Syringes, National Scientific) containing activated carbon, gypsum drying agent (Drierite, $CaSO_4$) and a piece of cotton sequentially packed from bottom to top, was attached to the inlet of the air pump.

A glass reaction cell designed by Dr. Abul Hussam with specific dimensions was used throughout the experiment. The glass cell has 1 mm thickness, 0.5 inch diameter and 1.75 inch vertical height. It is designed with two

0.5 inch long and 0.0625 inch diameter inlets on the top portion for AsH_3 and O_3 flow and a third outlet of the same size at the bottom for exit flow. Figure 3.1 shows the glass chemiluminescence reaction cell with its optical window mounted onto a photomultiplier tube. A waste vial made out of glass with Teflon septa screw top (40 mL, Supelco) filled halfway with activated carbon and linked by an FEP tube to the exit channel of the reaction cell and to a 0.5 L plastic bottle containing 2.0 g acidic potassium iodide (KI) in water, was used for waste drainage and residual ozone absorption.

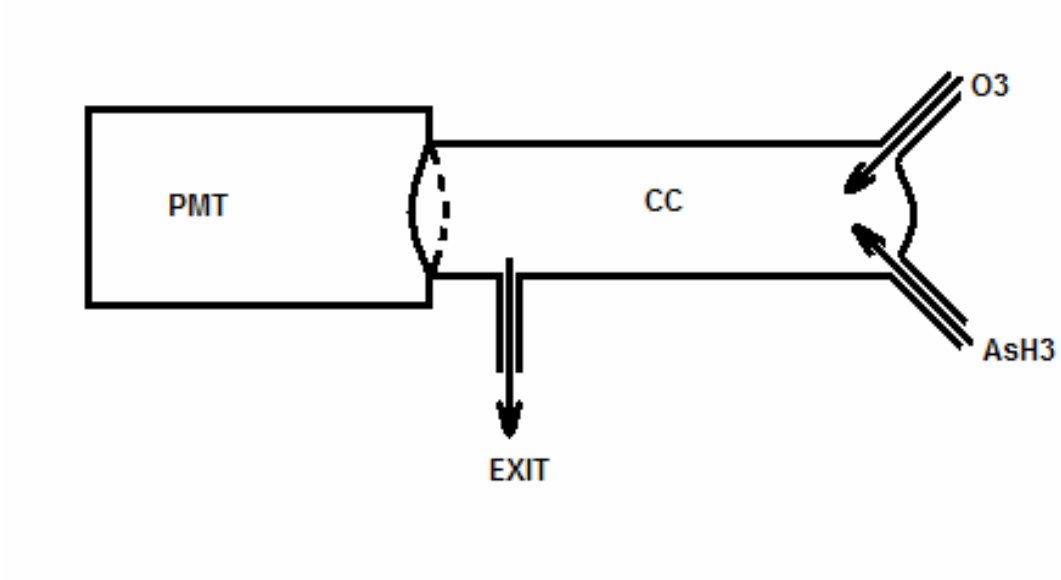


Figure 3.1. Chemiluminescence Chamber (CC) with O_3 and AsH_3 inlets and an exit connection with its optical window mounted on the PMT.

A photomultiplier tube (PMT, Hamamatsu photodetector Model H5784, Japan) was used to sense and amplify the emission of chemiluminescence (CL) resulting from the AsH_3-O_3 reaction. A black opaque polyethylene box, 20 cm x 15 cm x 5 cm (l x w x h) was used to keep the reaction cell and the PMT attached together in the dark, so that light can not access and interfere with the CL detection. A circuit board with op-amps (operational amplifiers) with variable gain up to 10000 was implemented by a network of feedback resistors. The circuit has a filter with a 1 sec time constant to reduce the external noise such as the noise induced by air pump fluctuation. All data acquisition and control was maintained by a PMD USB-1408FS data card (Measurement Computing Inc. USA). The PMT gain was controlled by a 12 bit DAC and all data were acquired by a 14 bit ADC. Autoburet, syringe pump and PMT test with LED were controlled by the digital logic output from the data card. The software interface was written in house by using Delphi-6 (Borland Software Inc. USA), a Pascal programming language and it was loaded onto a Pentium 4, Windows XP class laptop PC.

3.2.2.2 System Schematic Representation

The schematic representation of the instrument using the reaction vial for batch measurement is shown in Figure 3.2.

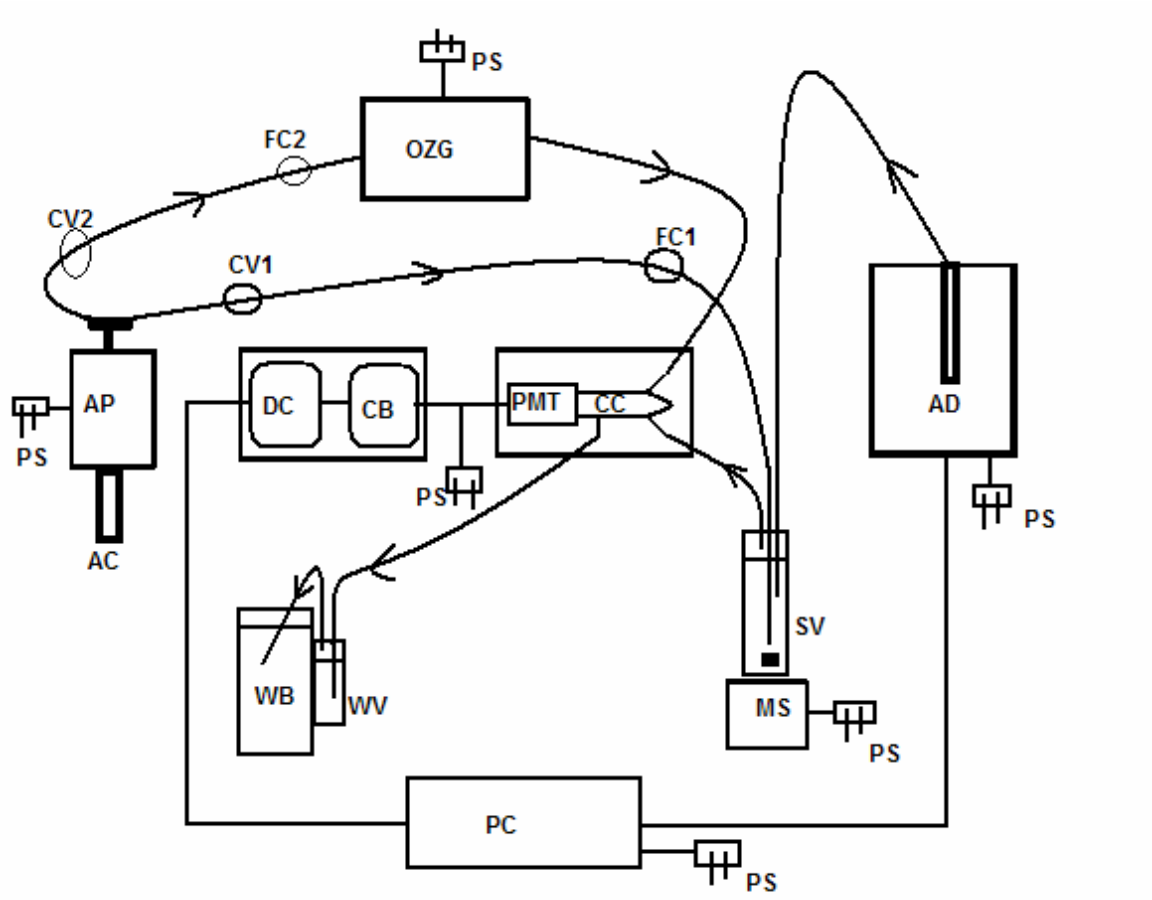


Figure 3.2. Schematic diagram of Gas-phase CL based arsenic analyzer. The abbreviations indicate: Power Supply (PS), Auto-Dispenser (AD), Sample Vial (SV), Magnetic Stirrer (MS), Flow Controller 1 & 2 (FC1, FC2), Check Valve 1 & 2 (CV1, CV2), Ozone Generator (OZG), Chemiluminscence Chamber (CC), Photomultiplier Tube (PMT), Circuit Board (CB), Data Card (DC), Air Pump (AP), Activated Carbon (AC), Waste Bottle (WB), Waste Vial (WV),and Personal Computer (PC).

Sample measurements begin with a mixture of 1.0 mL arsenic containing solution and 1.0 mL of 5.0 M H_2SO_4. They are introduced into the 7.0 mL glass vial with screw top Teflon line septa. Three holes are made on the tightly closed Teflon septa. (Note: Teflon Septa is leak resistant, but it is important to check for leaks frequently.) The first hole will be used for $NaBH_4$ inlet and it reaches midway into the vial. The second hole is the air flow inlet which is adjusted by an air flow controller (FC 1). This air flow tube nearly reaches the bottom of the vial for purging the generated arsine from the solution. The third hole is for AsH_3 gas outlet and it is set near the top of the septa. Unless stated otherwise, the three tubes are all Teflon FEP with 1/16 inch OD and 0.02 inch ID. The 10.0 mL auto-dispenser (Metrohm) or the 1 mL bidirectional auto-syringe (SYVA), indicated as AD on the schematic diagram, is connected to a switch on the circuit board (CB) through its serial port and powered by a 120 V AC power supply (PS). The syringe or dispenser, depending on which one is being used, is linked to the sample vial via one of the three FEP tubes.

The chemiluminescence cell, which is also known as the CL chamber (CC), is connected to the ozone generator by one of the two top inlets via a series of 1/16 and 1/4 inches OD tubes respectively. The other top inlet of the CC is connected to the AsH_3 gas flow purged from the sample vial. The third connection of the CC goes to the waste vial (WV) filled halfway with activated carbon. This outlet is located at the bottom portion of the CC and uses 1/16 inch

OD tube. The WV is further linked to the waste bottle (WB) containing water and dissolved potassium iodide (KI) via 1/4 inch OD Teflon FEP tube.

The air pump (AP) is located between the sample vial and the ozone generator (OZG). The main outlet of the AP is a bidirectional output leads to the ozone generator (OZG) and the other to the sample vial. Both of these lines go through unidirectional unions and two flow controllers, one on each side (FC1 and FC2). The connection between the AP and the sample vial involves successive 1/4, 1/8 and 1/16 inches OD tubes respectively. Two of these tubes are transparent PE tubes while the 1/16 inch OD tube that goes directly into the sample vial is black Teflon FEP. The other output from the AP is connected directly to the OZG using two successive 1/4 and 1/8 inch OD transparent PE tubes. (Note: These tubing sizes and connections were chosen after numerous experiments.) Table 3.1 summarizes the exact tube sizes and their connections used in the optimized system. A schematic diagram of the gas-phase chemiluminescence based arsenic analyzer is shown in Figure 3.2.

