AN ABSTRACT OF THE THESIS OF

Marcia Sakiko Ikeda for the degree of Master of Science in Chemistry presented on June 29, 1984.

Title: Speciation of Inorganic Arsenic by Hydride Generation Atomic Absorption Spectroscopy

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A sensitive technique for the determination of As(III) and As(V) at μg/L and sub-μg/L concentrations from a single sample aliquot has been developed. This technique is based on the hydride generation atomic absorption (HGAA) spectrophotometry. The hydrides of the arsenic species are generated in a reaction vessel by mixing the sample solution with $NaBH_4$. By controlling the pH of the reaction mixture, the hydrides of As(III) and As(V) are sequentially produced. The hydrides are swept out of the solution with hydrogen and directed to the atomization cell. A flame-in-a-tube technique is employed for atomization. The hydrogen/hydride mixture passes through a hydrogen/air flame where hydrogen radicals convert the arsine to atomic arsenic. The atomic vapor then passes into a 20-cm

long quartz observation tube where the atomic absorbance of arsenic is measured at 193.7 nm. The flame-in-a-tube technique was found to produce more consistent results than atomization based on electrothermal heating of a quartz tube to 900° C.

The parameters affecting the atomic absorption signal of arsenic that are independent of the oxidation state of arsenic were optimized using a 10 µg/L As(III) standard in 0.15 M citrate buffer. Optimization studies of the parameters that affect the speciation of inorganic arsenic were conducted on standards containing 10 µg/L of both As(III) and As(V) in a 0.15 M citrate buffer.

The optimized speciation procedure is based on measuring the peak absorbance signal due to As(III) in a 1-mL sample in 0.15 M citrate after an initial injection of 0.05 mL of 20% (w/v) $NaBH_4$. The pH of the remaining solution is lowered to zero by addition of 0.4 mL of concentrated HCl. The second injection of 0.1 mL of 20% (w/v) $NaBH_4$ results in a peak whose peak absorbance signal is due to As(V).

The method developed for the speciation of inorganic arsenic was applied to water and biological (hair) samples for the determination of As(III) and As(V). As expected from previous studies, only pentavalent arsenic was found in both water samples—0.1 µg/L in river water and 0.8 µg/L in tap water. Since only As(III) accumulates in the body tissues, a KOH digestion was employed on the hair sample to prevent the oxidation of the arsenic in the sample to the pentavalent form. In the hair sample, only the trivalent form was found at a concentration of 2.6 µg/g of hair.

The accuracy of the technique was tested through the use of NBS standard orchard leaves (SRM #1573). After drying the leaves in a muffle furnace at 450° C and dissolving the leaf ash in HCl, the diluted sample was analyzed for arsenic concentration by the developed procedure. A concentration of 10.6 μg As per gram of orchard leaves was found and agrees well with the NBS listed concentration of 10 ± 1μg/g of dried leaves.

For a 1-mL sample aliquot, the detection limits for As(III) and As(V) are 0.06 and 0.2 μg/L, respectively. The atomic absorption sensitivities for As(III) and As(V) are 0.2 and 0.4 μg/L, respectively. The time of analysis per sample or standard is approximately 1 min.

Speciation of Inorganic Arsenic by Hydride Generation Atomic Absorption Spectroscopy

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

Speciation of Inorganic Arsenic by Hydride Generation Atomic Absorption Spectroscopy

INTRODUCTION

Recently, science and the public have become increasingly concerned about the health hazards associated with metals and other species. One of the major reasons for this concern is the increased release of these species into the environment by man. Lack of information about the concentrations and their mechanisms of interaction in nature has prevented the reliable assessment of the public health hazards of released chemicals in the environment.

Studies of the interaction mechanism of various elements in nature has led to the discovery that the chemical form (e.g. oxidation state, complexed vs. non-complexed, etc.) of an element can affect its hazard to man. As a result, attention is turning to the development of instrumentation that allows studies and quantitation of the different forms of a particular element. The determination of the concentration of specific chemical forms of an element has been termed "speciation".

The research in this thesis is concerned with the element arsenic. The two oxidation states of concern are As(III) and As(V) which also denoted as the trivalent and pentavalent forms, respectively. These latter terms do not indicate the number of valence bonds. The toxicity of a given arsenical is related to its

rate of excretion from the body and degree of accumulation in certain body tissues. In general As(III) is more toxic than As(V). In addition to the inorganic arsenates and arsenites, organo-arsenic compounds are also formed, but their toxicity is considered to be less than that of As(V) (1). Arsenic causes toxicity by combining with the sulfhydryl (-SH) enzymes and thus interferes with the cellular oxidative processes (2). The pentavalent arsenicals have a very low affinity for the thiol groups and as a result are excreted rapidly from the body. The trivalent arsenicals have a much higher affinity for thiol groups and as a result are more toxic than the pentavalent forms (1). Arsenic is stored mainly in the liver, kidney, wall of the gastrointestinal tract, spleen, and lung. Because of the high number of disulfide bridges in elevated concentrations of arsenic are found in the hair and nails (3).

Arsenic can be released into the environment through several industrial processes such as from the smelting of copper, lead, zinc, and other ores; from the use of arsenic containing pesticides and herbicides; from the generation of power from coal; and from geothermal sources (4). Arsenic can also be a natural constituent in foods. Seafoods, pork, liver, and salt may contain high concentrations of arsenic (3). In general arsenic in the trivalent form is added to the environment, however, the pentavalent form is naturally occurring. Arsenite in water is slowly oxidized to arsenate by oxygen (5). The standard reduction potential of As(V) to As(III) is 0.559 V in acidic medium. In natural waters, the primary

As(III) species is in the form $As(OH)_3$ and the primary As(V) species are $H_2AsO_4^-$ and $HAsO_4^{2-}$.

Arsenic levels in natural water samples are usually in the low to sub µg/L range. The presence of arsenic of 50 µg/L in drinking water constitutes grounds for rejection of the supply (6). According to a study conducted by the United States Geological Survey in 1971 (7), 79% out of a total of 727 samples of water from around the U.S. contained total arsenic levels below 10 µg/L, 21% had arsenic levels greater than 10 µg/L, and 2% had levels greater than 50 µg/L.

The purpose of this research was to develop a simple method to determine the concentration of arsenic in the trivalent and pentavalent forms from a single, small aliquot of sample. The method developed is intended for use with water samples and biological samples. Sample preparation techniques must be chosen carefully in order not to change the As(III) to As(V) concentration ratio. Oxidative wet digestion methods (e.g. perchloric acid) or dry ashing methods in the presence of oxygen will cause oxidation of As(III) to As(V).

HISTORICAL

Introduction

There have been several methods developed for the determination of arsenic and a partial tabulation of these techniques is listed in Table I. Some of the elements in Groups IVA, VA and VA are capable of forming covalent hydrides that are volatile at room temperature. (See Figure 1). Several methods for the detection of these elements have been developed based on their ability to form volatile hydrides. These methods are the usual choice for these elements because of the added selectivity provided by removing the analyte from the sample matrix. Once the hydrides are formed, they can be swept out of the sample solution and into an atomizer (flame, graphite tube furnace, heated quartz tube, or a plasma) to produce a peak response.

Atomic absorption spectrophotometry (AAS) is the most popular method of detecting the volatile hydrides. Researchers have used a nitrogen/hydrogen/entrained air flame (13-15), a graphite tube furnace, (16-18), a heated quartz tube (19-22), or a hydrogen/oxygen flame in a quartz tube (23,24) to produce the atomic vapor. Although AAS is the most popular method, atomic emission using an inductively coupled plasma (ICP) (25,26) or a microwave induced plasma (MIP) (27,28) has been used to produce the excited atoms and to detect the hydrides. Atomic fluorescence spectrometry (AFS) has also been used by a few researchers as a detection method (29,30). A peak response

Table I. General Methods for As Determination

Technique	Detection Limit (μg/L)	Reference
Flame atomic absorption spectrophotometry	250	(8)
Neutron activation γ-ray spectrometry	1	(9)
Differential pulse polarography	3	(10)
Inductively coupled plasma emission spectrometry	40	(11)
Flame atomic fluorescence spectrometry	100	(12)

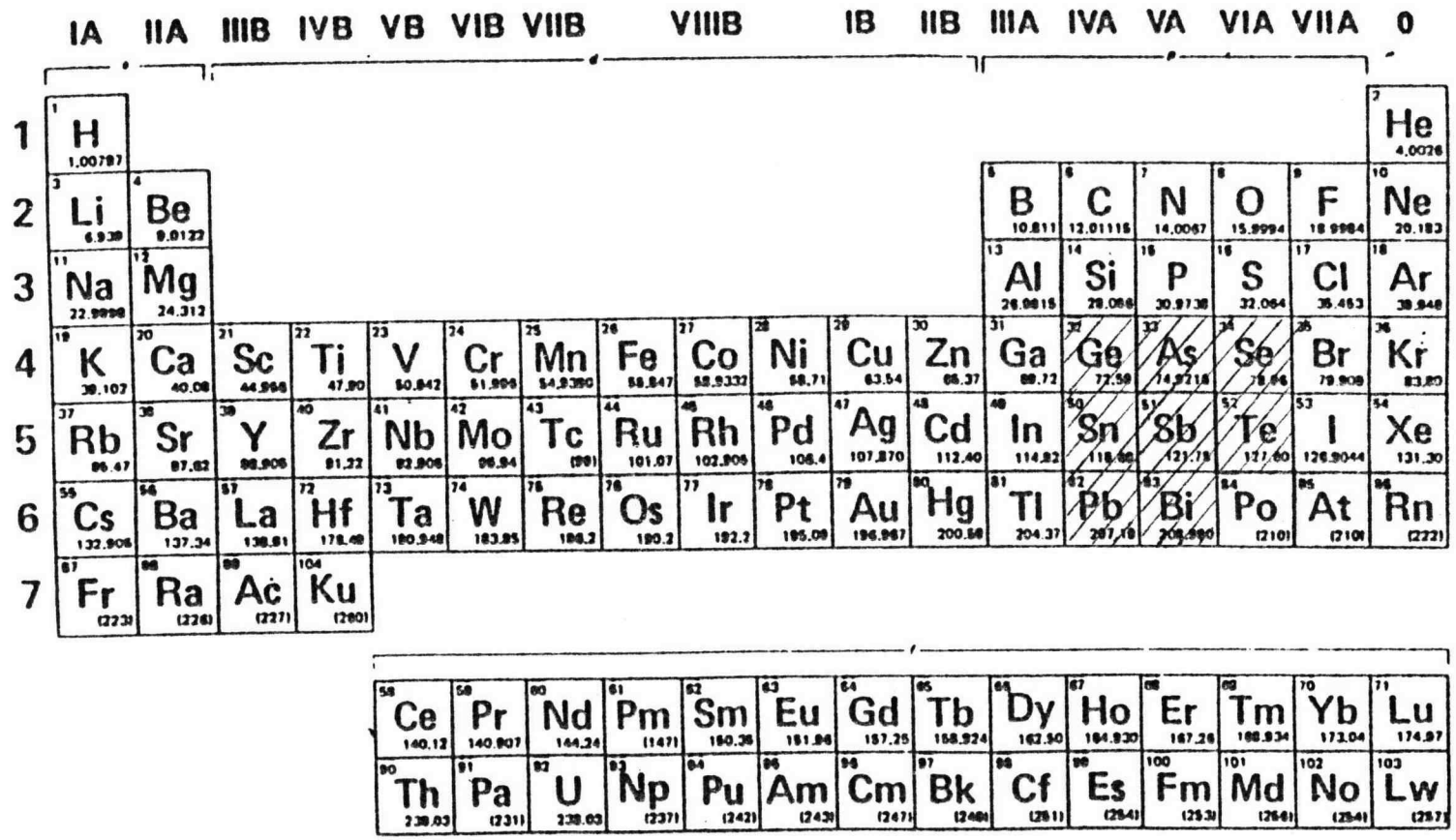

Figure 1. Elements that form covalent hydrides. Other elements form covalent hydrides, but the hydrides formed from the shaded elements are used for the determination of these elements.

results in the above mentioned methods, and either peak height or area is measured to obtain quantitative information.

The hydrides can also be directly swept into a solution to form a colored complex and molecular absorption spectrophotometry is used as the method of detection (31,32).

A partial tabulation of the methods used to determine arsenic hydrides is listed in Table II.

Hydride Generation

For over a 100 years, hydride generation has been utilized for qualitative and quantitative determinations of arsenic. The Marsh Reaction (33), a qualitative test for arsenic, forms the hydride by reaction of an arsenic solution with zinc and a mixture of HCl and H_2SO_4. The generated arsine is then combusted with excess oxygen to give the bluish flumes of As(III) oxide as shown in Equation 1.

$$2\ AsH_3 + 3\ O_2 \longrightarrow 3\ H_2O + As_2O_3 \qquad (1)$$

The Gutzeit Test (33) is a modification of the Marsh Reaction giving a quantitative test for arsenic. The arsine is formed in the same manner as in the Marsh Reaction, but is then bubbled into a 50% (w/v) solution of silver nitrate to give a yellow solid which can be weighed.

$$AsH_3 + 3\ NO_3^- + 6Ag^+ \longrightarrow Ag_3As \cdot 3AgNO_3 + 3\ H^+ \qquad (2)$$

The spectrophotometric determination of volatile metal hydrides began with the work of Kingsley and Schaffert (32) in 1951. Their

Table II. Methods for As Determination Coupled with Hydride Generation

Technique	Detection Limit (µg/L)	Reference
AAS (flame)	0.8	(13)
AAS (graphite furnace)	12	(18)
AAS (quartz tube)	1	(19)
AAS (flame-in-a-tube)	0.2	(23)
AFS	3	(30)
ICP emission spectrometry	0.8	(25)
Molecular absorption spectrophotometry	10	(31)

work described the production of arsine by a reaction of mossy zinc and a mixture of HCl and H_2SO_4 with an arsenic solution. The generated arsine is passed into a solution of ammonium molybdate hydrazine sulfate. Development of the molybdate heteropoly blue arsenic complex gave a characteristic absorption band, with an absorbance maximum at 865 nm.

The most popular colorimetric test for arsenic is based on the reagent silver diethyldithiocarbamate (31). The arsine is generated by zinc in an acidic solution. The arsine is then collected into an absorber tube containing silver diethyldithiocarbamate dissolved in pyridine. In the absorber solution, the arsenic reacts with the silver salt, forming a soluble red complex, with a absorbance maximum at 535 nm.

As a result of the rapid development of the flame atomic absorption (FAA) spectrophotometer in the 1960's, FAA became the method of choice for elemental analysis. There are problems, though, associated with the determination of arsenic and other hydride forming elements by FAA spectrophotometry using direct solution nebulization. A major problem is the 60% background absorption at the As analysis wavelength by the traditional air/acetylene flame. Also, the detection limit for As is only 250 µg/L (8).

In 1968, Kahn and Shallis (34) began the use of an argon/hydrogen/air entrained flame for the use with elements whose analytical absorption lines are in the low ultra-violet region of the spectra (As—193.7, Se—196.0 nm). They noted a reduction in the

background absorption to only 15% with the argon/hydrogen/air entrained flame.

With the popularity of AAS, the hydride generation technique was modified to be used in conjunction with an existing AA spectrophotometer (35). Hydride generation is used instead of direct solution nebulization because of its higher selectivity and better detection limits. With direct solution nebulization, the entire aqueous sample is aspirated into the atomizer. As a result, there are many atoms other than those of the analyte present in the atomizer, which leads to greater potential interference problems. In the hydride generation technique, only the hydride (and also any other volatile products) is swept into the atomizer by means of a carrier gas. Thus, the analyte is removed from the bulk of the sample matrix which reduces the potential for interferences.

Detection limits are better with hydride introduction because the amount of analyte delivered to the atomizer during the peak is much greater than the amount of analyte delivered per unit time with direct solution nebulization. All the analyte in a 1-50 mL sample is introduced into the atomizer in a few seconds with hydride generation. Using flame nebulization, the actual sample delivery rate to the flame is only about 0.01 mL/s.

Holak (35), in 1969, developed a gas sampling technique for the determination of arsenic by hydride generation atomic absorption (HGAA) spectrophotometry. The arsine, generated by a reaction of zinc and HCl with an arsenic solution, is passed through a $CaCl_2$

drying tube and frozen out in a liquid nitrogen trap. The trap consists of a piece of glass tubing bent into a U-shape, filled with small glass beads, and placed in liquid nitrogen. After all the arsenic is converted to arsine and collected, the trap is removed from the liquid nitrogen and rapidly warmed to room temperature. Nitrogen, at a flow rate of 50 mL/min, is delivered to the trap to help sweep the revolatilized arsine into the FAA spectrophotometer for detection using an argon/hydrogen/air entrained flame. Holak felt that his technique isolates the analyte from the sample matrix, so virtually all chemical interferences are eliminated.

From the beginning of the use of hydride generation for the detection of volative hydrides, two major reactions have been developed for their formation. The first method discovered was the Zn/acid reaction (32):

$$Zn + HCl \longrightarrow ZnCl_2 + H\cdot \xrightarrow{M^{m+}} MH_n + H_2 \qquad (3)$$

Mixtures of Mg and $TiCl_3$ with HCl/H_2SO_4 (36) and of aqueous Al slurry reaction with HCl (21) have been used less frequently to produce the hydrides.

One of the major disadvantages of the Zn/acid reaction is the low reaction rate. Reaction times on the order of 30 minutes are required for complete reduction to the hydrides. The characteristically low reaction rates result in broad analyte peak responses; therefore, many attempts were made to develop techniques to sharpen the analyte absorption peaks. Fernandez et al. (37) used a balloon

as a reservoir to collect the generated hydrides. Once the reaction is complete, the contents of the balloon are rapidly expelled into the atomizer. This technique was never widely used because the acid vapors reacted with the balloon causing cracks and leaks, thus shortening its life. Another collection method previously mentioned as a liquid nitrogen trap that has been used by several researchers (28, 35, 38).

