UNIVERSITY OF KWAZULU – NATAL

DETERMINATION OF ARSENIC IN SEAWEED KELP TABLETS BY HYDRIDE GENERATION – INDUCTIVELY COUPLED PLASMA ATOMIC EMISSION SPECTROSCOPY (ICP-AES)

by

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Dedication

To my mother – Tequabo Berhe and my father – the late Ghebreyohannes Teclu

Contents

Declaration

The research work described in this dissertation was carried out in the School of Physical and Chemical Sciences, University of KwaZulu Natal, Pietermaritzburg, from February 2002 to March 2004, under supervision of Dr. Colin Southway.

The work represents original work by the author and have not otherwise been submitted in any form for any degree or diploma to any University. Where use has been made of the work of others it is duly acknowledged.

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Abbreviations

AAS	Atomic absorption spectroscopy
AsB	Arsenobetaine
AsC	Arsenocholine
AsL	Arsenolipid
AsS	Arsenosugar
DCP-AES	Direct current plasma atomic emission spectroscopy
DMA	Dimethylarsinate
DMAA	Dimethylarsinic acid
EDTA	Ethylenediaminetetraacetic acid
FAAS	Flame atomic absorption spectroscopy
GC	Gas chromatography
HG	Hydride generation
HG-ICP-AES	Hydride generation inductively coupled plasma atomic emission spectroscopy
HPLC	High performance liquid chromatography
ICP-AES	Inductively coupled plasma atomic emission spectroscopy
MAA	Methylarsonic acid
MMA	Monomethyl arsonate
MMAA	Monomethyl arsonic acid
MS	Mass spectroscopy
$NaBH_4$	Sodium borohydride
PTFE	Polytetraflourethylene
RSH	Cysteine
TFM	Trifluoromethyl plastic (modified PTFE)
TMA	Trimethyl arsine

TMAO	Trimethylarsine oxide

Abstract

A study was carried out for the determination of arsenic in commercial seaweed kelp tablets by developing and optimizing a hydride generation method. The effects of pH and different reaction media were investigated. A continuous hydride generator that generates arsine using sodium borohydride as a reductant was coupled to an inductively coupled plasma atomic emission spectrometer as a detection system.

L-cysteine was used as a pre-reductant agent. L-cysteine enhances the As(III) signal at low acid concentration and fulfills other functions (such as reducing interferences). Interference studies were undertaken in different reaction media.

The methodology developed has a detection limit (based on 3σ) of 0.6 μg/l for As(III) in 0.1 M HCl reaction media and a relative standard deviation of 4 %.

By following the established method, arsenic levels were investigated in microwave - digested, dry - ashed and wet - digested commercial seaweed kelp tablets samples and the results were compared. The microwave assisted digestion procedure was found to give the highest arsenic concentrations (81.90 μg/g for Tablet A; 93.69 μg/g for Tablet B).

The open beaker wet digestion procedure gave values of 22.71 μg/g for Tablet A and 37.15 μg/g for Tablet B and the dry ashing procedure gave 4.63 μg/g for Tablet A and 5.95 μg/g for Tablet B.

The microwave procedure was found to be the preferred digestion procedure due to its efficiency, rapid, convenient and less prone to contamination.

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Chapter 1

Introduction

Seaweeds are marine plants which have a wide range of uses for human and animal consumption. They contain a valuable source of proteins, fats, carbohydrates, vitamins and minerals. An industrially produced seaweed kelp tablet is utilized as supplement for human consumption.[1] The nutritional use of seaweed kelp is also affected by the presence of trace elements. These trace elements and other major elements are absorbed by seaweed using different mechanisms from the environment in which the seaweed grows.[1]

One of the trace elements which exists in seaweed is arsenic. Arsenic is a ubiquitous element that exists in the atmosphere, the aquatic environment, in soils and sediments, and in organisms. Since natural and anthropogenic inputs vary geographically, the levels of arsenic show a wide range of concentrations. Arsenic contributes to environmental pollution and affects human health if the concentration exceeds the permissible level. The toxicity of arsenic depends on its type of species. For example As(III) and As(V) are highly toxic whereas arsenobetaine is relatively non-toxic.[2]

The objective of this study was to develop a method for the determination of arsenic in commercial seaweed kelp tablets. Arsenic is present in seaweed and it is necessary to study its concentration levels and chemical forms.

The most widely used method for the determination of arsenic is the reduction of

arsenic compounds to arsines followed by atomic spectroscopy for its detection.[3] This improves the sensitivity and detection limit of arsenic analysis. This is due to arsine formation as a preconcentration system. Early methods[3] for generation of arsine used dissolution of metals (Zn, Mg, Al) in mineral acids to form nascent hydrogen which reacts with As(III) to form AsH_3. Other compounds used for generating arsine are $TiCl_3$ or $SnCl_2$ in concentrated HCl[4] or Al in basic medium.[5,6] In this study $NaBH_4$ is used as a reducing agent for the formation of arsine.

There are two major modes of operation for hydride generation systems, batch and continuous modes.[3] Both modes involve reduction of the acidified sample and transport of the generated hydride into detector. In batch mode, acidified sample and reductant are injected into a reaction vessel and the hydride produced is purged and carried by an inert gas, whereas in continuous mode, the hydride is generated by continuously reacting the acidified sample and reductant. The hydride formed is separated from the liquid waste by gas - liquid separator. A continuous hydride generation system is used in this work, which has the advantage of less contamination problem and being less prone to transition element interferences.[3]

It is well known that As(III) and As(V) show different sensitivities in the continuous hydride generation system.[7] This is due to the kinetic difference that the two species show. The process of arsine formation from As(V) suggests that there are two steps:[7] the reduction of As(V) to As(III) and then formation of AsH_3. Therefore, a pre-reducing agent is necessary to reduce As(V) to As(III). In this investigation L-cysteine is used as a pre-reducing agent. It has the advantage of enhancing signal at low HCl concentration, reducing interferences from some elements and more importantly almost the same optimum response under the same HCl concentration can be obtained for As(III) and As(V).[8]

In order to achieve the separation of arsenic species a detailed investigation of differ-

ent reaction media, pHs and buffers are required. Anderson et al.[9] reported a detailed study on selective reduction of arsenic species using continuous hydride generation. In this study concentrations of $NaBH_4$, HCl (in presence and absence of L-cysteine), reaction coil length, reaction time and pH of buffered reaction media were optimized using the hydride generation system and ICP-AES detection system.

The developed HG-ICP-AES method was used to determine arsenic in commercial seaweed kelp tablets using three digestion procedures i.e. microwave - assisted, dry - ashing and wet - digestion and their efficiencies compared.

Chapter 2

Seaweed

Seaweeds are marine-algae. The algae contains some of the primitive members of the plant kingdom with simple structures that appeared long ago in the geological history with no change to the present day. Seaweeds are large algae or macro-algae. They are present in the sea attached to the seabed between the upper level of the intertidal zone and the maximum depth at which light for growth can penetrate. Seaweeds have interactions with other marine organisms and all interact with their physicochemical environment. Seaweeds show some features that make them different from land plants or phytoplanketers. Seaweeds are multicellular, which gives them the advantage of being able to grow extensively in the third dimension of the water column.[10] They usually grow vertically away from the substratum; which brings them to light, grow large without competition of space, and get nutrients from a large volume of water.[11] Seawater contains all the necessary nutrients that the seaweed requires to absorb and at the same time seawater gives support to the plant body. Seaweeds grow upward from the base not from the tip i.e. the oldest is at the top and the youngest at the base.[10] Moreover, support tissue is not required for this upward growth. Support tissue is metabolically expensive as it is nonphotosynthetic. However, seaweeds must have some strength and resilience to withstand water motion. Some of the larger seaweeds

like *Pterygophora* have stiff, massive stripes; and others like *Hormosira* use flotation to keep them upright.[11]

The flora of seaweeds change from region to region as terrestrial floras do. Salinity, light and temperature are the physical factors that limit seaweed distribution.[11]

The following figure gives a simplified picture of seaweeds with their major parts.

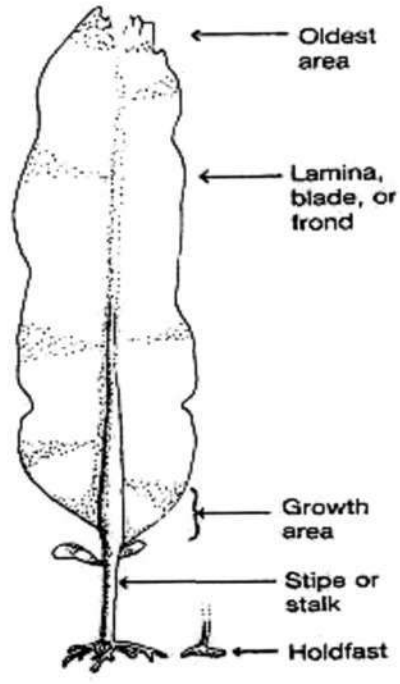

Figure 2.1: A Simplified Figure of Seaweed's Parts

Holdfast is the root- or disc like in shape which do not function as the root of land plants in taking up nourishments. Its main function is to hold the seaweed fast to the rock or stone surface. The holdfast may sometime creep over an area, thereby increasing the seaweed's spreading to the surrounding rock or shell surfaces.

The frond is the main part of the seaweed. It can vary its shape from a leaf-like flat blade to fine filaments that resembles hair. It may be single or branched into simple or complex shapes. At the base there may or may not be a stalk-like attachments to the holdfast.

The thallus is the whole seaweed plant. Unlike a land plant, each cell in the seaweed carries out the same function as any other cell.[10]

2.1 Varieties and Nature of Seaweeds

Seaweeds are classified as belonging to the plant sub-kingdom *Thallophyta*, in which there are eight classes and the species could be large weeds (macro algae) or micro algae. Some of the colour identifications of the species are:

Chlorophyceae – green algae (macro)

Phaeophyceae – brown algae (macro)

Rhodophyceae – red algae (macro)

Cyanophyceae – blue-green algae (micro)

The first three species are the main groups of seaweeds. The colour of each group is more or less definitive. All the species have chlorophyll as their photosynthetic base, therefore all are green, but the green is covered by another pigment such as brown or red. Shade can change with age of seaweed or season. Therefore, colour as an identification guide (light-absorbing characteristics) decides where in the zonation scheme the seaweeds are likely able to grow.

Chlorophyceae (green) absorb the long wavelengths of light which is red light and reflect green. These plants grow where there is strong light in shallow waters and store food just as land plants as starch. They are linked with land plants, being on the borderline between land and sea. Phycocyanin (blue), xanthophylls (yellow) and carotene are the pigments present in *chlorophyceae.*

Phaeophyceae (brown) are the largest and longest of the seaweeds. They absorb medium wavelength green light. Green light can penetrate deeper than red light, so brown seaweeds live deeper. Brown seaweeds show a preference for cooler water temperature. *Phaeophyceae* contain the pigment fucoxanthin.

Rhodophyceae (red) seaweeds absorb the blue and ultra violet light wavelengths. Red is the most common colour of seaweeds and lives up to depth of 600 meters in clear water areas. The pigments phycoerythrin (red) and phycocyanin (blue) are the main pigments of *Rhodophyceae.*[10] In terms of species composition, *Rhodophyceae* make up the largest component of seaweed communities.[12]

Cyanophyceae contain the same pigments as *Chlorophyceae.*

In different countries or within the same area, seaweeds can be named differently for the same species. Therefore, it is useful to use the scientific names when dealing with identification. All seaweeds have two names; the first name with an initial capital letter tells the family or genus the seaweed that belongs. The second name, which always starts with a small letter, is the plants own species name. In some cases, the names contain one or two names associated with the name. These refer to the scientist who first named the seaweed or if there is re-identification or revision of that plant, the first scientist's name will be bracketed, followed by the latest person's name.

For example; in the class *Chlorophyceae* (green seaweeds): *Enteromorpha intestinalis* (Linneaus) Link, *Enteromorpha* is the genus, *intestinalis* is the species; Linneaus first named this seaweed, but Link has revised the species.

Seaweeds do not have flowers, seeds or fruits. Seaweeds produce spores that can swim from their special parts of the frond known as sporangia. The spores are produced asexually and when they settle on the suitable rock new plants will grow into either male or female seaweeds. Egg cells from female seaweeds, which are remaining fixed in the frond, are developed, and male develop swimming sperm cells. Both eggs and sperms are released into the sea, where fertilization takes place. If sea conditions are favourable the fertilized eggs stick onto surfaces and grow to new plants. In some seaweeds the male and female plants are identical, others may show colour differences. Further variations in species can be brought about by sexual and asexual way of producing the species, which

can be identified as two completely different species. The alternation of generation is seaweed's specialty of producing species. All seaweeds do not follow this general pattern, some have very complex variations, others have merged reproductive activities, and a lot is unknown.[10]

2.2 Conditions for Growth of Seaweeds

Among the main factors affecting the growth of seaweeds are light, temperature, salinity, water motion, and nutrient availability. Each of these factors is briefly described below.

2.2.1 Light

Light is the major factor affecting plants growth and has a complex nature. The complexity of light arises primarily from the nature of light itself, and secondly the effects it has on plants. Seaweeds grow in dynamic and diverse light environment. The outward flow and flood of tides have impact on the quality and quantity of the sun's energy reaching seaweeds and the irradiance on the earth's (ocean's) surface. The use of light in seaweeds is to provide the energy for photosynthesis and for all biological processes. Inorganic carbon supply, temperature, pH and age of tissue affects rate of photosynthesis. Light is the signal for the events like reproduction, growth, distribution, etc throughout the life cycles of the algae. The quality and quantity of light greatly depends on the depth and number of particles in the water.[11]

2.2.2 Temperature

Temperature is the most fundamental factor for all organisms because of its effects on molecular activities and properties i.e. on most aspects of metabolism. Living organisms are rarely at thermal equilibrium with their environment[13] and the inside temperature

of the cell is more important than the surroundings. However, the inside temperature of the seaweed's and other organisms are almost near the temperature of their surfaces or of the surrounding water or air. Latitude and ocean currents affect seawater's surface temperature.

Molecular activity and structure in seaweeds is affected by temperature. If the temperature increases by 10^0C, the biochemical reaction rates would approximately double.[11] However, enzyme reactions show peak activities at optimum temperature and if the temperature changes there will be changes in tertiary or quaternary structure as a result inactivate and denature the enzymes. There are also optimum temperatures for the processes of photosynthesis, respiration, and growth that are consequences of enzyme reactions. The effects of temperatures are not uniform across all processes. The optimum temperatures vary within species. At complex levels other environmental variables have larger effects and may decrease the effects of temperature. A gradually changing temperature can make a metabolic rate to get used to the changing climate. Freezing kills many algae, especially if ice is formed inside the cells. However, many intertidal algae can tolerate temperatures below zero. Tropical algae on the other hand, are killed by low temperatures even above 0^0C.[11]

2.2.3 Salinity

Salinity is grams of salts per kilogram of solution (parts per thousand). This definition fails to confirm the physical, chemical, and biological complexity of this "factor". This complexity can be the relationships that seawater density, light refraction, and electrical conductivity bear to salinity (and also to temperature)[11]. Ion concentrations, density of seawater, and osmotic pressure are the biological aspects of salinity.

Natural salinity of marine and brackish waters range from about 10 to 70 part per thousand with 25 to 35 parts per thousand being the most common. The important

components of salinity to seaweed physiology are the total concentration of dissolved salts and the corresponding water potential, availability of specific ions such as calcium and bicarbonate. Because the cell walls of many seaweed's are rigid, the internal pressure is regulated by active movement of ions across membranes or by interconversion of monomeric and polymeric compounds.[11]

2.2.4 Water Motion

The waters of the sea are in constant motion. The motions can be great ocean currents, tidal currents, waves, and other small-scale circulations which are caused by density changes. Environmental factors affect the force of water motion. Also, water motion affects nutrient availability, light penetration, temperature and salinity changes. To understand the effects of water motion on seaweeds, it is necessary to understand the physical nature of waves and currents. Ocean waves are created by the movement of wind over the sea surface. Such characteristics of the wave: height, and wavelength depend on the speed and duration of the wind which blow on the distance of the open water.

Sediments may be moved by water motion which can have deleterious effect to algae due to the burial of some species.

Some seaweeds can change morphologically if the seaweeds are moved from rough to calm water. The interaction of genotype and wave environment has been elucidated in a *Laminaria* species.[11]

2.2.5 Nutrients

Seaweeds are photoautotrophic i.e. they use sunlight to produce organic matter using mineral ions and water. Many of the photosynthetic seaweeds are also auxotrophic which require small amounts of growth stimulators like vitamins for growth. There-

fore, the availability of nutrients is essential for regulating growth, reproduction and biochemistry of seaweeds.[14]

Seaweeds require inorganic carbon, water, light, and various mineral ions for photosynthesis and growth. All algae require the elements C, H, O, N, P, Mg, Fe, Cu, Mn, Zn and Mo.[14] S, K and Ca are required by all algae but can be replaced by other elements. Some algae require Na, Co, V, Si, Cl, B, and I. Macro-algae require all the major seawater constituents except Sr and F.[14]

For metabolic processes plants require around 21 elements, but more than double this number is present in seaweeds. The presence of an element in the seaweed does not signify that the element is essential nor does the relative amount of the element indicate the importance of the element. Generally, elements (essential or non-essential) are accumulated in the tissue of algae above their concentration in seawater which can give concentration factors of up to a thousand fold. Some of the elements are taken up in excess of the required whereas others are absorbed but not used. There are few elements such as nitrogen, phosphorus, iron and trace metals (e.g. cobalt and manganese) which limit the growth of seaweeds due to their low concentration in specific period of time. Nitrogen is the most frequently observed to limit seaweed growth. Nitrogen sources for seaweed are dissolved inorganic nitrogen (nitrate, nitrite, ammonium) and dissolved organic nitrogen (urea and amino acids). Ammonium more absorbed than other nitrogen compounds. Light, temperature, water motion, desiccation, and the ionic form affect uptake rate of nitrogen or other ions. Biological factors such as types of tissue, age of plant, nutritional past history and interplant variability also affect uptake.[11]

2.3 Chemical Compositions of Seaweeds

Seaweeds are composed of different types of elements and compounds. At least 56 elements have been reported to be present in seaweeds.[14] Some of the elements and

compounds that exist in *Chlorophyceae*, *Phaeophyceae* and *Rhodophyceae* are given in the following tables. From Table 2.1 it can be seen that C, H, O, K, N, S, P, Ca, and Mg are present in the seaweeds in excess of 1 mg/g dry weight.