The CC is attached to the inside wall of the black box (BB) with black electrical tape and mounted onto the PMT. The black box is sealed and secured in place with black electrical tape to prevent any ambient light from entering and reacting with the ozone or simply altering the chemiluminescence detection. The PMT is connected to the circuit board (CB) and shares a 12 volt DC power supply (PS) with the CB.

Table 3.1. Precise tubing sizes used in the optimized system

Connections	**Tubing Measurements**
Dispenser to sample Vial	44 cm long, 1.6 mm OD and 0.5 mm ID (FEP).
Sample vial to the AsH_3 inlet of the CC	12 cm long, 1.6 mm OD and 0.8 mm ID (FEP) → P-630 union → 24 cm long, 1.6 mm OD and 0.5 mm ID (FEP) → p-630 union → 30 cm long, 1.6 mm OD and 0.5 mm ID (FEP).
Sample vial to air pump	24 cm long, 1.6 mm OD and 0.5 mm ID (FEP) → P-446 FC → 24 cm long, 1.6 mm OD and 0.5 mm ID (FEP) → CV-300 union → 29 cm long, 3.2 mm OD and 1.6 mm ID (FEP) → O2-19CV0012N CV → 8 cm long, 6.3 mm OD and 4.8 mm ID (PE) → T connection → 6 cm long, 6.3 mm OD and 4.8 mm ID (PE).
CC exit to waste vial	112 cm long with a portion of it made into 8 cm diameter loop, 1.6 mm OD and 0.5 mm ID (FEP).
Waste vial to waste bottle	19 cm long, 3.2 mm OD and 1.6 mm ID (FEP).
Ozone generator to CC	8 cm long, 6.3 mm OD and 4.8 mm ID (PE) → U-665 union → 75 cm long, 1.6 mm OD and 0.5 mm ID (FEP).
Ozone generator to air pump	9 cm long, 6.3 mm OD and 4.8 mm ID (PE) → 28 cm long, 3.2 mm OD and 1.6 mm ID (FEP) → P-447 FC→ 29 cm long, 3.2 mm OD and 1.6 mm ID (FEP) → O2-19CV0012N CV → 22 cm long, 3.2 mm OD and 1.6 mm ID (FEP) → “T” connection → 6 cm long, 6.3mm OD and 4.8 mm ID (PE).

Experimental results that will be discussed later on in this chapter were obtained by applying the settings described above. The syringe pump was also replaced by a 6-port load / inject valve (V-450, Upchurch Scientific) which includes a 1.0 mL sampling loop and a peristaltic pump (Model 77120-62, Master Flex C/L).[88] A set of experimental data were collected by using the six port load / inject valve in flow injection system. In this case, the system was setup as a sequential flow injection analyzer (FIA).[89] The peristaltic pump (PP) continuously pumps 5.0 M H_2SO_4 and 4% $NaBH_4$ into the 6-port valve and the "T" connection respectively, using two identical polytetrafluoroethylene (PTFE) tubes (1/16 inch OD and 1/30 inch ID). The reactor (R) is a 30.0 mL disposable plastic syringe container inverted with the lower tip connected to 1/8 inch OD and 1/16 inch ID PTFE tube rotated into three loops and directed to a waste bottle. The top of the reactor is tightly capped with a rubber stopper with penetrations for two PTFE tubes. One of the inlets is for the sample flow that is mixed with H_2SO_4 and $NaBH_4$ in the "T" connection, pumped by the peristaltic pump. The second one is an outlet for AsH_3 that is being purged into the CC to react with O_3 for the chemiluminescence formation. The main difference in flow injection with the sample vial system described above is that, here the flow of acid and reducing agent are continuous and the generation of AsH_3 begins in the "T" connection before it gets to the reactor. Sequential orders of flow injection as well as Auto Dispenser systems are explained thoroughly in section 2.2.2.4. Table 3.2 shows the exact tubing sizes and their connections used in the optimized flow injection

system. The schematic diagram of the 6-port load / inject valve flow system coupled with gas-phase CL detection is shown in Figure 3.3.

Table 3.2. Precise tubing sizes used for the flow injection system coupled with CL detection

Connections	**Tubing Measurements**
Two rounds of loop between port 1 and 4	66 cm long, 10.5 cm loop diameter, 1.6 mm OD, 0.5 mm ID PEP.
Port 6 to “T” connection	14 cm long, 1.6 mm OD, 0.8 mm ID PTFE.
“T” connection to reactor	25 cm long, 1.6 mm OD, 0.8 mm ID PTFE
Peristaltic pump to “T” connection	95 cm long, 1.6 mm OD, 0.8 mm ID PTFE
Peristaltic pump to port 5	120 cm long, 1.6 mm OD, 0.8 mm ID PTFE
Reactor to CC	65 cm long, 1.6 mm OD, 0.8 mm ID PTFE
Port 3 to waste bottle	45 cm long, 1.6 mm OD, 0.8 mm ID PTFE
Reactor exit, three rounds of loop	200 cm long, 11 cm loop diameter, 3.2 mm OD, 1.6 mm ID PTFE

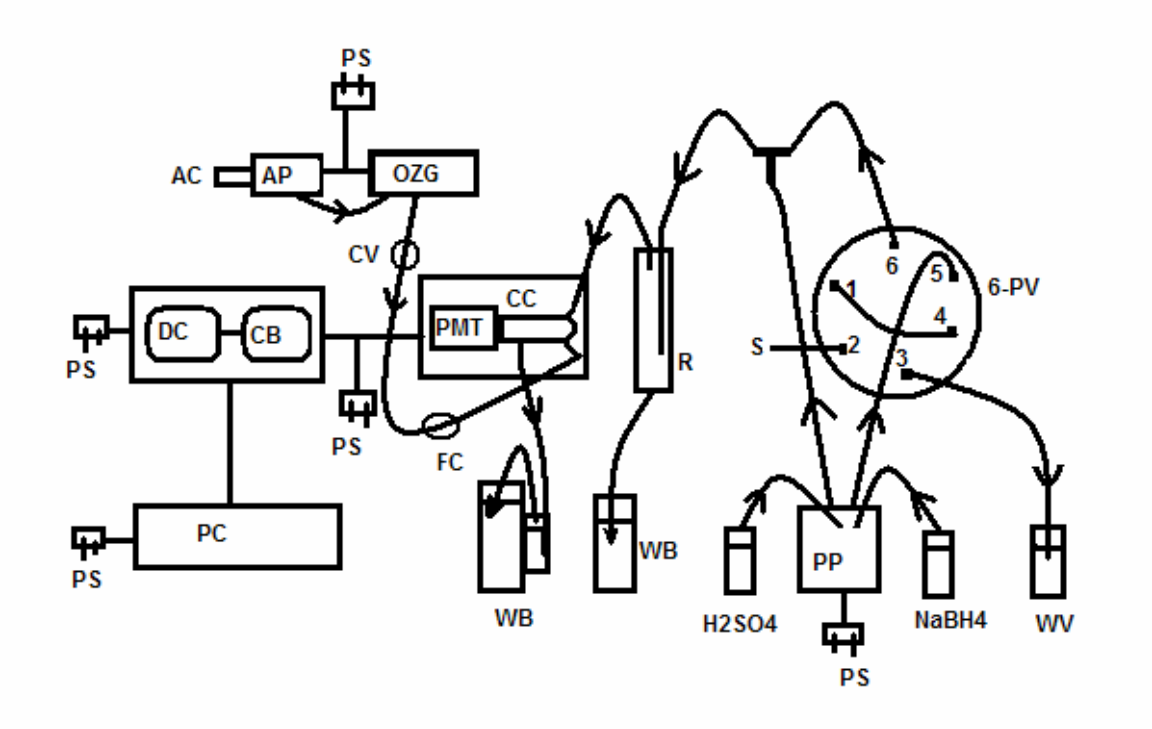


Figure 3.3. Schematic diagram of flow injection coupled with gas-phase CL based arsenic analyzer. The abbreviations indicate: Six-port Valve (6-PV), Sample Injecting Syringe (S), Peristaltic Pump (PP), Power Supply (PS), Waste Vial (WV), Reactor (R), Waste Bottle (WB), Chemiluminescence Chamber (CC), Photomultiplier Tube (PMT), Flow Controller (FC), Check Valve (CV), Ozone Generator (OZG), Air Pump (AP), Activated Carbon (AC), Circuit Board (CB), Data Card (DC) and Personal Computer (PC).

3.2.2.3 System Mechanization

The auto-dispenser is programmable to operate from its own resident memory or it can be controlled from a PC as needed. It is very important that the exact amount of required reagent is delivered at a specific time, so that there will

be enough time to collect data for the background signal as well as the actual chemiluminescence signal. In this experiment the program was setup to allow a 20 second delay for collecting background signal then activate the auto-dispenser to add $NaBH_4$ solution and continue collecting data for another 40 seconds until the signal reaches near the background level. In general 30% of the total allocated time for running a single experiment is devoted to background signal collection and 70% of the total time is spent collecting the chemiluminescence signal. The program subtracts the background signal from the total signal and displays the integrated signal (mV-s) in the user window. The integrated signal (I Signal) from several standard solutions can be used to calculate the calibration factor (calibration factor = I Signal (mV-s) / Conc. (µg/L)). Once a calibration factor (slope of the linear calibration curve) is known, the concentration of unknown samples can be automatically calculated and displayed in the user interface window in units of µg/L. The program has different adjustable parameters that are applicable for testing and troubleshooting the instrument with a blue LED that is also controlled by the computer interface. The full scale signal detection can be adjusted from 1 - 20 V with a signal amplifier gain up to 10000, and the PMT gain varied from 0 - 900 mV. The number of data points that can be collected in a single experiment and the interval between these data points can also be adjusted in the user interface. The chemiluminescence data acquisition user window indicating the adjustable parameters is shown on Figure 3.4. The program has the option to digitally filter

the data, enter a calibration factor for direct concentration display, reading data, printing data, copying and saving data files for post processing.