A very critical disadvantage of the zinc/acid reaction is that it is only capable of forming the hydrides of As, Se, and Sb. Also these elements do not form hydrides if the element is in its highest oxidation state. Thus a pre-reduction step is necessary for As, Se, and Sb to ensure that each has been reduced to its lower oxidation state prior to the hydride generation reaction.

In 1972 Braman and Foreback (28) introduced a second reaction for the formation of the volatile hydrides—the $NaBH_4$/acid reaction.

$$BH_4^- + H_2O + H^+ \longrightarrow H_3BO_3 + H\cdot \xrightarrow{M^{m+}} MH_n + H_2 \qquad (4)$$

The $NaBH_4$ can be added in pellet form or as a solution. The $NaBH_4$/acid reaction does have several advantages over the zinc/acid reaction. With $NaBH_4$ solutions, as opposed to pellets, the reduction reaction is more easily controlled and automated. One of the most popular methods to automate the technique is through the use of a proportioning pump to add the reagents. By choosing the appropriate tube diameters, the concentrations of the reagents and carrier gas can be optimized. The pump delivers the reagent to a mixing coil, mixing the sample, acid, and $NaBH_4$ to produce the

hydrides. The hydrides are removed from the sample solution through the use of a stripping column. The volative hydrides are then detected by any of the previously discussed methods.

Another advantage of the $NaBH_4$/acid reaction is that the reaction is complete on the order of 10-30 s, compared to approximately 30 minutes required for the Zn/acid reaction. Even though the reaction rate is much higher, the same collection techniques have been used to further sharpen the analyte peak response. The most important advantage of the $NaBH_4$/acid reaction, though, is that it is capable of forming the hydrides of Ge, As, Se, Sn, Sb, Te, Pb, and Bi.

Since Holak's initial work, there have been extensive studies on cations and anions that affect the formation of the volatile hydrides (39, 40). A few of the more commonly recognized interfering species include large excesses of Cu^{2+}, Ni^{2+}, Co^{2+}, and Sn^{2+}, but the concentrations of these cations that cause interference are much greater than environmental concentrations. Drinkwater (41) has used EDTA to complex Ni^{2+} prior to the addition of $NaBH_4$ to form hydrides which suggests that preferential reduction of Ni^{2+} to its elemental form occurs without the production of the hydrides. McDaniel et al. (42) suggest that increasing the concentration of $NaBH_4$ reduced the interference effects. This would also support the possibility of preferential reduction occurring.

Once generated, there are different ways to atomize the hydrides for detection. The first method utilized a argon/hydrogen/air entrained flame (35). In an effort to improve the detection limit,

flameless atomizers were used. Goulden and Brooksbank (39) used a graphite tube as the atomizer, but because of the extremely high atomization temperatures that were required, the graphite tube had a very limited lifetime.

In 1972, Chu (43) introduced a heated quartz tube to serve as the atomizer. The quartz tube is placed in the optical axis of an AA spectrophotometer, and is heated to approximately 900° C either electrothermally or with an air/acetylene flame. To date, three major instrument companies (Varian, Perkin-Elmer, and Allied Instruments) all use a heated quartz tube as their atomizer in their hydride generation accessory package.

Until 1980, it was widely thought that the atomization of the hydrides in a heated quartz tube is due to a simple thermal atomization process (44-46). Welz and Melcher (47) discovered that the atomization of the hydrides is in fact caused by collision with hydrogen free radicals. There were several factors that lead them to their suspicions that the atomization process of the hydrides was not caused by thermal decomposition. Firstly, in a heated quartz tube, temperatures of approximately 900° C are found to be optimum for atomization. Whereas, temperatures of 1700-1800° C are necessary to atomize the hydrides in a graphite tube furnace (18). Secondly, the addition of oxygen or air to the inert carrier gas increases the sensitivity (21).

Welz and Melcher were not able to obtain any measurable signal when pure arsine was passed into a heated (900° C) quartz tube that

is placed in the optical path of a FAA spectrophotometer. A sharp, narrow absorption peak results when a mixture of arsine and argon is injected between the reaction vessel and the heated quartz tube if H_2 is simultaneously generated in the reduction vessel by reaction of $NaBH_4$ in an acid solution. The hydrogen formed in the reaction vessel is swept out using argon as the carrier gas and then mixed with the injected arsine and argon prior to entering the heated quartz tube. The atomization efficiency, according to Welz and Melcher, is about 4 orders of magnitude lower in the absence of hydrogen than in the presence of hydrogen. Thus, they proposed the atomization mechanism of the volatile hydride-forming elements is as follows:

$$MH_x + H\cdot \longrightarrow MH_{x-1} + H_2 \qquad (5)$$

$$\vdots$$

$$MH + H\cdot \longrightarrow M + H_2 \qquad (6)$$

When air or oxygen is added to the carrier gas, the H radicals are most probably formed by the reaction proposed by Dedina and Rubeska (24)

$$H\cdot + O_2 \longrightarrow OH\cdot + O\cdot \qquad (7)$$

$$O\cdot + H_2 \longrightarrow OH\cdot + H\cdot \qquad (8)$$

$$OH\cdot + H_2 \longrightarrow H_2O + H\cdot \qquad (9)$$

In the absence of air or oxygen, the free radicals can be formed by the decomposition of hydrogen at the quartz surface. The unsaturated oxygen atoms at the quartz surface bind with the hydrogen molecules, resulting in hydrogen free radicals (47).

Welz and Melcher concluded that the flame-in-a-tube atomization technique gives the most sensitive results for arsenic. Here a

small hydrogen/oxygen flame is maintained inside a quartz tube. As the hydride passes though the flame, the hydride can react with the hydrogen free radicals that are produced in the flame. This technique was first used by Siemer and Hageman (48), but has not been utilized by many other researchers. It was not until the work by Dedina and Rubesta was published (24) that the reasons why this technique is capable of giving the most sensitive results for arsenic were known.

Arsenic Speciation

The speciation of inorganic arsenic began with the work by Braman and Foreback in 1973 (49). They reported that As(III), but not As(V), is reduced to arsine with $NaBH_4$ at a pH range of 4-9 and this has also been noted by other researchers (50, 51).

Braman and Foreback's method starts with a single aliquot of sample (50 mL) that has been buffered to pH 4-5 with potassium biphthalate. Sodium borohydride, added to the solution, reacts only with the As(III) to produce arsine under these conditions. Helium, the carrier gas, sweeps the volatile arsine into a liquid nitrogen trap for collection as previously described. After approximately 5 minutes, the reduction reaction is completed and the trap is removed from the liquid nitrogen and warmed rapidly to room temperature. The revolatilized arsine is swept through an He electrical discharge where the As emission at 234.9 nm is measured. To the remaining solution, oxalic acid and sodium cyanoborohydride are added. The

reduction of As(V) to As(III) by the sodium cyanoborohydride takes about 2 minutes for completion. Then $NaBH_4$ is again introduced, resulting in the formation of arsine, which is again collected in a liquid nitrogen trap and ultimately revolatilized and detected. The arsine produced in this step comes from only that of As(V) because the As(III) was removed with the first addition of $NaBH_4$.

Aggett and Aspell (50), in 1976, accomplished speciation of arsenic by a differential method. The total arsenic and the As(III) concentrations are determined separately and the As(V) concentration is calculated by difference. Sodium borohydride added to one aliquot of sample, maintained at a pH of 4-5 with a 0.5 M acetate buffer, produces the arsine due only to the As(III) that is present in the sample. A second aliquot, in 5 M HCl, is used to determine the total arsenic concentration. The arsine is either case is swept into a FAA spectrophotometer for detection where the absorbance at 193.7 nm is measured.

Howard and Arbab-Zavar (51) have studied the speciation of arsenic using the SCCD method as previously described. The pH of the sample is adjusted to 6.0 using a 0.25 M acetate buffer and placed in a hydride generation flask. An absorber tube filled with an SDDC solution is fitted to the flask. The arsine, generated by addition of $NaBH_4$, is collected for 15 minutes in the absorber tube. The absorbance of the resulting complex is measured at 525 nm to determine the As(III) concentration. The addition of 5 M HCl reduces the pH of solution in the hydride generation flask. Sodium borohydride is again injected into the flask and the resulting arsine

is bubbled into an absorber tube filled with fresh SDDC solution. The absorbance of the complex is measured to determine the concentration of As(V).

Yamamoto et al. (52) achieved speciation of arsenic by the use of a potassium iodide pre-reducing step. The As(III) concentration is determined from a solution controlled to pH 4-5 with a 40% citrate buffer. The evolved arsine is swept into a FAA spectrophotometer for an absorbance measurement at 193.7 nm. Potassium iodide is added as a pre-reductant to a second aliquot of sample in 2 M HCl. The reduction of As(V) to As(III) by the potassium iodide requires about 30 minutes. Heating of the sample could reduce the reaction time to approximately 5 minutes. The arsine is again generated by addition of $NaBH_4$ and detected by FAA. The As(V) concentration is then calculated by difference.

Braman and Foreback (49) also developed a method for the speciation of the methylated forms of arsenic. Their method is based on the difference in the boiling points of the corresponding arsines, as listed in Table III.

Table III. Boiling Points of Arsines

Compound	Boiling Point (°C)
Arsine	-55
Methylarsine	2
Dimethylarsine	55

Samples are buffered to pH 1-2 with a 10% oxalic acid solution. The corresponding arsines produced are frozen out in a liquid nitrogen trap. After total reduction to the arsines by $NaBH_4$, the trap is removed from the liquid nitrogen and allowed to warm under control of a Variac. The arsines are volatilized according to their boiling points and passed through an electrical discharge maintained in the He carrier gas and the atomic emission is measured at 234.9 nm. Since the arsine, methylarsine, and dimethylarsine pass through the discharge at different times, the readout appears similar to that of a gas chromatogram.

Howard and Arbab-Zavar (53) also studied the speciation of methylated arsenicals. Their technique is also based on the difference in the boiling points of the various arsine formed. After revolatilization from the liquid nitrogen trap, the arsines are swept into a heated quartz tube where the atomic absorbance is measured at 193.7 nm.

Andreae (54) used a liquid nitrogen trap to collect the various methylated arsines after $NaBH_4$ reduction. The revolatilized arsines are swept into a gas chromatograph for separation. The column is packed with 16.5% silicone oil DC-550 on 80-100 mesh Chromosorb W AW DMCS. Helium, at a rate of 80 mL/min, is the carrier gas. Both electron capture (ECD) and flame ionization (FID) detectors were studied. While the detection limits for the ECD are lower than that for the FID, the FID has a linear dynamic range of about 3 orders of magnitude larger than the ECD.

Talmi and Bostick (55) also used a gas chromatograph to separate the methylated arsenic species. They used a 5% Carbowax 20 M on 80-100 mesh Chromosorb 101 column to achieve separation. The eluants were detected by a microwave emission spectrometer, measuring the emission at 228.8 nm.

Ricci et al. (56) have used ion chromatography to separate the various inorganic and organic arsenic species prior to reduction to the corresponding arsines. The Dionex 3 X 500 mm anion separator with a gradient elution using a combination of $NaHCO_3$, Na_2CO_3, and $Na_2B_4O_7$, gives adequate separation to allow continuous arsine generation by addition of $NaBH_4$. The arsines are atomized by a heated quartz tube, and the As atoms are measured by AAS.

The major speciation techniques for arsenic species are based on their reduction to arsine and the various ways for detection have been presented. Speciation of arsenic has also been accomplished using ion-exchange columns with either X-ray fluorescence (57) or graphite furnace AA (58) for detection without reduction to the volatile arsines.

INSTRUMENTATION

A single beam AAS, originally developed by Hawley and Ingle (59) for cold vapor mercury determination, was modified for determination of arsenic by hydride generation atomic absorption (HGAA) spectroscopy. A block diagram of the instrument is shown in Figure 2. A list of the components and manufacturers is compiled in Table IV.

Arsine is generated in the reaction vessel and swept by hydrogen into the absorption cell where the atomization of the hydride occurs in the hydrogen/air flame located below the observation region of the cell. Radiation from an arsenic hollow cathode lamp (HCL) passes through the atomization cell and the 193.7 nm As line is selected for analysis by the Czerny-Turner configured monochromator (2 nm spectral bandpass). The photons transmitted through the atomization cell are detected by the photomultiplier tube (PMT). The photoanodic current of the PMT, which is proportional to the photon flux reaching the detector, is then converted to a voltage through the use of the current-to-voltage (I/V) converter circuit previously described by Hawley (59). The I/V converter circuit has a feedback resistance range of 10 kΩ to 100 MΩ and a feedback capacitor range of 100 pF to 1 μF. The output from the I/V converter enters a logarithmic amplifier circuit to give a direct absorbance readout. The signal finally passes through a low pass active filter to a strip chart recorder for a permanent record.

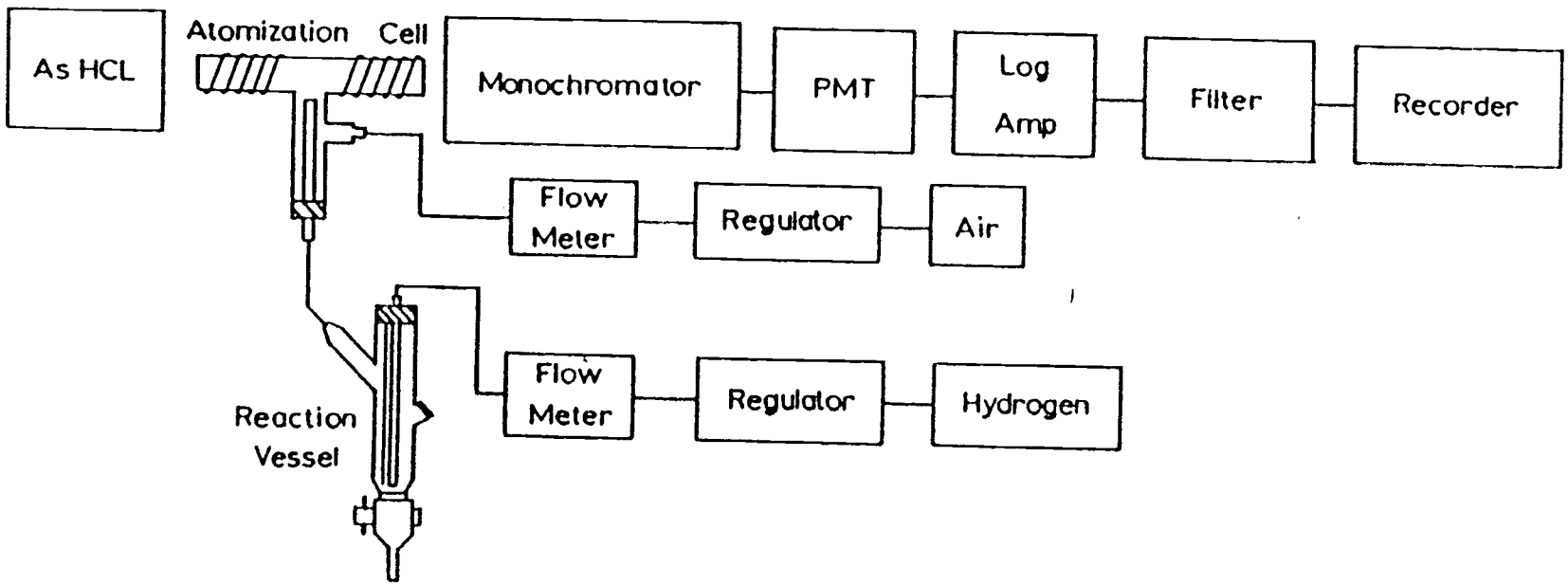

Figure 2. Block diagram of the HGAA spectrophotometer.

Table IV. Components of the Instrument

Item	Supplier and Model No.
1) Source and power supply	
As single element hollow cathode lamp (maximum current of 10 mA)	Westinghouse
HCL power supply (0-50 mA)	Heath EU 703-62
2) Monochromator	Heath EU 700
3) Photomultiplier tube (PMT), power supply and holder	
PMT	RCA 1P28
Power supply	Keithley 84489
PMT housing	Heath EU 701-93
4) Current to voltage (I/V) converter	
Operational amplifier	Analog Devices AD540
Dual power supply (± 15 V)	Analog Devices 915
5) Logarithmic amplifier	
Logarithmic amplifier	Analog Devices 755N
Dual power supply(± 15 V)	Analog Devices 915
6) Low-pass filter and amplifier	Spectrum 1021A
7) Strip chart recorder	Heath SR-204
8) Sample injector	Hamilton
Power supply	Heath EUW-17
9) Digital voltmeter	Analog Devices 2024
10) Flowmeters	Gilmont (size 2)

The reaction vessel (See Figure 3) was constructed by Mario Boschetto, OSU chemistry department glass-blower. The carrier gas is brought into the reaction vessel through a 0.25 in o.d. piece of glass tubing. The outlet of this aeration tube is placed at the bottom of the reaction vessel to allow mixing of the solutions by bubbling action and also to help force the volatile hydride out of the vessel and into the atomization cell. The side arm port for introduction of reagents with syringes was designed to be as short as possible to minimize the volume of the cell. The top of the reaction vessel is covered with a sleeve-type rubber septum (VWR 16170-167) and the aeration tube is brought through the middle of the septum. The side-arm port is also covered with a sleeve-type septum (Aldrich Z10,074-9). The Teflon stopcock at the bottom of the reaction vessel allows easy removal of the reaction mixture after a run. PVC Tygon tubing is used to connect the reaction vessel to the atomization cell (1/8 in i.d.) and for all gas connections (1/4 in i.d.).

The atomization cell, also constructed by Mario Boschetto, shown in Figure 4, utilizes the flame-in-a-tube atomization technique. This cell consists of an observation tube and a flame atomizer. The observation tube, placed in the optical axis of the AA spectrophotometer, is where the absorbance of the atomic arsenic is measured. The flame atomizer, located in the entry tube, atomizes the arsine as it passes through the flame.