Table 2.1: Some Elemental Concentrations in Seaweeds[14]

Element	Concentration in Dry Matter	
	Mean(μg/g)	Range (μg/g)
Macronutrients		
H	49 500	22 000 - 72 000
Mg	7 300	1 900 - 66 000
S	19 400	4 500 - 82 000
K	41 100	30 000 - 82 000
Ca	14 300	2 000 - 360 000
C	274 000	140 000 - 460 000
N	23 000	500 - 65 000
P	2 800	30 - 12 000
Micronutrients		
B	184	15 - 910
Zn	90	2 - 680
Fe	300	90 - 1 500
Cu	15	0.6 - 80
Mn	50	4 - 240

Extensive analysis on marine plankton for chemical composition shows the ratio of carbon, nitrogen and phosphorus to be 106 C : 16 N : 1 P (by atoms) which is called the Redfield ratio.[15] The ratio for seaweeds was about 550 : 30 : 1. The large value for carbon is thought to be due to their large amount of structural and storage carbon.

The average carbohydrate and protein content of seaweeds has been estimated at about 80% and 15%, respectively for the ash-free dry weight.[15]

Temperate seaweeds show variation in chemical composition with season due to the nitrogen limitation in the coastal waters in summer.

The function and compounds of the essential elements in seaweed are tabulated in the following table.

Table 2.2: The Function and Compounds of the Essential Elements in Seaweed[11]

Element	Probable Functions	Examples of Compounds
Nitrogen	Major metabolic importance as compounds	Amino acids, pyrimidines, porphyrins, amino sugars, amines
Phosphorus	Structural, energy transfer	ATP, GTP, etc., nucleic acids, phospholipids, coenzymes including Co-A, phosphoenolpyruvate
Potassium	Osmotic regulation, pH control, protein conformation and stability	Probably occurs predominantly in the ionic form
Calcium	Structural, enzyme activation, ion transport	Calcium alginate, calcium carbonate
Magnesium	Photosynthetic pigments, enzyme activation, ion transport, ribosome stability	Chlorophyll
Sulfur	Active groups in enzymes and coenzymes, structural	Methionine, cystine, glutathione, agar, carrageenan, sulfolipids, coenzyme A

Table 2.2: Continued...

Element	Probable Functions	Examples of Compounds
Iron	Active groups of porphyrin molecules and enzymes	Ferredoxin, cytochromes, nitrate reductase, nitrite reductase, catalase
Manganese	Electron transport in photosystem II, maintenance of chloroplast membrane structure	
Copper	Electron transport in photosynthesis, Enzymes	Plastocyanin, amine oxidase
Zinc	Enzymes, ribosome structure (?)	Carbonic anhydrase
Molybdenum	Nitrate reduction, ion absorption	Nitrate reductase
Sodium	Enzyme activation, water balance	Nitrate reductase
Chlorine	Photosystem II, secondary metabolites	Violacene
Boron	Regulation of carbon utilization (?), ribosome structure (?)	
Cobalt	Component of vitamin B_{12}	Vitamin B_{12}
Bromine[a] Iodine[a]	Toxicity of antibiotic compounds (?)	Wide range of halogenated compounds, especially in Rhodophycea

[a]Possibly an essential element in some seaweeds[2]

2.3.1 Arsenic Content of Seaweeds

Even though there is a constant arsenic concentration in seawater ranging from 1.0 to 1.5 μg/g,[16] the levels in seaweeds show a variation in arsenic concentration: *Rhodophyceae*, 1.43 μg/g; *Chlorophyceae*, 1.54 μg/g; *Phaeophyceae*, 10.3 μg/g.[11] This topic will be treated in chapter 4 for the different species of arsenic in different species of seaweeds.

2.4 Uses of Seaweeds

Seaweeds have a long history as a source of food for human consumption. According to Bonotto[17] there are 5 genera of green seaweeds including 27 species, 40 genera of brown seaweeds including 88 species, and 56 genera of red seaweeds including 344 species, totaling 101 genera and 459 species of seaweed that have economic value as food, manure, pharmaceutical, or industrial use.

The use of seaweeds as food, fodder, fertilizer and drug will be explained in the following paragraphs.

FOOD

Seaweeds have been used as food as early as 600 – 800 B.C. in China and undoubtedly seaweeds were used in prehistoric times. Seaweeds are consumed for their food value, flavours, colours, and textures and can be mixed with other types of food. Seaweeds are almost a complete food, readily assimilated as they contain K/Na (potassium/sodium) ratio the same as the human body.[10]

Many seaweeds contain significant amounts of protein, vitamins and minerals which are essential for human nutrition. Seaweeds also contain large amounts of polysaccharides that are not digestible by humans.[18]

The protein content of seaweeds range from 4 to 25% of the dry weight. The sweet

taste of some seaweeds is due to the relatively high content of free amino acids.[19] Protein and amino acid contents in seaweeds vary with season, habitat, age, plant part and growing conditions such as light, nutrients and salinity. There is also a nutritional variation among different species of seaweeds, especially fibre content.[10]

Edible seaweeds are good sources of vitamins[19] of which the content varies seasonally.[19]

The mineral and trace element composition of edible seaweeds makes them a dietary source of sodium, chlorine, potassium, phosphorus, magnesium and calcium in large quantities; iron, manganese, and iodine, needed in smaller amounts; and copper, zinc, molybdenum and cobalt needed in trace amounts[18,190] for human consumption.

Therefore, as a source of proteins, carbohydrates, and minerals, seaweeds are important for human nutrition.

FODDER

In an area where seaweeds are abundant, they have been used to feed cattle, horses, goats, pigs and poultry. Either the livestock are allowed to graze on the seashore to supplement their conventional food or confined in an area where they have little choice but to graze on seaweeds. Seaweeds have also been collected and transported to feeding sites. A meal of dried, chopped seaweed is added to a regular diet in feeding livestock far away from the shore. *Ascophyllum* is a species of seaweed used in this way. Seaweed and seaweed meal with other plants can be mixed to form silage. It was recommended to use seaweed as a supplement food for livestock as the high mineral content of seaweed may cause some difficulties if used as a sole diet.[19]

FERTILIZER

The use of seaweeds as fertilizers has been demonstrated repeatedly by coastal farmers that have access to seaweeds.[19] Seaweeds are collected or harvested for agricultural use. They are applied (whole or chopped) as wet or dry to composted, liquefied, supplemented, and extracted preparations. Application of burned seaweed ashes or 'kelp' can improve soil fertility.

Seaweeds as a fertilizer are rich in nitrogen and potassium but are low in phosphate and should be supplemented through other sources to be used for most crops. The composition of the species that are used as fertilizers varies seasonally and geographically. Seaweeds have high organic matter content which in turn improves the water retention and mechanical properties of the soil. The absence of weeds and spores of fungi that harm terrestrial crops add advantages on the seaweeds as fertilizers. Despite this advantage, deleterious effects of seaweed manures include excess manganese release in low pH soils, water-logging of soil fertilized with *Pachymenia*, inhibited growth and low availability of some nitrogen in the seaweeds.[19]

DRUGS

The extracts and preparation from some seaweed have been used for medicine. Brown algae which contain iodine can cure goiter. Seaweed can be used to make vermifuges, cough medicines, soothing lotions and cosmetics.[11]

Chapter 3

Environmental Arsenic Speciation

3.1 Arsenic

3.1.1 General

In the periodic table, the transition of non-metals to metalloids takes place in the 15^{th} Group. This group consists of nitrogen (non-metal), phosphorus and arsenic (metalloids), and antimony and bismuth (metals).[20] Therefore, arsenic belongs to the group 15 below phosphorus and above antimony with atomic number 33 and relative atomic mass 74.9216. Different arsenic isotopes with mass numbers 68 - 80 exist and mass number 75 is the stable natural isotope.

The ground state electronic configuration of arsenic (4s, 4p and 4d) is:

Arsenic is a ubiquitous element which is found in the aquatic environment, the atmosphere, in soils and sediments, and in organisms.[21] This wide distribution of arsenic

in the environment is attributed to its natural existence and its application as herbicides, pesticides, fungicides or its use in industries (such as wood preservation, etc.).[22] Arsenic ranks 20^{th} in abundance in the earth's crust and constitutes $5x10^{-4}$ % of the earth's crust.[23] Arsenic in the earth crust and igneous rocks is about 3 mg/kg (10^{-6} g per 1 000 g), in coal between 0.5 and 93 mg/kg with mean value of 17.7 mg/kg, and in brown coal about 1,500 mg/kg.[24]

Arsenopyrite - FeAsS is the most abundant ore for arsenic. Arsenolite, As_2O_3; mimetite, $Pb_5Cl(AsO_4)_3$; olivenite, Cu_2OHAsO_4; cobalite, CoAsS; proustite, Ag_3AsS_3;[25] orpiment, As_2O_3; realgar, As_4S_4[23] etc are some of the ores for arsenic. There are different ways of preparing arsenic from its ore. Arsenic can be extracted from its ore by heating the ore in the absence of air, in which arsenic sublimes.[25]

$$FeAsS \rightarrow FeS + As \tag{3.1}$$

In another method of preparing arsenic, the sulfide ores are first roasted to the oxide:

$$2As_2S_3 + 9O_2 \rightarrow 2As_2O_3 + 6SO_2 \tag{3.2}$$

The arsenic oxide reacts with either carbon or hydrogen to give arsenic:

$$As_2O_3 + 3C \rightarrow 2As + 3CO \tag{3.3}$$

$$As_2O_3 + 3H_2 \rightarrow 2As + 3H_2O \tag{3.4}$$

Arsenic exists as As_4 molecules in the vapour state at 800^0C and As_2 at 1 750^0C. Above 300^0C, arsenic vapour condenses as grey arsenic, but at 100 - 200^0C, glassy modifications result.[26] Metallic or grey arsenic and yellow arsenic are the elemental arsenic which exists at room temperature (25^0C).[25] Metallic arsenic is not soluble in common solvents. At 25^0C, the thermodynamically stable rhombohedral, grey arsenic forms six membered rings of arsenic atoms with sheets. The van-der Waals distance is 4.0 Å(10^{-10} meter).[20]

The stable grey arsenic has a density of 5.73 g/cm^3, a melting point temperature of 814^{0}C at 36.5 bar pressure, specific heat of 24.2 Jmol^{-1}K^{-1} at 28^{0}C, and vapour pressure of 1 bar at 604^{0}C.[25] Yellow arsenic is formed by quenching the vapour consisting As_4 in CS_2.[20] Yellow arsenic has density of 2.03 g/cm^3 at 18^{0}C. It is more volatile than grey arsenic. Heat, light, and catalysts such as iodine and bromine facilitate the transformation of yellow arsenic to more stable forms of arsenic.[25]

The chemistry of arsenic is so complex that there are many inorganic and organic compounds of arsenic.[16] It can be seen from its electronic configuration that arsenic has three half-filled orbital which could be used to form three covalent bonds. Other intermediate bond types are also possible. The following examples show some of the compounds of arsenic formed by different bond types.[20]

Covalent bonds: AsH_3, $AsCl_3$, $As(C_6H_5)_3$, As_4

Covalent and ionic bonds: AsH_2^-, in $NaAsH_2$

Ionic bond: As^{3-} in Na_3As

Arsenic shows different valence states, -3, +3 and +5 and has both cationic and anionic forms.[25]

The majority of elements in the periodic table react with arsenic to form binary compounds. The metal arsenides can be prepared by reacting the metal and arsenic. Lithium, sodium, and potassium form arsenides of the formula M_3As. These arsenides react with water to give arsine, AsH_3. Similarly, the alkaline-earth elements react with arsenic to from the arsenides of the formula M_3As_2 and on hydrolysis gives arsine.[23]

Arsenic can also exist as oxides and oxyacides. Two types of arsenic (III) oxide exist. They are arsenolite (M.P. 278^{0}C) and claudetite (M.P. 312^{0}C). Arsenolite (a lattice of As_4O_6 molecules) is formed by condensation of the vapour or by crystallization from solution. Claudetite has a simple hexagonal layer structure in which arsenic atoms are joined by oxygen atoms. Arsenic oxide has uses in the manufacture of pesticides and

weed-killer.[23,25]

Concentrated nitric acid oxidizes arsenic to the triprotonic arsenic (V) acid, H_3AsO_4 with the following dissociation constants:[23]

$$K_1 = 5.6 \times 10^{-3}, \qquad K_2 = 1.7 \times 10^{-7}, \qquad K_3 = 3 \times 10^{-12}$$

A black precipitate of arsenic is formed when arsenic in either +3 or +5 state reacts with stannous ion in concentrated hydrochloric acid which can be used as detection (test) method for arsenic.

In low concentration, arsenic (III) acid and arsenic (V) acid may be reduced to arsine by active metals in acid solutions.[23]

$$3Zn + 6H^+ + H_3AsO_3 \rightarrow AsH_3 + 3H_2O + 3Zn^{2+} \tag{3.5}$$

$$4Zn + 8H^+ + H_3AsO_4 \rightarrow AsH_3 + 4H_2O + 4Zn^{2+} \tag{3.6}$$

A mirror of elementary arsenic can be used to show the presence of an extremely small amount of arsine when it is thermally decomposed.

$$AsH_3 \rightarrow As + \frac{3}{2}H_2 \tag{3.7}$$

In this project the formation of arsine by a reducing agent and using an ICP-AES detection method will be employed to identify arsenic species.

The emission of arsenic into the atmosphere is effected by the natural phenomenon of weathering, volcanic and biological activities and anthropogenic activities. The arsenic then will be redistributed by rain and dry deposition.[21]

In the environment, arsenic is mainly transported by water. Aluminum and iron may sometime play a role in the sedimentation of arsenic.[16] The concentration in aquatic and soil/sediment by the dissolution of arsenic in water is controlled by the variety of input and removal mechanisms.

The mobilization of arsenic by anthropogenic processes is mainly due to smelting activities and fossil - fuel combustion. The ratio of anthropogenic to natural atmospheric inputs of arsenic was at about 40:60[27] in the studies done on 1980s.

The setting of typical level of arsenic in the environment cannot be precisely known. This is due to the differences in the input of arsenic in the environment by anthropogenic as well as natural depends on different conditions such as geographical location. Therefore, it is only possible to set-up a level whether the arsenic concentration is in the ppt, ppb, or ppm range.[21]

3.2 Arsenic Speciation

The interest in the environmental speciation of trace metals has been greatly increased in the past years due to the impacts that the species have on the environment; as a result of their chemical form and/or oxidation state that affects their toxicity and biological functions.[28,29] In toxicology, the various species of an element show great differences in toxic properties because these species follow different metabolic pathways.[30]

Speciation of an element may be defined as the determination of the concentration of the individual physico-chemical forms of the element that make up the total concentration of the element in a given sample.[31] The individual physico-chemical forms include:

1. Gaseous compounds which contain particulate material
2. Solid forms or phases such as soil or sediment
3. Aqueous phases and a liquid sample which comprises of particulate and colloids material as in natural water.

The determination of the total concentration of an element previously was considered to be sufficient for clinical and environmental conditions. Even though it is important

to know the total concentration of an element, the determination of species is a useful task, because the level of toxic species in an element are more important in setting of environmental and biological standards than the total concentration of the element alone.[3] But the identification of element species presents many analytical challenges.[2] This may be due to the the fact that the species are often unstable and the concentration is very low in the various environmental matrices.[32]

The main problem in speciation studies is the danger that exists because of contamination and loss of the species in the sample preparation for analysis[3] such as in extraction, derivatization, separation, detection etc.[32] and the disruption of the labile equilibria.[33] Therefore, the collection, treatment and preservation of samples for speciation studies need careful consideration and planning.[3]

The necessary requirement for the techniques in the studies of environmental speciation is sensitivity and selectivity.[34] Sensitivity is a particular problem if the concentrations of the species are below the detection limit of the technique used.

The choice of the method in environmental speciation depends on many factors. One of the main factors is the technique should involve little sample manipulation with less sample contamination. Other considerations include the concentration of the metals in the sample, interference and the information required.[33] The widely used technique for metal speciation is to couple the separatory power of chromatography with the detection capacity of atomic spectroscopy.[35]

As previously stated, arsenic enters the environment from different sources, such as smelting, coal-fired power plants, application of agricultural organoarsenical herbicide, etc.[36] Therefore, this wide use and the following behaviour[30] of arsenic makes it necessary to have methods to study the environmental and occupational exposure to toxic arsenic compounds.[37]

1. Arsenic has different oxidation states (As(III))/As(V)) which are interchangeable

under certain conditions

2. Arsenic forms small organometallic molecules which are indicative of exposure, as it is known that the inorganic arsenic species are changed/or metabolized to MMAA and DMAA as a detoxifying mechanism.

3. Arsenic is part of the unchanged organic arsenicals in the body such as AsC and AsB, AsS, AsL, etc.

4. Arsenic forms macromolecular biomolecules, the same as the arsenate bound to transferrin and hemoglobin.

It is known that many organisms including man transform arsenic.[36] Inorganic arsenite and arsenate ions, MAA, DMA, phenylarsonic acid, several organic esters of arsenic oxyacids, and volatile alkyl arsines are some of the biologically transformed products.[38] Biomethylation of inorganic arsenic is regarded as the detoxifying of the harmful species. These species can be excreted or stored.[39]

The determination of total arsenic concentration cannot give the toxicity of arsenic alone because total arsenic concentration can be high while the more toxic forms of arsenic may be present in low concentrations.[40] The chemical species of arsenic depends on its source (inorganic arsenic from minerals, industrial discharge and insecticides; organic arsenic from industrial discharges, insecticides and biological action of inorganic arsenic). The chemical forms can be inorganic arsenic as As(III) and As(V) which are most toxic. The methylated arsenicals such as MMA, DMA and TMAO which are relatively toxic. The quaternary arsonium compounds, AsC, AsB and the TMA are non-toxic.[34] The naturally occurring compounds which are called arseno sugars have not been adequately evaluated for their toxicity levels.[41]

The chemical formulas for some of the different arsenic species in the environment are given in the following figure.