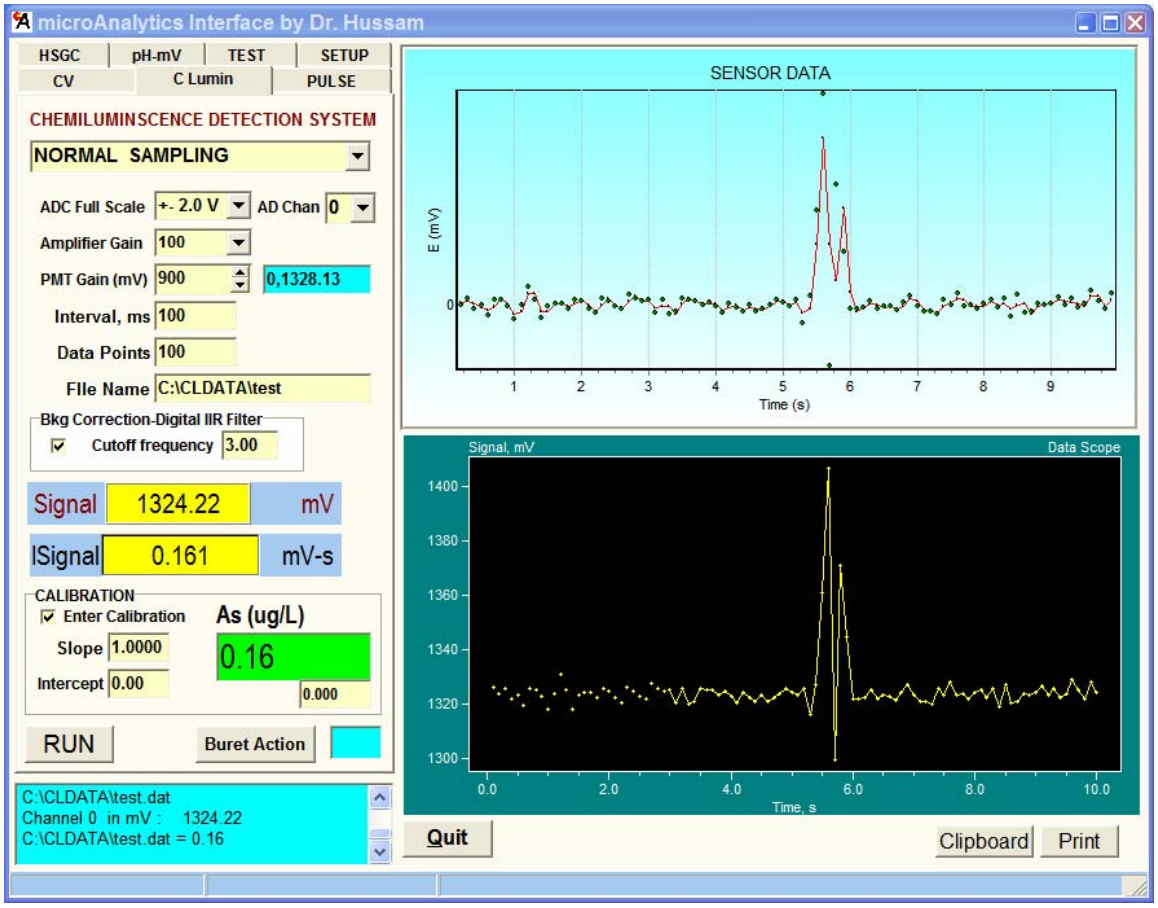


Figure 3.4. Data acquisition user window for gas-phase chemiluminescence based arsenic analyzer. Two real-time data plot windows are shown. The top window displays processed data and the bottom window displays the raw data.

3.2.2.4 Operational Order

The first step is the preparation of the sample solution and required reagents. A 1.0 mL arsenic containing solution is mixed with 1.0 mL of 5.0 M H_2SO_4 for typical sample analysis. The reducing agent, 4% $NaBH_4$ was prepared from 1.0 g $NaBH_4$ and 0.37 g Na_2EDTA in 25.0 mL of 0.5 M NaOH. Before testing a sample, the LED signal detection is performed to check functionality of the PMT and the integrity of the data acquisition system. In a typical operational order, 0.5 mL $NaBH_4$ is added to the arsenic containing solution described above at a speed of 0.5 mL/s by the auto-dispenser following 20 seconds of background signal detection. Dispensing $NaBH_4$ into an acidic sample that contains arsenic instantly causes reduction of As (V) to As (III) to form arsine gas and hydrogen. The hydrogen gas, with the help of the air pump, purges the arsine gas into the CC. Arsine reacts with ozone to give CL that can be measured by the PMT. The total time for one complete run is approximately one minute.

In the flow injection system (FIS), the first step is to load the sample into a 1.0 mL sample loop through port-2 of the 6-port load / inject valve then inject the sample. Inside the loop, the sample mixes with 5.0 M H_2SO_4 that is being continuously pumped by the peristaltic pump into the 6-port load / inject valve via port-5. The excess sample is drained through port-3 into a waste container when the sampling loop fills up. The acidic sample leaves the 6-port load / inject valve via port-6 to the “T” connection where 4 % $NaBH_4$ is continuously pumped by the peristaltic pump. The generation of hydride begins in the “T” and reduction of

any As (V) to As (III) continues along the way to the reactor. Liquid and gas separation takes place in the reactor once the solution (sample + 4% $NaBH_4$ + 5 M H_2SO_4) exits the "T" connection and enters the reactor. The arsine gas is then purged into the CC with the help of the flowing hydrogen gas that has been generated in the process. Unlike the batch auto-dispenser system described above, the flow injection system does not require additional force from an air pump because the hydrogen gas efficiently pushes the arsine gas. The rest of operational order is the same for both flow injection and auto-dispenser systems.

3.2.2.5 Light Detection and Processing

Chemiluminescence is a very weak intensity light that can be detected best by a sensitive PMT. The detection begins with the metal package PMT, a low-power consumption high-voltage supply and a low noise amplifier.[90] In general, a PMT contains a glass vacuum tube, a photocathode, a series of dynodes and an anode. The entry window of the PMT contains a thin deposit of a photocathode. Electrons emission occurs as a result of the photoelectric effect upon the strike of photons on the photocathode deposit. The electrons are then accelerated towards the more positive potential dynodes and additional electrons are produced at each dynode. This cascading effect produces as many as 10^7 electrons for each photon striking the photocathode. Finally the accumulated charges reach the anode where it can be measured as current pulse.[91] Figure 3.5 shows the schematic diagram of the PMT.

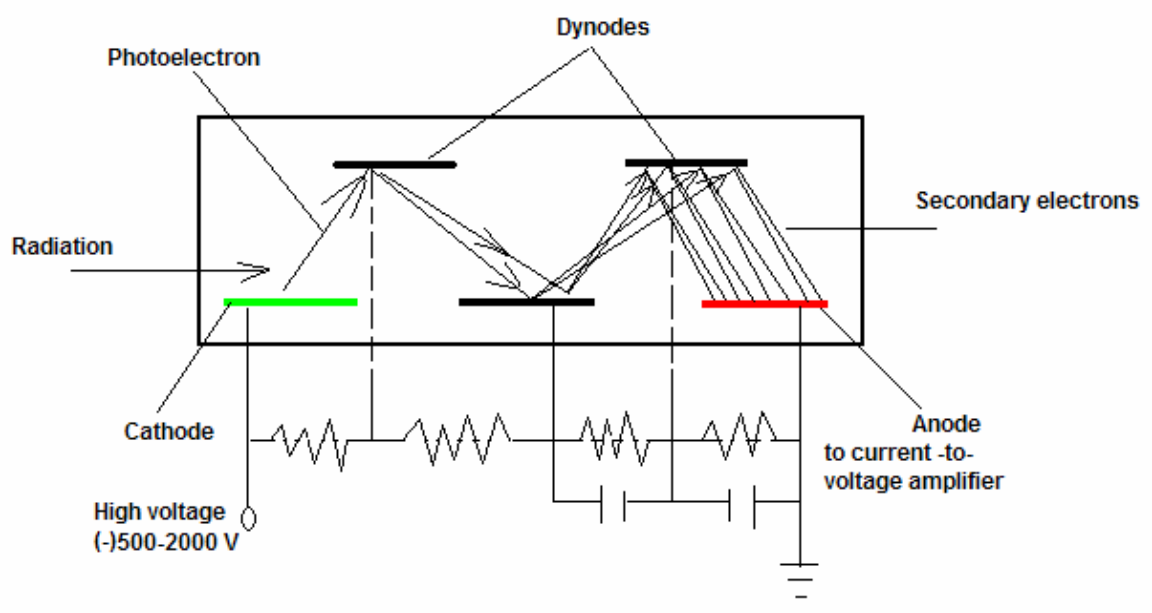


Figure 3.5. Schematic diagram of a Photomultiplier Tube (PMT).

In this experiment a PMT for general application in the visible range was operated in the range of 300 – 650 nm, with ± 12 V external power supply to convert the output current into voltage. The specifications of the PMT are summarized in Table 3.3. The PMT gain was controlled by a DAC voltage control from the data card and was set to 800 – 900 mV for most CL work. The signal was further amplified by a two junction gate field-effect transistor (JFET)-input operational amplifiers (op-amps-OPA604AP). The first op-amps gains can be adjusted to 10X, 100X, 1000X and 10000X through four resistors in a current-to-voltage configuration. The second stage has a gain of 10 and a 1 – 10 μF capacitor to maintain a 1 sec filter time constant. The analog signal from the

PMT, which was further amplified by the op-amps, was then converted to a digital signal by an analog to digital converter (ADC) data card (USB-1408FS) and concurrently acquired by the data acquisition software.

Table 3.3. Specifications of the PMT (Hamamatsu, Module H5784, Japan)[92]

Parameters	**Specifications**
Input Voltage	± 11.5 to ± 15.5 V
Max. Input Voltage	± 18.0 V
Max. Input Current	+9/-1 mA
Max. Output Signal Voltage	+10.0 V (Load Resistance 10 kΩ)
Max. Control Voltage	+1.0 V (Input Impedance 100 kΩ)
Control Voltage Adjustment	+0.25 to 0.90 V
Current to Voltage Conversion Factor	1 V/μA
Effective Area	8 mm
Peak Sensitivity Wavelength	420 nm
Sensitivity Adjustment Range	$1:10^4$ x
Offset Voltage	± 3 mV
Noise (peak to peak)	2 mV
Settling Time	2 s
Operating Temp.	+5.0 to 50.0 ^{o}C
Weight	100 g

3.3 Results and Discussion

This section describes the basic CL formation from the $AsH_3 – O_3$ reaction in a reflective CC, the effect of silvering the CC, generation of hydride from $NaBH_4$, electrochemical hydride generation, ozone and air flow, system response, determination of arsenic in standard solutions and unknown solutions, signal reproducibility, unique field water sample analysis and validation of results with other analytical techniques.

3.3.1 Chemiluminescence (CL) Formation

Chemiluminescence (CL) is the generation of electromagnetic radiation as light by the release of energy from chemical reactions. Electronically excited intermediates or products donate their energy to another nearby molecule and luminesce after the desired chemical reaction takes place. In principle, the light can be emitted in the visible, ultraviolet or infrared regions, but the visible light is the most common and detectable.[93]

It has long been known that the reactions of ozone with hydrides of arsenic (As), tin (Sn), selenium (Se) and antimony (Sb) produce simultaneous CL but, the arsine-ozone ($AsH_3 – O_3$) reaction generates a more intense CL than the others.[94] The pioneers, Kitao Fujiwara *et. al,* described the concept of the gas-phase chemiluminescence detection from arsine-ozone reaction for the first time in 1982.[95] The use of AsH_3-O_3 reaction-based CL generation for the measurement of arsenic has not been fully pursued for at least 25 years because

of the complexity of the technique and its dependability on an expensive luminometer.[96] In this project, a gas-phase chemiluminescence reaction of AsH_3-O_3 is studied under improved laboratory conditions and some of the problems are discussed.