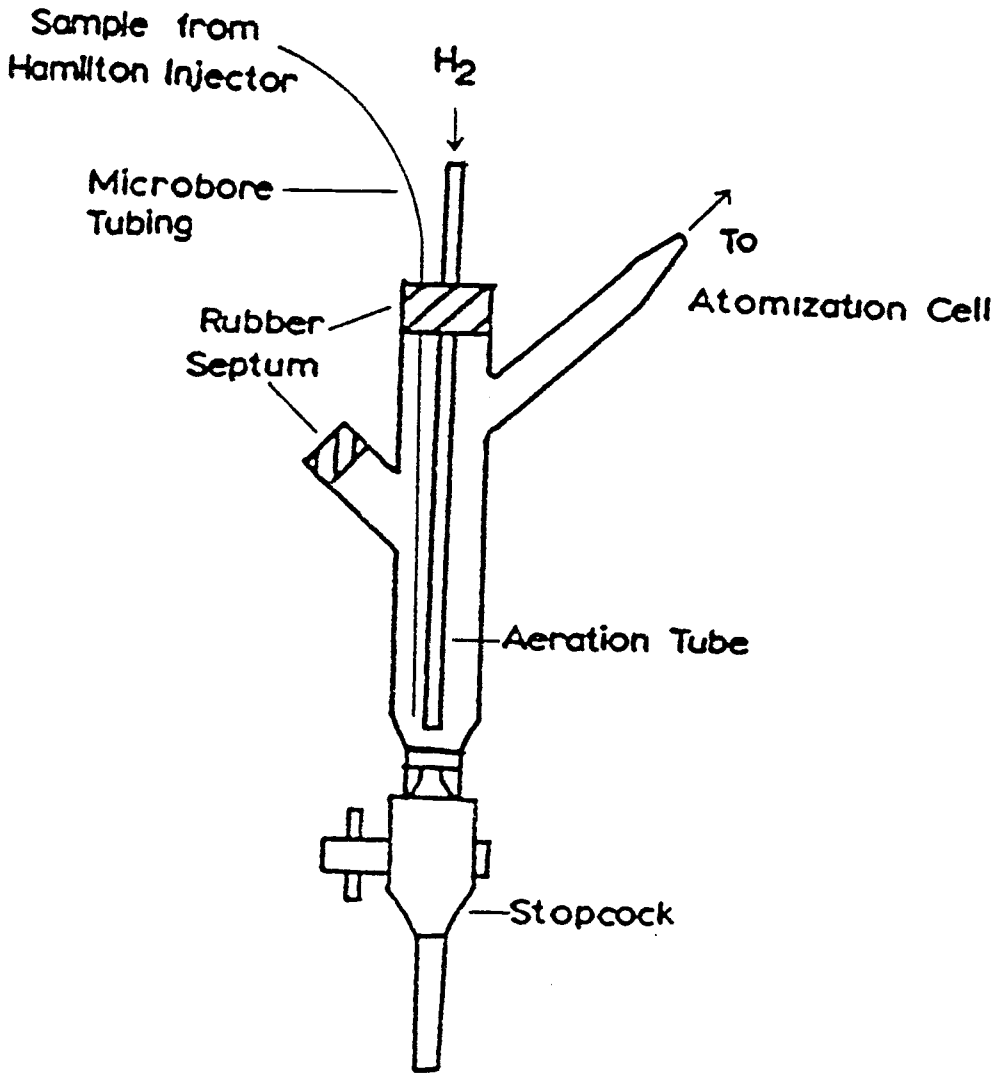

Figure 3. Reaction vessel (See Figure 6 for dimensions).

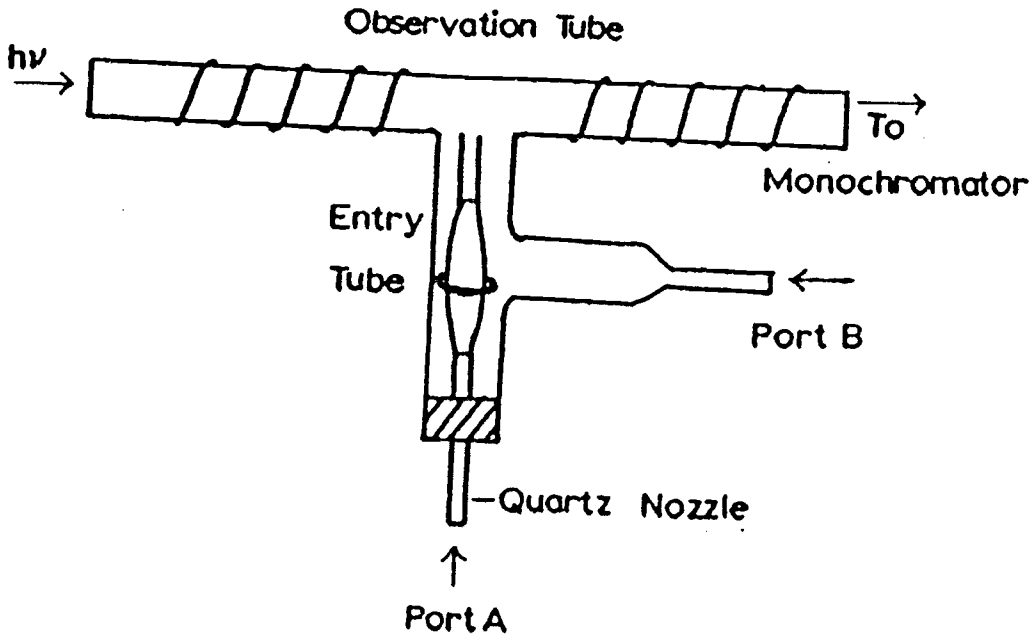

Figure 4. Atomization cell (See Figure 10 for dimensions). For clarity, Port B is shown parallel to the observation tube. The cell is actually designed so that Port B is perpendicular with respect to the observation tube.

The observation tube is wrapped with 26 gauge Nichrome wire and covered with asbestos tape. A Variac is used to control the temperature of the tube. The hydrogen carrier gas from the reaction vessel enters the atomization cell through the quartz nozzle in the center of the entry tube. Air is introduced into the entry tube through Port B, resulting in a hydrogen/air flame located at the tip of the quartz nozzle.

This researcher, with the help of Bill Pesklak, former OSU chemistry department graduate student, constructed the logarithmic amplifier circuit using the components specified in Table IV. The circuit with appropriate switches and connectors was placed in a metal box. The operation of the logarithmic amplifier is described by equation

$$E_o = k \log_{10} (i_s/i_r) \qquad (10)$$

where

E_o = output voltage, V

k = logarithmic slope, 1-2 V/decade

i_s = input photoanodic current from the PMT anode

i_r = reference current, internally fixed to 10 μA

Before beginning an analysis, the PMT voltage is adjusted so that the photoanodic current from the PMT matches the internal reference current (10 μA), thus producing an E_o of 0 V.

A Hamilton precision liquid dispenser is used to introduce the sample into the reaction vessel through the top septum (See Figure 3), with 1/8 in microbore tubing. Using a 1 mL sample

volume, the dispenser has a delivery precision of ± 0.01 mL. Art Ambrose, former OSU chemistry department graduate student, modified the Hamilton dispenser to allow triggering with a 5 V TTL pulse. A SPST switch was added to provide the trigger pulse. Gas-Tight Hamilton syringes are used for manual addition of discrete volumes of all other reagents into the reaction vessel.

The Spectrum 1021A low-pass active filter is used to reduce the high frequency noise and increase the signal to noise ratio of the peak signal measurement. The filter has a cut-off frequency range of 5 to 0.01 Hz and also has the ability to attenuate or amplify the signal.

EXPERIMENTAL

Introduction

This section covers the solution and glassware preparation, instrumental parameters used for the HGAA spectrophotometer, and the actual analysis procedure. The procedure used for a storage study is also presented in this section. The preparation of real samples (tap water, Willamette River water, NBS standard orchard leaves, and human hair) is discussed.

Solution and Glassware Preparation

All solutions were prepared with deionized water from a Millipore Mill-Q system attached to the in-house deionized water lines. The water delivered has a resistance of about 18 MΩ.

Glassware utilized in this work was all borosilicate glass and was initially cleaned in a Brasonic 52 sonic cleaner with a 2% (v/v) nitric acid solution. Copious amounts of deionized water were then used to rinse the glassware prior to usage. For each standard solution, the same glassware was used throughout the studies. After the initial acid cleaning, the standard solutions are prepared and allowed to stand in the designated glassware for a day, then discarded. The glassware was again rinsed with copious amounts of deionized water and then used to prepare and store the standard As solutions used for analysis.

All amounts of less than 5 g were weighed using a Mettler Type H balance which has an accuracy of $\pm$ 0.2 mg. Weighings of

more than 5 g were made using a Sartorious Type 1106 top-loading balance which can weigh to 0.01 g.

All chemicals used are of reagent grade quality. Separate stock solutions of 1000 mg/L of As(III) and As(V) are made according to the procedure in Table V. Dilutions of the stock solutions were made using Eppendorf pipets. No dilutions greater than 1:1000 were used for analysis.

Table V. Preparation of Stock Solutions

As(III)	Stock Solution (1000 mg/L As) - 1.3204 g of A_2O_3 (Mallinckrodt) dissolved in 500 mL of 0.05 M NaOH, neutralized with HCl, and diluted to 1000 mL with deionized water.
As(V)	Stock Solution (1000 mg/L As) - 1.6028 g As_2O_5 (Alpha Products) dissolved in one liter of deionized water.

The reducing agent, a 20% (w/v) $NaBH_4$ solution, was made by dissolving 20.0 g of $NaBH_4$ (Alpha Products) in 100 mL of 0.05% (w/v) KOH (J.T. Baker Chemicals) solution. A cloudy solution resulted which was then filtered through a medium porosity sintered glass crucible, giving a clear solution. This

procedure has been noted by Bye (60) to increase the stability of the $NaBH_4$ solution. Once the filtration was completed, the $NaBH_4$ solution was stored in the refrigerator.

A citrate buffer was used for the speciation of inorganic arsenic. A 0.75 M solution of citric acid was made by dissolving 63.03 g of anhydrous citric acid (J.T. Baker Chemicals) in approximately 150 mL of deionized water in a beaker. The citric acid solution was then transferred into a 200 mL volumetric flask. A magnetic stirbar was placed into the flask and KOH pellets (J.T. Baker chemicals) were added while stirring until the desired pH was reached. A Chemtrix, model 60 A pH meter and a Altex combination pH electrode were used for the measurements, after calibrating with commercial pH 4.0 and 7.0 buffers.

The molarity of the citrate buffer is defined as the concentration of all of the chemical forms of citrate present at a certain pH (totally unprotonated, partially protonated, or totally unprotonated citrates). This same definition will be used whenever referring to the concentration of a buffer.

Interference Study

Since several researchers have already done extensive interference studies, this type of study was not conducted. An artificial water sample was made to contain Mg^{2+}, Ca^{2+}, Na^{+}, K^{+}, Fe^{3+}, and Cu^{2+} at normal environmental levels

(See Table VI), to test for any possible interferences from these elements at environmental levels. Standard solutions containing 1 μg/L As(III) and As(V) in deionized water were compared with samples of the same concentration of As(III) and As(V) diluted with the artificial water.

Real Sample Analysis

To test the performance of the HGAA spectrophotometer, river water, tap water, and human hair samples were analyzed for their As(III) and As(V) concentrations. An NBS standard was used to test the accuracy of the technique for total As.

The tap water was taken from a lab in Gilbert Hall, on the campus of Oregon State University. The river water was taken from the Willamette River at the OSU crew docks in Corvallis, Oregon. Before the water samples were acquired, two 500 mL brown glass bottles were cleaned with sonic cleaner as previously described. A solution of 1 μg/L As was stored in the bottles for 1 day to reduce the possibility of loss of As by adsorption onto the glass surface. The bottles were then rinsed with copious amounts of deionized water before sampling. The river water samples were taken towards the middle of the river to reduce the possible effects of the land nearby. Once both water samples were acquired (tap and river water), they were acidified to pH 2 by the addition of HCl and then stored in the refrigerator. Analyses of these samples were completed within

Table VI. Composition of Artificial Water

Cation	Concentration of Ion	Source
Na^+	10 mg/L	NaCl
K^+	10 mg/L	KCl
Ca^{2+}	10 mg/L	$CaCl_2 \cdot 2H_2O$
Mg^{2+}	10 mg/L	$Mg(NO_3)_2 \cdot 6H_2O$
Cu^{2+}	10 µg/L	$Cu(NO_3)_2 \cdot 3H_2O$
Fe^{3+}	100 µg/L	$Fe(NO_3)_3 \cdot 9H_2O$

(All the above chemicals were from J.T. Baker)

24 hours of the collection time. Before analysis, 20 mL of the stock citrate buffer was diluted to 100 mL with the water samples.

Because arsenic does accumulate in hair, the researcher's hair was used as a sample to test for possible accumulation of arsenic in her hair. In June of 1983, the arsenic concentration in the researcher's hair was determined by an independent lab (Mineralab in Hayward, CA). The lab used a perchloric/nitric acid digestion and aspirated the solution into an inductively coupled plasma emission spectrometer for measurement.

In order to maintain the possibility for speciation of the arsenic in hair, a KOH digestion was employed in this study. One gram of hair in 25 mL of 5 M KOH was placed into a tightly capped vial and put into a hot water bath. Complete digestion of the hair took approximately 15-20 minutes, resulting in a clear, but brown-colored solution. The solution was neutralized into a 100 mL volumetric flask, and 20 mL of the stock citrate buffer were added before dilution to 100 mL. Upon addition of the citrate buffer, a fluffy brown precipitate formed. The solution was filtered through a medium porosity sintered glass crucible before analysis.

NBS orchard leaves (SRM #1571) were used to test for the accuracy of the technique. The NBS lists the arsenic concentration as 10 ug As per gram of orchard leaves. The bottle of orchard leaves was placed in an oven at 100^{o} C to

dry the leaves before weighing. The bottle, placed in a desiccator, was allowed to cool to room temperature. Two beakers containing 100 mg of the dried orchard leaves were placed in a muffle furnace at 400° C (rather than the typical 500° C to prevent volatilization of arsenic) for approximately 24 hours until a white ash resulted. The ash was dissolved in 5 mL of 50% (v/v) HCl, and they heated slightly to complete dissolution. The contents of the beakers were then quantitatively transferred into 100 mL volumetric flasks. Next 20 mL of the stock citrate buffer was added before dilution to 100 mL with deionized water.

The standard addition method was applied to all samples to test for interferences from the sample matrix that could offset the calibration curve slope. A 3-point standard addition curve was plotted and the As(III) and As(V) concentrations were calculated for each sample.

The tap and Willamette River water samples were spiked with 1000 µg/L As resulting in an increase of 1.0 and 2.0 µg/L As to the sample solution. The procedure used to make the standards is outlined in Table VII. The solutions were then diluted to 100 mL with either the tap or Willamette River water.

The hair solution resulting after digestion was diluted by a factor of 10 for the analysis. The concentration of arsenic in the samples was increased by 2.0 and 4.0 µg/L using spikes of a 1000 µg/L As solution. Table VIII lists the solution

Table VII. Standard Addition Procedure for Water Samples

Volume of 1000 µg/L As added (mL)	Volume of 0.75 M Buffer added (mL)
0	20
0.1	20
0.2	20

Table VIII. Standard Addition Procedure for Hair Sample

Volume of 1000 µg/L As added (µL)	Volume of 0.75 M Buffer added (mL)
0	2.0
20	2.0
40	2.0

Table IX. Standard Addition Procedure for NBS Sample

Volume of 100 mg/L As added (µL)	Volume of 0.75 M Buffer added (mL)
0	2.0
20	2.0
40	2.0

preparation for the standard addition method. The above solutions were brought to a total volume of 10 mL with diluted hair solution.

The NBS standard orchard leaves were spiked with a 10 mg/L As solution to increase the arsenic concentration by 20 and 40 µg/L As. The procedure used to make the standards is outlined in Table IX. Each of the above solutions were then diluted to 10 mL with the orchard leave sample solution.

Storage Study

Two 100 mL solutions containing 1 µg/L of both As(III) and As(V) were made and brought to pH 2 for stabilization. The glassware utilized has already been dedicated to 1 µg/mL arsenic concentrations, so it was only necessary to rinse with deionized water prior to usage. One solution was stored in the refrigerator, while the other was kept at room temperature. Both solutions were compared to freshly made samples of 1 µg/L As(III) and As(V). Comparisons were made every day for 3 days, then continued once a week for 4 weeks.

Analysis Procedure

Standards for the calibration curve were made directly prior to analysis. Each standard or unknown was adjusted to 0.15 M citrate by dilution of the stock citrate buffer. The $NaBH_4$ solution is placed in a flask in an ice bath of help reduce possible decomposition during analysis time.

Normal instrumental parameters used are listed in Table X. The PMT voltage was varied to match the 10 μA reference current needed by the I/V converter as previously described. A warm-up period of about 20 minutes is necessary for the As HCL.

To light the hydrogen/air flame, the hydrogen flow is set to its optimum value with the air flow turned off. The hydrogen is then mixed with the ambient air and is ignited using a match at one end of the observation tube. Once the hydrogen is burning at the tube ends, the air flow rate is slowly increased causing the flame to burn back and ignite at the quartz nozzle tip in the entry tube. Hydrogen is usually still burning at the ends of the observation tube, so that the air flow rate must be increased until the flame is totally contained at the tip of the quartz nozzle. Once this condition is reached, the air flow rate can be lowered to its optimum value.

Once the solutions were prepared and the instrumental parameters set as in Table X, the following steps were used for the determination of the As(III) and As(V) concentrations from the sample.