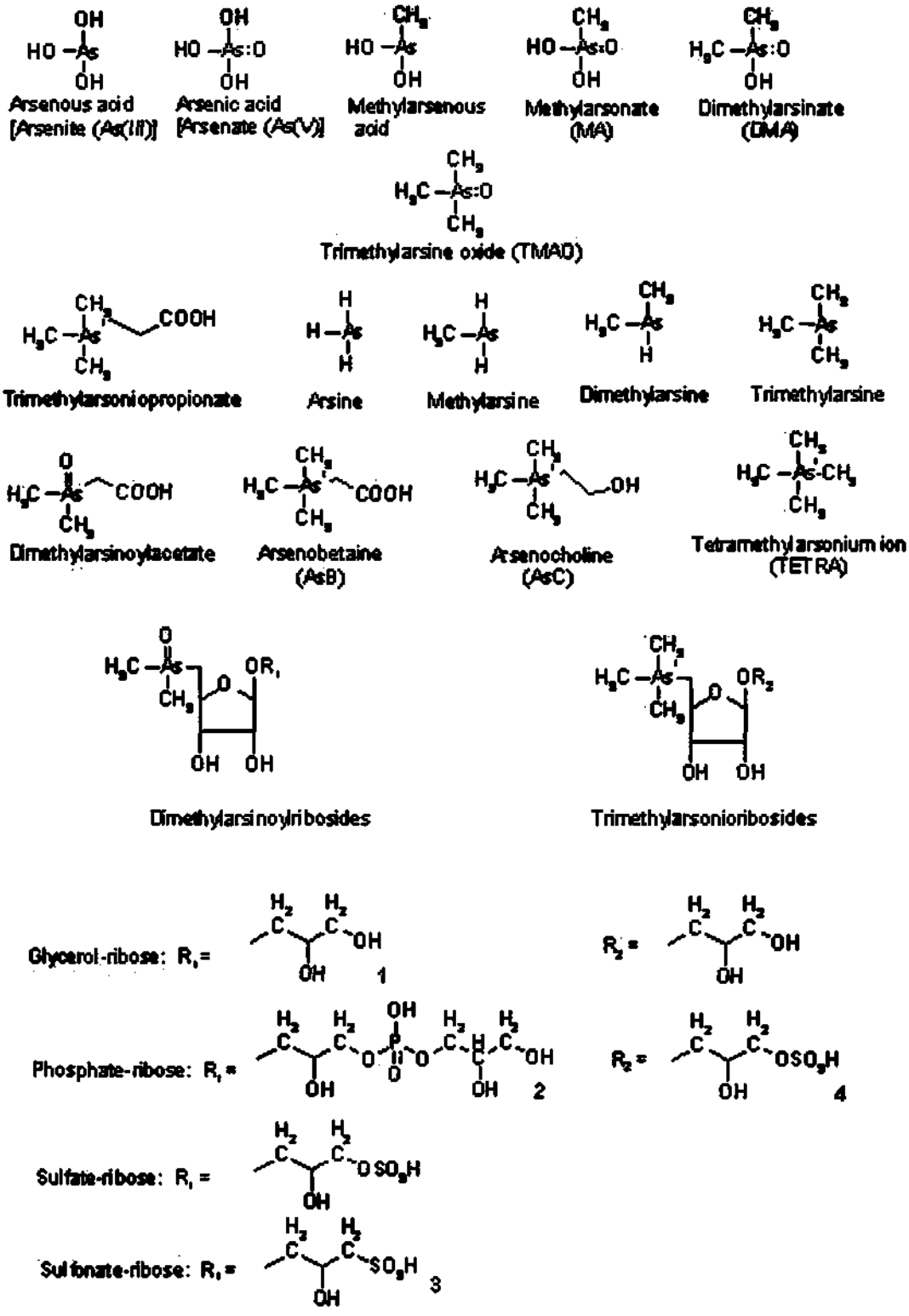

Figure 3.1: Environmental Arsenic Compounds[42]

[a]Compounds 1, 2, 3 and 4 refer to Table 4.2

3.2.1 Predictions of Arsenic Species using Thermodynamics

The presence of species in a specific environment conditions is controlled by different factors such as pH, redox potential etc.

The controls for the distribution and speciation of arsenic in the environment can be identified using geochemical modelling. But this way of identifying the conditions is hampered by the lack of full thermodynamic data for the different species and it is only applicable for systems at equilibrium. Despite this lack of information, it gives useful predictions of the occurrence, absence, or fate of various dissolved and solid arsenic species in the environment. Biological intervention or anthropogenic sources could cause deviations from the expected distribution of arsenicals. If the system is disturbed, the direction can be predicted but not the rate.

Inorganic arsenic compounds have more thermodynamic data than their counter part arsenoorganic compounds. Arsenoorganicals response to different environmental conditions are predicted on known chemical properties.[21]

In the following sub-topics inorganic arsenicals and organoarsenic compounds will be discussed by giving special attention to the existence of their different species in an environment with different condition i.e. pH and redox potentials.

INORGANIC ARSENIC COMPOUNDS

There are different inorganic arsenic species present in the environment. For example, $HAsO_2$, $pK_{a1} = 9.23$; H_3AsO_4, $pK_{a1} = 2.20$, $pK_{a2} = 6.97$, $pK_{a3} = 11.53$. About 70% of arsenic in the environment exists as inorganic arsenic salts such as $NaAsO_2$, $Pb_3(AsO_4)$, arsenides, sulfides and oxides.[43]

The predominant soluble species and solids in an environment can be indicated by drawing E_H (thermodynamic potential) versus pH diagrams. The hypothetical electron affinity, pE can sometimes be used instead of E_H. The two parameters can be related

by the equation:

$$pE = \frac{F}{2.3RT} E_H \tag{3.8}$$

where T is absolute temperature, F is Faraday constant (96 485 coulombs) and R is the gas constant (8.315 J K^{-1} mol^{-1}).[44]

In preparing E_H - pH diagrams, various systems should have to be taken into consideration. The stability diagram for arsenicals in the environment in the presence of oxygen and water, and oxygen, water and sulfur are shown below. Figure 3.2 (a) shows

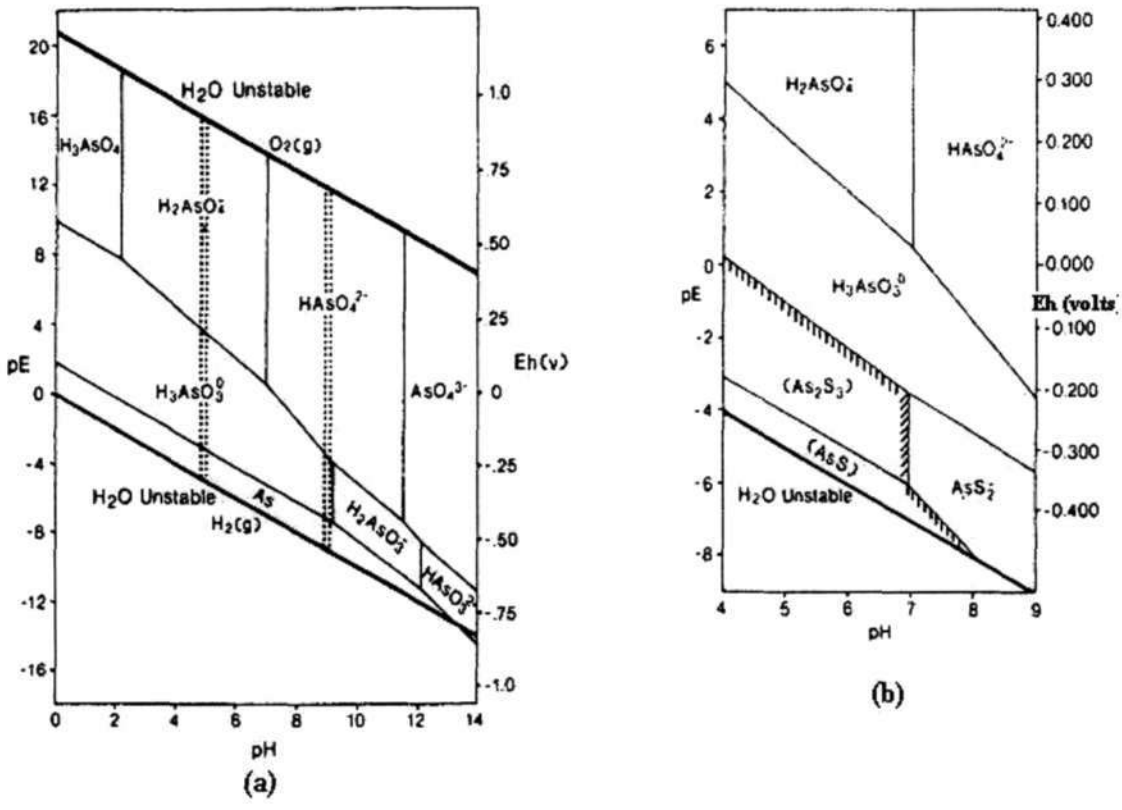

Figure 3.2: pE-pH Diagrams[21]

pE-pH diagram for the As-H_2O system at 25^0C. Total dissolved As species set at 50ppb. The area within the vertical bars represents the common pE-pH for natural water and Figure 3.2(b) shows pE-pH diagram for the As-S-H_2O system at 25^0C with total dissolved As and S species set at 50 ppb and 32 ppm respectively. The area within the

hatched lines denotes that the solid phases are predominant (i.e., total dissolved As species <5 ppb).

At the line separating the fields, the species have equal activities; whereas the species which is found within its field is the dominant species.

In drawing the stability diagrams, the species are within the stability field of water. For example, in natural water (pH = 4-10), pE is approximately 17 to -10. Beyond this limit water will either be oxidized to oxygen or reduced to H_2.[45]

Different values have been calculated for redox levels in oxic natural water systems. Turner et al[46] proposed the equation pE = 20.6 - pH, for oxygenated water.

From Figure 3.2(a), it can be shown that for oxygenated water, arsenic (V) acid species - H_3AsO_4, $H_2AsO_4^-$, $HAsO_4^{2-}$ and AsO_4^{3-} are stable. In reducing condition and lower pH arsenic (III) acid becomes stable, mainly as H_3AsO_3.[21]

On thermodynamic grounds arsenic (As(V)) mainly $HAsO_4^{2-}$ should dominate over As(III) in oxygenated water at pH ranges for fresh waters 5 - 9[47] and seawater 7.5 - 8.3.[48] At high pH of seawaters, H_3AsO_3 hydrolyze to a significant amount of $H_4AsO_4^-$ (i.e. $As(OH)_4^-$).[46]

Unlike the equilibrium speciation of many elements, arsenic forms additional complexes with the significant water constituents. For example, at high fluoride concentration (1.58 mg/L), the species $HAsO_3F^-$ and $AsO_3F_2^-$ comprise 10% of the total As(V).[41] In oxygen deficient systems, the action of bacteria will make pE value to decrease as it is governed by the reduction of SO_4^{2-} to S^{2-} and HCO_3^- and CH_4.[46] Turner et al.[46] reported a lower limit for natural waters to be:

$$pE = \frac{34 - 9(pH)}{8} \tag{3.9}$$

Large values of pE (= -2.9) and precipitation with iron are reported[49] for the variable concentration of sulfide in reducing conditions. These condition makes As(III) more thermodynamically important (Figure 3.2(b)). In the presence of sulfur, arsenic forms

the insoluble arsenic sulfides. Orpiment (As_2S_3) and/or realgar precipitate in acidic conditions. At higher pH (neutral or alkaline) the more soluble thioarsenite species are important.[48,50]

From Figure 3.2(a), it is evident that under reducing conditions if pH is decreased there is an increasing possibility that the more toxic arsenite will be the predominant species. There is discrepancy in the ratio of As(V)/As(III) in which the predicted one is 10^{15} - 10^{26} but actually it is found in the range of 0.1 - 250. This is due to the action of algal/bacterial transformations. In environmental systems it is observed that bacteria plays a role in both oxidizing and reducing inorganic arsenic.[21]

ORGANIC ARSENIC COMPOUNDS

Organoarsenic compounds are part of the organo-metallics which are formed by the combination of the element (metal/metalloid) to carbon. Unless the arsenic is directly attached to the carbon, it is not an organo-metallic compound.[25]

There are many organoarsenic compounds in the environment and their chemistry is extensive.[16] Organoarsenic compounds are widely distributed in the atmosphere, aquatic systems, soils and sediments, and biological tissue. Different organoarsenicals are produced for commercial use as biocides in agriculture and forestry. The amount of these organoarsenic compounds that are introduced by human activities in the environment is generally small.[21]

The bonds As-C in organoarsenic are stable in different environmental conditions such as pH and oxidation potential.[16]

Organoarsenicals can be produced from inorganic arsenicals using the process of biological methylation. Craig[51] notes that there is little evidence that the As-C in the environment is formed by chemical means. Therefore, the synthesis of organoarsenic involves the living organisms in which arsenic is involved in the metabolic pathways of

the cell.[21]

Bacteria, fungi, algae, invertebrates, vertebrates and man can transform inorganic arsenic into organoarsenic compounds. The transformation could be either through the stepwise reduction and methylation of arsenic via methane arsonic and dimethylarisinic acid to the methylated arsines or the other method could be the formation of complex organoarsenic compounds like arsenobetaine and arsenocholine (possess methyl group) which are characteristic of the aquatic biota.[51] The higher organoarsenic compounds are very resistant to chemical degradation.[16]

The acidity (pH) plays a role in the rate of methylation in which the highest rates are from pH 3.5 to 5.5. The dissolution of inorganic arsenic into the overlaying waters from sediments and enhanced biomethylation could be observed by the increase of the acidity of the system.[52]

The following table gives some of the methylation products.

Table 3.1: Biomethylation Products of Some Important Organoarsenicals[22]

Compound	Comments
$(CH_3)_4As^+$	has only been found in tissues to date, but is likely to be more widely distributed
$(CH_3)_3AsO$	limited detection in environment may be due to facile reduction to $(CH_3)_3As$; reacts with H_2S to give $(CH_3)_3AsS$
$(CH_3)_3As$	easily oxidized to $(CH_3)_3AsO$ and $(CH_3)_2AsO(OH)$; may escape detection by some analytical procedures
$(CH_3)_2AsO(OH)$	stable and water soluble, easily detected by conventional methods, can be reduced to $(CH_3)_2AsH$, and reacts with RSH to $(CH_3)_2As(SR)$; pKa = 6.2[22]
$(CH_3)_2AsH$	easily oxidized to $(CH_3)_2AsO(OH)$

continued...

Table 3.1: continued...

Compound	Comments
$CH_3AsO(OH)_2$	stable and water soluble, easily detected, can be reduced to CH_3AsH_2, and reacts with RSH to give $CH_3As(SR)_2$; pKa= 3.6, 8.2[22]
$(CH_3)_2AsSR$	may be formed in anoxic soils and sediments; reacts with O_2 to give $(CH_3)_2AsO(OH)$
$CH_3As(SR)_2$	may be formed in anoxic soils and sediments; reacts with O_2 to give $CH_3AsO(OH)_2$
Arsenosugars	found mainly in algae, stable at neutral pH, and reacts with acid/base to give $(CH_3)_2AsO(OH)$
$(CH_3)_2As(O)CH_2CH_2OH$	(dimethylarsinoyl)ethanol
$(CH_3)_3As^+CH_2CH_2OH$	arsenocholine; easily converted to arsenobetaine
$(CH_3)_3As^+CH_2COO^-$	arsenobetaine; can give $(CH_3)_3AsO$ and $(CH_3)_2AsO(OH)$ on reaction with base

The above biomethylation products could be interrelated by the following scheme.

From the scheme, (a) indicates the compounds that undergo pH-sensitive reactions with sodium borohydride to give volatile products that can be detected by gas chromatography (GC)/atomic absorption spectroscopy (AAS). The compounds designated by (b) are products of biomethylation in which their distribution is limited by reactivity toward oxygen.[12] The species of arsenic that are found in the anoxic waters and sediments are not known fully, but there are arsenic sulfides which are produced by the reaction with environmental reagents.[21] Under anaerobic conditions, dimethylarsine is produced as a result of biomethylation of arsenic.[53]

The biological involvement makes the prediction of organoarsenic compounds in the

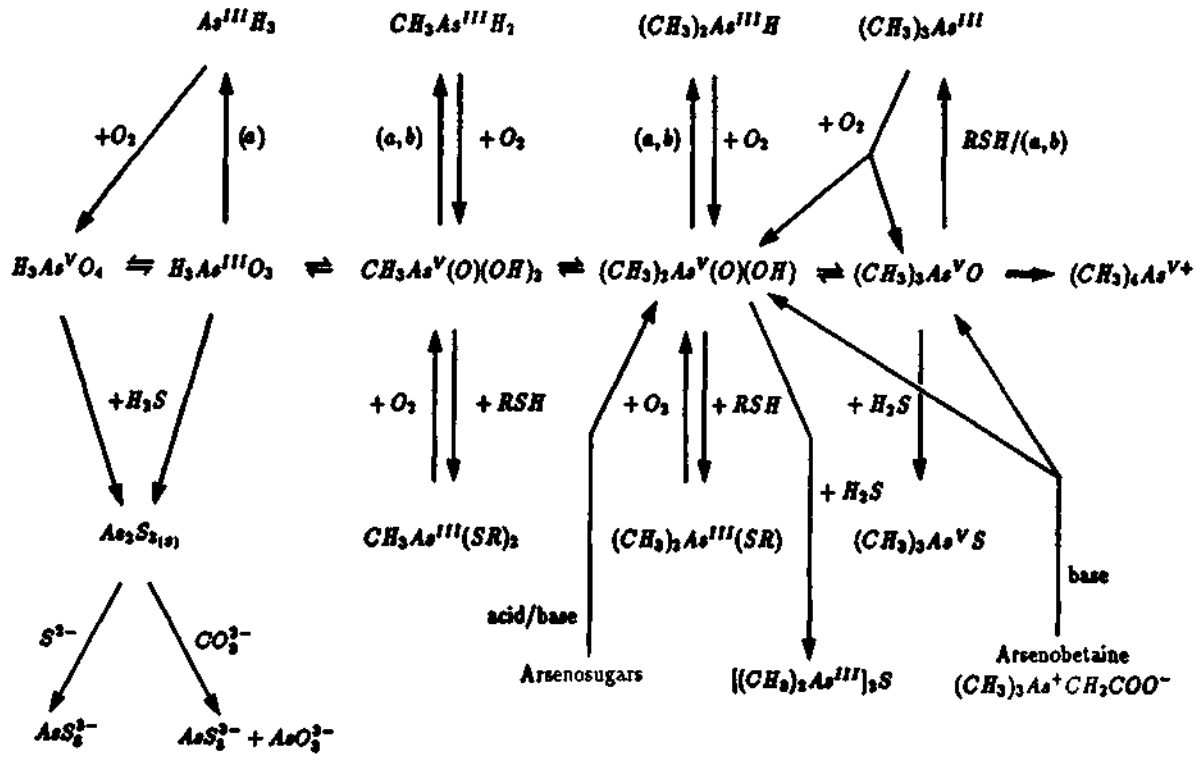

Figure 3.3: Interrelationships Among Different Arsenic Compounds[22]

environment difficult. Biomethylation products can be listed but their stability in the environment can not be given. Oxidation, reduction, hydrolysis and reaction with sulfur compounds can affect their distribution, volatility, and mobility of the biologically produced species.[21]

Due to the lack of thermodynamic data for organoarsenic compound, no stability diagrams were constructed for the species.[21] Previously it was suggested[21] that the organoarsenic compounds are unstable at low E_H, but this suggestion is inconsistent with their existence in the environment.

Many organometallics are kinetically stable but all organometallics thermodynamically are unstable with respect to their constituent elements and their decomposition products.[54]

3.3 Factors for Speciation

There are a lot of factors that control speciation in the environment. The presence of different species in the environment can be governed by the environment in which it presents.[55]

Under anoxic conditions (lacking of oxygen) like in some interstitial waters of sediments, deep waters, sewage, and closed basins as fjords, the speciation of the element can change completely.

Redox reactions are important parameters for the determination of species that may exist in the environment. They can determine the oxidation state of some metal ions, the occurrence of different ligating or precipitating anions.[56] For example, Fe(III) can be reduced to Fe(II), As(V) to As(III), etc. These changes of oxidation states can greatly influence the bioavailability and toxicity of the element. In reducing conditions for example, the bioavailability of Hg and Cd is reduced due to association of these elements with sulfur which would form precipitate of sulfide salts.