The reaction of arsine and ozone is represented as follows. The extended CL emission is proved to be due to the excimer (excited molecule by a photon formation, $(AsO)_2$).[97]

$$AsH_3 + O_3 \rightarrow AsO^* + AsO^* \leftrightarrow (AsO)_2 \rightarrow 2AsO + h\upsilon \qquad \text{eq.1}$$

The CL due to this reaction is superimposed on a background, over and above the electronic noise. We suspect this background was due to CL reaction of isoprenes and light olefins with ozone. These impurities are sometimes naturally occurring and were not trapped by the granular activated carbon trap at the end of the pump. A typical CL signal generated from 50 ppb SRM solution is shown in Figure 3.6. Figures 3.7 and 3.8 show the same signal with applied 3 point median filter smoothing and 7 point moving average smoothing, respectively.

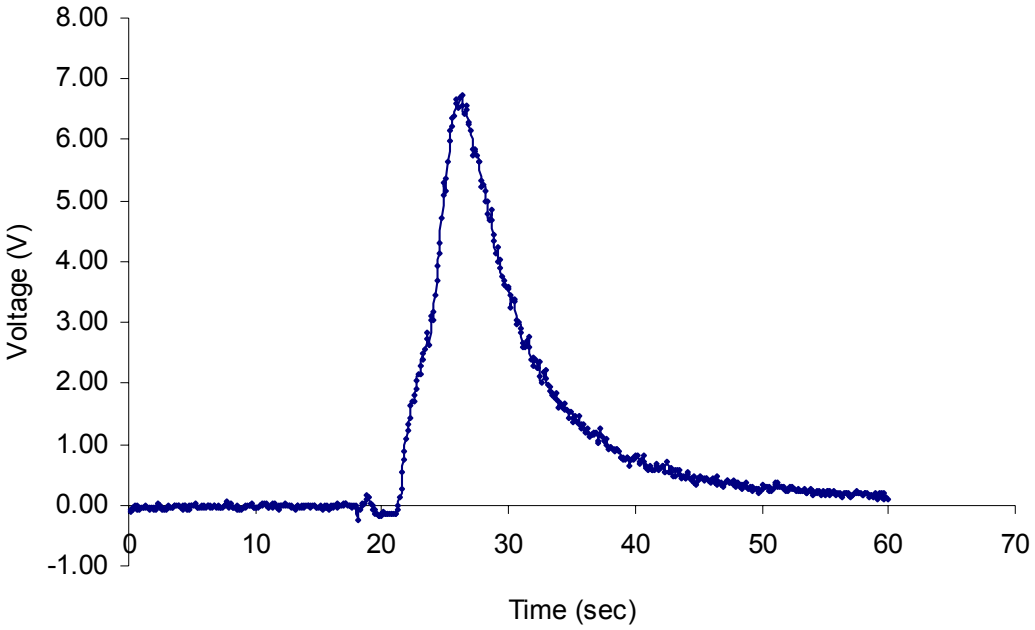


Figure 3.6. CL signal for 50 ppb As_{total} SRM solution.

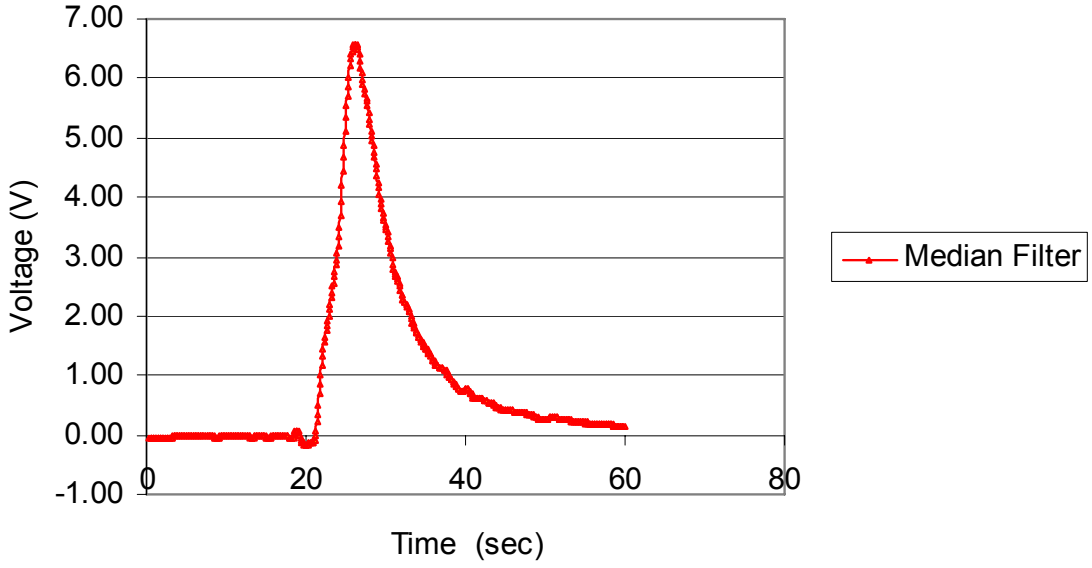


Figure 3.7. Median filter smoothened CL signal.

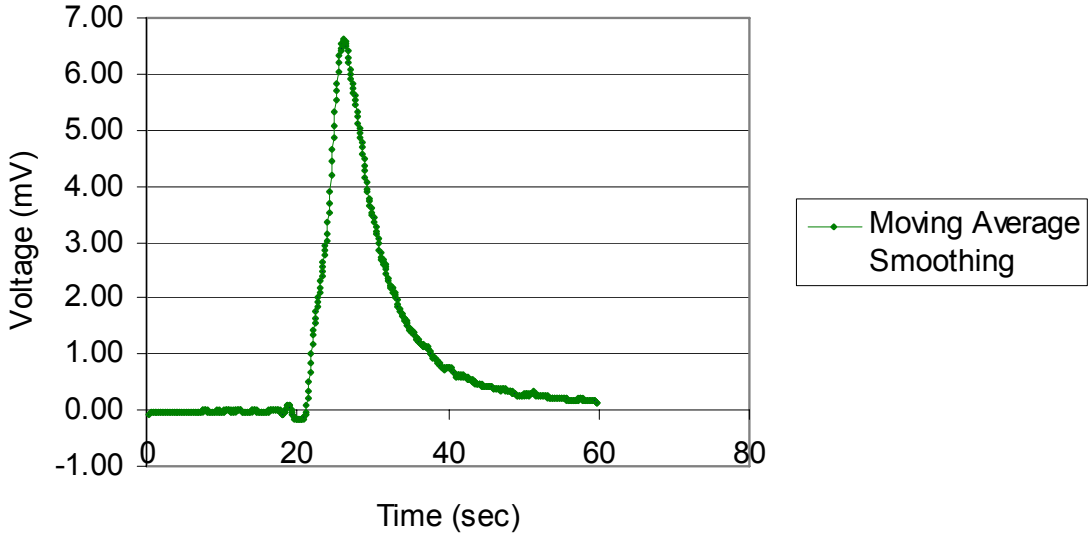


Figure 3.8. Moving average smoothened CL signal.

The rate of the signal decay represents the kinetics of arsine removal from the solution during purging assuming the CL reaction is instantaneous. The signal decay shown in Figure 3.8 suggests first order kinetic with a half-life of 5.82 (ln(2)/k) and experimental rate constant k_{exp} of 0.119 sec^{-1} (-slope). As shown in Figure 3.9, the data fits a first order reaction.

$$Rate = k[O_3]^y [AsH_3]^z = -0.119\ (As_{total}) + 4.289 \qquad \text{eq. 2}$$

The signal reaches its maximum when all of the arsine completely reacts with ozone and decreases when there is no more arsine is flowing into the CC. The ozone concentration, measured iodometrically was found to be 6.25×10^{-5} M. Notice that the concentration of ozone does not change and the rate depends solely on the arsine concentration. This means $k[O_3]^y$ remains constant over the

course of the reaction because $[O_3] >> [AsH_3]$. The experimental rate constant, k_{exp} can be determined as $\ln ([AsH_3]_0 / [AsH_3])$ for first order reactions, but it is not the true rate constant that appears in the general rate law. The true rate constant can be calculated from k_{exp} using the experimental data and the ozone concentration as follows.

$$k_{exp} = k[O_3] \quad so, \qquad \text{eq. 3}$$

$$k = k_{exp} / [O_3] = (0.119\ s^{-1}) / (6.25 \times 10^{-5}) = 1.9 \times 10^{3}\ M^{-1}\ s^{-1} \qquad \text{eq. 4}$$

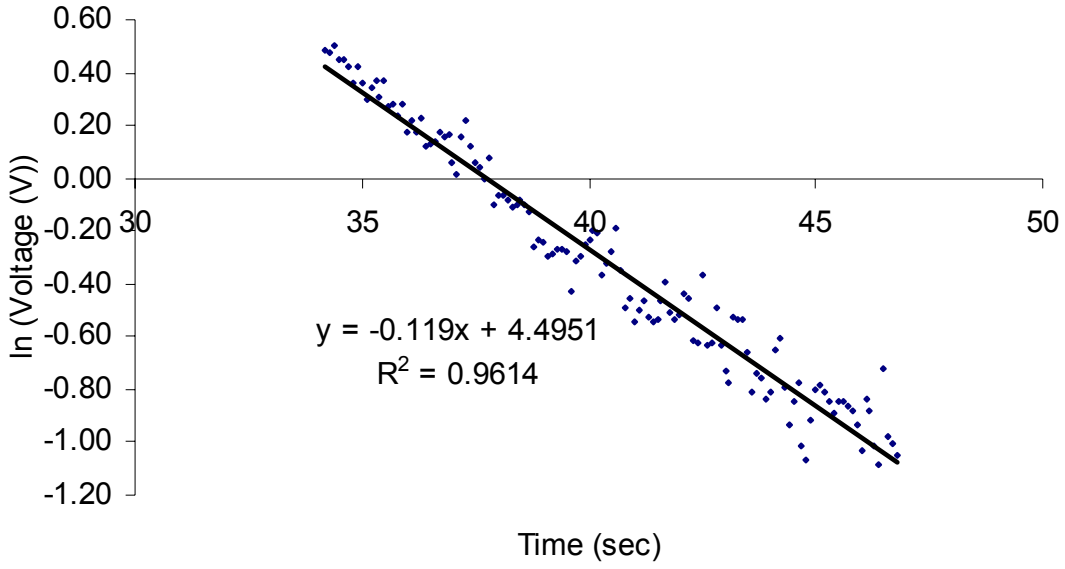


Figure 3.9. CL signal decaying for 50 ppb SRM As_{total} solution, showing first order reaction.