1) Inject 1.0 mL of sample through the top septum using the Hamilton automatic liquid dispenser.
2) Add 0.05 mL of 20% (w/v) $NaBH_4$ through the side arm septum.
3) Add 0.3 mL of concentrated HCl through the top septum.
4) Add 0.1 mL of 20% (w/v) $NaBH_4$ through the side arm septum.

Table X. Instrumental Parameters

As lamp	10 mA
H_2 flow (Gilmont #2)	30 on the scale (1100 mL/min)
Air flow (Gilmont #2)	15 on the scale (150 mL/min)
Wavelength	193.7 nm
Slitwidth	1000 μm
PMT voltage	-420 V
I/V converter	
R_f	100 M
C_f	0.001 μF
Active cut-off filter	
Cut-off frequency	0.05 Hz
Gain	X5, X10
Strip chart recorder	
Scale	100 mv
Speed	1 in/min.
Cell temperature	200° C
Logarithmic amplifier	1 V/decade

Once the above sequence is completed, the contents of the reaction vessel are emptied through the stopcock and into a waste beaker. The next sample is injected into the reaction vessel and emptied two more times to ensure that there is no unreacted $NaBH_4$ left in the reaction vessel when the concentration of As(III) and As(V) is to be measured in the next sample. The top and side arm septa need to be replaced after some time. If an injection can be made through the septa without feeling a slight resistance, they should be replaced.

RESULTS AND DISCUSSION

Introduction

In this section, the construction of critical instrumental components, the optimization of instrumental and experimental variables, and the application of the HGAA instrument for analysis of samples are presented. Firstly, the development of the reaction vessel and atomization cell is discussed in detail. Secondly, the optimization of the various instrumental parameters that affect the production and atomization of arsine is described. For the speciation of inorganic arsenic, the reagents used and their concentrations are critical; therefore, the optimization of solution variables for the best detection limit for both arsenic species is next presented. The results from a storage study, relating loss of arsenic and change of As(III) to As(V), are described. The speciation method developed is then shown to be applicable to water and biological (hair) samples. Finally the accuracy of the technique is tested from the analysis of NBS standard orchard leaves.

Development of the Reaction Vessel

Two basic designs of the reaction vessel were studied. The first one was the same utilized by Jim Hawley (59) in his thesis work on cold vapor mercury determinations. His reaction vessel is shown in Figure 5. The carrier gas, after being reduced to the appropriate flow rate by using a Gilmont flowmeter, is brought into the vessel through Port A. The force of the gas on the porous frit (course

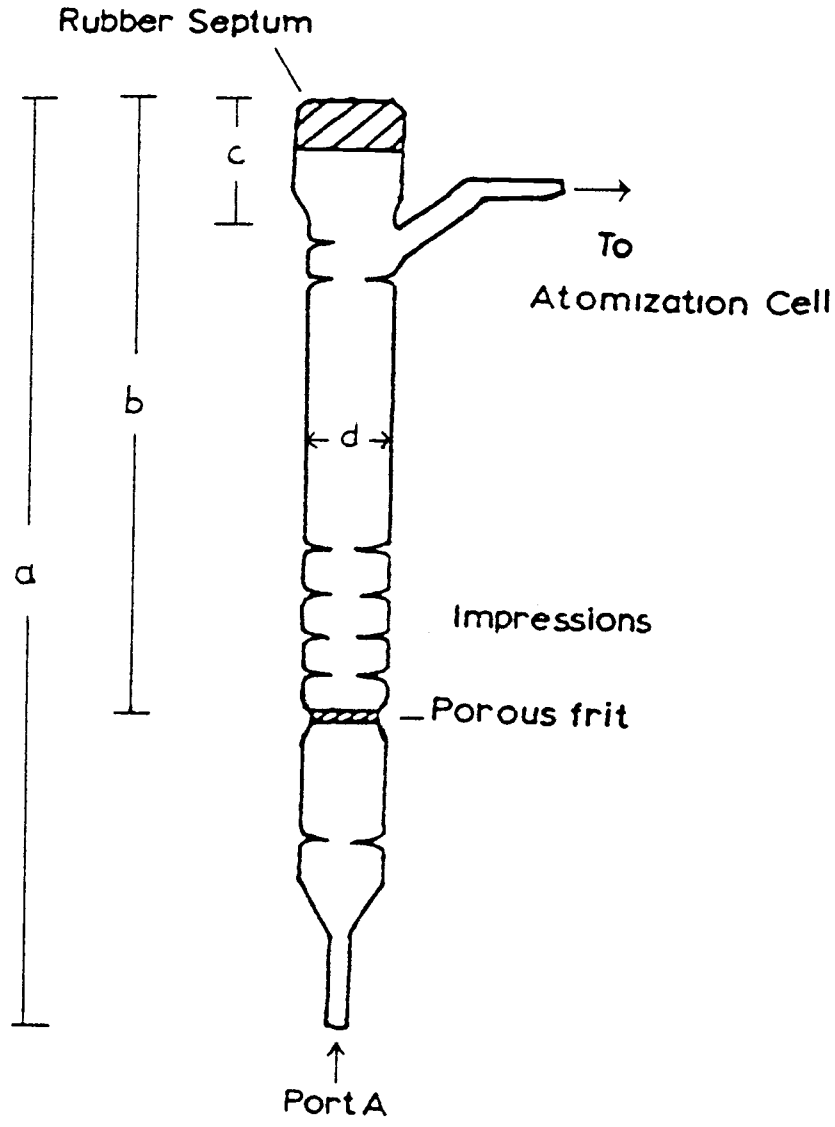

Figure 5. Original reaction vessel. a, 15 cm; b, 10 cm; c, 7.5 cm; d, 1.5 cm.

porosity) keeps the sample from passing through the frit and also causes the sample solution to bubble. This bubbling of the sample solution results in better mixing of the sample and the reducing agent. Port A is also attached to a vacuum line to evacuate the contents of the reaction vessel into a waste flask. By choice of the position of the 3-way valve, either the carrier gas or a vacuum is brought to Port A. The sample and the $NaBH_4$ solutions are added manually in discrete volumes using Gas-tight Hamilton syringes through the septum.

The bubbling of the sample solution became a problem when the arsenic solutions were made in a buffer instead of 5% (v/v) HCl which was used for the initial studies. The sample foamed and was not controlled by the impressions that were designed to break any bubbles formed. As a result, water mist accompanied the volatile hydride and carrier gas into the atomizer. A glass wool trap was added to help minimize the mist entering the atomizer. There was so such mist exiting the reaction vessel, however, that the glass wool trap soon became saturated and had to be replaced after approximately 10 runs.

A reaction cell was constructed using the same design, but instead had a medium porosity frit. The flow rate of the carrier gas had to be increased to obtain acceptable bubbling and caused a decrease in the arsenic signal due to greater dilution by the carrier gas. The medium porosity frit did reduce the foaming problem, but a glass wool trap was still required. The trap had to be replaced about once a day.

As a result of the foaming problems, a reaction vessel without a porous frit was constructed as shown in Figure 6. The carrier gas is brought into the reaction vessel from the glass aeration tube through the top septum. Impressions are not needed in this reaction vessel as foaming is not a problem. The side-arm port is used for the addition of the $NaBH_4$ solution. The HCl is added through the top septum because the residual $NaBH_4$ solution on the side-arm septum reacted with the acid on the end of the syringe causing cracks and leaks in the septum. A 3-in syringe needle is used for the addition of the HCl to ensure that the acid reaches the solution and does adhere to the walls of the reaction vessel.

The reaction vessel was designed to handle small sample volumes (1 mL). Other researchers have utilized reaction vessels capable of holding 10-100 mL of sample. The reaction vessel was also designed for easy addition of the sample solution by using the sample injector and the rapid removal of the sample solution between runs by using the Teflon stopcock.

Development of the Quartz Atomization Cell

A significant portion of the research was spent on the development of a quartz atomization cell that would give reproducible results. As noted in the historical section, most commercial and research instrumentation for HGAA have employed the heated quartz tube atomization technique. This atomization method was chosen for study first because of its ease of construction and low cost. Gas

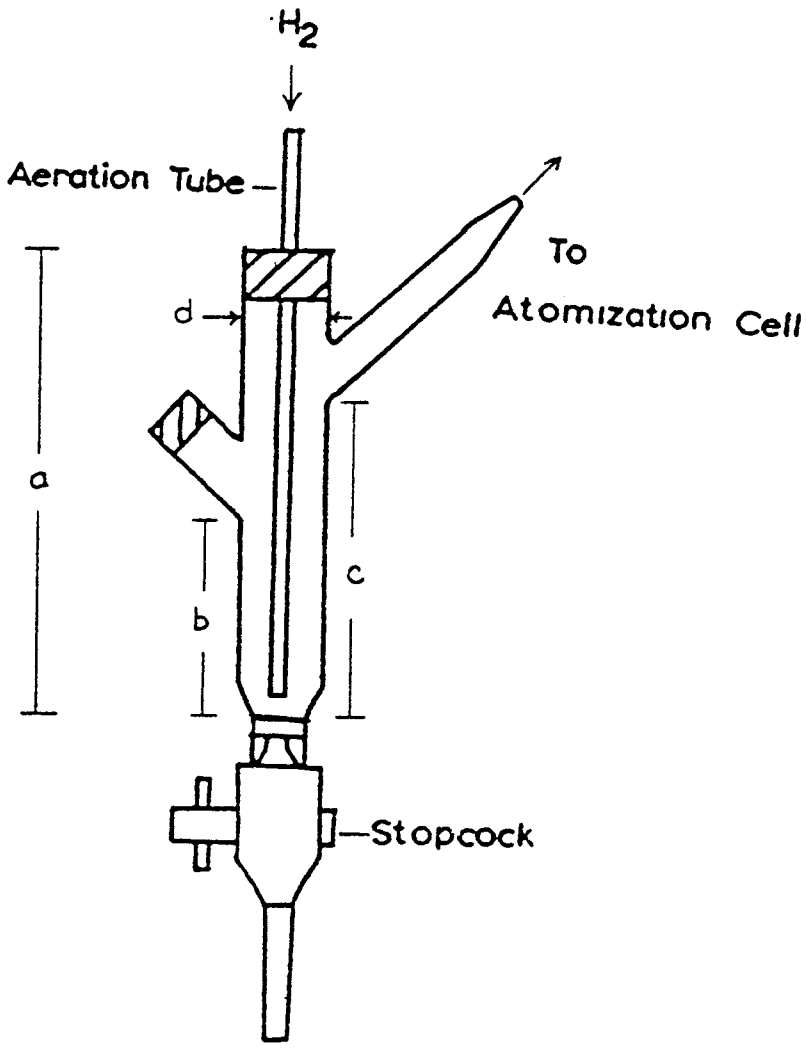

Figure 6. Final reaction vessel. a, 11 cm; b, 5 cm; c, 7.5 cm; d, 1.5 cm.

flow rates on the order of 100-1000 mL/min are needed instead of the much larger flow rates required for a conventional FAA spectrophotometer. Electrothermal heating of the quartz tube, as opposed to heating by a flame, was chosen because of its low cost. The use of electrothermal heating also allowed more versatility in the construction of the instrument.

The first heated quartz atomization cell had a T-configuration as shown in Figure 7. The cell was wrapped with wire for electrothermal heating. The high temperatures required for the atomization of arsine necessitates the use of Nichrome wire because of its resistance to oxidation. The 28 gauge Nichrome wire could not be used because the current required to obtain enough power to heat the quartz tube to 900° C caused breakage of the wire. The 24 gauge wire was harder to wind around the quartz tube and would not form to the quartz tube's outer diameter. As a result, 26 gauge Nichrome wire was wrapped around the cell until a total resistance of approximately 35 Ω was achieved.

Because of the direct proportionality between absorbance and cell path length, a 60 cm length and 0.14 cm i.d. cell was tried to increase the sensitivity of the technique. Most researchers have used a 10 cm cell so that it can easily fit into a conventional FAA spectrophotometer.

There were many problems associated with this cell design. The cell was mounted on the optical rail using 2 tube holders. After use at 900° C, the cell started to warp, causing a decrease in the

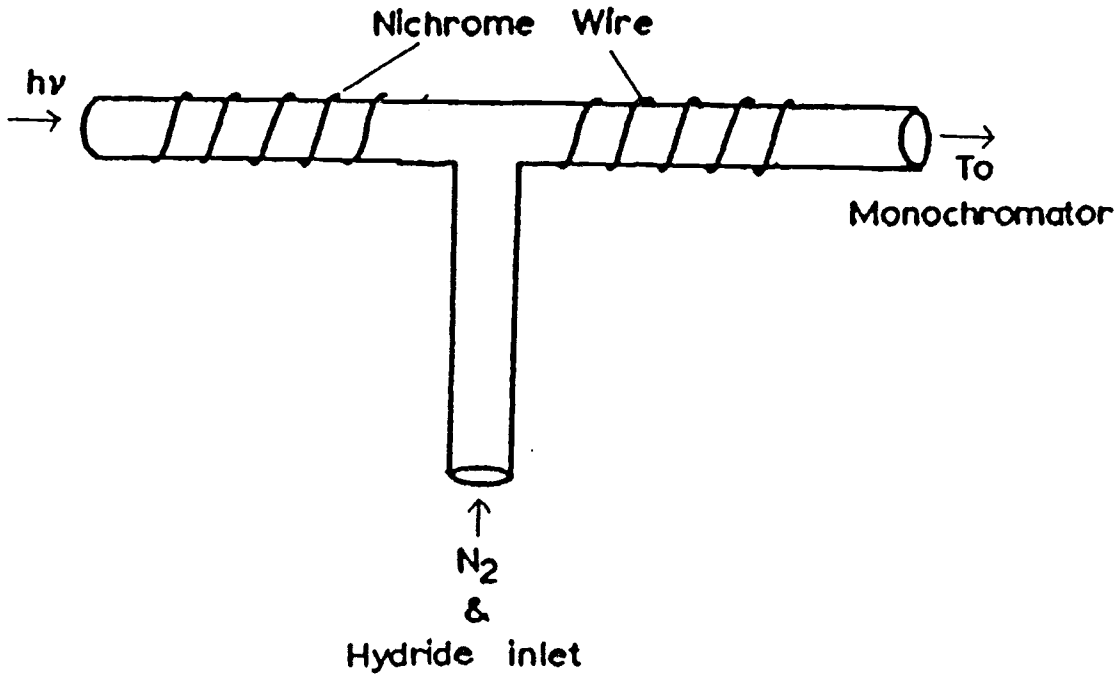

Figure 7. First atomization cell. The quartz atomization cell is 60 cm x 0.4 cm i.d. The carrier gas inlet is 8 cm x 0.4 cm i.d. The ends of the Nichrome wire are attached to a Variac.

fraction of light passed through the tube to the monochromator. A stainless steel bed was constructed by the OSU machinist to support the quartz tube and to minimize the warping.

The most troublesome problem with the use of the heated quartz tube was the breaking of the Nichrome wire due to its increase oxidation rate at high temperatures. Quartz tubes were placed around the heated quartz cell as shown in Figure 8 so that the Nichrome wire was exposed to an atmosphere of nitrogen instead of air. This modification reduced the frequency of breaking of the Nichrome wire, but did not totally eliminate the problem.

The small diameter of the atomization tube could have caused extra strain on the Nichrome wire, so a shorter quartz tube with a larger diameter (20 x 2 cm i.d.) was constructed in the same T-configuration. The dimensions of the shorter cell were chosen so that its volume matched that of the 60 cm cell. The larger diameter cell virtually eliminated the Nichrome wire breakage.

Another problem with the heated quartz tube approach was observed with both the 20 and 60 cm cells. Decomposition of $NaBH_4$ and release of hydrogen (H_2) occurred when the $NaBH_4$ solution is injected in sample because the pH is lowered. The addition of hydrogen to the otherwise inert carrier gas results in combustion of the hydrogen when it mixed with the ambient air at the open ends of the tube. The absorbing flame and the increased blank signal resulted in larger detection limits.

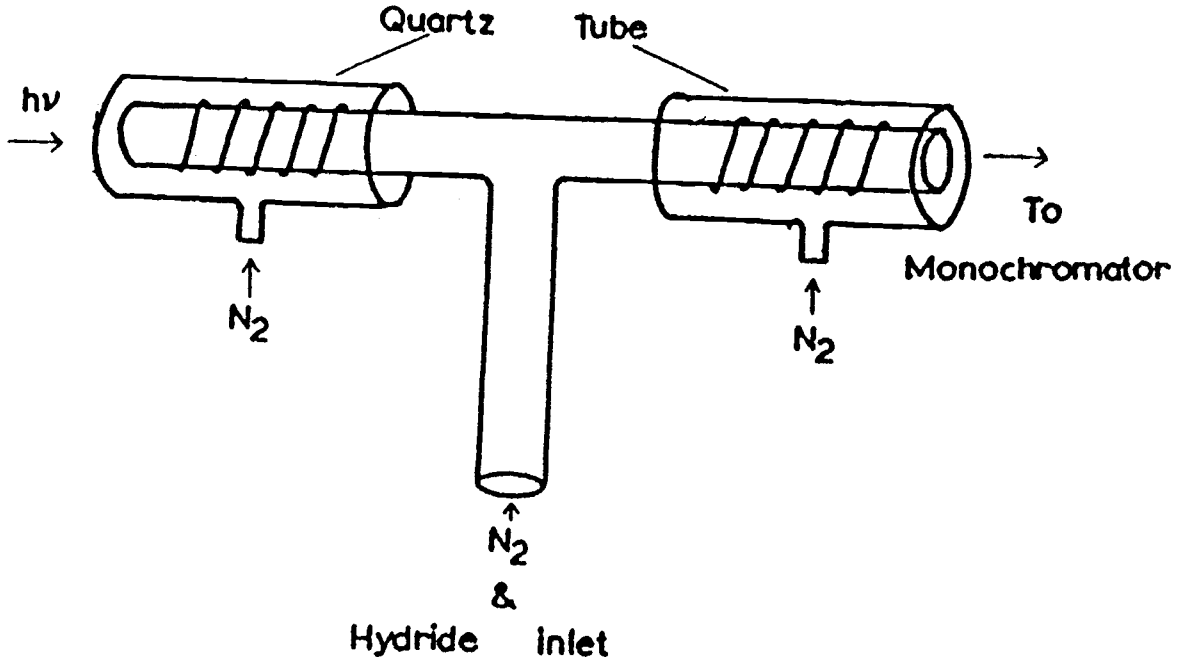

Figure 8. Modification of the first atomization cell. The quartz tubes are approximately 30 cm x 2 cm i.d.