In sediments of natural waters the action of microorganisms reduces Mn salts, Fe hydroxides, and sulfate. The product of these reductions, specially, Fe(II) and sulfide, can react with As(V) and Cr(VI) to produce As(III) and Cr(III).[57] Many redox reactions are slow, therefore the concentrations of the oxidizable or reducible may be different from those predicted thermodynamically.[56] The presence of other elements affects the speciation of elements. Alkaline earth cations in high concentrations in natural waters can compete with heavy metals for the inorganic and/or organic groups. But this competition is not strong.

pH also affects speciation by either shifting the acid - base equilibrium and redox reactions or by competition between protons and metal ions for ligand groups and /or for the surface groups in adsorption processes.[57]

Metal mobilization in the environment, toxicity of drinking water, growth and re-

production of aquatic organisms, leaching of nutrients from soil, increased availability and toxicity of metals, etc are the problems which are caused by acidity.

Change in pressure and temperature on their part play a role in speciation. Temperature and pressure affect stability, and rate constants and the specific process of photosynthesis and growth. The type of bioorganisms present in the ocean could be drastically affected by a change of temperature from about 25 to 9^0C and pressure from 1 to 1000 atm. i.e. from the surface to the bottom.

Inorganic solids and dead or live organic matter can be a substrate for adsorption. Most of the suspended matter in natural water is accounted for by inorganic solids. They consist of clay minerals, carbonates, feldspars and quartz. As these materials are usually coated with hydrous iron and manganese oxides, the adsorption process is appreciably changed.

The net charge of elemental chemical species is a function of its oxidation state. Most metals and metalloids have variable oxidation state. For example, arsenic may exist as As(III) or As(V). When the soil-water system is in oxidized state, the environment is considered electron deficient, and the highest oxidation state is the most stable one. Therefore, behavior of elements or substances can be determined by their oxidation states.[58]

The elements of Group 15 (As, Sb, Se, etc) in aquatic chemistry are dominated by the formation of oxyanions (like arsenate, AsO_4^{3-}). Due to the chemical similarities between the nutrient anion species of the non-metals such as phosphate and nitrate, the oxyanions of Group 15 elements enter the metabolic pathways of the nutrients. As a result they will have toxic effects, and return back into water in a chemically changed form.

3.4 Source and Effect on Environment

As stated previously, arsenic can exist naturally or be introduced into the environment through human beings activities. The natural sources could be from rocks, sediments and soils.[7]

Soils which are uncontaminated contains between 0.2 and 40 mg/kg, while soils that are treated with arsenic compounds could contain up to 550 mg/kg.[16]

Sediments usually contain below 10 mg/kg dry weight arsenic.[16] Inorganic and organic compounds of arsenic were found in airborne particulate matter.[16]

In unpolluted surface waters, the level of arsenic is a few micrograms per liter (mg/l) or less. Penrose et al[59] reported that seawater contains from 0.001 - 0.008 mg/l arsenic concentrations. High levels of arsenic exist in areas where thermal activities are undergone.[16]

Foods can contain arsenic which could be derived either naturally or be introduced through the use of arsenic in feeds and pesticides.[60] Marine origin foods contains more arsenic than other[3] and humans could be exposed to large amounts of organic compounds through eating sea-food.[16] Due to biotransformation and accumulation, arsenic is present in organic forms and the concentration ranges from 1 to 100 μg/g in marine animals and algae.[3]

Arsenic is widely distributed in the biosphere primarily by anthropogenic activities where mobilization of the element is three fold higher than that of natural sources such as weathering, volcanic, etc. Therefore, the distribution of the element in aquatic ecosystems and body fluids and tissues would be mainly from anthropogenic activities.[28,61]

The effect of arsenic on the environment depends on the type of (or species) of arsenic presents in the environment, the route of exposure, the rate and duration of exposure, etc.

Arsenic in soils and rivers is present as As(V) and As(III). These two types of species

undergo chemically and or microbiologically mediated oxidation or reduction changes to form organic arsenic species like simple methylated species (MMAA and DMAA), or complex compounds (AsB, AsC, AsS, AsL, etc).[28] Inorganic arsenic species (arsenate and arsenite) are present in larger amounts than the main organic species in water.

The oxidation state of arsenic in the surface water is largely unknown.[16] Braman and Foreback[62] found the ratio of trivalent to pentavalent arsenic in uncontaminated surface water with concentration between 0.0025 and 0.0030 mg/l is from 0.06 to 6.7.

Man and animals are prone to arsenic poisoning. Arsenic is a protoplasmic poison.[63] It slows down the activity of many enzymes such as cellular metabolism and respiration by reacting with sulfhydryl groups. Most of the time arsenic compounds change to trivalent form as an arsenite or arsenoxide (R-As=O). This form then reacts with sulfydryl group of the enzyme in which it forms R-As-(S-protein)$_2$.[60] This could be the reason why As(III) is more toxic than As(V). Much of the toxicity of arsenicals is also due to the intramolecular reactions which forms a six-member ring compound that occur when arsenic combines with both sulfhydryl group of a-lipoic (thioctic) acid.[60]

Other elements could react with arsenic to make the compound more water - soluble which enhances its readily absorption through the gastro intestinal tract, lungs and skin. The inorganic and organic pentavalent form of arsenic is absorbed more than the trivalent form.

Arsenic has various health effects in humans and animals:[16]

- Arsenic can affect different organs in man and animals. But there is no data that can interrelate the effect with either tissue or blood concentration. There is no specific organ that can be correlated with intoxication of arsenic as the kidney is considered for the intoxication of cadmium and the central nervous system for the methyl mercury intoxication.

- Acute and subacute effects of arsenic may involve respiratory, gastro intestinal,

cardiovascular, nervous, and haematopoietic systems.

- Exposure to high levels of arsenic, especially in the smelting industry could cause lesions of the upper respiratory tract including perforation of the nasal septum, laryngitis, pharyngitis, and bronchitis.

- Inorganic arsenic can give rise to skin lesions in man, especially palm - plantar hyperkeratosis, disturbance of liver function and can exert chronic effects on the peripheral nervous system.

- A chromosomal aberration has been found in persons exposed to arsenic through medication. Studies have indicated that inorganic arsenic affects DNA repair mechanisms.

- No data exists for the teratogenicity of inorganic arsenic but high levels of both trivalent and pentavalent inorganic arsenic in hamsters, rats and mice induce teratogenic effects.

- Inorganic arsenic is a potential carcinogen in smelter environments as shown by the epidemiological studies. The intake of several grams of arsenic for a long time and ingestion of arsenic in drinking water can cause skin cancer of tumours of low malignancy.

- Side effects such as encephalopathy and optic atrophy on the central nervous system could be induced by taking the medication with some organic compounds such as [4-[2-amino-2 oxoethyl]-amino]-phenyl arsonic acid (tryparsamide).

- No carcinogenicity case has been reported by the organic arsenic compounds tested on experimental animals.

Chapter 4

Arsenic Species in Seaweeds

In chapter 2, it has been stated briefly that arsenic is present in the major seaweed divisions. In this topic a more detailed review of the concentrations, types and sources of arsenic in seaweeds will be presented.

4.1 Types of Arsenic Species in Seaweeds

Seaweeds contain different species of arsenic. It is difficult to say by whom the first analysis of trace of arsenic in marine algae was performed. But for the same purpose A.C. Chapman[64] has examined samples of Irish moss, agar and other edible seaweeds. The first published paper concerning this subject was by A.J. Jones[65] in 1922. In his paper titled "The arsenic content of some of the Marine Algae", this author gave a result for arsenic (as arsenious oxide on the dry substance) for 11 varieties of seaweeds ranging from 6 to 125 ppm. This result was later confirmed by A.C. Chapman.[64] Both A.J. Jones and A.C. Chapman analyzed seaweeds for a complex organic arsenic compound with no poisonous properties. The complex organic arsenic was later found to be arsenobetaine.[21] In other reports,[42] dimethylarsinoyl ribosides were found to be the major arsenic compounds present in marine algae. Currently, about 25 different

organic arsenic compounds have been identified in seaweeds.[42]

A group of researchers in West Australia[66,67] have done the most significant work in the field of arsenic in marine algae and plants. In an experiment performed by Morita et al.[68] in samples of Hiziki seaweeds, they were successful in identifying As(III), As(V), and AsB. But no quantitative analysis was performed.

Marine algae have been shown to contain arsenic phospholipids and AsL due to the chemical similarity between arsenic and phosphorus where arsenic replaces phosphorus. This condition, particularly has been observed in phosphate deficient waters.[42,69] The organoarsenic species: AsB and AsC have been also related to arsenic phospholipids and AsL in which their presence has been confirmed in marine crustacean,[69,70] and fish.[11,69]

One of the major groups of arsenic compounds in seaweed is AsS[42,66,71] (e.g. in brown kelp (*Eklonia*) AsS are the major form of arsenic[72]). AsS contain hydroxyl, phosphate and/or sulfate groups[21,42,66,71] and exist as uncharged or anionic species at neutral pH.[71] The esterification of AsS may give arseno-lipids in marine and biota.

4.2 Sources, Accumulation and Concentration of Arsenic Species in Seaweeds

Previously it has been shown that arsenic in marine organisms and seaweeds is in the form of lipid soluble and water-soluble arseno organic compounds.[1,73,74] These compounds can be possibly produced either by marine animals and plants using inorganic arsenic that is taken-up from their surroundings or there may be a group or groups of microorganisms that can synthesis these compounds. The second case can be routed via the food chain from the simple organisms to the more advanced organisms. A combination of the two methods may be also possible for the formation of arseno organic compounds.[75]

Klump[76] studied the factors that play a role in taking-up of As(III) and As(V) by two species of seaweed namely *Fucus spiralis* and *Ascophylum nodusum.* The factors studied were temperature, pH, salinity, KCN, photosynthetic inhibitors and phosphate. He observed that As(V) was more readily accumulated than As(III) and the uptake of either form were proportional to the external concentration of up to 1 mg/l arsenic. He also observed increased As(V) uptake at higher temperatures but the uptake was not affected by variation of salinity (9 - 36 parts per thousand) or pH (7 - 9). KCN which inhibits respiration was found to decrease uptake of As(V) in a concentration - dependent fashion, whereas uptake was not affected by inhibitors of photosynthesis. This suggests that the uptake of As(V) requires energy derived from respiration rather than photosynthesis. The competitive inhibition of uptake of As(V) by phosphate was observed at phosphate concentration ranges of 40 - 400 mM.[76]

The mode of accumulation of compounds by marine algae can be principally by adsorption or by a secondary processes. Studies using radioisotopes indicate that there are two states involved for uptake:

1. Accumulation using adsorption on the exterior surface of the marine algae followed by

2. A slower uptake depending on metabolism.

The first stage is independent of metabolism regulating factors such as light and temperature while the second stage depends on these factors. Therefore, chemical uptakes depend on metabolism of specific species and are influenced by physical parameters within the environment. Metabolic processes can make for some compounds to accumulate and the degradation or exclusion of others. The variations in geographical location and season have influence in metabolism as they affect factors for metabolism such as temperature, light availability, salinity and degree of desiccation. Structural considerations also play a role in chemical uptake. Many seaweeds are entirely in direct

contact with water.[12]

Seaweeds are known to accumulate metals, hydrocarbons, polychlorinated biphenyls (PCBs), and numerous other compounds from the sea where they grow,[12] and has been used as a bioindicators or monitors of the seawater pollution. The first reference material of seaweed is NIES [National Institute for Environmental Studies] No. 9 Sargasso and the second is No. 15 Hijiki.[77]

V.Vlachos et al[78] have studied 40 types of seaweeds for different metal concentration from the KwaZulu - Natal Coast (Palm Beach, Ispingo Beach and Mission Rocks) using x-ray fluorescence. The data for the highest concentration of arsenic (in ppm) in 6 seaweeds from the study were shown below.

Table 4.1: Arsenic Concentration in ppm in Seaweeds of KwaZulu Natal[78]

Halimeda Cuneata	*Stypocaulon funiculare*	*Osmundaria serrata*	*Prionitis nodifera*	*Sargrassum incisifolium*	*Zonaria subarhculata*
66 - 119	102 - 120	900 - 1428	38 - 223	<24 - 109	91 - 92

Marine algae and seaweed contain appreciable amount of arsenic.[16] Because of bioaccumulation and the accumulation of arsenic is present in organic forms, the concentration ranges from 1 to 100 μg/g.

Arsenic in the marine food chain has been identified in the presently assumed nontoxic compounds such as AsB, AsC and AsS.[79]

The Laminarials species, *Eckloria radiata* and *Laminaria japonica* contain low concentrations of inorganic arsenic.[21,80] *Hizikia fusiforme* (Hiziki in Japan) of the Fucales order contain half its arsenic burden as sugar derivatives and half as arsenate.[81] Therefore, the difference in order as in Laminariales and Fucales may account for the variation in chemical composition.[80]

Arsenic species in some selected seaweed species with their respective concentration ranges from reference 35 are tabulated in the following table.

Table 4.2: Arsenic Compounds in Some Seaweed Species[a,42]

Species	Location	As Conc (μg/g)	As-sugar 1[b]	As-sugar 2[b]	As-sugar 3[b]	As-sugar 4[b]	Other As-sugars	As(V)	DMA	Other cpds. & unknowns
Green algae										
Broyopsis maxima	Japan	19.4 dry	minor	sig	nd	nd	nd	nd	nd	sig
Codium fragile	Japan	0.6 wet	major	sig	nd	nd	nd	nd	minor	minor
Ulva pertusa	Japan	17.1 dry	sig	minor	nd	nd	nd	nd	nd	sig
Red algae										
Ahnfeltia paradoxa	Japan	11.7 dry	minor	sig	nd	minor	nd	nd	nd	sig
Gigartina intermedia	Japan	19.8 dry	minor	sig	nd	nd	nd	nd	nd	sig
Gloipoeltis furcata	Japan	25.0 dry	sig	major	nd	nd	nd	nd	nd	minor
Hypnea japonica	Japan	9.9 dry	nd	major	nd	nd	nd	nd	nd	sig
Laurencia okamurai	Japan	19.2 dry	minor	sig	nd	sig	nd	nd	nd	minor

continued...

Table 4.2: continued...

Species	Location	As Conc (μg/g)	As-sugar 1[b]	As-sugar 2[b]	As-sugar 3[b]	As-sugar 4[b]	Other As-sugars	As(V)	DMA	Other cpds. & unknowns
Palmaria palmate	Japan	7.6 dry	major	minor	nd	nd	nd	nd	minor	nd
Porphyta tenera	Japan	7.6 dry	major	sig	nd	nd	nd	nd	nd	nd
Porphyta tenera	China	16 dry	major	sig	nd	nd	nd	nd	nd	nd
Porphyta tenera	Taiwan	21 dry	major	sig	nd	nd	nd	nd	nd	minor
Schizmenia dubyi	Japan	12.0 dry	minor	major	nd	nd	nd	nd	nd	minor
Brown algae										
Ecklonia radiata	Australia	10 wet	sig	nr	major	nr	nr	nr	nr	nr 114
Eisenia bicyclis	Japan	15 dry	sig	minor	major	nd	nd	nd	sig	nd
Fucus gardneri	Canada	9-17 dry	sig	nd	major	sig	nd	nd	minor	nd
Fucus serratus	Denmark	7 wet	minor	minor	major	sig	nd	tr	tr	tr

continued...

Table 4.2: continued...

Species	Location	As Conc (μg/g)	As-sugar 1[b]	As-sugar 2[b]	As-sugar 3[b]	As-sugar 4[b]	Other As-sugars	As(V)	DMA	Other cpds. & unknowns
Fucus spiralis	Denmark	31.5 dry	nr	sig	sig	major	nd	nd	tr	minor, prob.As sugar 1
Fucus vesiculosis	England	140 dry	nr	minor	major	sig	nr	nr	nr	nr
Hizikia fusiforme	Japan	10 wet	nr	tr	minor	sig	tr	major	nr	nr
Laminaria digitata	Denmark	43 dry	nr	sig	major	nd	nr	nr	nr	nr
Laminaria digitata	Scotland	72.1 dry	minor	sig	major	nd	nd	nd	nd	nd
Laminaria japonica	Japan	4 wet	minor	sig	major	nr	nr	nr	nr	nr
Undaria pinnatifida	Japan	2.8 wet	sig	sig	major	nr	sig, cpd 5	nr	nr	nr
Undaria pinnatifida	Japan	33.8 dry	minor	sig	sig	nd	nr	nd	nd	nd

[a]Major = major arsenic constituent, >50% of extractable arsenic; sig = significant arsenic constituent, >10-50% of extractable arsenic; minor = minor arsenic constituent, 1-10% of extractable arsenic; tr = trace arsenic constituent, <1% of extractable arsenic; nr = not recorded; nd = not detected.
[b]See Figure 3.2

Chapter 5

Determination of Arsenic Species

5.1 Background

Arsenic is one of the most poisonous elements known for a long time. The determination of the total arsenic has also a long history.

Before 1836 no satisfactory method for detecting arsenic was available. The means of detecting a poison were presumptive and inadequate before the nineteenth century. The only means of detecting arsenic at that time was to throw a suspected food or stomach into a red-hot coal, the presence of arsenic could be recognized by the production of a garlic odour. But this observation was sometime frustrated by the idea that the dead man had lately taken onions![82]

Scheele's green (copper arsenite) provided one of the earliest of the purely chemical tests for arsenic.[83] Another method of identifying arsenic was the formation of a yellow arsenic sulphide with ammonical silver nitrate, known as Hume's test.[85] Berzelius[84] devised a reduction test for the yellow arsenic sulfide precipitate that was formed by Hume's test or any suspected matter to contain arsenic which were mixed with black flux (potassium carbonate and charcoal) and heated in a drawn-out glass tube. If arsenic was present a metallic stain or mirror appeared on the cooler parts of the tube.

Even though some toxicologists at that time objected of its lack of sensitivity, Berzelius was the first to suggest a simplified method for the precipitation tests. The Marsh test[86] was introduced into the world in 1836 in order to avoid the uncertainty associated with the use of black flux method. The apparatus was constructed from 1.9 cm diameter glass tubing bent into the form of a syphon with limbs about 12.7 and 20.3 cm long. A small zinc rod was placed in the short limb and kept from slipping into the other limb by the insertion of a short glass rod. Hydrogen for testing was collected by pouring dilute sulphuric acid through the limb. A blank test was performed by burning hydrogen and allowing the flame to play on a piece of crown glass. If no stain appeared on the glass, more hydrogen was collected and burned from the material under test either in solution or suspended in water that was added through the open limb.

The stains produced on a cold glass in Marsh's test gave only qualitative results. Berzelius in 1837 made a step to evaluate arsenic quantitatively, when he passed mixtures of arsine and hydrogen through a glass tube, heated in the middle before ignition at the jet; the mirrors of arsenic formed could be evaluated quantitatively.