3.3.2 Chemiluminescence (CL) Chamber

Optimizing the reflection of the intense CL in the CL chamber (CC) is as crucial as the formation of the CL itself. To maximize the CL detection, the cell was silvered on the exterior with silver nitrate solution in accordance to silvering protocol and painted black on the top with black epoxy metal paint.[98] At the same time, a similar reaction cell was wrapped with shinny silver paper (simply used for gift wrapping purposes) and secured on top using several rounds of white tape and black electrical tape. The two reaction cells were compared for optimum CL generation. The sensitivity of the silver paper wrapped and white / black taped reaction cell was 50% - 60% more superior than the silvered and black painted CL cell. The silvering was conducted on three similar CL cells and the resultant signals from all three CL cells were similarly lower than the silver-paper-wrapped CC. When a reaction cell is silvered from the outside, the inside will be just like a mirror whereas wrapping a cell with silver paper does not have the same effect. This experiment suggests that intense CL from AsH_3 - O_3 reaction is better reflected in shiny but not mirror-like surroundings. Figure 3.10 compares the results for silvered and silver-paper wrapped reaction cells.

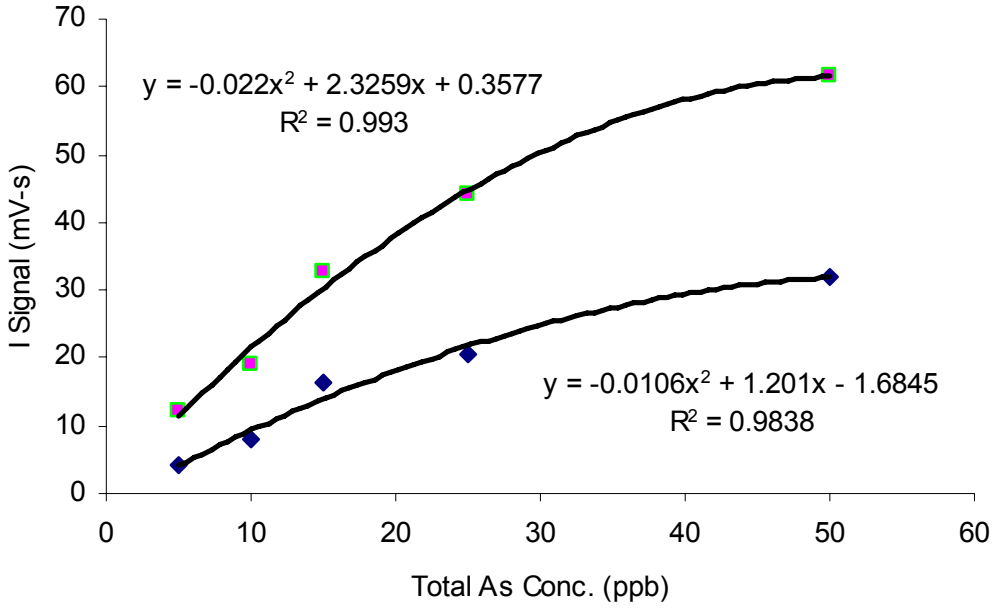


Figure 3.10. CL Signal response for 50.0, 25.0, 15.0, 10.0 and 5.0 ppb total arsenic concentration in silvered and silver-paper-wrapped glass reaction cells. The top polynomial regression represents the silver-paper-wrapped reaction cell and the bottom one represents the reaction cell silvered using $AgNO_3$ solution.

3.3.3 Sodium Borohydride ($NaBH_4$) Induced Hydride Generation

Arsenic forms a volatile hydride (arsine, AsH_3) when reduced with $NaBH_4$ under controlled pH.[99] $NaBH_4$ induced reduction of arsenic is unique among the other reducing agents such as Zn and HCl. It is exceptionally capable of differentiating arsenic oxidation states.[100] The overall hydride generation reaction including As (V) reduction to As (III), can be represented by the following chemical equations respectively.

$As(O)(OH)_3 + H^+ + BH_4^- \rightarrow As(OH)_3 + BH_3 + H_2O$ eq. 4

$As(OH)_3 + 3BH_4^- + 3H^+ \rightarrow AsH_3 + 3BH_3 + 3H_2O$ eq. 5

$BH_3 + H_2O \rightarrow H_3BO_3 + H_2$ eq. 6

A combination of 4.0 % $NaBH_4$ in 0.5 M NaOH and 1.0 mM Na_2EDTA at room temperature was used as a reducing agent. This combination was chosen to stabilize $NaBH_4$ for extended periods of time. One of the problems with using $NaBH_4$ is that it is very unstable. With the above combination we were able to use the solution for 25 - 30 days. Addition of more concentrated NaOH could help extend the stability beyond a month but it also increases the overall alkalinity. To reverse this effect more acid or buffer solution can to be added to the sample. Increasing the amount of acid in the sample is not our interest because it undermines the hydride generation. Besides, more solution in the sampling vial could take up more volume and it may cause moisture transport into the CL cell. If moisture manages to get into the CL glass cell, it may absorb the intense CL and no signal would be detected by the PMT. This problem had been encountered previously before we determined the specifics of sample quantity and reagents based on numerous experiments. (1.0 mL sample, 1.0 mL 5.0 M H_2SO_4 and 0.5 mL $NaBH_4$). In the flow injection system, the simultaneous addition of more NaOH to the $NaBH_4$ solution to increase stability and addition of buffer solution to the sample to adjust the pH to its original value makes the measurement of arsenic at controlled pH very difficult. It has been established that $NaBH_4$ is a major contributor for the alkalinity.

The hydrolysis rate of $NaBH_4$ to BH_4^- and H^+ was previously reported as first order in other studies. It was suggested that impurities may increase this first order hydrolysis rate. Metallic catalysts like nickel and cobalt accelerate the hydrolysis reaction for hydrogen generation that could be used for H_2 fuel cells.[101] The two major disadvantages of $NaBH_4$ are the cost and long term stability; 4% $NaBH_4$ in 0.5 M NaOH / 1.0 mM Na_2EDTA is only stable for a maximum of one month. The use of $NaBH_4$ in developing countries or for continuous onsite arsenic monitoring could be very expensive. Electrochemical hydride generation (EHG) is a good alternative which can solve these problems. EHG avoids the potential contaminants in $NaBH_4$ and does not require frequent preparation of chemicals. Literature indicates that EHG has been applied previously in flow injection configuration and also coupled with a number of spectrophotometer techniques.[102-103]

3.3.4 Ozone and Air Flow

The maximum air flow of 1000 mL/min was applied to push the AsH_3 from the sample vial to the CC. The excess hydrogen generated in the reaction cell and the hydride has the capability to push itself once it is separated from the liquid, but our experiments show that the optimum results can be achieved by pumping air into the sample solution. In the flow injection system, air is not pumped into the reactor because the AsH_3 already starts to be generated in the "T" connection when the acidified sample from the 6-port valve joins the

continuous flow of $NaBH_4$ from the peristaltic pump. By the time the pre-reduced arsenic solution reaches the reactor (R), it will be efficiently separated and purge itself into the CL chamber through the designated tube. The ozone flow was set at a steady state of at least 100 mL/min but it can be adjusted as desired using the ozone flow controller. The maximum ozone output was 300 mg/h or (6.25×10^{-5} M) in this flow rate. When the ozone generator is engaged, the PMT output voltage increases from 3.98 mV to 4.02 mV. This small increase in signal was due to the background CL reactions. It was also proven that only the dark voltage of the PMT is detected when AsH_3 is still flowing and the ozone flow is turned off. A spike due to the CL signal is not detected.

3.3.5 System Response

The analyzer has been optimized to give the best possible signal based on a large number of experiments. We identified different factors that could contribute to compromised CL signal. One of the problems was unwanted access of light into the black box where the CC and PMT are held together. Due to high sensitivity of the PMT, any stray light that gets into the black box can be detected and may alter the result from the CL signal. This external light increases the background noise and decreases the signal noise (S/N) ratio. As a result, the detection limit cannot be dependable or easily determined. All tubing connections of the CC were purposely made black in color to prevent external light contact. Transparent tubing used in previous experiments displayed a noisy

signal. In one instance the CL signal improved noticeably when an experiment was run in the dark with the room light turned off.

The position of the CC is also a factor affecting the CL detection. Improved results were obtained when the exit connection of the CC was at least 45^{o} inclined. This provides for a better drainage of the reacted AsH_3-O_3 from the CC before a subsequent reaction takes place. The issue of moisture accumulation inside the CC and its effect on the signal may also be resolved by this inclination. Accumulation of AsH_3 - O_3 on the surface of the inner CC may form a molecular or atomic film (adsorbate) that could adsorb the intense CL before it is detected by the PMT.

Some of the connections could become vulnerable for leaks due to the relatively high pressured flow of the gas or liquid. To check leaks on various connections should be the first trouble shooting step when CL signal is diminished. Commercial soap water (Snoop ® Leak Detector) has been the preferred method for minuscule leak detections.

Addition of a magnetic stirrer greatly improved the CL signal detection by forcing more AsH_3 to be eluted from the sample vial. A sample with a magnetic stirrer spinning at ~400 rpm gave ~25 - 50 % improved CL signal value and reproducibility when compared with a tested sample without a magnetic stirrer. Table 3.3 shows integrated signal variations for identical samples with stirrer and without stirrer.

Table 3.4. Effect of magnetic stirrer on integrated signal

As Conc.	I Signal (mV-s) without stirrer	Calibration Factor	I Signal (mV-s) with stirrer	Calibration Factor
5ppb 1	5.657	1.131	12.044	2.409
5ppb 2	5.811	1.162	12.96	2.592
5ppb 3	5.685	1.137	11.561	2.312
% rsd	1.4347		5.83	
10ppb 1	8.67	0.867	19.174	1.917
10ppb 2	8.722	0.872	19.87	1.987
10ppb 3	8.618	0.862	18.911	1.891
% rsd	0.5998		2.565	
15ppb 1	7.287	0.486	32.824	2.188
15ppb 2	5.295	0.353	31.635	2.109
15ppb 3	5.419	0.361	27.666	1.844
% rsd	1.8436		0.7545	
25ppb 1	8.316	0.333	47.615	1.905
25ppb 2	8.237	0.329	48.332	1.933
25ppb 3	8.024	0.321	47.89	1.916
% rsd	1.8436		0.7545	
50ppb 1	32.16	0.643	61.022	1.22
50ppb 2	33.946	0.679	61.614	1.232
50ppb 3	32.347	0.647	62.011	1.24
50ppb 4	34.504	0.69	60.989	1.22
50ppb 5	33.676	0.674	61.252	1.225
50ppb 6	32.11	0.642	63.621	1.272
50ppb 7	34.287	0.686	60.059	1.201
50ppb 8	31.477	0.63	63.231	1.265
50ppb 9	30.742	0.615	62.029	1.241
50ppb 10	30.07	0.601	61.554	1.231
% rsd	4.6962		1.7169	

Signal disturbance due to vibration was avoided by securely packaging the circuit board and data card together in an aluminum box. Previously 1 μF and 45 μF capacitors were used for signal amplification, but when replaced with

a 10 μF capacitor, the reproducibility of results greatly improved due to noise spike filtration. The 10 μF capacitor was chosen because the 45 μF capacitor was too big and it retains extra charge from previous signals and adds those charges to the successive signal. On the contrary, the 1 μF capacitor was too small to remove excess noise.