To eliminate the flame from the optical axis of the instrument, the quartz atomization cell design was changed as shown in Figure 9. With the outlet now out of the optical axis, the flame formed did not interfere with the analysis. High quality ultra-violet transparent quartz windows (Heraeus Amersil) were attached to the ends of the quartz tube by using a high temperature adhesive, Ultra-Bond™ 552 (Aremco Products, Inc.). The new design of the quartz atomization cell allowed the use of a ceramic tube furnace as the heating device. The quartz tube was not, however, completely covered by the tube furnace; therefore, Nichrome wire and a Variac were used to heat the exposed portion.

This new design overcame the mechanical problems associated with the electrothermal heating of the quartz tubing to a temperature of 900° C and allowed the study of the determination of smaller quantities of arsenic (10-50 μg/L). However, the signal observed from an arsenic solution decreased rapidly with time. A calibration curve could not be obtained before the signal of the initial arsenic standard decreased by a factor of two. Cleaning the quartz tube with hydrofluoric acid (HF) restored the signal to its original value. The effect of the HF cleaning of the quartz tube on the signals of arsenic solutions has been noted by other researchers (47). Within hours of the cleaning with HF, the signal would again start to decrease.

Another method was sought to obtain lower detection limits because the cleaning of the quartz tube with HF entailed a lengthy curing process for the reattachment of the quartz windows onto the

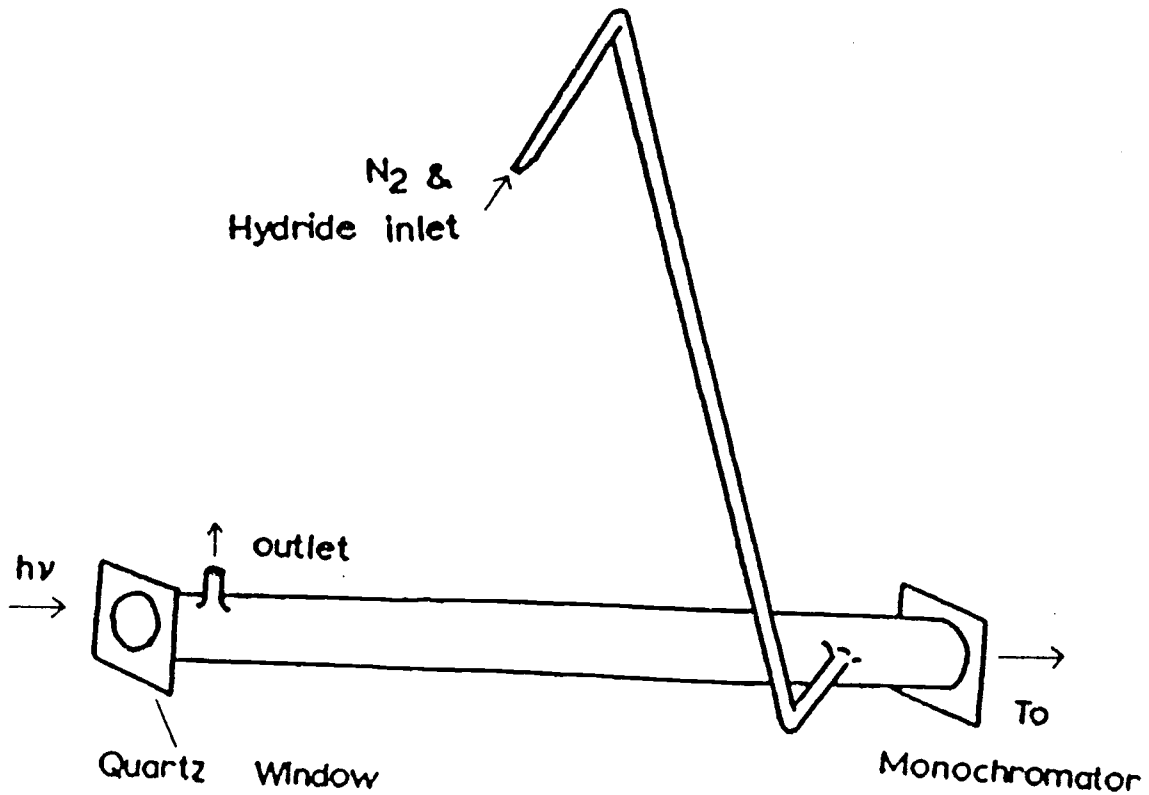

Figure 9. Second atomization cell. The main body of the cell is 20 cm x 1 cm i.d. The inlet and outlet tubes are 0.4 cm i.d.

tube ends. It was noted by Goulden et al. (21) that the addition of oxygen or air to the inert (N_2 or Ar) carrier gas caused an increase in sensitivity. While the addition of air or oxygen did result in an increase of the signal of the arsenic solution, the reproducibility was lowered. The signals from a 50 µg/L solution of As(III) varied by as much as a factor of two for repetitive runs.

Soon after the publication by Welz and Melcher (47) which indicated that H radicals were responsible for the atomization of the volatile hydrides at temperatures below 1700° C, it was decided to change the atomization technique to that of a flame-in-a-tube. The new quartz atomization cell as shown in Figure 10 became the atomization cell that is used for the remainder of the studies. The atomization cell consists of two parts, an observation tube with a central entry tube and a nozzle assembly that fits into the entry tube.

Hydrogen is now used as the carrier gas and air is brought in through Port B. A small hydrogen/air flame is maintained at the nozzle Point C. The observation tube is wrapped with Nichrome wire and asbestos tape to keep the quartz tube at a temperature of 200° C to evaporate the water that is formed in the combustion reaction of the flame. A quartz nozzle brings the carrier gas and the hydride through Port A from the reaction vessel to the combustion region of the cell. The quartz nozzle is held into place by use of an O-ring. Teflon tape is wrapped around the quartz nozzle, and the O-ring is then slipped around the Teflon tape. This procedure stabilizes the nozzle and helps ensure that the nozzle stays in the

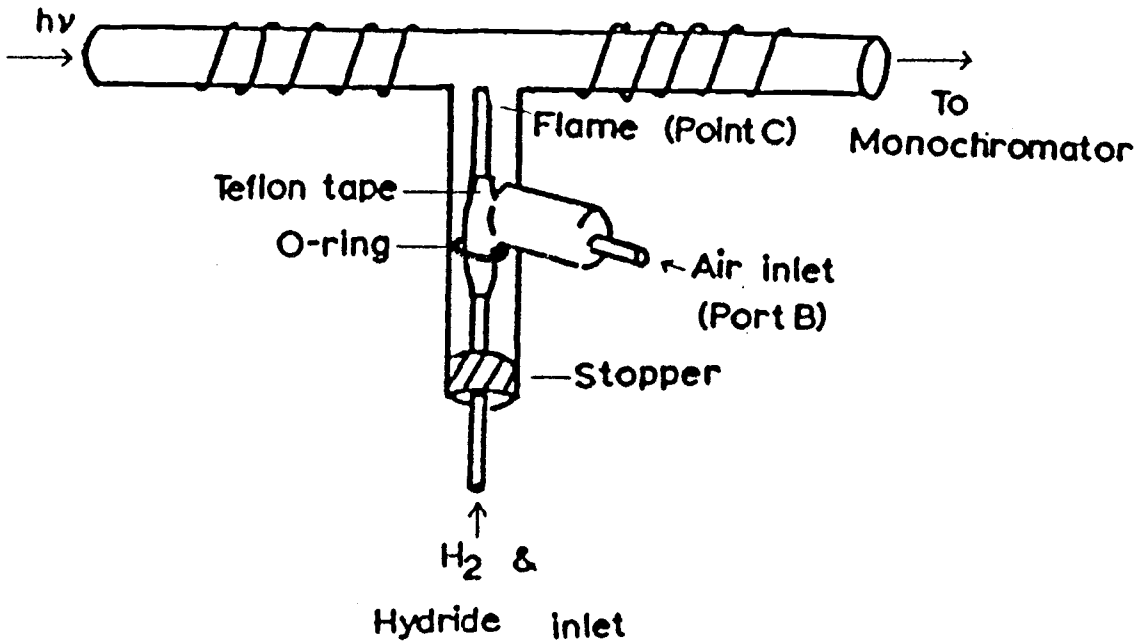

Figure 10. Final atomization cell. The observation tube is 20 cm x 1 cm i.d. The entry tube is 6 cm x 1 cm i.d., with Port B extending out from the entry tube approximately half-way down.

center of the entry tube. The quartz nozzle goes through a rubber stopper which then allows the height of the nozzle in the entry tube to be adjusted.

Optimization Studies

The optimization studies were conducted after preliminary studies were done so that each variable was at its near optimum value. For each study, the one variable alone was changed while the others were kept at their previously optimized values.

Production and atomization of arsine

This section covers the optimization of the experimental parameters for the production of arsine from the sample and the atomization of arsine to elemental arsenic. The samples used for optimization of the production of arsine were 10 µg/L As(III) in 0.15 M citrate buffer unless otherwise stated. A 1-mL sample volume was used with 0.05 mL of 20% (w/v) $NaBH_4$ injected into the sample.

Reaction vessel optimization. For optimum production of arsine from the sample, good mixing of the reagents is necessary. The position of the aeration tube is critical to the efficiency of the reaction and stripping of arsine from the reaction solution. When the $NaBH_4$ solution is injected into the sample solution, it is desired to have the arsine concentrated in a small plug of carrier gas.

The effect of aeration tube position on the peak absorbance of a 10 µg/L As(III) solution was studied. The standard solution (1 mL) was added to the reaction vessel, such that the depth of the solution

approximately 3 cm. The aeration tube was placed directly above the sample solution and an absorbance of 0.09 resulted after the injection of $NaBH_4$. The tube was then moved down to the center of the sample volume. The resulting absorbance was 0.12. The aeration tube was then moved to the bottom of the reaction vessel and the absorbance was 0.14. The aeration tube was kept in this position for further studies, thus optimizing the efficiency of the reduction reaction and also the efficiency of sweeping out the hydride to the atomization cell.

Flame optimization. The research previously published using the flame-in-a-tube atomization technique employed a hydrogen/oxygen flame burning in the quartz tube (23, 24). Since it has already been discovered that the reason for the flame is only for the production of the H radical, a hydrogen/air flame was tried first. The burning velocity of the hydrogen/air flame (440 cm/s) is much less than that of the hydrogen/oxygen flame (1190 cm/s), so there is less of a chance of flashbacks occurring. The slower burning velocity also allows the gas flow rates to be lower which reduces dilution of the arsine.

Oxygen was tried as the oxidant for the flame and a large background absorption interference was noted. The signal for the blank could not be distinguished from a 100 µg/L As(III) solution. Hence, it was decided to use air as the oxidant for the flame.

Hydrogen was chosen as the carrier gas for safety reasons. If air is used as the carrier gas, then air and hydrogen produced in the

reduction reaction are mixed before the nozzle, which could result in potential flashback problems.

The quartz atomization cell has 2 gas entry ports (See Figure 10). Hydrogen can be introduced through the quartz nozzle (Port A) or through Port B. With traditional total consumption burners, the sample is aspirated into the center of the flame. To keep the sample in the center of the flame, hydrogen and the resulting hydride were brought into the entry tube through the quartz nozzle. Air was then added through Port B. The position of the gasses was then switched so that air was brought into the entry tube through the quartz nozzle and the hydrogen and hydride were brought in through Port B. This change resulted in a decrease of the peak absorbance for a 10 µg/L As(III) solution of approximately 30%. The decrease in absorbance is probably due to less efficient interaction of the hydride with the flame, thus decreasing the atomization efficiency.

The flow rates of the hydrogen and air play a critical role in obtaining optimum conditions for the atomization of the hydride. The previous publication on the flame-in-a-tube atomization technique (24) indicates that a fuel rich flame is desired. Figure 11 shows the optimization curves for the gas flow rates. Adjustment of the air flow rate to be as low as possible produces the maximum signal. An air flow rate of 150 mL/min was found to be optimum. An air flow rate of less than 100 mL/min results in an unstable flame and excess hydrogen generated in the reaction vessel would often extinguish the flame.

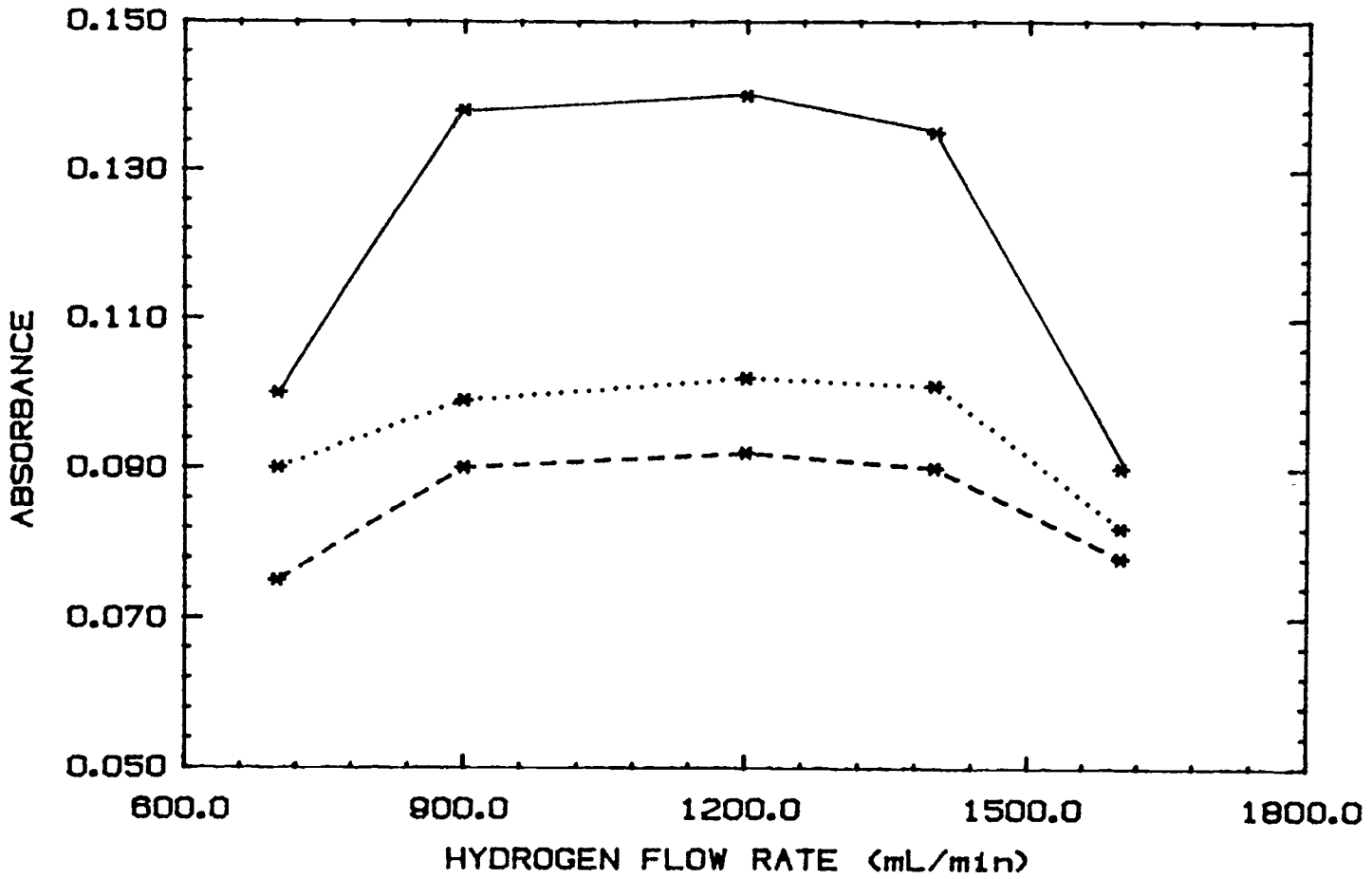

Figure 11. Optimization of hydrogen and air flow rates. Solid line—air at 150 mL/min, dotted line—air at 250 mL/min, dashed line—air at 350 mL/min.

A hydrogen flow rate above 1400 mL/min caused a more severe foaming problem and thus necessitated the use of a glass wool trap in the tubing between the reaction vessel and atomization cell. Also the higher hydrogen flow rates dilute the arsine, causing a decrease in the absorbance peak height. A hydrogen flow rate of 1100 mL/min was used for the remainder of the studies. This flow rate was chosen because slight fluctuations in the flow rate do not affect the peak absorbances. Figure 11 shows that there is a broad maximum at 900-1300 mL/min hydrogen, with an air flow rate of 150 mL/min. The flame under optimized conditions exhibits a ring-shape with what appears to be a hollow center. The flame can only be seen in a darkened room.

Cell Temperature. The temperature of the atomization cell was varied to see if it had an affect on the peak absorbance. Table XI shows the temperature effect on the peak absorbance for a 10 µg/L As(III) solution. The temperature was measured 3 in from one end of the observation tube. Above 170° C, the peak absorbance was independent of temperature. These data agree with Welz and Melcher's findings (47) that the atomization is complete in the hydrogen/air flame located in the entry port of the atomization cell. If the observation tube was allowed to cool to below 100° C, the water formed as a product of the flame combustion does not evaporate and causes a decrease in signal. The cell temperature was maintained at approximately 200° C for the remainder of the studies. This is achieved with a Variac setting of about 20 VAC.