An alternative to Marsh's test was introduced by the publication of 1841 Hugo Reinsch's[87] test. In the test black stain of arsenic was deposited on copper foil from hydrochloric acid solutions.

In 1874 Mayerçon and Bergeret suggested the use of mercuric chloride as a reagent for arsine, and a convenient form of apparatus for holding the filter-paper in the gas stream was devised by Gutzeit[88] in 1879.

5.2 Analytical Procedure for Arsenic Speciation

The aim of this sub-topic is not to present all the available techniques for arsenic speciation, but the intention is to summarize the main analytical procedures, as well as to present some of the new techniques which could give useful information. The summary

is organized in the following sub-topics according to main steps such as sample pretreatment and dissolution, solution stability, preconcentration, isolation and determination.[89]

The basic techniques for speciation analysis were developed in the early 1980s. The methods include: sample pretreatment steps, derivatization techniques (e.g. hydride generation), separation steps (GC or HPLC) and detection (e.g. AAS, ICP, MS).[90] Some of the errors that are included in the specific analytical procedure are given in the following table.

Table 5.1: Steps in Determination of Element Speciation and Errors Observed[57]

Steps	Methods	Sources of Error
Storage	Wet Storage	Instability of compounds (volatilization, degradation)
Drying procedure	Freeze-drying	Instability of compounds (volatilization, degradation)
Pretreatment	Extraction	Incomplete extraction, change of original speciation, losses in cleanup
Derivatization	Hydride generation, ethylation, cold vapour	Inhibition, incomplete transformation, decomposition
Separation	GC, HPLC, Cold trapping	Decomposition of the species, adsorption on column
Detection	Element specific (AAS, ICP-AES, MS) or non-specific (FID, ECD) procedure	Interferences, atomization, ionization problems

One of the most widely used analytical procedures for speciation analysis couples the separatory power of chromatography with the detection ability of atomic spectrometry.

There is no specific method for element speciation that can give high accuracy of

results. There is no certified reference material available for trace element (speciation) determination in biological and environmental samples. The reference material is certified only for total element content.[9,91]

Contamination and losses of element in the preparative steps of a sample for analysis are the major problems in speciation studies. The errors of contamination could be excluded by using highly pure materials and chemicals.[3]

The arsenic species that are present in the environment and living organisms show large differences in their metabolism and toxicity[3] that make arsenic speciation studies of critical importance. Since arsenic species occur at a very low level, a reliable results is achieved by employing a very sensitive methods of analysis.

The most often determined species of arsenic in environmental waters, soils and sediments are As(III) and As(V) while in biological tissues and fluids, organic arsenic species are the most common constituents. Since the concentration of these species is low, it is required to perform a preconcentration step as the detectors available lack sensitivity for direct determination. Hyphenated techniques (such as GC-ICP-AES, HPLC-ICP-AES, etc) are the most popular analytical methods used for arsenic speciation.[3]

By controlling appropriate experimental conditions, a selective reduction could be performed to achieve simple and rapid method of arsenic speciation.[92]

Errors may arise in sampling, sample pretreatment and measurement in arsenic analysis. Improper sampling and storage could change the form of arsenic present in the sample. Due to low levels of arsenic in biological samples, the analysis for arsenic in environmental media is difficult. Moreover, many arsenic compounds are volatile and therefore could be lost easily from the sample, also contamination, even from ultrapure chemicals, could complicate the analysis.[75]

5.3 Pretreatment and Dissolution of Sample

Qualitative and quantitative determination of species of one or several elements requires careful consideration and planning when collection, treatment and preservation of samples were performed. This task is different from procedures for 'total' element analysis. Any method that is adopted for sampling and preservation of sample between collection and analyses in the laboratory should not disturb the equilibrium established among the species.[3,79,93] This treatment of the sample depends on the analysis required.[51] Introduction of biological contamination is undesirable, especially if the sample has to be stored for long periods; in the presence of microorganisms and oxygen the sample could deteriorate, producing alterations between species.[3]

In the determination of arsenic in biological material, it is necessary to guard the loss by volatilization during the destruction of organic matter. This is the case in marine plants in which they contain chloride even after careful washing.[94]

Most of the methods for the determination of arsenic require the removal of organic matter in the sample. In many cases, before measuring arsenic, it must be liberated from organic compound. This chemical disruption changes the oxidation state of some or all the arsenic; therefore, it is only possible to measure total arsenic concentration.

There are four ways of librating arsenic from its matrix.[89]

A. WET ASHING

In this type of ashing, nitric acid is used to digest samples such as vegetables, animal muscles, powdered coal and sewage. In a sample which has been digested by nitric acid prior to evaporation to dryness at 180^0C, a complete recovery of arsenic is possible.

Considerable loss of arsenic can be experienced in wet oxidation procedures, for example, that uses a mixtures of nitric, sulphuric and perchloric acid without a reflux condenser.[94] The three acid mixtures nitric, sulphuric, and perchloric acid are used for samples requiring more aggressive digestion. The sample is first refluxed with hot

sulfuric and nitric acids before oxidation is completed with perchloric acid.

Tracer experiments on the seaweed *Fucus serratus* for the losses of arsenic by wet ashing using a mixture of nitric acid, sulfuric acid and perchloric acid and which was then evaporated showed a high loss of arsenic. If a mixture of nitric and sulphuric acids were used, it appeared that arsenic began to volatilize in appreciable amounts even before sulfur trioxide fumes appeared.[94]

B. Microwave-Assisted Digestion

Microwave-assisted digestions are used in environmental analysis of solid samples. It has the advantage of:[95]

- Shorter digestion times
- Lower risks of contamination
- Smaller amounts of reagents requirement
- Lower risk of losses of analyte

Moreover, since the digestion is performed at high temperature and pressure there is a complete mineralization of the sample

C. DRY ASHING

Dry ashing at 450^0C can lead to loss of most of the arsenic present in a sample,[94,96] and this loss is partially prevented by ashing with magnesium nitrate as described by Evans and Bandermer.[97]

D. OXYGEN COMBUSTION

In the Schöniger combustion method, biological samples are digested by igniting them with oxygen in a closed flask. The combustion products are then absorbed in HCl solution.[89]

E. FUSION

Arsenic may be leached from minerals by fusion with NaOH in a silver or nickel crucible. Essentially all arsenic is recovered in the leach liquid even when a residue is present.[89]

5.3.1 Derivatization

There are some environmental compounds which have high molecular weight or contain polar functional groups that can not be analysed by GC because either they are nonvolatile or tail badly or are strongly attracted to the stationary phase or they can decompose at the running temperature of the GC. Therefore, a derivatization procedure is employed to convert the analyte to a volatile form. Grignard reagent is the mostly used compound for chemical derivatization. They have the structure: Organic moiety - Mg - halogen, the organic moiety may be alkyl or aryl group. The organic moiety replaces the anion in the analyte species thereby changing the analyte into volatile compound. Careful selection of the Grignard reagent is necessary in order to get correct results.

Some derivatization procedures may not be reproducible. This may arise from partial destruction of derivatizing agent or impurities in the agent. As a result, the data obtained could be inaccurate and/or imprecise. Regardless of these problems, derivatization is a reliable method in speciation studies by employing successful procedure.[98]

HYDRIDE GENERATION

Trihalides of arsenic ($AsBr_3$ and $AsCl_3$) or as a single or substituted trihalide (arsine) are the volatilized forms of arsenic which have analytical purposes.[89] Volatilization of arsenic has the advantage of isolation of arsenic from sample matrix in the form of gaseous arsine. This method requires a preliminary reduction of arsenic in the sample to As(III) by either $SnCl_2$, $TiCl_3$, KI.[89] Arsine can be generated using a second reducing

agent such as Mg^0, or Zn^0 from arsenite ion. Alternatively, arsine can be generated using $NaBH_4$ without prior reduction of As(V).[89]

The following equation shows the reduction of arsenic species into arsine.[99]

$$NaBH_4 + 3H_2O + HCl \rightarrow H_3BO_3 + NaCl + 8H^{\cdot} \quad \xrightarrow{As^{m+}} \quad AsH_3 + H_2(\text{excess}) \qquad (5.1)$$

where As^{m+} is the analyte of interest and m may or may not equal 3.

Sodium borohydride is used as reductant and hydride transfer in the hydride generation process to yield the hydride of metals and metalloids. The advantage of this reaction is that metal - carbon cleavage does not occur and as a result speciation is maintained. As, Ge, Sb, Se and Sn can be determined by hydride generation method. The yield from the hydride can be restricted by selecting a proper pH in the reaction vessel. For example, in the case of arsenic, at pH 1 the arsine can be from arsenite (III) and arsenate (V) while at pH 5 only arsenite (III) is reduced. Hydride generation presents drawbacks in case of high contents of organic compounds in the matrix.[32]

Papers have been published concerning arsenic determination by arsine generation combined with AAS,[100–104] ICP-AES,[101,102] and DCP-AES.[105] Arsenate (V) and arsenite (III) show different behaviour and sensitivities in the arsine generation process. Various workers have developed a method for the determination of arsenite (III) in the presence of arsenate (V).[9,106–108] If the oxidation state of arsenic has been less important, workers have preferred to reduce arsenate (V) to arsenite (III) by various prereducing agents before arsine generation.[109] The most important prereductant is potassium iodide (KI). The prereductant KI is used with ascorbic acid, which can prevent the oxidation of iodide to triiodide by air, or with oxidants such as iron (III) and copper (II).[110] However, potassium iodide is used in strong acid media. In acid concentration of less than 0.3 M, iodide could not reduce arsenate (V) to arsenite (III) completely.[9,110] Furthermore, it took as long as 4 - 5 hour to reduce arsenate (V) to arsenite (III) completely at room temperature as reported by Haring et al.[110]

Even though hydride generation technique is relatively simple and sensitive, it suffers from interferences that arise from certain metal ions in the sample solution.[109,111–113]

There are two sources that interfere in arsenic speciation. One of the sources is the concentration of different species in the sample and the second is the efficiency of hydride generation of the different species.[92]

The presence of different ions has been shown to affect the arsine generated in the analysis of arsenic species. The interferent could consume the reductant, slowing the reduction kinetics or the formation of other hydrides such as hydrogen selenide can reduce the result.[114]

Several proposals have been made to reduce the effects of interferent in the arsine generation procedure. Some of the methods are: time-consuming matrix separation,[115] increasing the acidity of the reaction medium[116] and the addition of chemical modifiers such as KI,[115] EDTA[117] or other chelating agents.[118]

Braman et al.[107] found that the reduction of arsenic compounds was pH dependent and related to the pKa of the individual arsenic acids when treated with sodium borohydirde. The relationship between pH and the fraction of undissociated arsenic acid present at equilibrium in aqueous solution is given by:[9]

$$\alpha = \frac{[H^+]}{[H^+] + K_1} \tag{5.2}$$

where α = fraction of undissociated arsenic species present at equilibrium K_1 = first dissociation constant of the arsenic acids and $[H^+]$ = hydrogen ion concentration

The following figure shows the fraction of undissociated arsenic versus pH for arsenic species based on the above equation.

It can be seen from the figure that the determination of As(III) and As(V) can be complicated by the presence of DMAA and MMAA.

The efficiency of hydride generation can be affected by different operating parameters. The concentration of acids, type and concentration of preductants, temperature

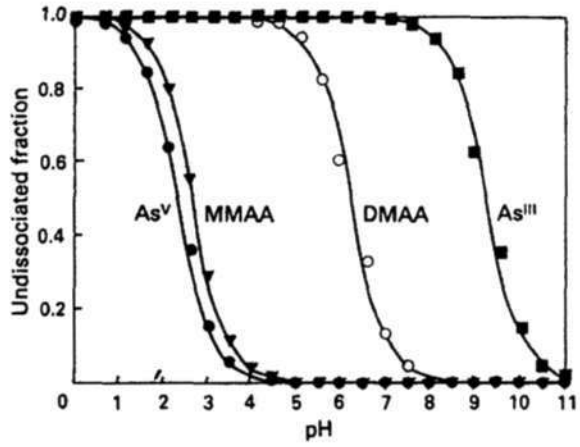

Figure 5.1: Relationship Between Fraction of Undissociated Arsenic Acids Present at Equilibrium and pH.[9]

and volume of mixing coil and the concentration of sodium borohydride, the flow rate of the carrier gas and any auxiliary oxidant can be used to optimize conditions for hydride generator.[119]

A successful separation of arsenic species by hydride generation method can be achieved by a proper selection of reaction condition by changing the buffers, pHs and reducing media of the reaction media.[92]

The disadvantage of using the reducing medium $NaBH_4$ in alkaline solution is, it is only stable for few days even when it is stored refrigerated and should be prepared everyday. The main disadvantage in using $NaBH_4$ is the interference caused by the transition metals, mainly those of groups 8,9,10 and 11.[111,112,120]

Many environmentally and biologically important organoarsenic compounds such as AsB, AsC, tetramethyl arsonium ion, AsS, etc do not form stable volatile arsine as the binding bond in these molecules is simply too strong[32] and can not be detected[3] upon treatment with sodium borohydride. Thus, they are unlikely to interfere with the determination of the species (As(III), As(V), MMAA and DMAA) that is given by the specificity of hydride generation.[40] To solve this problem, an appropriate decomposition

procedure such as microwave digestion with potassium persulfate and sodium hydroxide may be required[71] which convert the arsenic species into arsenate.

In the presence of 2% L-cysteine in aqueous arsenic solution for the determination of As(III), As(V), MMAA and DMAA using the pH dependence of hydride generation the optimum acid concentration was observed to shift to a much lower level ($\sim$ 0.3 - 0.5 M HCl). It seems that L-cysteine enhances the rate of arsine generation.[8]

The presence of thiol group (-SH) in L-cysteine and thioglycerol have effect on the hydride generation procedure. Cullen et al.[8] have shown that methylarsenicals can be reduced by thiols and dithiols including cysteine, glutathione, dithiothreitol, 2-3-dimercapto propanol, and lipoic acid. Chen et al.[7] have shown that arsenate can be reduced by L-cysteine. Therefore, the following mechanism can be proposed for the reduction of arsenic compounds in the presence of thiol compounds such as L-cysteine:

$$\begin{array}{lcll} As(OH)_3 & & As(SR)_3 & & AsH_3 \\ AsO(OH)_3 & & As(SR)_3 & & AsH_3 \\ CH_3AsO(OH)_2 & \overrightarrow{RSH} & CH_3As(SR)_2 & \overrightarrow{NaBH_4} & CH_3AsH_2 \\ (CH_3)_2AsO(OH) & & (CH_3)_2As(SR) & & (CH_3)_2AsH \end{array}$$

where R = - $CH_2CH(NH_2)COOH$ for cysteine and - $CH_2CH(OH)CH_2OH$ for thioglycerol.

Initially the arsenic compounds react with the thiols to form the intermediate compounds of $As(SR)_3$, $CH_3As(SR)_2$, and $(CH_3)_2As(SR)$ which are all in the As(III) state. Even though there are arsenic sulfur compounds in the intermediate, the final products do not contain cysteine. This is experimentally proven using a modified[8] hydride generation - cold trap gas chromatography with atomic absorption detection which is the same technique as used by Braman et al.,[107] Andreae,[121] and Vien and Fry.[122]

The above mechanism suggested that the pentavalent arsenic, As(V), MMAA and

DMAA should have first to be reduced to As(III) by L-cysteine prior to reaction with sodium borohydride. Consequently, faster reaction can be expected of pre-reduced arsenic species with sodium borohydride. It is known that[62,101,107,109,121,123–126] the arsine generation from arsenite is faster than from pentavalent arsenicals. In the absence of L-cysteine or KI, both the pre-reduction and hydride formation would be performed by reacting with sodium borohydride

5.3.2 Preconcentration and Isolation of Arsenic Species

The principal methods used to preconcentrate arsenic species are using coprecipitation and adsorption, volatilization and extraction.

The main methods of preconcentration and isolation of As(III) and/or As(V) from solution are shown in the following table. All entries but the last[75] are summarized from the review by Talmi and Fieldman.[127]

Table 5.2: Methods for Preconcentration and Isolation of Arsenic

Method	Reagents and Conditions	Comment
Coprecipitaiton	$Fe(OH)_3$, pH 7	99% recovery of As at 2ng/ml
Coprecipitation	Ce, Zn, In, Fe, Ti, Al, Hydrodoxides, pH 7	As(V) is quantitatively carried down
Coprecipitation	In, Zn, hydroxides, pH 8.5	As(III) is 95% carried down
Adsorption	Hydrated MnO_2	Quantitative adsorption of As(V) up to 272 mg As(V)/g MnO_2

Continued...

Table 5.2: Continued...

Method	Reagents and Conditions	Comment
Chelation-precipitation	Thionalide	95% recovery of As(III)
Liquid-liquid extraction	Diethylammonium diethyldithiocarbamate in chloroform	As(III) but not As(V) was extracted
Liquid-liquid extraction	Ammonium pyrrolidine dithiocarbamate in methylisobutyl ketone	As(III) but not As(V) was extracted
Liquid-liquid extraction	Diethyldithiophosphoric acid, low pH	As(III) extracted
Liquid-liquid extraction	2:3 CCl_4 and 2-butanone	$AsCl_3$ extracted from 6 M HCl
Liquid-liquid extraction	Benzene	Arsenite extracted quantitatively from 8 M HCl; germanium is not left behind
Volatilization	H_2SO_4 HNO_3 oxidation add KBr	$AsBr_5$ distilled off; some PBr_5 and $SbBr_5$ also distilled over
Volatilization	Zn^0 and HCl	AsH_3 produced and flushed out[a]
Volatilization	Mg^0 and acid	AsH_3 produced and flushed out[a]
Volatilization	$NaBH_4$ pH 1.5	Only As(III) AsH_3

Continued...

Table 5.2: Continued...

Method	Reagents and Conditions	Comment
Volatilization	$NaBH_4$ pH 1.5	As(V) is reduced to As(III) which is then converted to AsH_3
Ion Exchange	Strong base anion exchange resin in Cl^- form (e.g. Amberlite IRA 400), pH 5	Quantitative adsorption of As(III) from distilled water or acid mine leachate at or below 0.46 ppm

[a]Hydrogen sulfide and mercaptans combine with As to inhibit the release of AsH_3; such compounds must be oxidatively destroyed before AsH_3 is generated, or volatilized during HCl digestion open to the air. Metals such as Co, Ni, Ag, Hg, Cu interfere in the generation of arsine as they tend to form insoluble arsenides. Thus, if present in large amounts, they must be complexed with EDTA or citrate or be removed before arsine generated.[60]

COPRECIPITATION AND ADSORPTION

The hydroxides of transition elements were used to preconcentrate As(V), for example the arsenate ion is collected by coprecipitation with $Fe(OH)_3$ at pH 7 in concentrations as low as 2 - 4 ng/ml. The hydroxides of La and Mg can coprecipitate As(V). The hydroxides of In and Zr at pH 8.5 are capable of coprecipitating As(III).