Extending the tube length between OZG and CC has also provided an improved CL signal. Longer tubing offers sufficient time for light olefins, such as ethane and isoprene, to react fully with O_3 before reaching the CC. Last but not least, the CL signal was remarkably optimized when a loop was included on the tube connecting the CC-exit to the waste vial. The loop exerts a back pressure in such a manner that the AsH_3-O_3 can not leave the CC without being detected.

3.3.6 Determination of Total Arsenic, Arsenic (III) and Arsenic (V)

Total arsenic concentration was determined in strongly acidic conditions at pH $\leq$ 1.0. In principle, both As (III) and As (V) convert to AsH_3 upon the addition of $NaBH_4$ in acidic media. However, residual arsenic concentrations were encountered in the sample vial following each first run. When the experiment first began, only 40-45% of the total arsenic was being extracted as AsH_3 and a residue of 55-60% still remained in the sample vial. After a large number of experiments and system adjustments, it is now possible to extract at least 85 % of the AsH_3. For example an integrated signal of 10 V for the first run of a sample will result in the residue arsenic giving a signal of $\leq$ 2 V in the second run

of the same solution. In the flow injection system it is not possible to determine the residue arsenic content left undetected because the sample is not retained after each run because of the continuous flow system, rather the sample goes directly into the waste vial. However, the likelihood that 100% of arsenic could be extracted from the injected sample in the form of AsH_3 is essentially nonexistent. This being the case, as long as the resultant data are reproducible and a superior calibration is applied, the analyzer still determines arsenic concentration of the desired sample efficiently and reproducibly, regardless of the fact that some residue may still be left in the sample. Below is a linear calibration equation used for determination of arsenic concentration in some unknown samples.

$$I\ Signal\ (mV\text{-}s) = (1.0958 \pm 0.02)\ As_{(tot)}\ \mu g/L + (9.58 \pm 0.02) \qquad \text{eq. 7}$$

$$r^2 = 0.9752$$

A blank sample essentially gives no signal as shown in the bottom signal Figure 3.11. For a series of arsenic containing samples tested, signal peak height and peak area increased sequentially according to their concentration as expected. The reproducibility of the results is > 90 % in all cases. Easily identifiable signals were detected in samples containing < 1.0 ppb As. The limit of detection (LOD) was 0.146 ppb (µg/L or 146 ppt), determined based on 10 runs of 50.0 ppb As_{total} SRM solution (standard reference material). Figure 3.11 shows the actual graph of signal response for a series of arsenic containing samples.

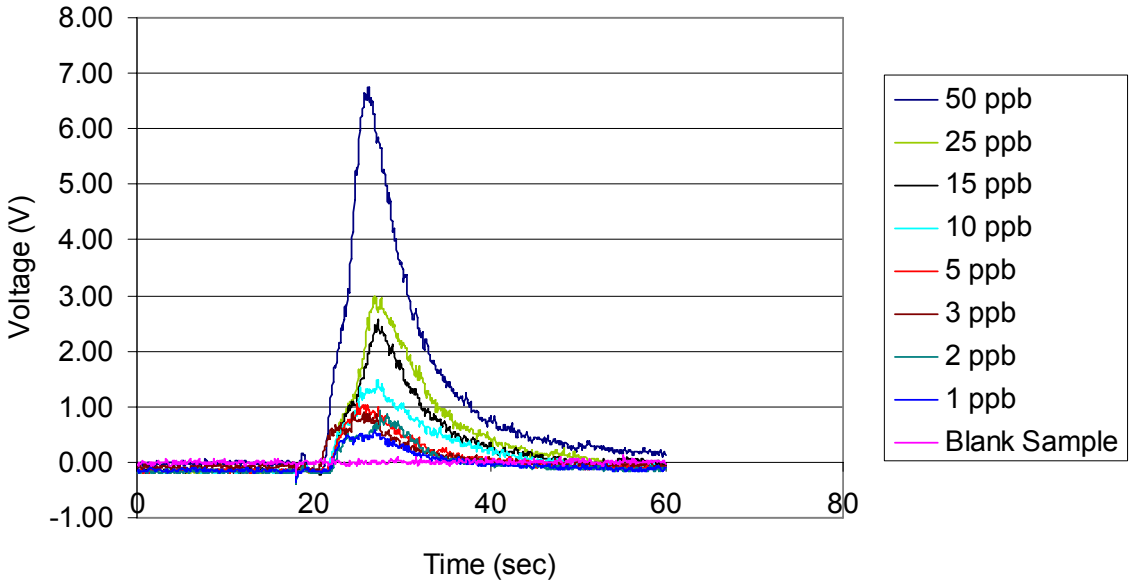


Figure 3.11. Comparison of incremental CL signals for 1.0, 2.0, 3.0, 5.0, 10.0, 15.0, 25.0 and 50.0 ppb As_{total} determinations.

Table 3.5 shows the results for 0.00, 10.0, 15.0, 25.0 and 50.0 ppb total arsenic standard samples. Table 3.6 shows previously analyzed blind samples (A = 40.0 ppb, B = 30.0 ppb, C = 20.0 ppb, D = 60.0 ppb and E = 30.0 ppb). Each unknown sample was tested 5 times and standard deviations of 0.5, 0.6, 0.4, 0.7 and 0.45 were resulted for samples A, B, C, D and E respectively. (Errors were calculated based on "t" test at 90% confidence level). The results indicate a trend of increasing integrated signals as the arsenic concentration increases in each sample. The concentrations of arsenic determined from the gas-phase CL detection fairly agree with the standard concentrations. Table 3.7

shows the reproducibility of results for 5.0, 10.0, 15.0, 25.0 and 50.0 ppb total arsenic samples prepared from 400 ppb stock solution.

Table 3.5. Total arsenic determination for standard samples in ppb

As_{total} Standard (ppb)	**I Signal (mV-s)**	**Calculated Conc. (ppb)**
0	0.004 ± 0.248	0.000
10.0	19.174 ± 0.231	8.755 ± 0.162
15.0	28.563 ± 0.329	17.320 ± 0.431
25.0	41.035 ± 0.267	28.705 ± 0.570
50.0	62.156 ± 0.293	47.272 ± 0.890

Table 3.6. Total arsenic determination for unknown samples in ppb

Unknown Samples	**I Signal (mV-s)**	**Calculated Conc. (ppb)**
A	51.520 ± 0.476	38.273 ± 0.814
B	43.986 ± 0.571	31.398 ± 0.769
C	33.201 ± 0.381	21.556 ± 0.521
D	72.123 ± 0.659	57.075 ± 1.190
E	44.802 ± 0.420	32.142 ± 0.694

Table 3.7. Reproducibility of Integrated Signals for Consecutive Arsenic Containing Samples

Replicate Runs	**50 ppb**	**25 ppb**	**15 ppb**	**10 ppb**	**5 ppb**
1	61.022	47.615	32.563	19.174	12.044
2	61.614	48.332	32.824	19.870	12.960
3	62.011	47.890	33.281	18.911	12.242
4	60.989	47.980	32.662	18.670	11.561
5	61.252	48.215	32.25	18.825	11.883
Average	61.377	48.006	32.716	19.090	12.138
STDEV	0.433	0.281	0.378	0.472	0.522
90% Confidence Interval	$\pm$ 0.412	$\pm$ 0.268	$\pm$ 0.361	$\pm$ 0.450	$\pm$ 0.498

A citric acid buffer (pH of 4.5) was employed for the determination of isolated As (III). A diluted solution of 50 ppb As_{total} prepared from a stock solution containing 50% As (III) and 50% As (V) was tested in citric acid. The addition of $NaBH_4$ adjusted the pH to 5 - 7. In this setting, the sample was determined to contain 73% of As (III). This shows a 23% increase of As (III) from

the original sample. This means reduction of As (V) to As (III) had taken place prior to the test. The sample solution was prepared with 2.0 M H_2SO_4 solution which might have contributed to a lower pH than it was anticipated from the citric acid buffer. Literature reports indicate that significant first order kinetic reduction of arsenic occurs in acidic media.[104] It appears that the pH control of the final reaction solution is critical. An average of 38.213 $\pm$ 0.38 µg/L (ppb) As (III) was determined in a 50.0 ppb total arsenic solution. Standard deviation for 5 consecutive runs was 0.4 and error was calculated based on 90% confidence level. Once the As (III) was determined, the remaining As (V) was calculated by simply subtracting 38.213 $\pm$ 0.38 from the total arsenic concentration.

3.3.7 Determination of Arsenic in Unique Water Samples from Ethiopia and Tap Water sample from Fairfax, VA

Unique water samples collected from six different regions of Ethiopia and tap water from Fairfax, VA were tested for arsenic measurements. The exact locations where these samples were collected are listed below in Table 3.8. Latitude and longitude are listed in degree, minutes, and seconds (DMS).

Table 3.8. List of unique field water samples and tap water sample

Field Sample	Latitude (DMS)	Longitude (DMS)
Nile-Fall water. Gojam, Ethiopia.[105]	11° 10' 60'' N	39° 52' 60'' E.
Akaki deep-well water. Akaki Beseka, Ethiopia.[106]	8° 52' 60'' N	38° 46' 60'' E.
Lalibela spring-water and groundwater. Lalibela, Ethiopia.[107]	12° 1' 60'' N	39° 1' 60'' E
Awasa-Lake water. Awasa, Ethiopia.[108]	7° 2' 60'' N	38° 28' 0'' E
Legedadi deep-well water. Legedadi, Ethiopia.[109]	9° 4' 60'' N	38° 55' 0'' E
Fairfax city tap water. Fairfax, VA. USA.[110]	38° 50' 46'' N	77° 58' 24'' W

All of the samples showed $\leq$ 1.0 ppb As concentration or none at all upon first examination with the CL detection system. However when the samples were tested using a Hydride Generation Atomic Fluorescence Spectrometer (HG-AFS), two out of seven showed significant arsenic concentrations. The findings indicate that pre-reduction of arsenic is required for these samples prior to CL

detection. The hydride generation was compromised by the lack of As (III) in these real samples, so that enough CL could not be generated. Reduction of As (V) to As (III) may take some time when complex organic matter is present in the solution, which we suspect is the case behind the poor CL generation for these field samples. In HG-AFS, the samples were subjected to potassium iodide (KI) induced reduction for 45 to 60 minutes prior to testing. Pre-reduction of As (V) to As (III) is mandatory for HG-AFS because the system uses only 0.7% $NaBH_4$ as a reducing agent.[111] It was expected the 4% $NaBH_4$ used in the gas-phase CL detection system would be sufficient to reduce all of the As (V) to As (III) paralleling the standard solutions prepared in the laboratory. However the field samples do not behave as standard samples and may require longer periods of time for reduction. Table 3.9 shows real sample measurements in µg/L, after pre-reduction with KI. Absolute uncertainties were calculated based on 3 replicate runs.