Table XI. Cell Temperature Study

Variac Voltage (V)	Temperature °C	Absorbance
5	100	0.140
10	175	0.179
20	235	0.178
30	300	0.179
40	415	0.177
Off	60	0.085

Quartz nozzle position. The position of the quartz nozzle in the entry tube has a significant effect on the resulting peak absorbances for As(III). The position of the nozzle is changed by moving the rubber stopper up or down along the nozzle itself. The dependence of peak absorbance from a 10 µg/L As(III) solution on nozzle position is shown in Table XII. If the top of the quartz nozzle is placed too close to the observation tube, the blank signal increases due to the additional hydrogen from the decomposition of the $NaBH_4$ that enters the ignition area of the entry tube. If the top of the nozzle is placed too far from the observation tube, the arsenic atomic population that reaches the observation tube for the absorbance measurement is greatly reduced. A distance of 0.5 cm from the top of the entry tube to the top of the quartz nozzle was found to be optimal.

Instrumental parameters

This section describes the optimization of the instrumental parameters that are independent of the production or atomization of the arsine.

As HCL current. The As HCL current plays a dramatic role in the sensitivity of the technique. Table XIII shows the absorbance for a 10 µg/L As(III) solution at various lamp currents. The table

Table XII. Quartz Nozzle Position

Distance[a] (cm)	Blank Absorbance	As Absorbance
2.0	0.000	0.006
1.5	0.000	0.019
1.0	0.000	0.103
0.5	0.001	0.143
0.0	0.010	0.145

[a] Distance measured from the top of the entry tube to the top of the quartz nozzle.

Table XIII. Study of the As HCL current

As HCL current (mA)	Absorbance
3	0.014
6	0.048
10	0.110
12	0.152

indicates that the current should be maintained at the maximum current as specified by the manufacturer. After many hours of lamp usage, crystal build-up occurs in the cathode region of the HCL. The crystal build-up can eventually cause an electrical short resulting in an interruption of the hollow cathode discharge. The crystal build-up can be easily removed by tapping the side of the HCL while being held in a vertical position. Once the crystals are disloged, the hollow cathode discharge can be resumed.

Active filter setting. The Spectrum 1021A filter and amplifier is used to filter out the high frequency noise. The cut-off frequency of the filter must be low enough to minimize the baseline noise, yet high enough to allow the pen of the strip chart recorder to respond fully to the peak absorbance signal. The baseline peak-to-peak (p-p) noise for each filter setting was recorded along with the corresponding absorbance peak for a 10 µg/L As(III) solution. Table XIV lists the peak absorbance signal, the p-p noise and the signal-to-

Table XIV. Cut-off Filter Setting Study

S/N	Absorbance	Noise (p-p)	Filter Setting (Hz)
25	0.178	0.007	5
29	0.176	0.006	1
28	0.170	0.006	0.5
50	0.150	0.003	0.1
70	0.140	0.002	0.05
35	0.070	0.002	0.02
40	0.040	0.001	0.01

noise ratio (S/N) for each filter setting. The S/N is taken as the ratio of the peak absorbance to the p-p noise. It was decided that the filter set at a cut-off frequency of 0.05 Hz gives the best compromise of filtering out the high frequency noise and obtaining the highest peak absorbance for the 10 μg/L As(III) solution.

Speciation of inorganic arsenic

In this section, the optimization of the parameters that affect the speciation of inorganic arsenic is presented. The selection of the buffer is discussed in detail. The concentration and volume of the reducing agent ($NaBH_4$) and its effect on the peak absorbances for each arsenic species are examined. The volume of HCl needed to reduce the pH of the sample and thus maximize the peak absorbance due to the As(V) in the sample is discussed. In an effort to decrease the peak width due to As(V), several pre-reducing agents were tried and the results of these studies are presented in this section.

Selection of the buffer. For speciation of inorganic arsenic by HGAA spectrophotometry, 3 buffers have been utilized by other researchers—acetate (52, 53), citrate (54), and biphthlate (49) buffers. The 3 buffers were studied to determine which one would give the largest calibration sensitivity. The corresponding acids of the buffers have dissociation constants that are relatively similar (See Table XV).

Table XV. Dissociation Constants of Acids

Acid	K_{a1}	K_{a2}	K_{a3}
Acetic	1.75×10^{-5}	—	—
Citric	7.4×10^{-4}	1.7×10^{-5}	4.0×10^{-7}
Phthalate	1.2×10^{-3}	3.9×10^{-6}	—

An acetate buffer was chosen to be studied first because it had been the most widely used for the speciation of inorganic arsenic (52, 53). Initial studies showed that a 0.15 M acetate buffer (0.075 M HAc and 0.075 M NaAc) at pH 4.75 gave reproducible signals for a 10 µg/L As(III) solution upon the initial injection of 0.05 mL of $NaBH_4$. Addition of 0.4 mL of concentrated HCl into the reaction volume of 1.05 mL, followed by a second injection of $NaBH_4$, gave reproducible response for the 10 µg/L As(V) solution.

But upon making a new acetate buffer with acetic acid from a new bottle, the signal due to the As(V) in the sample decreased by about an order of magnitude. The volume of HCl was increased to 1.0 mL, but the peak absorbance signal did not increase. The buffer was "cleaned up" by passing it through a Chelex 100 ion-exchange resin to trap any metal ions in the new acetate buffer that could be interfering with the reduction of As(V) to arsine. The clean up step with the ion-exchange resin failed to produce any increase in the absorbance

peak. Acetic acid from other source (Corco) was used to prepare the buffer and the same reduction in the peak absorbance signal of As(V) was also noted.

At this time, it was decided to try the biphthlate buffer that was utilized by Braman and Foreback (49) in their speciation work. A 4% (w/v) potassium biphthlate (KHP) solution was made by dissolving 4.0 g of KHP in 100 mL of deionized water. The resulting pH of the solution was 4.0. The buffer did not work for the determination of As(V) when the pH of the solution was lowered to 0 with an addition of 0.4 mL of concentrated HCl. At a pH much lower than 2, a significant portion of the biphthlate is converted to phthalic acid which is considerably less soluble in water. As a result, upon addition of the HCl, the phthalic acid precipitated out in the reaction vessel. The formation of the precipitate interfered with the determination of the As(V) concentration in the sample. Also the precipitate made the cleaning of the reaction vessel between runs much more difficult.

As a result of the problems from the acetate buffer and biphthlate buffer, it was decided to try the citrate buffer. Citric acid does have the advantage that two pKa's are within the desired pH range for the speciation studies.

The concentration and initial pH of the buffer. The concentration of the buffer must be large enough to neutralize the base that is added from the $NaBH_4$ solution, but small enough to allow the pH to be reduced below 0 with a minimum amount of acid for the reduction of

the As(V) to arsine. A buffer concentration of 0.15 M was determined to be the optimal value for the citrate buffer. A citrate buffer concentration of 0.075 M resulted in a relative standard deviation (RSD) for a 10 µg/L As(III) solution of approximately 20%. The pH of the solution jumped to 10 upon the addition of the $NaBH_4$ solution. The significant increase in the pH would account for the large RSD. The RSD for a 10 µg/L As(III) solution decreased to 3% when the buffer concentration was increased to 0.15 M. The pH after addition of the $NaBH_4$ solution was 7. An increase of the buffer concentration to 0.3 M did not significantly decrease the RSD for the 10 µg/L As(III) solution. As a result, it was decided to use a buffer concentration of 0.15 M for the speciation studies.

The initial pH of the buffer is critical to minimize any cross-interference from the As(V) that is present in the sample. If too low of an initial pH is chosen (pH less than 2), a significant portion of the As(V) in the sample is also reduced from the initial injection of the $NaBH_4$ solution. This initial pH results in a falsely high As(III) concentration from a given sample. Yet if the initial pH of the buffer is too high, the reduction of the As(III) to arsine will not be complete.

The effect of the initial pH of the citrate buffer was studied by measurement of the peak absorbance for 10 µg/L of each arsenic species in solutions buffered at the two pKa's of the citric acid. The peak absorbance of the 10 µg/L As(III) solution in the buffer was compared to a 10 µg/L As(III) solution in 5% (v/v) HCl. It is

assumed that all the As(III) in the 5% HCl solution is converted to arsine. The results of the comparison are listed in Table XVI.

The cross-interference from a 100 µg/L As(V) solution in 0.15 M buffer was compared to the signal from a 10 µg/L As(III) solution in 0.15 M buffer at the same pH. The results of the cross-interference study are listed in Table XVII. The lower pH buffer was chosen for further speciation studies because there was no significant difference in peak absorbance between the As(III) solution in the 0.15 M citrate buffer at pH 3.1 compared to that in the 5% (v/v) HCl. The cross interference is worse with the lower pH buffer, but still minimal, since a As(V) solution yields a signal equal to only about 2% of the equivalent As(III) concentration. Therefore, only one correction factor is needed at pH 3.1; whereas both the As(III) and As(V) would have to be corrected at the higher pH 4.7 citrate buffer. At pH 4.7, not all the As(III) (10 µg/L) is not converted to arsine from the initial injection of $NaBH_4$. At pH 3.1, all the As(III) was converted to arsine after the initial injection of $NaBH_4$. Only a peak absorbance equal to that of the blank was observed after addition of the HCl and $NaBH_4$.

Reducing agent. The concentration of the $NaBH_4$ solution should be large enough that only a small injection volume is required for the initial injection to obtain the peak absorbance signal for the As(III) concentration in the sample. A small volume of $NaBH_4$ reduces the dilution effects on the determination of the As(V) concentration from the same sample aliquot. A solution of 20% (w/v)

Table XVI. Initial pH of the Citrate Buffer

Sample	Absorbance
10 µg/L As(III) in 0.15 M Citrate (pH = 4.7)	0.120
10 µg/L As(III) in 0.15 M Citrate (pH = 3.1)	0.139
10 µg/L As(III) in 5% (v/v) HCl	0.140

Table XVII. Cross-interference Study

Sample (0.15 M Citrate)	Absorbance
10 µg/L As(III) (pH = 4.7)	0.120
100 µg/L As(V) (pH = 4.7)	0.011
10 µg/L As(III) (pH = 3.1)	0.139
100 µg/L As(V) (pH = 3.1)	0.032

$NaBH_4$, which is near the solubility limit for $NaBH_4$ in water, was prepared in 0.05% (w/v) KOH. The $NaBH_4$ solution was filtered before use to increase its stability as noted by Bye (60). The filtering of the $NaBH_4$ solution decreased the blank absorbance signal for As(III) from 0.14 to 0.002 absorbance units. A similar decrease in blank absorbance was noted for As(V).

The injection volume of the reducing agent was varied from 0.01 mL to 0.5 mL of 20% (w/v) $NaBH_4$ and the resulting peak absorbance from a 10 µg/L As(III) solution in 0.15 M citrate buffer are shown in Table XVIII. The data in Table XVIII indicate that if insufficient $NaBH_4$ is added to the sample, complete reduction to arsine is not achieved for a 10 µg/L As(III) solution. This could in part be due to the volume of $NaBH_4$ that actually reacts with the sample compared to the volume that is injected. Some of the $NaBH_4$ solution could adhere to the walls of the reaction vessel and thereby not react with the sample. The reduced peak absorbance could also be due to the decomposition of $NaBH_4$. The decomposition may be completed before the reduction of the trivalent arsenic to arsine is complete.

The data in Table XVIII also indicate that if too large of an injection volume of $NaBH_4$ is used, a reduced peak is also observed. This decrease in absorbance is due either to a dilution of the sample volume by up to 50%, or the dilution of the plug of arsine that enters the atomization cell by excess H_2 produced in the reduction reaction.

Table XVIII. Volume of Reducing Agent

Volume of $NaBH_4$ (mL)	Absorbance
0.01	0.009
0.02	0.015
0.03	0.055
0.04	0.123
0.05	0.125
0.06	0.120
0.08	0.105
0.1	0.100
0.2	0.100
0.5	0.095

In a separate experiment, 0.1, 0.2, and 0.4 ml of 10, 5, and 2.5% (w/v) $NaBH_4$, respectively, were used. These volumes keep the total amount of $NaBH_4$ added constant. No effect on the peak absorbance of a 10 µg/L As(III) solution in 0.15 M citrate was observed. To minimize the volume of $NaBH_4$ added, 0.05 mL of 20% (w/v) $NaBH_4$ was chosen for the concentration and volume of the reducing agent required for the initial injection to determine the As(III) concentration in the sample. This yields the maximum absorbance as shown in Table XVIII. The volume of $NaBH_4$ required for the determination of As(V) had to be doubled to obtain the maximum absorbance signal.

Hydrochloric Acid. Hydrochloric acid was used to lower the pH of the sample solution for the determination of the As(V) concentration. The sample, containing 10 µg/L of As(V) only, was injected with the initial 0.05 mL of 20% (w/v) $NaBH_4$ and no observable peak resulted. The volume of the concentrated HCl was varied and the resulting peak absorbance values for the 10 µg/L As(V) are listed in Table XIX. A volume of 0.4 ml of concentrated HCl was chosen as

Table XIX. Addition of Hydrochloric Acid

Volume of HCl (mL)	Absorbance
0.1	0.037
0.2	0.041
0.3	0.065
0.4	0.070
0.5	0.068
1.0	0.049

it sufficiently lowered the pH of the solution to allow the As(V) to be reduced to arsine and yielded the maximum As(V) signal. The final pH is below 0 after addition of 0.4 mL of concentrated HCl.

Pre-reductant studies. In an effort to further increase the peak absorbance due to the As(V) concentration in the sample, 3 pre-reducing agents were tried. The absorbance peaks for As(V) and for As(III) took about 20 and 10 s, respectively to return to the baseline. It was hoped that the pre-reducing agents would reduce the As(V) in the sample to As(III) within a reasonable length of time and allow the elimination of the acid step. Potassium iodide, stannous chloride, and sodium cyanoborohydride were studied as pre-reducing agents. Both the potassium iodide and sodium cyanoborohydride did not have any effect on the reduction of As(V) to As(III) after 5 minutes. Stannous chloride caused a large blank interference and thus could not be used. As a result, it was decided not to use a pre-reducing agent in the speciation procedure.

Calibration Curves and Detection Limits for As(III) and As(V)

Calibration curves for both As(III) and As(V) were obtained by using standards containing equal concentrations of each species under the optimized conditions discussed in the previous section. Five repetitive standard measurements were made followed by five blank measurements. The average blank corrected peak absorbance for each standard is calculated by subtracting the average peak absorbance of the blank solution from the average total peak absorbance of the

corresponding arsenic species. The resulting peak height was used to calculate the peak absorbance for each sample. Figure 12 depicts the peaks obtained from a standard containing 10 µg/L of both As(III) and As(V).

Table XX lists the standard As(III) concentrations with their average blank corrected absorbances and standard deviations. Table XXI lists the blank corrected absorbances and standard deviations for the corresponding standard As(V) concentrations.

The error bars for the calibration curves were calculated with Equation 11.

$$A \pm ts / \sqrt{n} \qquad (11)$$

where

A = average blank corrected peak absorbance

t = t statistic from table

n = number of measurements

s = standard deviation (SD of the blank corrected absorbance)

The t statistic is 2.776 using a 2-tailed test at the 95% confidence level and n = 5. The SD of the blank corrected absorbance is calculated by using Equation 12.

$$s_a = (s_t^2 + s_b^2)^{1/2} \qquad (12)$$

where

s_a = SD of the blank corrected absorbance

s_t = SD of the total analytical absorbance signal

s_b = SD of the blank absorbance signal

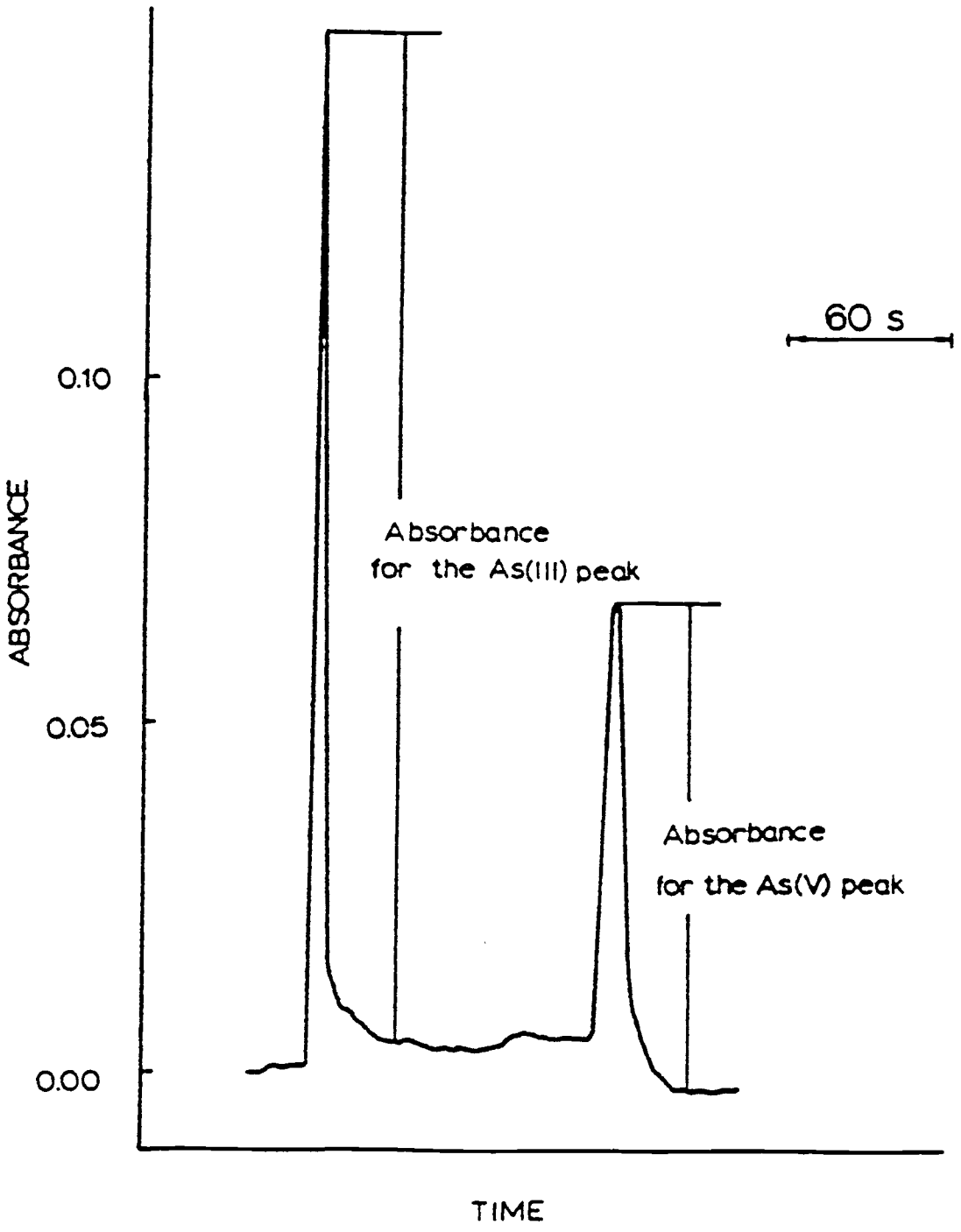

Figure 12. Typical absorbance peaks for speciation of arsenic. The peak absorbance is calculated by measuring the change in absorbance from the top of the peak to the baseline following it.