Organic reagents have been also employed to preconcentrate arsenic by coprecipitation. Such an organic compound is thionalide which has been utilized by Portman and Riley[94] to coprecipitate As(III) from sea water and digested biological samples. Since only As(III) is coprecipitated using thionalide, As(V) has to be reduced to As(III) by ascorbic acid at boiling temperature.[89]

EXTRACTION

Solvent and liquid - liquid extraction have advantages in the determination of arsenic species. The method can separate the analyte from the complex and potential interfering matrix.[9]

The extraction should be done without contaminating or losing the analyte from the matrix. Extraction should also not change the speciation and reduce interferences from the matrix.

Different acid extraction (such as hydrochloric acid, acetic acid, hydrochloric - acetic acid mixture etc) procedures have been employed for sediments and biota analyses. Organic solvent extraction e.g. dichloromethane, chloroform, toluene, hexane etc can be done for techniques involving GC or HPLC.[32] Extraction methods have to be assessed for their quality by spike recovery. This is done by spiking a sample with a known content of analyte. The spiked sample is left to equilibrate and finally after the extraction the analyte is determined. The major disadvantage is that the spike may not bind the same as the naturally occurring compounds. If the extraction method does not alter the matrix composition and appearance, recovery can be performed on the previously extracted sample.

Certified reference materials may be used as a test for accuracy of the recovery.[32]

Trivalent arsenic can be extracted using liquid - liquid extraction whereas As(V) not. Several interfering elements may be eliminated by extracting (e.g. using cupferon) arsenic in the pentavalent state after which a reduction of As(V) to As(III) is performed to be extracted using different reagents. Various dithiocarbamates, such as diethylammonium dithiocarbamate (in $CHCl_3$) or ammonium pyrrolidine dithiocarbamate can extract As(III) at pH 2.6 from aqueous solution.[89]

Depending on the type of sample, low and variable percentage of organoarsenicals can be extracted using solvents such as methanol in marine samples.[21]

5.3.3 Detection

There are different detection systems employed in the determination of arsenic species. These detection systems must be rapid and inexpensive and must have the capability of giving a reasonable accuracy and precision at the concentration levels encountered.[120]

Different detection methods have been devised for total arsenic and of the various methylated and inorganic forms in air, solid, water and biological materials.[16,25]

Some of the detectors used in total arsenic or species of arsenic determination are briefly summarized as follows.

A. MOLECULAR ABSORBTION SPECTROPHOTOMETRY

This detection system is a widespread technique for arsenic determination because of its technical simplicity, excellent accuracy and precision and its low cost. Samples like urine, biological materials etc have been analysed using this method.

Two of the compounds which are universally accepted in the determination of arsenic using spectrophotometric method are silver - diethyl dithiocarbamate (Ag - DDC) and ammonium molybdate. Arsine which is produced by arsenic volatilization method is passed through 0.5 % Ag - DDC solution in pyridine to form a red chromopore whose intensity is read at 533 nm.[89] The reaction between arsenate ions with acidified molybdate forms an arsenomolybdate, when reduced this compound produces a blue complex compound where its absorbance is monitored at 866 nm.[89]

B. RADIOCHEMICAL TECHNIQUES

NEUTRON ACTIVATION ANALYSIS (NAA)

NAA is nondestructive and sensitive method, with a detection limit of 0.1 ng using a thermal neutron flux of 10-12 neut.cm^{-2}.sec^{-1}. The method is applicable at the sub-ppm

level and requires a smaller sample size than the spectrophotometry technique. NAA has been used in the analysis of biological and marine samples,[89,128] plant tissues,[89] water samples[129] and many others.

C. X-RAY FLUORESENCE SPECTORSCOPY

Arsenic in complex matrices as in rocks, soils, river and sea sediments can be determined by x-ray fluorescence spectroscopic detection. Preconcentration is required in water analysis using x-ray fluorescence due to the lack of sensitivity. One of the preconcentration procedure,[89] is to extract arsenic using $CHCl_3$ after reacting arsenic with ammonium pyrrolidine dicarbamate. After evaporating $CHCl_3$ on a filter paper disk, it is detected by x-ray fluorescence down to 50 ng/ml with a precision and accuracy of $\pm$ 10 %. Alternatively, arsenic could be precipitated with either diethyldithiocarbamate (DDTC) or 1-(2-pyridylazo-2-naphthol) (PAN) and collected for analysis on Millipore filter.[81] PAN was efficient co-precipitant for samples of low quantities of Fe.

Results from Yair Talmi and D.T. Bostick[89] show that the thionalide cocrystalization method is suitable for x-ray fluorescence detection with little spectral interference from Fe, Cu and S. Since the thionalide cocrystalization is efficient, the determination of arsenic depends on the performance of the x-ray fluorescence detection system.

D. ATOMIC SPECTROSCOPY

I. ATOMIC EMISSION SPECTROSCOPY

The conventional flame emission technique is not sensitive enough for the low arsenic concentration in the environment. More energetic excitation sources, i.e. plasma discharge spectrometric sources are used to get higher sensitivities.[89]

II. ATOMIC ABSORPTION SPECTROMETRY

Flame absorption spectrometric sources are generally very reliable and simple to operate. However, sensitivity is limited and extensive interferences are observed from common ions unless a separated N_2O - C_2H_2 flame is used[130] for arsenic analysis.

Higher sensitivity can be provided by using non-flame atomic sources such as electrically heated absorption tubes, hollow cathode discharges, and graphite and tantalum resistor heated atomizers. But the sample size available for analysis is reduced (1 - 50 ml).[89]

In environmental samples for arsenic analysis, the most widely used procedure is the combination of FAAS with hydride generation technique.[42]

E. CHROMATOGRAPHIC METHODS

I. TOTAL ARSENIC

One of the insensitive methods employed for the determination of arsenic by GC is to form a volatile trichloride, trifluoride and trimethylsilyl derivatives. The volatile triphenylarsine formed by the extraction of arsenic as its diethyldithiocarbamate complex and phenylation of the complex with Grignard reagent after solvent evaporation was separated and detected by GC.[89]

II. SPECIATION OF ORGANO ARSENIC COMPOUNDS

Thin layer chromatography, thin layer electrophoresis and paper chromatography was applied earlier to separate and detect MMAA and DMAA. But accurate speciation analyses of these compounds are based on their volatile derivatives.[89]

Ion - exchange procedure separate common arsenic species by making use of the large differences in acid - dissociation constants of the species (acids), separation of

arsenic species by open - column using both anion - and cation - exchange resins have been reported by Henry and Thrope,[131] and others.[69,132,133]

5.3.4 Interfaced/Hyphenated Techniques

Different studies were undertaken to interface chromatographic system with a selective and sensitive detector in element speciation analysis. But these methods are not optimized for each other and needs compromise for each of them.[56]

In the analytical methods of arsenic speciation, coupling of the separating power of chromatography with the sensitive and selective detectors was employed. Some of them use coupling of liquid chromatography and inductively coupled plasma atomic emission spectroscopy (ICP-AES).[68,134–136] Sample atomization occurs within spatially limited plasma characterized by high temperature (6 000 - 10 000 K).[137]

Inductively coupled plasma atomic emission spectrometry offers good sensitivity, a dynamic range of over five orders of magnitude and multi - element detection abilities if it is coupled with high - performance liquid chromatography. If pneumatic nebulizers are used in the interface of HPLC - ICP - AES, the method suffers poor transport efficiency. However, the coupling will cause low tolerance of plasma to the organic solvents used in the HPLC's mobile phase. Workers investigated the performance of HPLC - ICP - AES methods by using different mobile phase composition, flow rates of mobile phase, stationary phase, pH and gradient elution.[71,138,139]

Morita et al.[68] reported the separation of arsenite, arsenate, methylarsonic acid, dimethyl arsinic acid and arsenobetaine with anion - and cation - exchange chromatography using phosphate buffer as a mobile phase.

Application of hydride generation (which gives lower detection limits[3]) after chromatography and before the detector ICP - AES allows the determination of As(III), As(V), MMA and DMA by reducing the species to arsine.[134] The major problem of us-

ing ICP - AES is its lower sensitivity for environmental analysis particularly as arsenic wavelengths are in the low UV - region.[140]

A high sensitivity and multi element capability can be obtained by coupling HPLC with ICP - MS. ICP - MS avoids the post column derivatization as in ICP - AES. The coupling of HPLC with ICP - MS was investigated by many workers such as Dean et al.[141]

Other couplings are GC - ICP - AES which was first reported in the late 1970s. Windsor and Deton[142] explored the ability of ICP - AES in studying elemental analysis in organic compounds. Chong and Houk[143] reported the first coupling of GC - ICP - MS in 1987.

HPLC - HG - AAS[144–146] and HPLC - ETAAS[147] are some of the common couplings used in arsenic speciation. When coupling techniques are designed care must be taken in matching the flow rate of a gas or liquid through the chromatographic column to the gas or liquid uptake rate of particular detector.[79]

Generally there are very diverse analytical methods for the analysis of arsenic species in the environment. The different methods are schematically presented in the following figure. Each process has advantage as well as disadvantage with the scope of study and the laboratory facilities available.[3]

In this project the analytical method chosen was hydride generation technique with ICP - AES detection system.

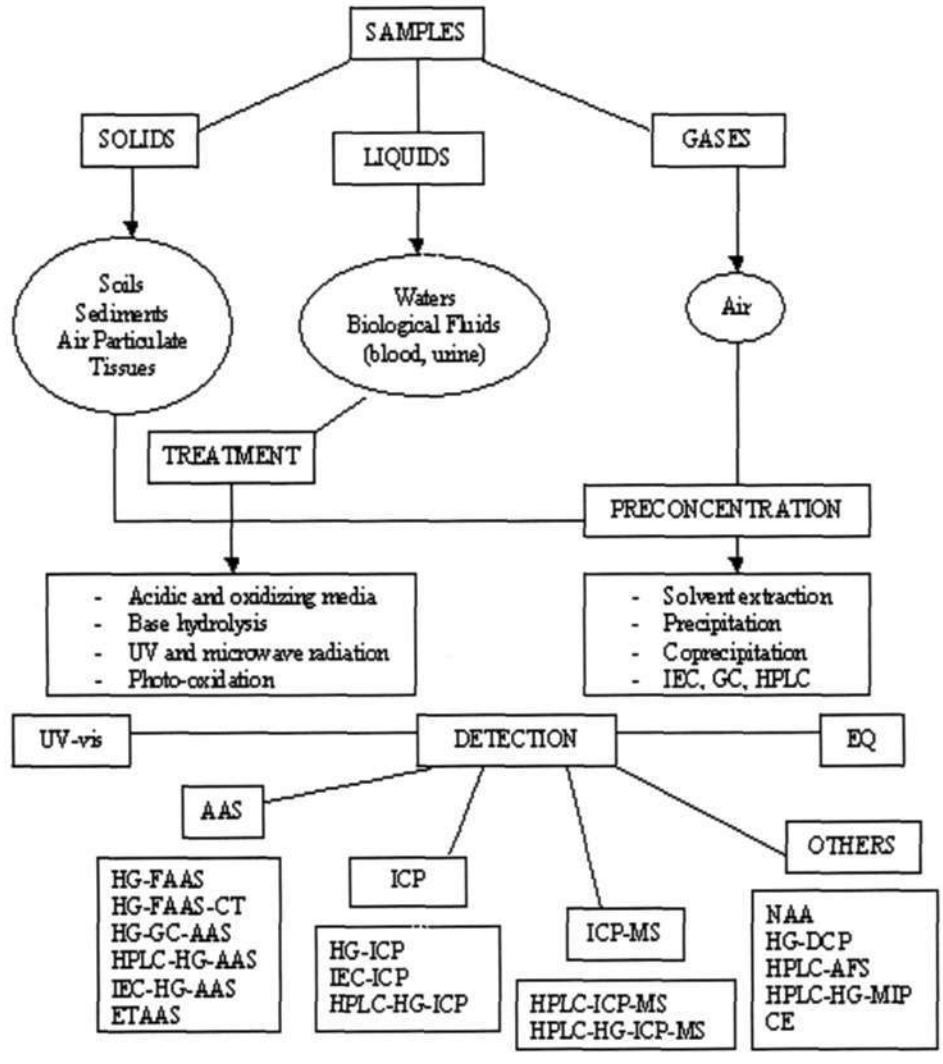

Figure 5.2: Procedures for Arsenic Speciation in Different Matrixes[3]

UV-vis = Spectrophotometry; **EQ** = Electrochemical Methods; **AAS** = Atomic Absorption Spectrometry; **ICP-AES** = Inductively Coupled Plasma Atomic Emission Spectrometry; **ICP- MS** = Inductively Coupled Plasma Mass Spectrometry; **HG** = Hydride Generation; **FAAS** = Flame Atomic Absorption Spectrometry; **CT** = Cryogenic Trapping; **GC** = Gas Chromatography; **HPLC** = High Performance Liquid Chromatography; **IEC** = Ion Exchange Chromatography; **ETAAS** = Electrothermal Atomic Absorption Spectrometry; **NAA** = Neutron Activation Analysis; **DCP** = Direct Current Plasma; **AFS** = Atomic Fluorescence Spectrometry; **MIP** = Microwave Induced Plasma; **CE** = Capillary Electrophoresis

Chapter 6

Experimental

6.1 Apparatus and Instrumentation

The apparatus and instrumentation can be considered to consist of two sections which are the arsine generator and the detector. A simple glass U-shaped gas-liquid separator (Figure 6.1) was used and the generated arsine was carried out in a continuous mode to the detector.

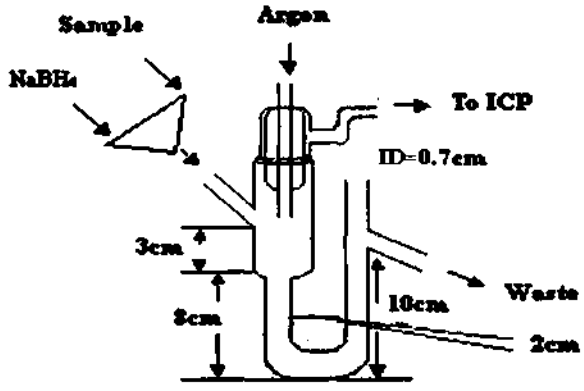

Figure 6.1: Gas Liquid Separator Apparatus

For HG-ICP-AES, the instrumental set-up is represented schematically in Figure 6.2.

It consists of a peristaltic pump (Gilson Minipuls 2) Gilson Medical Electronics S.A. (Villiers-le-Bel, France), uptake tubes for acidified sample and $NaBH_4$ solution, transport tube for argon and arsine (Nalgene tubing), a gas-liquid separator and ICP-AES spectrometer (Varian Liberty 150AX Turbo) Varian Australia Pty Ltd (Mulgrave, Australia) equipped with computer that controls instrument operation and data handling and data out-put printer (Epson LX-300).

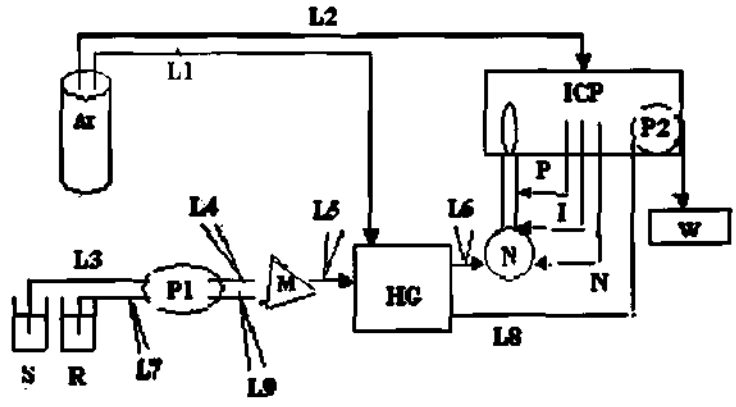

Figure 6.2: Schematic Diagram for HG-ICP-AES Set-Up

In Figure 6.2, the following designations were used:

- L1 = Argon delivery line (PVC tubing Nalgene 180 with pressure regulator) to gas-liquid separator
- L2 = Argon delivery line to ICP-AES
- L3 = Sample delivery line to Gilson Minipuls 2 peristaltic pump (1.5mm)
- L4 = Sample delivery line from Gilson Minipuls 2 peristaltic pump to mixer (0.5 mm PTFE)
- L5 = Mixed acidified sample and $NaBH_4$ delivery line (0.5 mm PTFE) from mixer to gas-liquid separator

- L6 = Generated arsine delivery line (PVC tubing Nalgene 180) to nebulizer
- L7 = $NaBH_4$ delivery line to Gilson Minipuls 2 peristaltic pump (1.0 mm)
- L8 = Waste liquid delivery line to ICP peristaltic pump
- L9 = $NaBH_4$ delivery line from Gilson Minipuls 2 peristaltic pump to mixer (0.5mm PTFE)
- P = Argon delivery line to plasma of ICP-AES
- P1 = Gilson Minipuls 2 peristaltic pump
- P2 = ICP-AES peristaltic pump
- S = Sample
- R = Reductant ($NaBH_4$)
- M = Mixer
- HG = Gas-liquid separator apparatus
- ICP = ICP-AES instrument
- Ar = Argon
- I = Intermediate argon delivery line
- N = Argon delivery line to nebulizer of ICP-AES
- W = Waste delivery line

6.2 Reagents

All solutions were prepared from the analytical or other purity grade chemicals. Ultrapure water that was prepared from a Modulab (US Filter Corporation, USA) water purification system was used for all solution preparation and dilution.

For all routine sample analysis a 2% (m/v) $NaBH_4$ (Chemically pure, Saarchem, South Africa) stabilized in 0.1 M NaOH (Saarchem, South Africa) was prepared daily. Sodium borohydride in water decompose steadily, with evolution of hydrogen, that loses its reducing capacity thereby creates a decrease in sensitivity of the method.[120]

The following arsenic reagents were used:

1. Sodium arsenate hydrate [$Na_2HAsO_4.7H_2O$], laboratory reagent, BDH, Poole England

2. Arsenic trioxide [As_2O_3], analytical reagent, BDH, Poole England

Approximately 838.3 ppm solution of As(III) and 999.9 ppm of As(V) stock solutions were prepared by weighing using Mettler AJ100 Mettler Toledo (Columbus, USA) balance appropriate amount (0.5534 g of As_2O_3 and 2.0821 g of $Na_2HAsO_4.7H_2O$) of the above reagents in 1 ml of concentrated HNO_3 (chemically pure reagent, SMM Chemicals (pty) limited, South Africa) and 0.2021 g and 0.2024 g NaOH respectively for the two arsenic reagents and diluted to 500 ml with ultrapure water.

Initially, the arsenic content of the reagents was checked by conventional ICP-AES.

The stock solutions were diluted using ultrapure water to give a working solutions and lower concentrations immediately before use.

The following chemicals were also used in the study.