Table 3.9. Unique sample arsenic determinations

Sample ID	Total As Conc. (µg/L)
Nile-Fall	0.960 ± 0.337
Akaki Deep-Well	BDL
Lalibela Spring	BDL
Awasa Lake	14.589 ± 0.421
Legedadi Deep-Well	6.664 ± 0.370
Lalibela Ground	BDL
Fairfax Tap	BDL

The results in Table 3.9 indicate that Awasa Lake water has the highest arsenic concentration followed by Legedadi Deep-Well water. Awasa Lake has a surface area of ~92 km^2 and maximum depth of ~23 m.[112] For a lake of this size, the measured 14.589 ppb As_{total} concentration in 1mL sample is significantly high. (Note: The EPA maximum limit for arsenic concentration in drinking water is 10.0 ppb.) The distribution of arsenic in the entire lake may not be uniform for different reasons. More water samples need to be tested from diverse portions of the lake in order to determine the final arsenic concentration.

The arsenic contamination in Awasa Lake is possibly linked to industrial waste or the hot springs running parallel to the Awasa basin in the Rift Valley

axis. (Note: Hot springs and volcanic activity can introduce potentially toxic high arsenic concentrations.)[113] The lake is fed by a number of streams and the Tikur Wuha River which may also have contributed to the arsenic contamination. Legedadi Deep-Well water samples showed an average As_{total} concentration of 6.64 $\pm$ 0.370 ppb. Even though the concentration is below the EPA limit of 10.0 ppb, the water will not be safe to use as a potable water source for extended periods of time. Sample analysis reports for Awasa Lake and Legedadi Deep-Well water are shown in Appendix B. The other four samples from Ethiopia and the tap water sample from Fairfax, VA, did not show any arsenic content. (Note: BDL (Below Detection Limit) implies < 107 ppt, the As detection limit of AFS.)

3.3.8 Validation with Other Techniques: Comparison Studies

Two popular laboratory instruments were used to validate our results from the gas-phase chemiluminescence based arsenic analyzer. The first one was Flow Injection Hydride Generation Atomic Absorption Spectrophotometer (FI-HG-AAS, Perkin-Elmer Model 5100, equipped with GEM software). The same six-port valve flow system used for the CL detection analyzer was coupled with the AAS. The second instrument used for validation was fully automated Atomic Fluorescence Spectrometer (AFS, PS Analytical, Millennium System software). Table 3.10 and Table 3.11 show total arsenic measurements with FI-HG-AAS and AFS respectively. Upon comparing the gas-phase CL analyzer with those of FI-HG-AAS and AFS, the data for both agreed in the range of $\geq$ 90%.

Table 3.10. Total arsenic determination with FI-HG-AAS

Standard As_{total} Conc. (ppb)	**FI-HG-AAS Absorbance**	**Calculated As_{total} Conc. (ppb)**
5.0	0.006 ± 0.000	6.093 ± 0.218
10.0	0.010 ± 0.001	10.155 ± 0.156
20.0	0.019 ± 0.000	19.903 ± 0.213
30.0	0.028 ± 0.000	28.738 ± 0.696
40.0	0.040 ± 0.001	40.620 ± 0.537

Table 3.11. Total arsenic determination with AFS

Standard As_{total} Conc. (ppb)	**AFS Peak Height (au)**	**Calculated As_{total} Conc. (ppb)**
1.0	55.933 ± 2.879	1.029 ± 0.030
2.0	108.232 ± 2.392	2.177 ± 0.110
3.0	154.796 ± 2.697	3.300 ± 0.230
4.0	191.725 ± 1.676	4.157 ± 0.120
5.0	249.335 ± 3.377	5.493 ± 0.287
10.0	497.078 ± 2.825	11.239 ± 0.520

3.4 Conclusion

Arsenic has been classified as one of the most deadly human carcinogens by the International Agency for Research on Cancer (IARC) and the United States Environmental Protection Agency (U.S. EPA). Previous studies suggest that the lifetime risk at levels of only 50.0 µg/L would lead as many as 13 additional cancer-related deaths per thousand people.[114] It is a moral responsibility rather than academic interest to save countless lives whose water supplies are contaminated with high levels of the odorless, colorless and tasteless arsenic, which accumulates in the body to cause sores, nerve damage, cancer and too often, death. Among the estimated 137 million people around the world, at least 50 million people in Bangladesh alone are at high risk of arsenic poisoning.[115]

In 2001, the U.S. EPA announced the maximum permissible level of arsenic in drinking water to be lowered from 50.0 to 10.0 µg/L and it became effective by 2006.[116] However, the Canadian Federal Government has recently proposed a revised 5.0 µg/L arsenic limit for the Federal Drinking Water Guidelines. According to the guideline, the estimated lifetime cancer risk associated with the consumption of drinking water containing arsenic at 10.0 µg/L is greater than the range that is considered generally to be "essentially negligible".[117]

The development of cost-effective, robust, portable and environmentally friendly arsenic detection and remediation techniques continue to be of interests

to researchers, international governments and non-governmental organizations (NGOs) around the globe. On February 5, 2005, the National Academies of Engineering (NAE) launched the Grainger Challenge prize for one million dollars plus for Sustainability to find a solution to the arsenic poisoning crisis. The full text of the challenge is shown in Appendix A.[118]

The first chapter of this Thesis Project discusses the history, occurrence, industrial applications, environmental and biological impacts, toxicity, hazards, and removal methods of arsenic. The second chapter discusses several analytical methods for the detection of arsenic, including spectrophotometers, electrochemical methods, field techniques, biosensors and chemiluminescence based methods. The third chapter discusses the development of a gas-phase chemiluminescence detection system for the measurement of arsenic in environmental and biological samples.

In this endeavor, we have developed a fully automated gas-phase chemiluminescence based arsenic analyzer. The field ready arsenic analyzer described in this Thesis Project is sensitive, robust, affordable and environmentally friendly. It can readily be packaged into a size of 45 cm x 30 cm x 45 cm (l x w x h) wood or metal box for field use and can be powered by a car battery. Figure 3.12 shows the picture of all the components of the analyzer in our laboratory setting.

We believe the gas-phase CL based technique is promising for arsenic detection in remote fields that may require continuous arsenic monitoring. It is

also a great addition to our state-of-the-art Anodic Stripping Voltammetry (ASV), electrochemical analyzer. Appendex C shows related publications on arsenic studies.

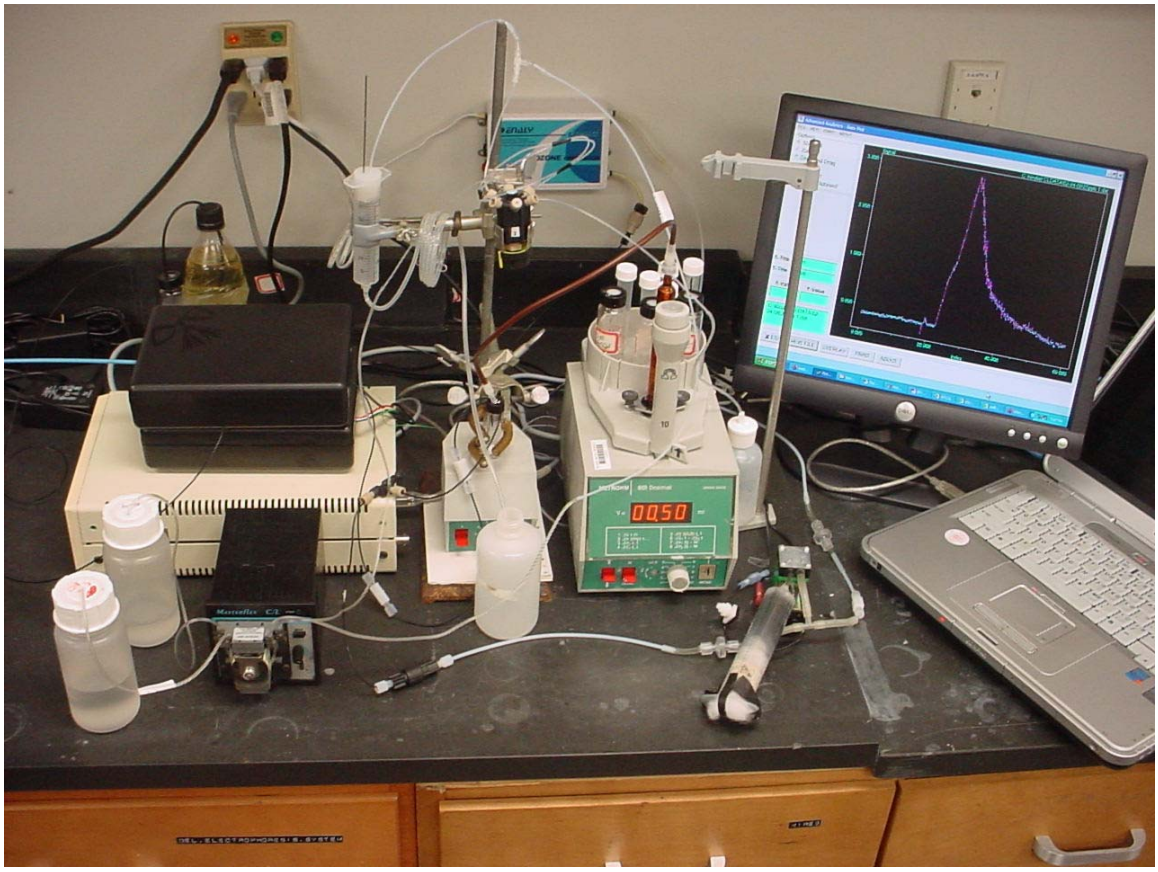


Figure 3.12. The gas-phase CL-based Arsenic Analyzer in the laboratory

3.5 Appendices

Appendix A: The Grainger Challenge

Introduction

The National Academy of Engineering (NAE), supported by The Grainger Foundation, has established the Grainger Challenge Prize for Sustainability. The primary purpose of the prize competition is to accelerate the development and dissemination of technologies to enhance social and environmental sustainability for the benefit of current and future generations. A complementary goal of the prize competition is to increase awareness among the U.S. engineering community of the importance of designing and engineering for sustainability, particularly in an international context, and to encourage and showcase efforts by U.S. engineers to bring sustainable technologies to the marketplace and to promote green design philosophies.

The Challenge

The specific goal of this competition, which may be followed by future prize competitions in like amounts for comparable goals, is the development of a household or multiple household scale treatment system to significantly lower the arsenic content in groundwater from tube wells as found in many developing countries. The system must have a low life cycle cost, be technically robust,

reliable, maintainable, socially acceptable and affordable, be capable of being largely manufactured and serviced in a developing country, and must not degrade other water quality characteristics.

Arsenic contamination has affected millions of people, in rural Bangladesh, and also in eastern India, Nepal, and several other countries. In Bangladesh, the arsenic is an unintended consequence of an aggressive international program to control the spread of cholera (prevalent in surface waters) by installing thousands of tube wells. Unfortunately, the tube wells tapped into aquifers, usually within 100 meters of the surface, containing hundreds of micrograms per liter (µg/L) of naturally occurring arsenic, well beyond the international standard of 10 µg/L.