Table XX. Calibration Data for As(III)[a]

As(III) (µg/L)	Mean Absorbance	Standard Deviation[b]
0.25	0.0051	0.0008
0.50	0.0093	0.0007
1.0	0.0182	0.0009
3.0	0.0519	0.0007
5.0	0.0868	0.0015
10.0	0.1423	0.0048

a The slope and intercept of the least squares fitted line using the first five points are 0.0172 AU $(\mu g/L)^{-1}$ ((0.0001) and 0.0008 AU (0.0002), respectively, with the standard error given in parentheses.

b Calculated from 5 repetitive measurements.

Table XXI. Calibration Data for As(V)[a]

As(V) (µg/L)	Mean Absorbance	Standard Deviation[b]
0.5	0.0052	0.0008
1.0	0.0101	0.0008
3.0	0.0288	0.0007
5.0	0.0503	0.0016
10.0	0.0673	0.0038

a The slope and y-intercept of the least squares fitted line using the first four points are 0.0100 AU $(\mu g/L)^{-1}$ (0.0001) and 0.0002 AU (0.0001), respectively, with the standard error given in parenthesis.

b Calculated from 5 repetitive measurements.

The calibration curves for As(III) and As(V) are shown in Figures 13 and 14, respectively. The error bars are shown on the calibration curves only when they exceed the width of the pen.

The detection limit (DL) and sensitivity were calculated for both As(III) and As(V) and are listed in Table XXII.

Table XXII. Detection Limits and Sensitivities

	DL (μg/L)[a]	Sensitivity (μg/L)
As(III)	0.06	0.2
As(V)	0.2	0.4

[a] The SD of the blank equals 0.0005 AU for ten measurements.

The DL is defined as the concentration which produces a net signal equal to twice the standard deviation of the blank. The SD of the 10 blank signals were used to calculate the detection limits. The atomic absorption sensitivity is defined as the concentration of the analyte which yields a 1% absorption or an absorbance of 0.0044. The relative standard deviation in the 1-5 μg/L range is typically 2-5%.

Linearity extends to about 6 and 5 μg/L for As(III) and As(V), respectively, yielding a dynamic range of about 25 for As(V) and 100 for As(III). This linear dynamic range of the calibration curves is small and comparable to that typically achieved for arsenic with a FAA spectrophotometer. This small dynamic range has been noted by

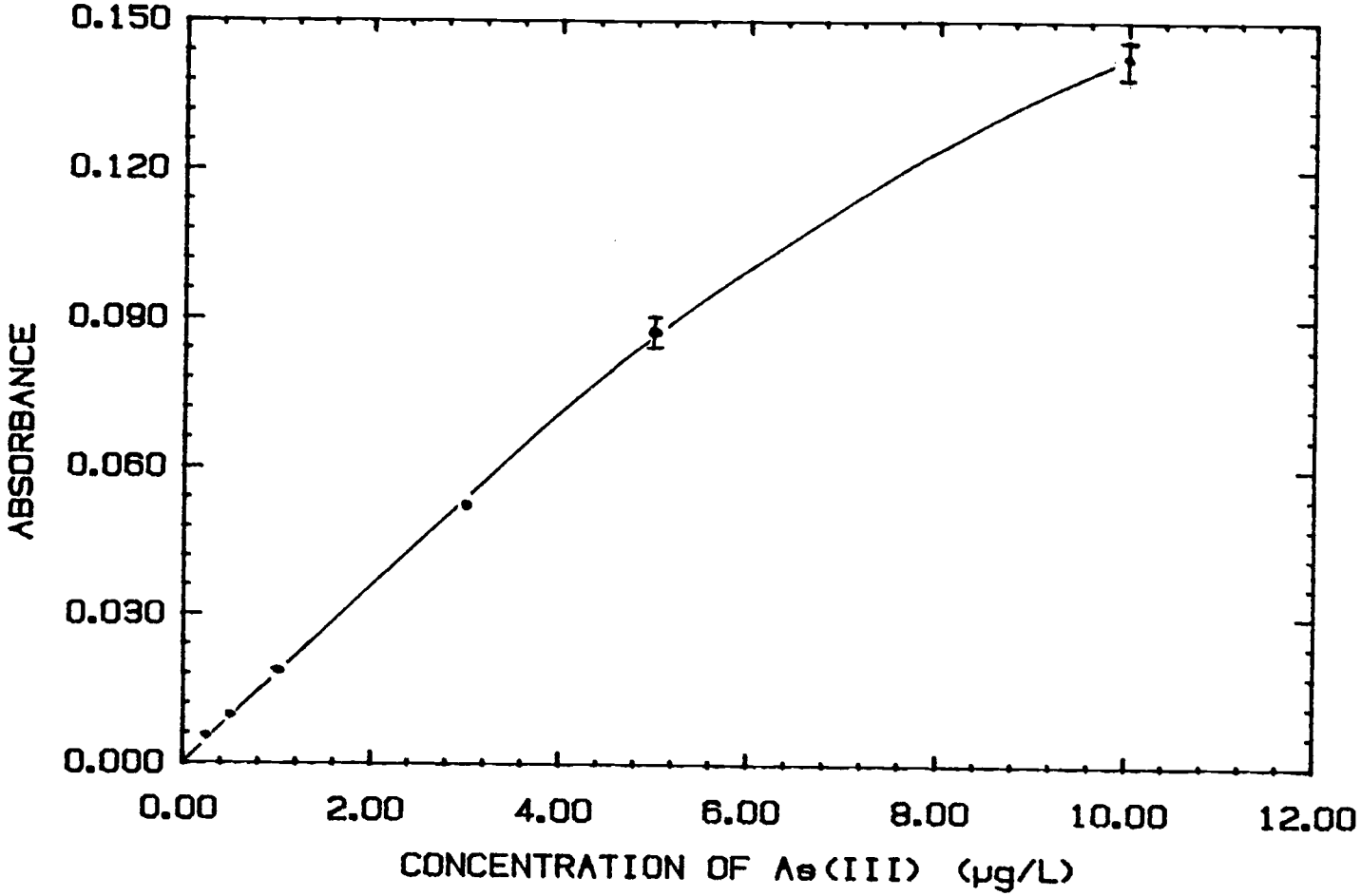

Figure 13. Calibration curve for As(III).

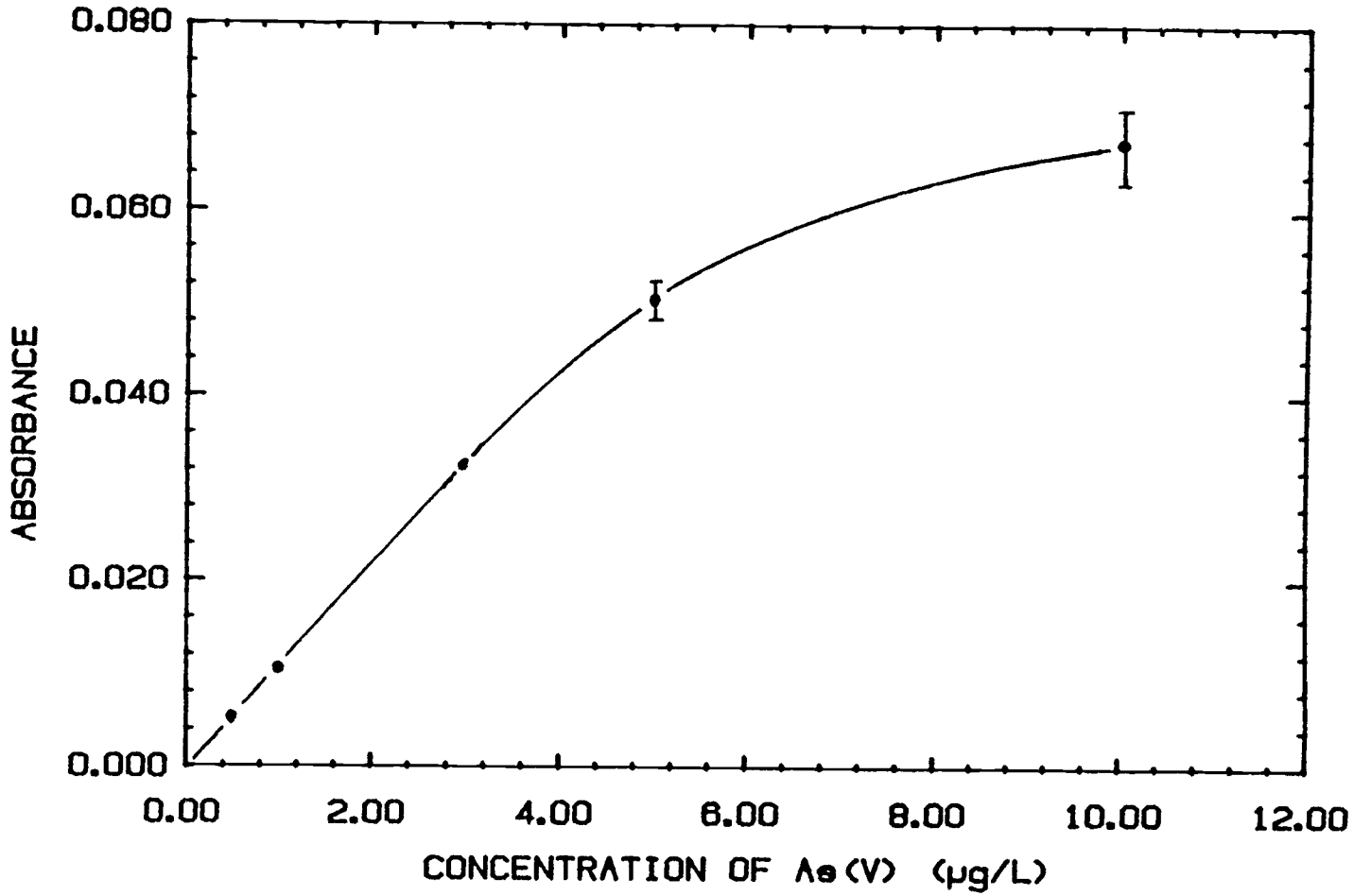

Figure 14. Calibration curve for As(V).

other researchers (61) using HGAA. The calibration curves become non-linear at absorbances about 0.1. This absorbance value is low when comparison to other elements determined by AAS. Some possible reasons for the decreased linear dynamic range for As include unresolved, non-absorbing lines that reach the detector or variation of the absorptivity over the source profile.

Interference Study

The results from the interference study on a 1 µg/L As(III) and 1 µg/L As(V) are listed in Table XXIII. Using a 2-tailed t test at the 95% confidence level, there is no significant difference between the peak absorbances obtained for the arsenic solutions diluted with deionized water and those diluted with the artificial water. Two dilutions of each sample were prepared. Therefore, it is believed that there should be no interferences that will cause error in the calculated arsenic concentration in either tap water or river water using the direct calibration curve method of analysis.

Storage Study

The results of the 4-week storage study indicates that the arsenic species are stable and that no loss of arsenic is observed for at least a month. There was no significant difference between freshly made standards of 1 µg/L As(III) and As(V) compared with either sample (refrigerated or non-refrigerated) at pH 2 any time during the study. These findings agree with those of other researchers (5).

Table XXIII. Interference Study

Solution	Abs for 1 µg/L As(III)	Abs for 1 µg/L As(V)
deionized water I	0.0185 (0.0010)	0.0097 (0.0011)
deionized water II	0.0182 (0.0007)	0.0100 (0.0012)
artificial water I	0.0190 (0.0011)	0.0095 (0.0009)
artificial water II	0.0186 (0.0008)	0.0098 (0.0010)

The standard deviations of the blank corrected absorbances for 5 repetitive runs are in the parentheses.

Real Sample Analysis

Water (tap and river), digested hair solution and the NBS standard orchard leaves were analyzed for their As(III) and As(V) concentration using the direct calibration curve method as well as the standard addition method. The results are shown in Table XXIV. Each sample and reagent blank were run 5 times to obtain the average blank corrected absorbance for the arsenic concentration. The slope of the calibration curve using external standards changes from day to day. Therefore, a calibration curve was always obtained directly preceding the analysis of a sample.

The tap and river water were shown to not need the standard addition method. According to the 2-tailed t test at the 95% confidence level, the arsenic concentrations determined from the external standard calibration curve are not statistically different from those using the standard addition method. The standard addition curves for the tap and river water samples are shown in Figure 15 and 16, respectively. The As(III) concentration in both water samples is below the detection limit of 0.06 μg/L. Other researchers have reported the arsenic in water samples to be mostly in the pentavalent form. The As(V) concentration is within the safe limits set by the EPA for water samples.

As noted earlier, the trivalent form of arsenic is more toxic than the other forms because it accumulates in the body, especially in the hair and nails. The KOH digestion procedure should not change the arsenic species found in hair. The independent lab used a

Table XXIV. Real Sample Analysis Results

Sample	Species	Ext. Std. Conc. (µg/L)[a]	Std. Add. Conc. (µg/L)[b]
Tap Water 1	As(V)	0.7 (0.6 - 0.9)	0.8 (0.3 - 1.4)
Tap Water 2	As(V)	0.7 (0.6 - 0.9)	0.7 (0.2 - 1.3)
River Water 1	As(V)	1.1 (1.0 - 1.3)	1.0 (0.5 - 1.6)
River Water 2	As(V)	1.2 (1.1 - 1.4)	1.0 (0.5 - 1.6)
Hair 1	As(III)	0.15 (0.13-0.18)	2.6 (2.1 - 3.2)
Hair 2	As(III)	0.17 (0.15-0.20)	2.8 (2.3 - 3.1)
NBS 1	As(V)	2.5 (2.3 - 2.6)	10.6 (9.1 - 12.2)
NBS 2	As(V)	2.6 (2.4 - 2.7)	10.1 (8.6 - 11.7)

a Samples' arsenic concentration determined using external standards.

b Samples' arsenic concentration determined using standard additions.

The 95% confidence interval for each concentration is listed in parentheses (See Reference 62).

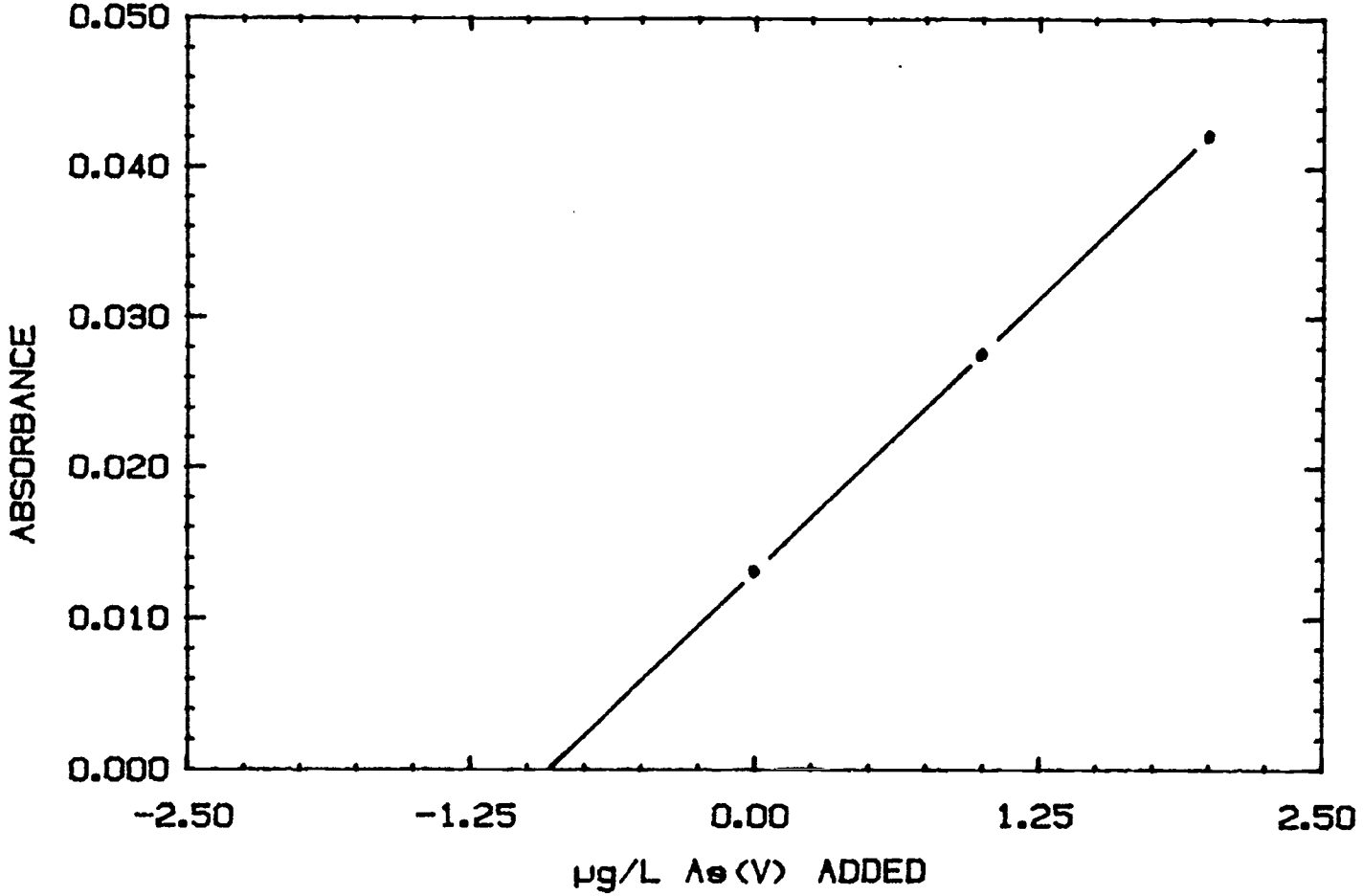

Figure 15. Standard addition curve for tap water sample analysis.