1. Hydrochloric acid (HCl), 32%, Chemically pure reagent (SMM Instruments, South Africa)

2. Argon (Ar), (Afrox, South Africa)

3. Glacial Acetic Acid (CH_3COOH), (Saarchem, South Africa)

4. Sodium Acetate trihydrate ($CH_3COONa.3H_2O$), (Saarchem, South Africa)

5. Citric Acid ($HOOC(OH)C(CH_2COOH)_2.H_2O$, Analytical reagent (Hopkin and Williams, South Africa)

6. Sodium Citrate ($Na_3C_6H_5O_7.5\frac{1}{2}H_2O$), Certified reagent (Fisher Scientific Company, USA)

7. EDTA $[CH_2N(CH_2COOH)CH_2COONa]_2.2H_2O$, Analytical reagent (BDH, England)

8. L-Cysteine ($C_3H_7NO_2S$), (Fluka) Sigma - Aldrich (Pty) Ltd (Vorna Valley, South Africa)

9. Nitric Acid (HNO_3), 65%, pro-analysis, Merck

10. Copper chloride ($CuCl_2.2H_2O$), (Saarchem, South Africa)

11. Nickel chloride ($NiCl_2.H_2O$), Analyzed reagent (Saarchem, South Africa)

12. Selenium oxide (SeO_2), Laboratory reagent (BDH, England)

13. Iron (III) chloride ($FeCl_3.H_2O$) (Associated Chemical Enterprise, South Africa)

14. Manganese chloride ($MnCl_2$), Analytical reagent (Associated Chemicals Enterprises, South Africa)

15. Magnesium nitrate hexahydrate ($Mg(NO_3)_2.6H_2O$), pro analysis (Merck, South Africa)

Sodium - citrate buffer and sodium-acetate buffer was prepared to the appropriate pH (measured by Crison MicropH 2000, Crison Instruments (Barcelona, Spain).

Standard metal solutions (i.e. Fe(III), Ni(II), Se(IV), Cu(II) and Mn(II)) that were used in the studies of interferences were prepared from the metal chloride and oxide chemicals.

Approximately (75 μg/l) of arsenic species (As(III) and As(V)) were used during the analytical method development procedures.

6.3 Hydride Generation Procedure

The hydride generation procedure for HG-ICP-AES can be explained briefly as follows.

An acidified sample solution or buffered solution was pumped by the Gilson Minipuls 2 peristaltic pump and was reduced when it came into contact with $NaBH_4$ solution at the mixer and the mixing coil.

The generated gas-liquid mixture was transferred to the gas-liquid separator (Figure 6.1) and the gas phase (arsine and hydrogen) was continuously swept by the inert gas argon. The gas phase was then detected for arsine by introducing into the plasma of the ICP-AES spectrometer and the signal was recorded. The liquid phase waste in the HG-ICP-AES was expelled forcibly by using a pump that had a rate greater than the liquid inflow rate. For each test solution, responses were recorded by using a constant concentration of the arsenic species ($\sim$ 75 μg/l) but varying the concentration of the other reagents.

6.4 Hydride Generation Conditions

In the present study, determination of arsenic in commercial seaweed kelp tablet, a detailed investigation was performed under different conditions to achieve the optimal conditions for the hydride generation. The response profiles of As(III) and As(V) under different conditions have been well documented by various researchers.[7–9,119,120]

The continuous hydride generation system that was shown in Figure 6.2 was employed to study the operating conditions. The parameters studied in this work include concentration of acid (HCl), $NaBH_4$ and L-cysteine, length of reaction coil, time for formation of arsine, buffer solutions and pH of the reaction media. These factors were optimized by the one-factor-at-a-time method. The signal from the ICP-AES was used as a response.

6.5 Sample Preparation

Two types of commercial seaweed kelp tablets were purchased from a supermarket in Pietermaritzburg, South Africa. The kelp tablets were digested using the following three digestion procedures.

Microwave Digestion

About 0.5 g of powdered kelp tablet was weighed and put into one of six (6) TFM digestion vessels. Five (5) ml of conc. HNO_3 (Riedel de Haën, Sigma - Aldrich Laborchemikalien GmbH (Seelze, Germany) was added and the sample was digested using the Multiwave Microwave sample preparation system (PerkinElmer/Paar Physica) (Anton Paar GmbH, Graz, Austria) at 260^0C and 28.75 bar for 35 minutes. After cooling, each of the solutions was diluted to 100 ml using ultrapure water. Ten (10) ml of the diluted sample, (and sufficient HCl and L-cysteine solutions to make the final concentrations 0.1 M and 0.5% respectively) were added to a 50 ml volumetric flasks and made up with ultrapure water. The solution was then ready for analysis using HG-ICP-AES.

The following table shows the program used for the microwave digestion (see Appendix 1).

Table 6.1: Microwave Digestion Program

Phase	Intial Power (W)	Final Power (W)	Time (min)
1	500	500	5:00
2	500	1 000	5:00
3	1 000	1 000	10:00
4	0	0	15:00 (cooling)

Dry Ashing

Approximately 0.25 g of the seaweed kelp tablet was weighted on porcelain crucible dish and 8 ml of 50% $Mg(NO_3)_2.6H_2O$ was added. The samples were evaporated to dryness in oven (Gallenkamp, England) at 105^0C overnight. The samples were then transferred to 100^0C warmed electric furnace (Gallenkamp, England). The temperature was kept increasing by 50^0C and heating for 30 minutes at the higher temperature until a final temperature of 500^0C was reached. After cooling, the samples were dissolved in 2 M HCl and made to 50 ml mark. Twenty five (25) ml of the sample solutions were then diluted to 50 ml in the presence of 0.5% L-cysteine using ultrapure water. Method blank was also prepared according the procedure. Then, the sample solutions were ready for analysis.

Wet Digestion

One g of a seaweed kelp tablet was weighed on a digestion vessel (8 cm X 4 cm borosilicate glass). Ten ml of 1:1 conc. HNO_3 was added and the mixture was heated for 15 minutes covered with a watch glass in boiling water bath. After cooling, 5 ml of conc HNO_3 was added and refluxed for 30 minutes. This process of adding 5 ml conc. HNO_3 was repeated four times until the evolution of brown fumes decreased. The solution was heated for almost 2 hours until the final volume in the vessel was approximately 5 ml.

After cooling and filtering (using Whatman 41 filter paper, Whatman Ltd (England)) the solutions were made to 100 ml mark using ultrapure water. In 25 ml volumetric flask, 5 ml of sample was made to the mark using ultrapure water after sufficient HCl and L-cysteine had been added to give a final concentration of 0.1 M and 0.5% respectively.

Citrate Buffered Sample

A wet-digested samples (the above procedure) were used to prepare a citrate buffered reaction media using the following procedure.

Ten ml of wet-digested samples were transferred to 50 ml volumetric flasks and sufficient amounts of sodium citrate (0.6 M) and citric acid were added to make the final solutions of pH 4.8. The solutions were then ready for analysis.

The following figure gives a summary of the sample digestion procedures used in this work.

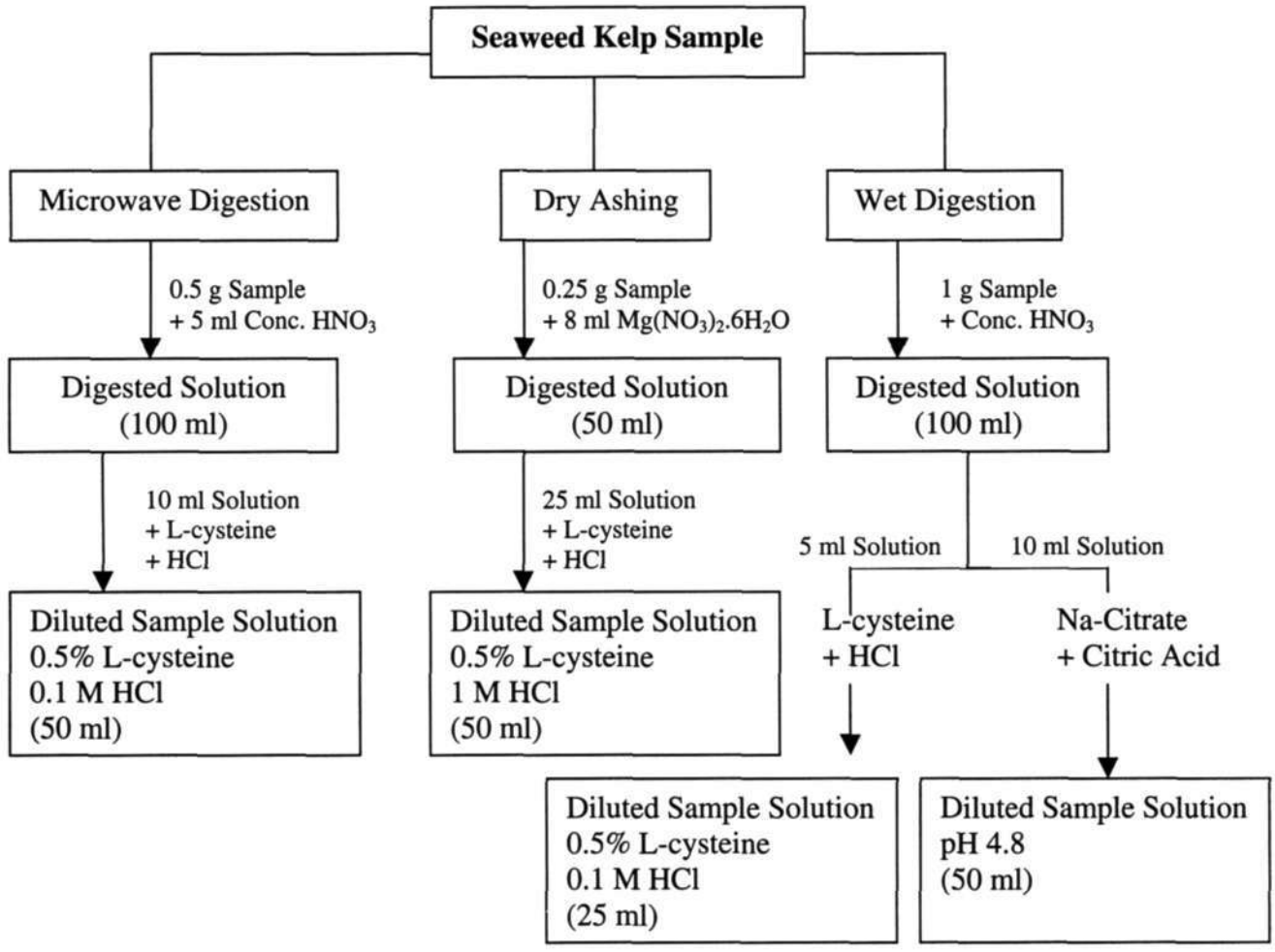

Figure 6.3: Flowchart for Sample Digestion Procedure

6.6 Results and Discussion

Before performing the experiments on optimizing the hydride generation conditions, the following studies were undertaken:

Checking of Arsenic Concentration Using Conventional ICP-AES

The arsenic species used in this study was checked by conventional ICP-AES with the experimental conditions given in Table 6.2 for their arsenic concentration and Figure 6.4 show the results.

Table 6.2: Experimental Conditions for ICP-AES

ICP Spectrometer	Varian Libery 150 AX Turbo
Power, kW	1.00
Ar Plasma Flow, L/min	15.0
Auxiliary Ar Flow, L/min	1.50
Photomultiplier, V	800
Integration Time, sec	1
Nebulizer, kPa	240
Wavelength, nm	193.7

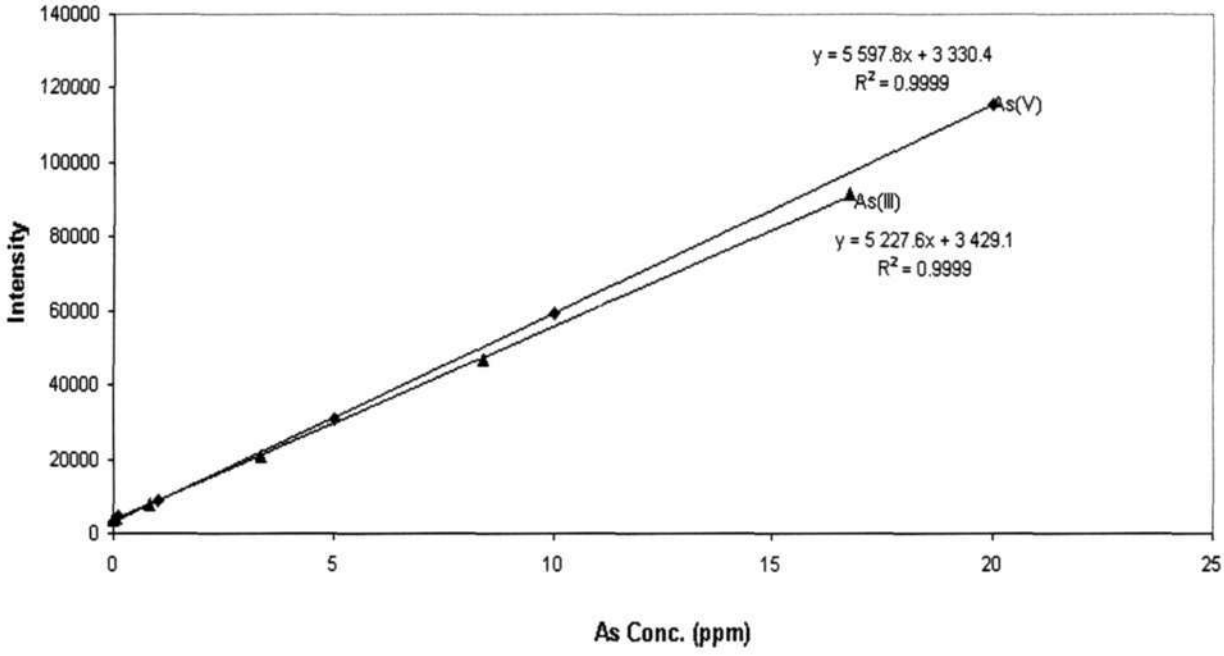

Figure 6.4: Calibration Graph for Arsenic Species Using Conventional ICP-AES

Calibrating the Gilson Minipuls 2 Peristaltic Pump

Different tube sizes were used in calibrating the pump. The tubes outside diameters (OD) range from 1 - 3 mm. The flow rate was measured using graduated cylinder and a stopwatch (Oregon Scientific). The results are shown in Table 6.3 and Figure 6.5.

Table 6.3: Flow Rate Results in ml/min

No.	Tube Size	000	100	200	400	500	600	800	1000
1	1.0 mm OD	0.16	0.40	0.70	1.30	1.60	2.00	2.60	3.40
2	1.5 mm OD	0.25	0.50	0.90	1.90	2.40	3.00	4.00	5.00
3	2.0 mm OD	0.34	1.00	2.00	4.20	5.40	6.50	8.60	10.50
4	3.0 mm OD	0.35	1.30	2.60	5.40	6.90	8.20	11.00	13.50

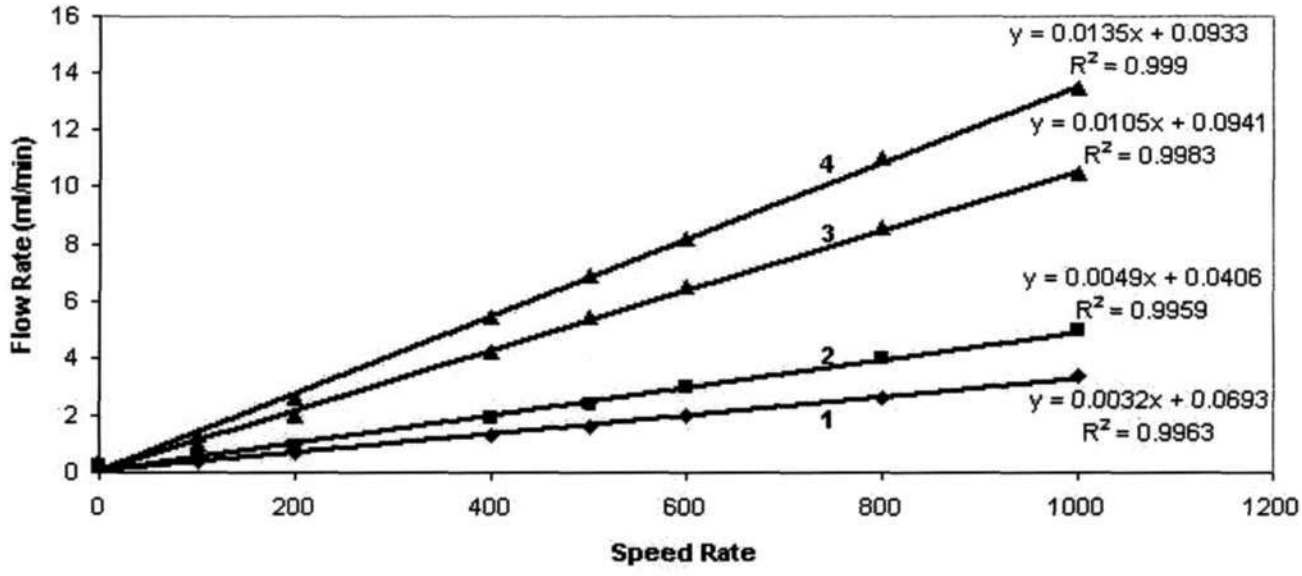

Figure 6.5: Flow Rate versus Pump Speed

Tube sizes (3) and (4) were excluded from further use, because their diameters were too large to fit the pump's channel and the free flow of the solutions were restricted.

When the pump increased (beyond pump speed rate 600), the plasma started to flicker. A flow rate of pump speed 500 was chosen for the sample and reductant using tube size number 1 for reductant ($NABH_4$) and tube size number 2 for sample for the rest of the study.

The plasma conditions for the conventional ICP-AES was used for the HG-ICP-AES.

The stability of plasma was checked using a five (5) minutes time display program and showed a constant intensity reading through out the specified time when the HG-ICP-AES system was operating. Therefore, for the rest of the work 1.60 ml/min $NaBH_4$, 2.40 ml/min sample flow rates and 193.7 nm wavelength for arsenic was used.

6.6.1 Study of Experimental Conditions

The continuous HG-ICP-AES system as depicted in Figure 6.2 was employed to study the selection of the optimum hydride generation conditions for the development of the method. Freshly prepared 50 ml test solutions of 75 μg/l in As(III) and As(V) were used to investigate the intensities of As(III) and As(V) in different media. The parameters studied in an attempt to establish the optimum conditions were: HCl concentration, $NaBH_4$ concentration, L-cysteine concentration, reaction coil length, reaction time, composition of buffer solutions and the pH of the reaction media.