Efforts to solve this problem have been under way for a decade, but no single solution has been implemented on a widespread scale. Laboratory tests have been conducted on technologies to determine if they are affordable, robust, and meet local water quality standards for a treatment system that can be used either in individual homes or several homes located adjacent to a single tube well. The intent of the NAE/Grainger Foundation competition is to encourage the American engineering community to become engaged in finding a solution to this specific challenge.

International scientists were invited to submit their arsenic filtration unit after a careful review of their proposal providing the full details of their stepwise process and basic program plan for the challenge. More than 70 entries were submitted from different countries for the first testing phase by potential competitors. Dr. Hussam's filtration unit was given the number "29". Between April and June 2006, fifteen of the entries were selected for further extensive testing phases of the competition. In February 2007, the prize winners were publicly announced and the prizes presented at a public forum in Washington, DC. The first prize winner for a million dollar Gold Award was number "29", Dr. Abul Hussam, Professor of Chemistry at George Mason University and the director of this Thesis Project.

Dr. Hussam does not intend to keep the money. He plans to give 70% to help distribute the system to the people of Bangladesh, 25% for research funding and 5% for George Mason University. When asked why he was giving away the money, he said, "We need money to do research and students to do the fundamental work." Time magazine, in the October 2007 issue, announced Dr. Abul Hussam as one of the "global heroes" in the scientists and innovators category. The award was conferred upon him on October 25, 2007 in London, England.[119]

Dr. Hussam is a native of Bangladesh and his own home in Kushtia, West Bengal has a deep-well potable water source containing 150 - 175 μg/L As.[120] Many of the wells around his native neighborhood are also contaminated with

arsenic up to 40 times higher than the maximum amount considered safe. The first challenge for Dr. Hussam was to develop a precise method of detecting traces of arsenic in the groundwater. He developed a computer-controlled electrochemical analyzer to measure arsenic before his efforts in developing the "SONO Filter®", a simple sustainable green system for arsenic remediation. Dr. Hussam always emphasizes the importance of sensitive and dependable measurement technique. He says, "Precise measurement is the key."

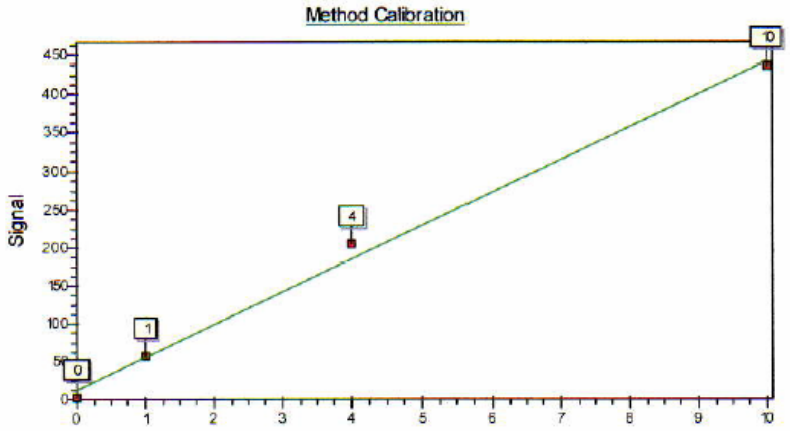
Figure 3.13. Picture taken from Time Magazine, April 2, 2007 issue.

Appendix B

AFS Analysis of Awasa Lake and Legedadi Deep-Well Water Samples

Calibration Details

Measured By :	
Fit Type :	Least Squares
Unit :	ng/ml
Slope :	43.115582
Y Intercept :	12.486461
Correlation Coefficient :	0.997781
Reslope %:	0.000000

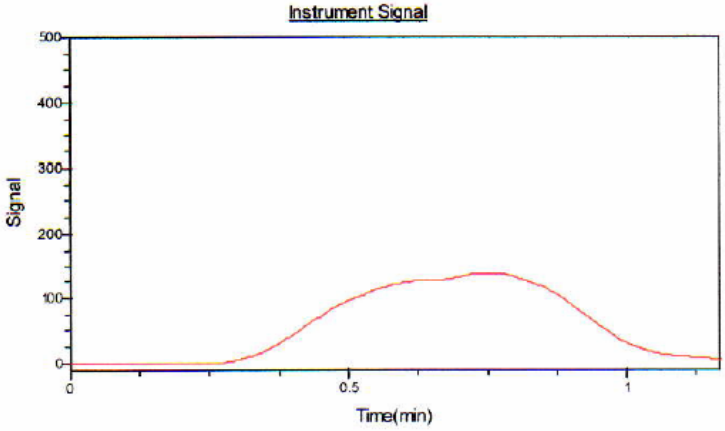


Single Analysis Report - C:\Program Files\P S Analytical\Millennium\Results\KIrubel samples.rsf

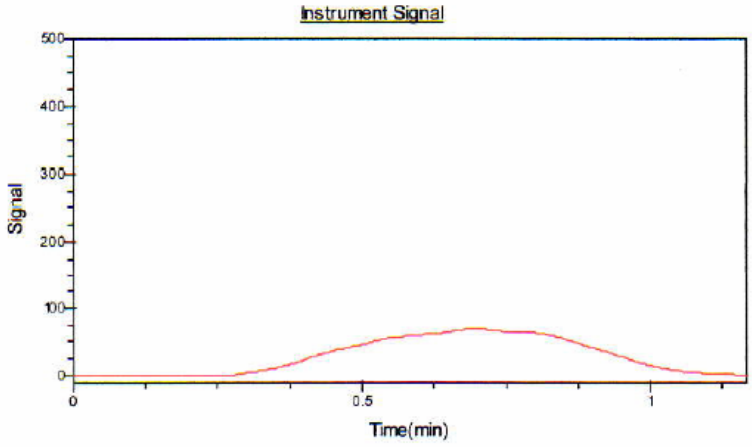
General Details

Method Name : Default0-1.5ppbAs

Name	*ID*	*Conc*	*Pk Ht*	*Pk Area*	*BaseLine*
	Awasa Lake	14.589648	138.294693	3959.141846	0.159471



Name	*ID*	*Conc*	*Pk Ht*	*Pk Area*	*BaseLine*
	Legedadi Deep Well 6	64886	69.958542	1961.352417	-0.402288



Appendix C

Related Publications

As	Estuarine waters	MS;Hy-ICP;L	Samples pre-reduced with KI–ascorbic acid; added to 20% HCl carrier solution. Hydride generated with $NaBH_4$. Arsenic detected by ICP-MS (92 pg As^{III})	236
As	Surface water	AFS;Hy;L AAS;Hy-F;L	Comparison of hydride generation AAS and flow injection hydride generation AFS for determination of Se and As in surface water. Detection levels for FIA–HG–AFS were a few ppt	237
As	Drinking water	AAS;Hy-F;L	Arsine cold trapped then released by electrical heating into heated quartz cell for detection by AAS. Arsenic species determined controlled by reaction conditions used to generate the arsine initially	238
As	Water	MS;ICP;L	Identification and quantification of organic and inorganic As compounds by HPLC–ICP-MS. Arsenic species separated by reversed phase ion-pairing HLPC using microbore column	239
As	High and low salinity seawater	AAS;ETA;L	Different modifiers compared for direct determination of As in seawater by ETAAS. LOD for As = 1.1–3 $\mu g\,ml^{-1}$ using different modifiers	240
As	Saline water samples	MS;Hy-ICP;L	Quantitative determination of As species by ion chromatography linked to hydride generator with a membrane separator and ICP-MS. Short analysis time, good reproducibility and pg detection limits	241
As	Lake, river and waste water	MS;ICP;L	Anionic and cationic ion chromatography columns coupled in series and interfaced to ICP-MS for simultaneous speciation of arsenite, arsenate, dimethylarsinate, monomethylarsonate, arsenocholine and arsenobetaine. Eluent carbonate buffer–nitric acid gradient. No interference from $AsCl^+$	242
As	Drinking water	AFS;F;L AA;ETA;L AE;ICP;L	Several hyphenated systems developed for As speciation including HPLC–ultrasonic nebulisation–AFS, HPLC–HG–ultrasonic nebulisation–AFS *etc.* Results for four arsenic species in water samples compared to ETAAS and ICP-AES data	243
As	Water	AA;Hy-F;L	Continuous flow and FI–HG–AAS methods described. Hydrides collected in cold trap (10–20 pg ml^{-1})	244
As	Groundwater and drinking water	MS;ICP;L	Low pressure FI system with anion exchanger used to separate As^{III} and As^{V} species. Determination by ICP-MS. ArCl interference not found	245
As	River, tap and mineral waters	AA;Hy-F;L	L-Cysteine used as reductant with sample prior to FI–HG. Determination of As by atomisation of hydride in a heated quartz tube and detection by AAS. LOD = 0.01 $\mu g\,l^{-1}$ for 0.5 ml sample	246
As	Seawater	AA;ETA;G	Electrochemical HG by Pb cathode. Hydrides trapped in Pd-coated graphite furnace for atomisation. Determination by ETAAS (84 pg)	160
As	Natural waters	MS;ICP;L	Separation and determination of seven As species in waters by HPLC–ICP-MS using a mixed HPLC column (Spherisorb 5 ODS Amino) and 5 mM phosphate buffer (pH5). ArCl interference removed: Cl^- separated from As species on column	247
As	Mineral waters	MS;ICP;L	Speciation of six As compounds using anion exchange column coupled with ICP-MS. In-house developed thermospray nebuliser for sample introduction (0.04–0.12 $\mu g\,l^{-1}$)	248
As	Seawater	AA;F-Hy;L	Four As species separated by ion exchange chromatography on Dowex 1-X8 and Dowex 50-X8 resins. Hydride generation then detection by AAS. Interferences investigated. LOD $\approx$ 0.3 $\mu g\,l^{-1}$ for As species	249
As	River water	MS;Hy-ICP;L	Sample boiled with 1 ml concentrated nitric acid, pH adjusted to 3.5 and applied to Chelex 100 chelating resin. Arsenic eluted off and sample analysed by FI–HG–ICP-MS (0.03 ng ml^{-1})	250
As	Tap water	AFS;F;L MS;—;L	Arsenobetaine and arsenocholine determined with an HPLC-ultrasonic nebulisation flame-AFS system. LOD for arsenobetaine = 6.7 ng; for arsenocholine = 8.2 ng	251
As	Saline water	MS;ETV-ICP;L	Advantages and shortcomings of use of ETV for sample introduction of small samples into ICP-MS. Direct analysis of As in saline waters discussed	252
As	Deionized water	AA;F;L	Stability of As species in deionized water stored under various conditions investigated by HPLC microwave assisted oxidation hydride generation flame-AAS	191
As	Water	AFS;F;L	Chromatography column (25mm × 4.5 mm id,10 μm C_{18} Rutin) prepared by coating with dimethyldioctylammonium bromide(I). Four As species separated. Detection of As by FI–HG–flame-AFS (0.4–1.2 ng ml^{-1})	253

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