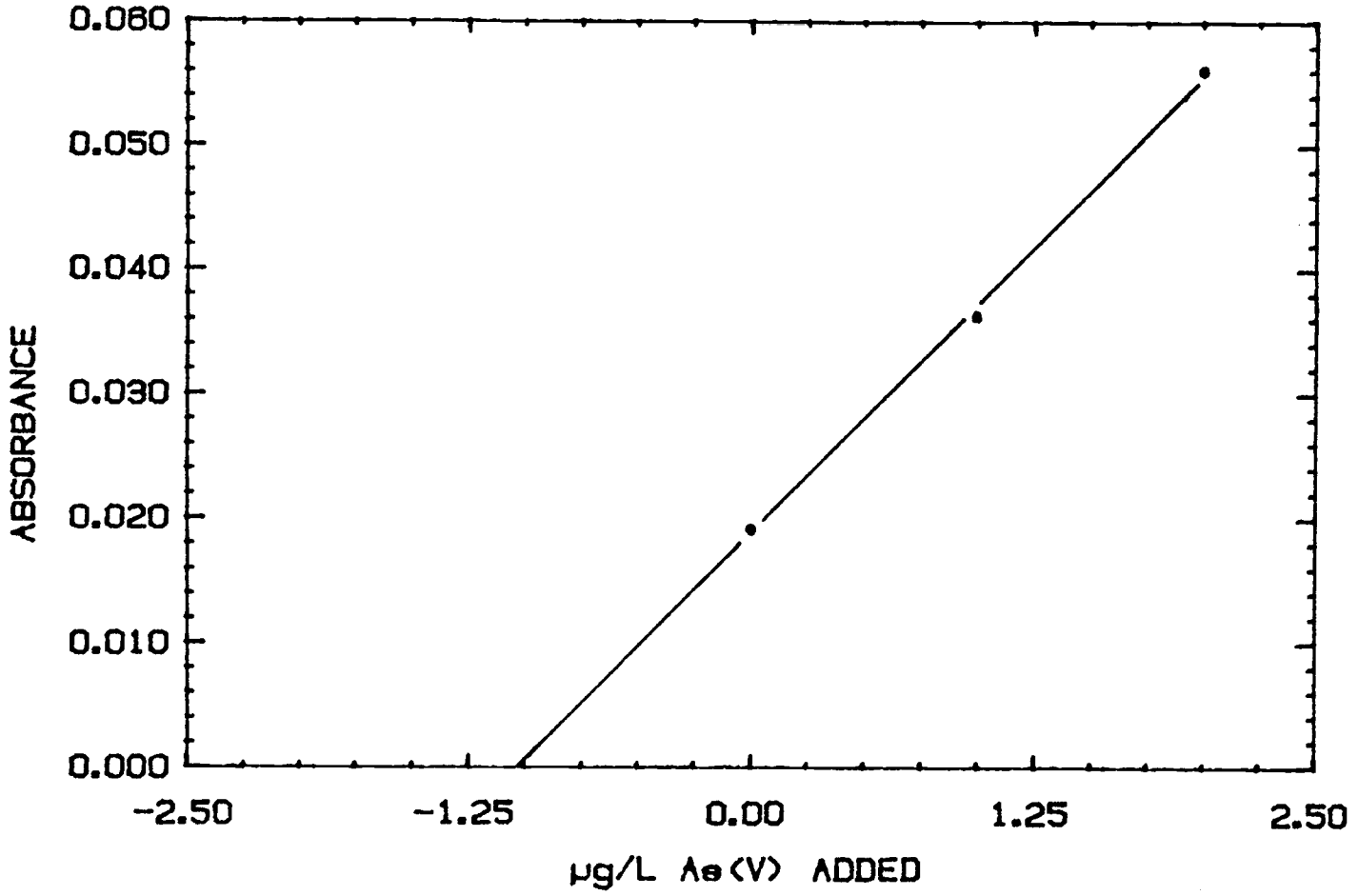

Figure 16. Standard addition curve for river water sample analysis.

perchloric/nitric acid digestion, so only the total arsenic was determined. The hair sampled was taken from the nape of the researcher's neck, and thus would represent new growth of hair and then could be used in comparison to determine if the researcher had actually accumulated a measurable amount of arsenic in her body since her hair was analyzed in June, 1983.

Upon analysis of the hair samples, only As(III) was detected as expected. The As(III) concentration using the direct calibration curve (0.15 µg/L) was found to be statistically different from the As(III) concentration from the standard addition method (2.6 µg/L) using the 2-tailed t test at the 95% confidence level. Figure 17 shows the standard addition curve for hair sample 1. The 2.6 µg/g of hair does show an increase over the 1.9 µg/g that was determined by the independent lab. A statistical comparison can not be made between the 2 samples because the details of the analysis procedure by the independent lab is not known. The lab does list the arsenic concentration in hair as:

Low	0-2 µg/g
Medium	2-3 µg/g
High	3- µg/g

NBS Standard. Since the NBS standard orchard leaves were dry ashed, it was assumed that all the arsenic present in the samples would be oxidized to the pentavalent form. No As(III) was detected in either NBS sample. The As(V) concentration using external standards was calculated to be 2.5 µg/L or 2.5 µg/g of leaves for NBS sample 1.

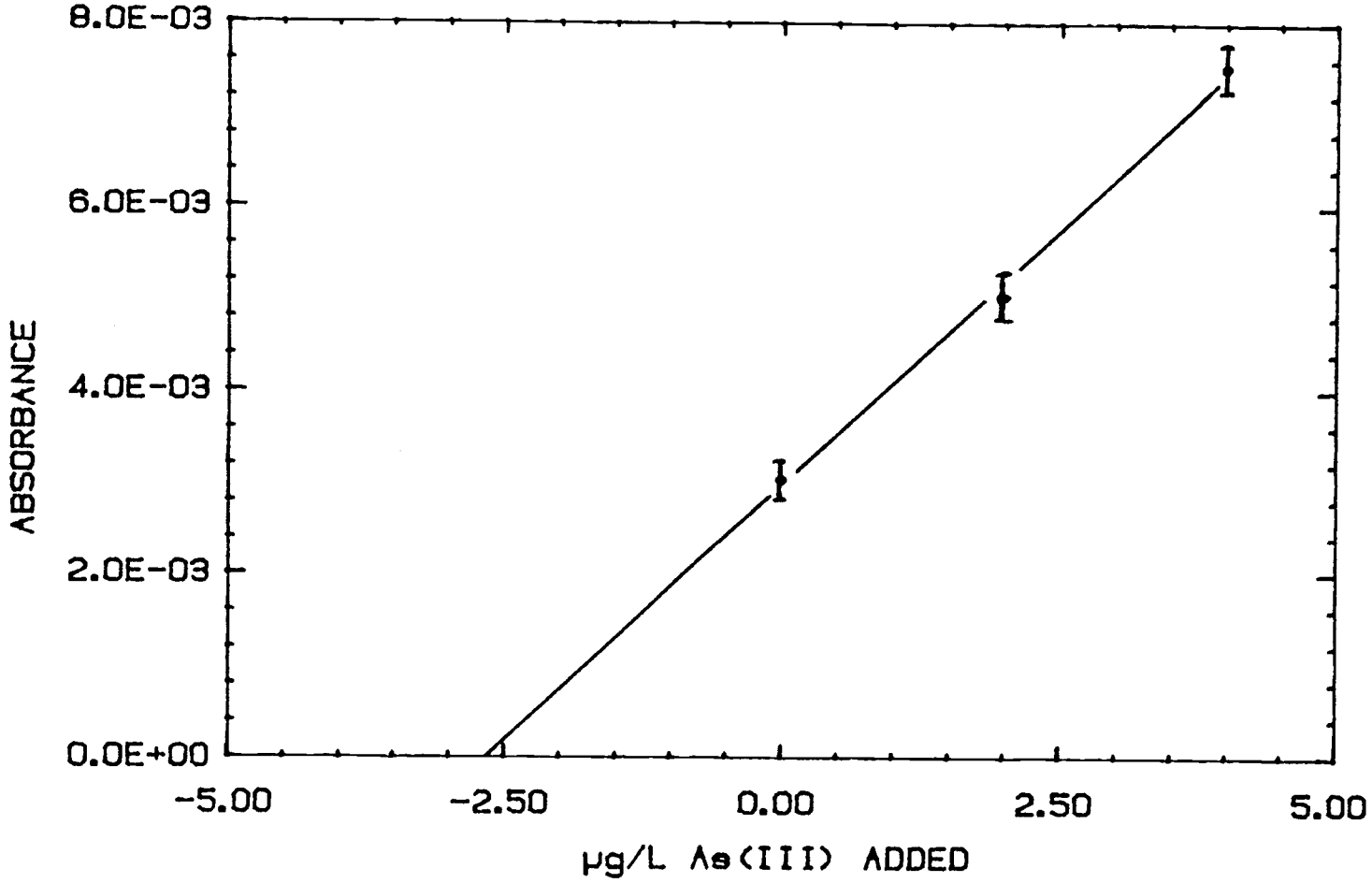

Figure 17. Standard addition curve for hair sample analysis.

This concentration is well below the 10 ± 1 μg/g of total arsenic as listed by the NBS.

The standard addition method did overcome the interference that caused a decrease in the calibration curve slope. Using standard additions (See Figure 18), the As(V) concentration was calculated to be 10.6 μg/L or 10.6 μg/g of leaves for NBS sample 1. This concentration agrees with the concentration of total arsenic given by the NBS.

While the use of the NBS standard could not test the accuracy of the speciation technique, the standard was used to test the accuracy of the HGAA spectrophotometric method for total arsenic. Though there are no standards which can be used to test the accuracy of a speciation technique for arsenic, the oxidation state of arsenic found in each of the samples was as expected.

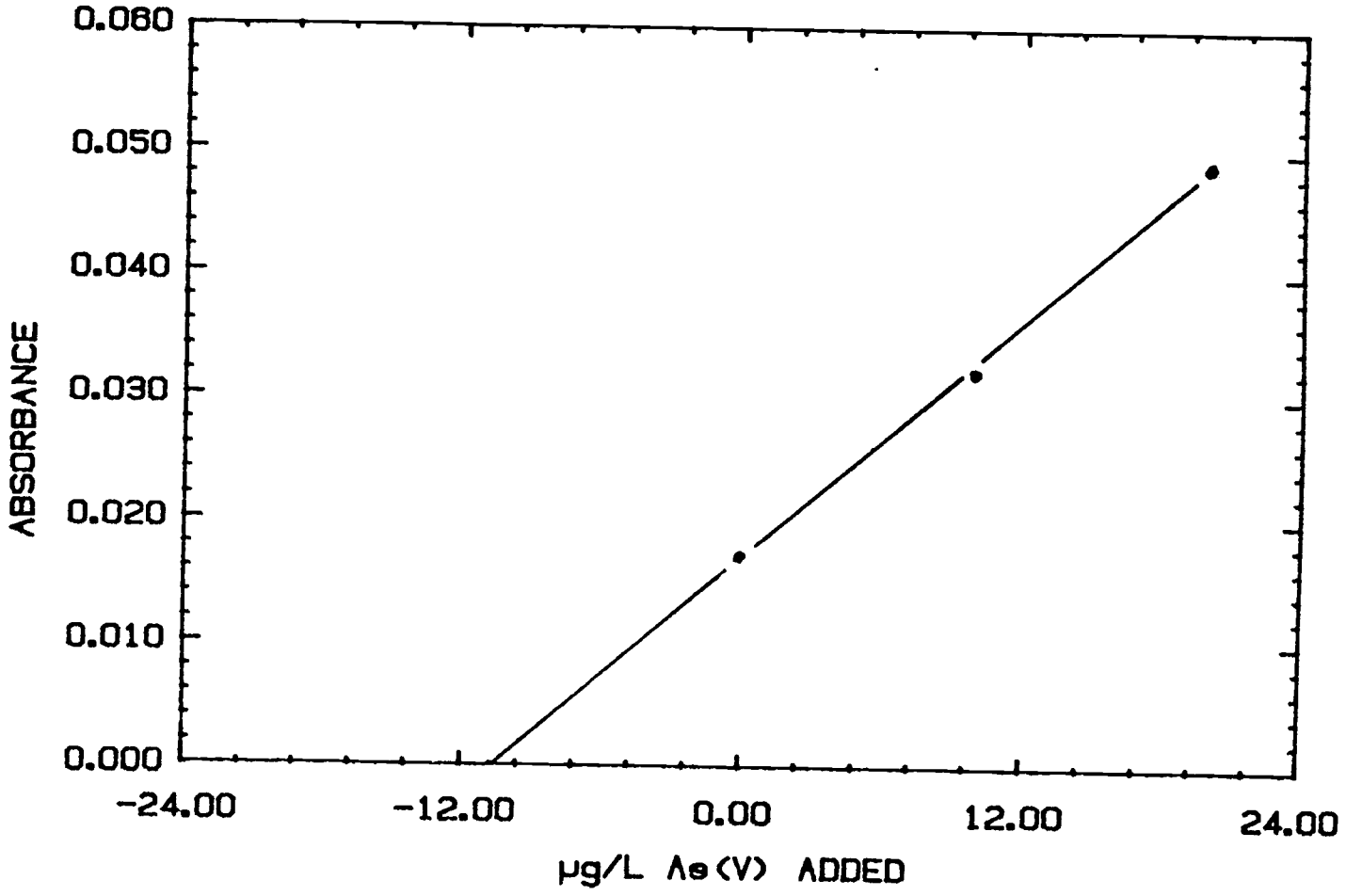

Figure 18. Standard addition curve for NBS sample analysis.

CONCLUSIONS

Speciation of inorganic arsenic can be accomplished with a single aliquot of sample by the use of a HGAA spectrophotometer and the analysis procedure developed. The method developed offers low detection limits (0.06 µg/L for As(III) and 0.02 µg/L for As(V) from a small sample volume (1 mL). The detection limits are better than those reported by other researchers who used volumes ranging from 10 to 100 mL with the HGAA technique. The small sample volume and design of the reaction vessel allow the absorbances from As(III) and As(V) in one solution to be measured in approximately 1 min.

The flame-in-a-tube atomization method offers advantages over the more frequently used atomization methods based on a heated (900° C) quartz tube or a hydrogen/nitrogen/entrained air flame. The flame-in-a-tube atomization method offers the advantage of a more consistent production of hydrogen radicals as compared to a heated quartz tube atomization method. In the heated quartz tube atomization method, the quality of the surface of the quartz tube plays a dramatic role in the efficiency of the atomization of the hydride. The hydrogen radicals needed to atomize the hydride at temperatures below 1700° C, are formed by reaction of the hydrogen produced in the reduction reaction and the unprotonated Si-O sites at the quartz surface. The heated quartz tube's surface needs to be cleaned at least once a day to obtain sufficient production of the hydrogen radicals necessary for the complete atomization of the hydride.

The flame-in-a-tube method provides much better detection limits than the traditional hydrogen/nitrogen/entrained air flame atomization method. The higher flow rates required to maintain the flame dilute the atomic arsenic.

The method developed for the determination of arsenic by HGAA spectrophotometry has proven to be accurate by determination of total arsenic in NBS standard orchard leaves. The sequential reduction scheme for the determination of As(III) and As(V) based on selective reduction of As(III) at higher pH was shown to be applicable to water and biological samples. The results for As(V) could be in error because of the organo-arsenic compounds present in the sample. The organo-arsenic compounds should, according to Braman and Foreback (49), also be converted to their corresponding arsine at the low pH, thus interfering with the determination of As(V). Braman and Foreback used a solution of sodium cyanoborohydride to prevent the formation of the organo-arsine.

Further research is needed to determine if the organo-arsines do interfere with the determination of As(V) as many techniques already employed for speciation of inorganic arsenic do not consider the possibility of the organo-arsenic compounds interfering with the determination of As(V). Previous studies indicate that the organo-arsenic compounds have concentrations on the same order as the inorganic As(V) found in water samples (49). Even if the organo-arsenic compounds do interfere with the determination of As(V), the toxicity difference between the two forms of arsenic is not great.

There is a great difference, however, between the toxicity of As(III) and As(V), as As(III) accumulates in the body tissues by combining with the sulfhydryl enzymes.

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