$NaBH_4$

The effects of changing the concentration of $NaBH_4$ stabilized with 0.1 M NaOH on the intensities of As(III) and As(V) is shown in Figure 6.6. The flow rate employed for $NaBH_4$ was 1.60 ml/min and that of the test solution was 2.40 ml/min. From the figure, it can be seen that the intensities of both As(III) and As(V) increase up to 1% $NaBH_4$. At concentrations greater than 2%, sensitivities start to decrease due to the dilution effects from the evolution of more hydrogen gas and/or changes in plasma conditions. When 4% $NaBH_4$ was used, the plasma was easily extinguished. This was also confirmed by R.Rubio et al.[134] in previous work. Therefore, a concentration of 2% was adopted for further work.

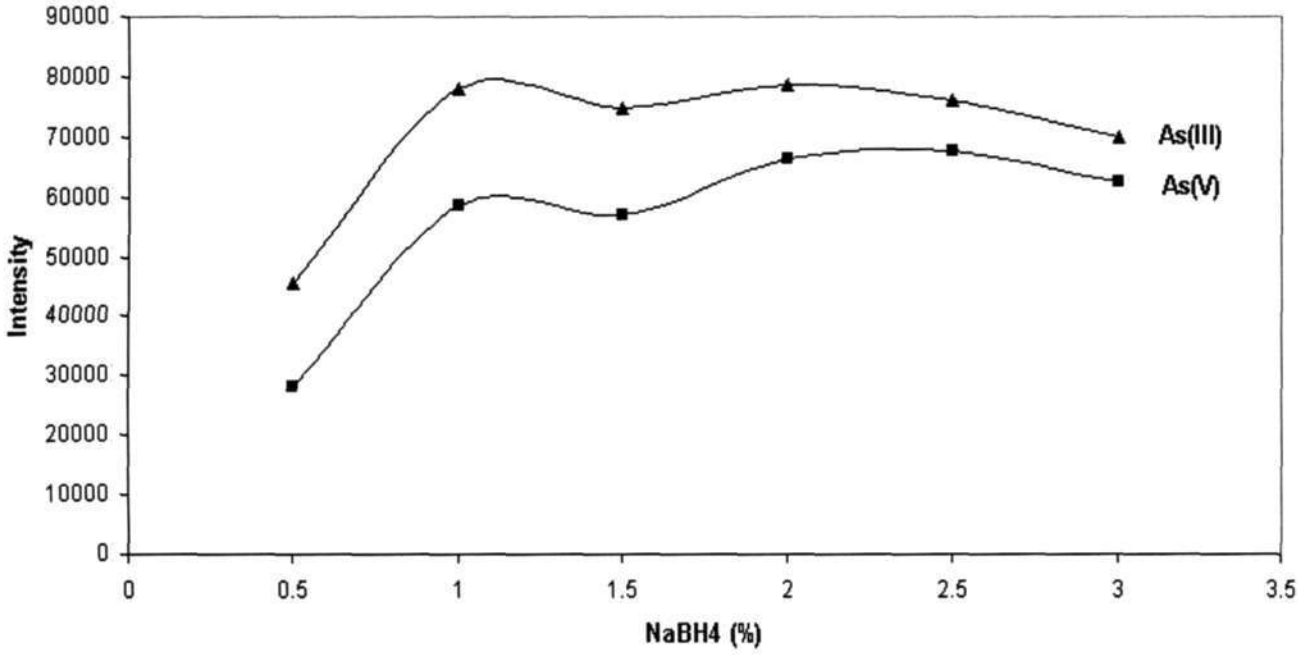

Figure 6.6: Effect of $NaBH_4$ on the Response of As(III) and As(V)

L-Cysteine

L-cysteine was used as a pre-reducing agent in this study. Figure 6.7 shows the effects of L-cysteine on the intensities of As(III) and As(V). From the figure it can be shown that the intensities fall above 0.5% L-cysteine. For further work, 0.5% L-cysteine was taken.

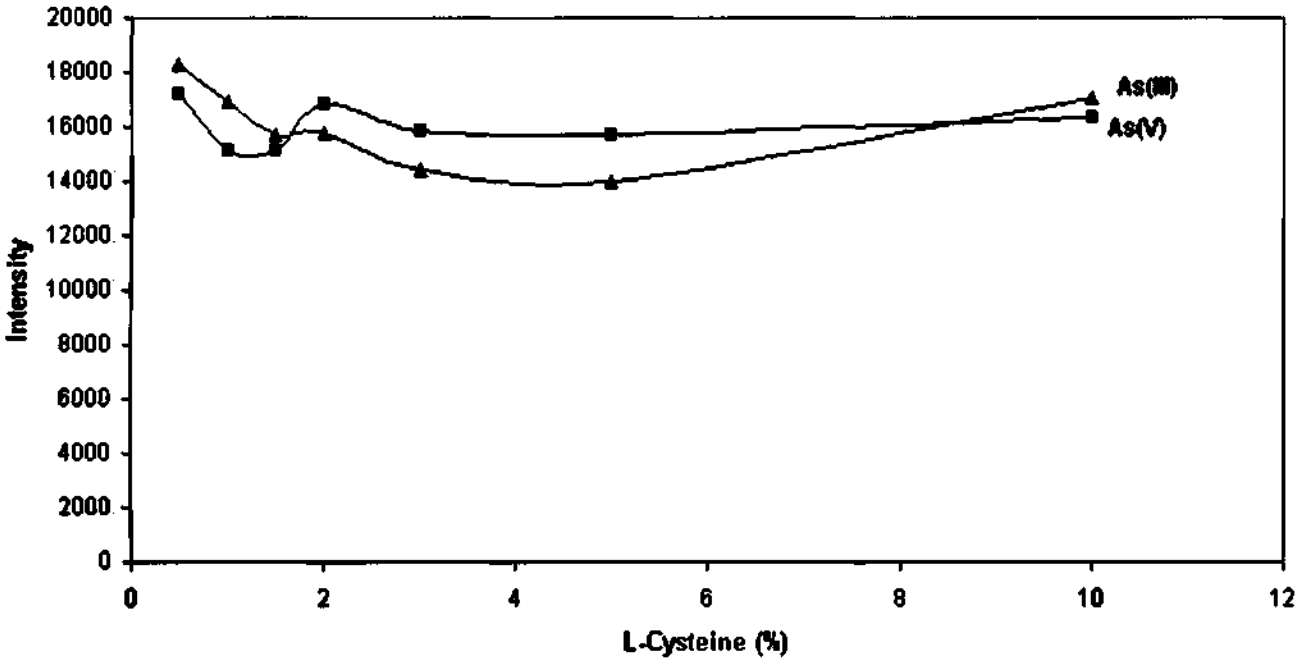

Figure 6.7: Effect of L-Cysteine on the Responses of As(III) and As(V)

HCl

Acid concentration, HCl in this study, is a critical factor in the determination of arsenic by hydride generation. The effect of HCl concentration on the intensities of As(III) and As(V) were studied in the absence and presence of L-cysteine. Hence, these two effects were investigated separately by using 2% $NaBH_4$, and $NaBH_4$ and 0.5% L-cysteine.

Absence of L-Cysteine

The use of hydride generation for arsenic speciation is pH dependent. The results in Figure 6.8 show the effect of HCl concentration on intensities of As(III) and As(V). It can be seen that the intensities of As(III) and As(V) increase with the increase of HCl concentration and reach a maximum at about 1 M for As(III) and 2 M for As(V). This result was similar in trend to that reported by R.K. Anderson[9] and X.C. Le et al.[8] As

can be seen clearly from the results in the figure, the optimum responses or intensities exhibited by As(III) and As(V) show differently on the HCl concentration. That is, there is no a single HCl concentration at which the two species show equal intensities.

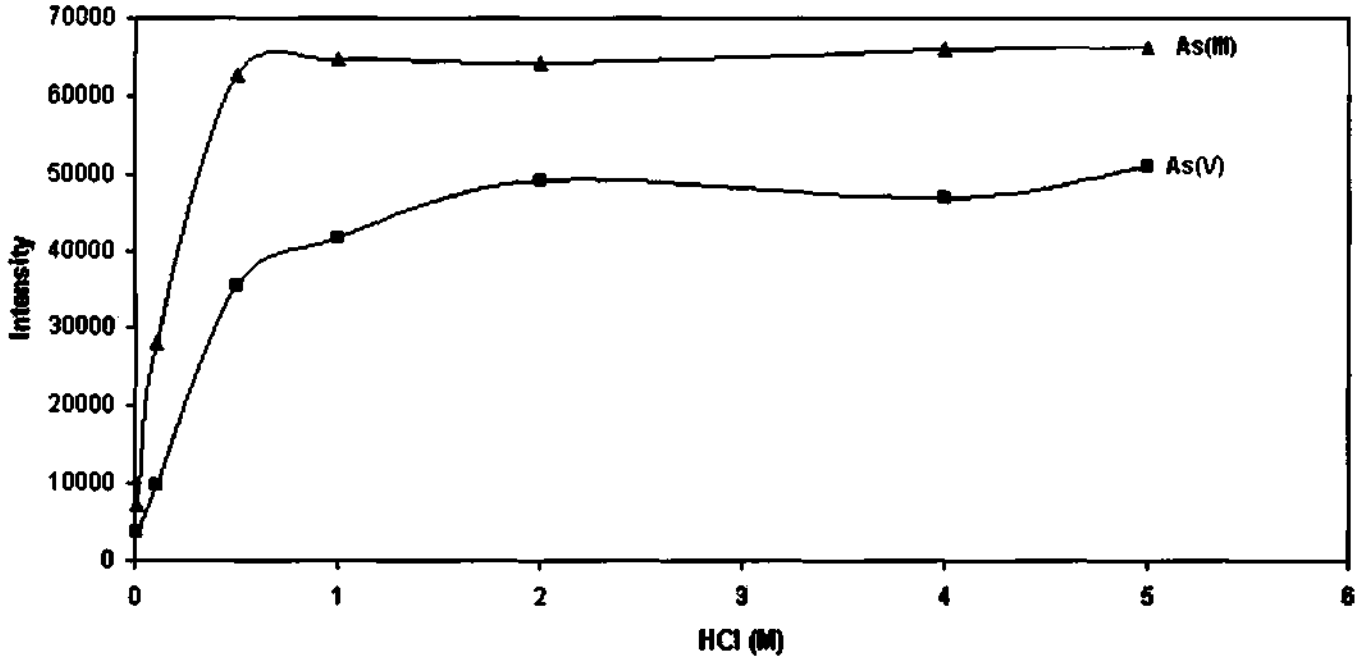

Figure 6.8: Effect of HCl (Without L-Cysteine) on the Responses of As(III) and As(V)

These results and those reported[9,124] show that each arsenic species has its own HCl requirement for achieving optimal response.

Moreover, Figure 6.8 shows the intensity of As(V) is lower than that of As(III) suggesting that arsine formation from As(V) is kinetically slow and the intensity being increased at higher HCl concentration to a level slightly less than that of As(III).

Presence of 0.5% L-Cysteine

The hydride generation conditions in the presence of L-cysteine are different from those in the absence of L-cysteine. In this study, the intensities of As(III) and As(V) were investigated in the presence of 0.5% L-cysteine and the results are shown in Figure 6.9. From the figure, the optimum HCl concentration is reached at about 0.1 M.

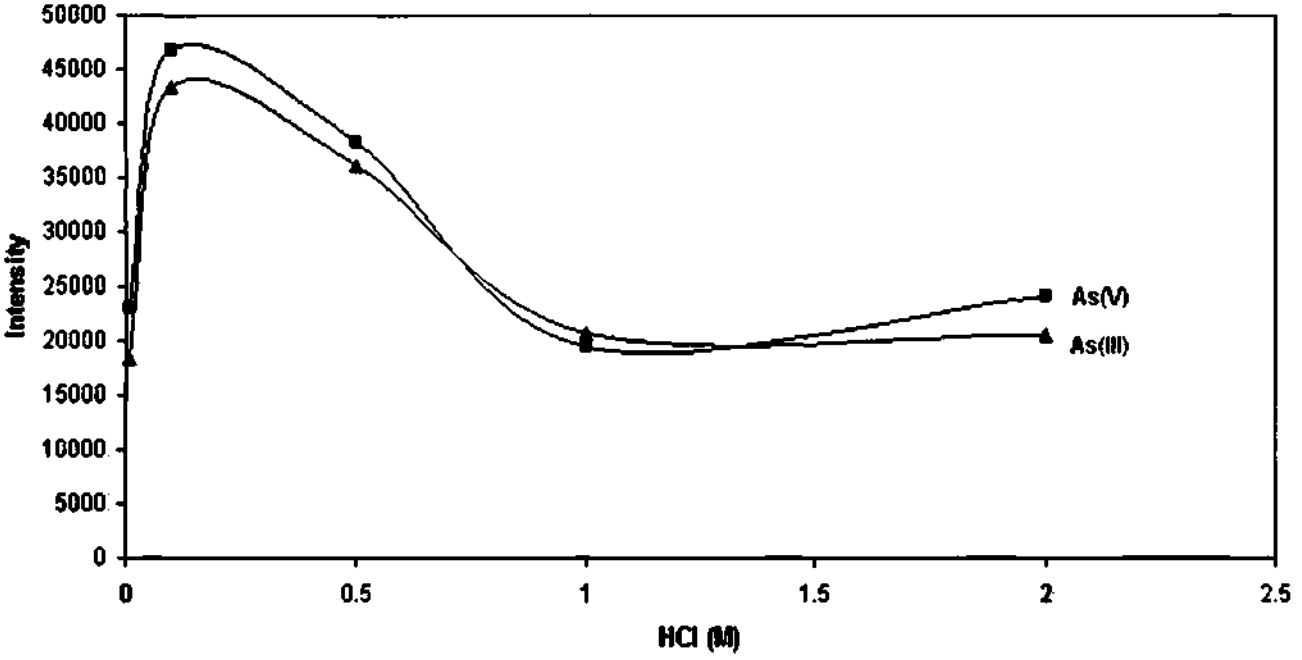

Figure 6.9: Effect of HCl in the Presence of 0.5% L-Cysteine on the Responses of As(III) and As(V)

Comparing Figure 6.8 and Figure 6.9, it can be seen that the arsine generated in the presence of L-cysteine reaches a maximum at 0.1 M HCl while in the absence of L-cysteine it is at about 1.0 - 2.0 M. The low acid (HCl) concentration in the presence of 0.5% L-cysteine is advantageous for the ICP-AES. This is due to the small amount of hydrogen produced as compared to the hydrogen released at high HCl concentrations. Moreover, Figure 6.9 clearly shows that the enhancement of the intensities at the low

HCl concentration in the presence of 0.5% L-cysteine were about 55% for As(III) and 380% for As(V) compared to the intensities obtained in the same HCl concentration in the absence of 0.5% L-cysteine. Thus, the error encountered in the absence of L-cysteine can be eliminated in the determination of total arsenic by adding 0.5% L-cysteine prior to hydride generation. This is due to exhibition of almost identical responses (intensities) obtained from the two arsenic species in the low HCl concentration. And this is clearly shown in Figure 6.9. As a result a single arsenic species (As(III)) can be used for calibration. Anderson et al.[9] have obtained identical results using mercaptoacetic acid as a pre-reductant.

Reaction Coil Length

The effect of changing the reaction coil length was studied on the intensities of As(III) and As(V) and the results are shown in the following figure.

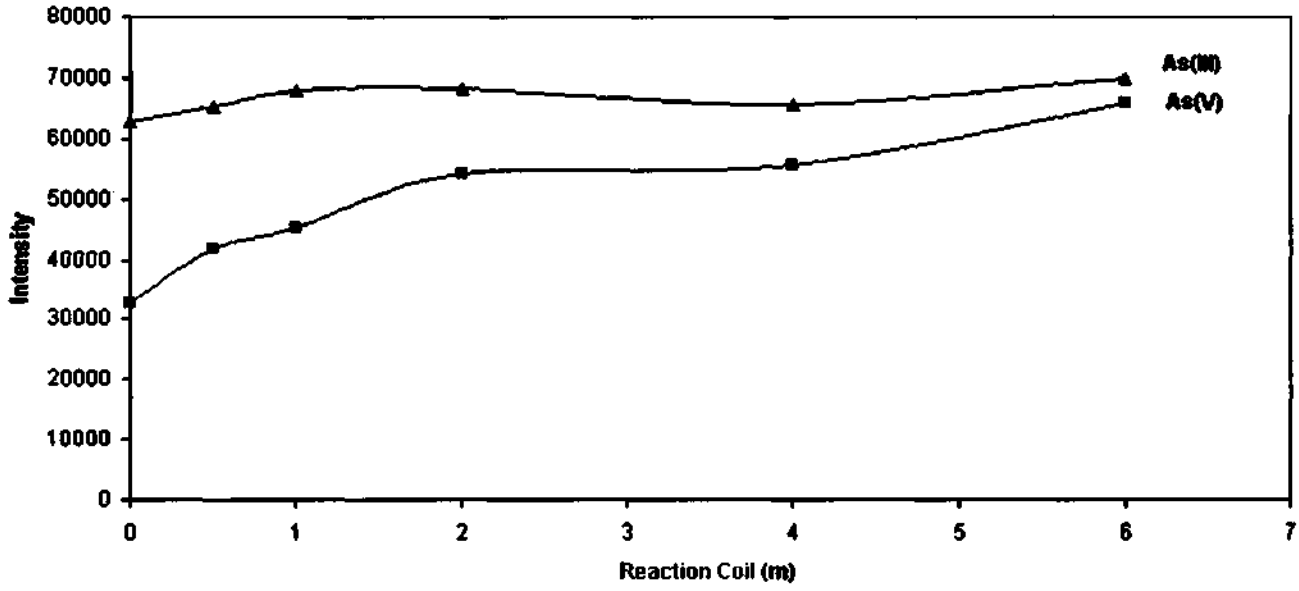

Figure 6.10: Effect of Changing Reaction Coil Length on the Responses of As(III) and As(V)

From the figure, changing reaction coil length did not have as much effect on the intensity of As(III) as on that of As(V). There is a 11% change in signal by changing the reaction coil from no reaction coil to 6 m reaction coil for As(III) whereas it 102% change for As(V). This is due to the very fast hydride forming reactions that occur with As(III) and the slow reaction that occurs in reducing As(V) to As(III) which precedes the hydride generation reaction. Even though pentavalent arsenic shows high sensitivities using few meters long reaction coil, in this study it was preferred to reduce As(V) to As(III) prior to hydride generation and short coil lengths of about 10 cm was used. This short coil has the advantage of suppressing interferences as hydride formation is usually fast reaction and the use of short reaction coil suppress interference which reacts slowly with $NaBH_4$ slowly.

Reaction Time

A test solution of 75 μg/l of As(V) was made to react with 0.5% L-cysteine by standing at different times before the solution was reduced by $NaBH_4$. Figure 6.11 shows the effect of reaction time on the intensity of As(V) using 0.5% L-cysteine and less than 10 cm reaction coil.

There is an increase of about 190% in intensity when the arsine generated was compared between the instant (almost zero standing time) reaction and standing for about one (1) hour. Hence, one (1) hour was chosen for the standing time before the solution was made to react with $NaBH_4$ as there was not much improvement in intensity in lengthening the standing time beyond one (1) hour.

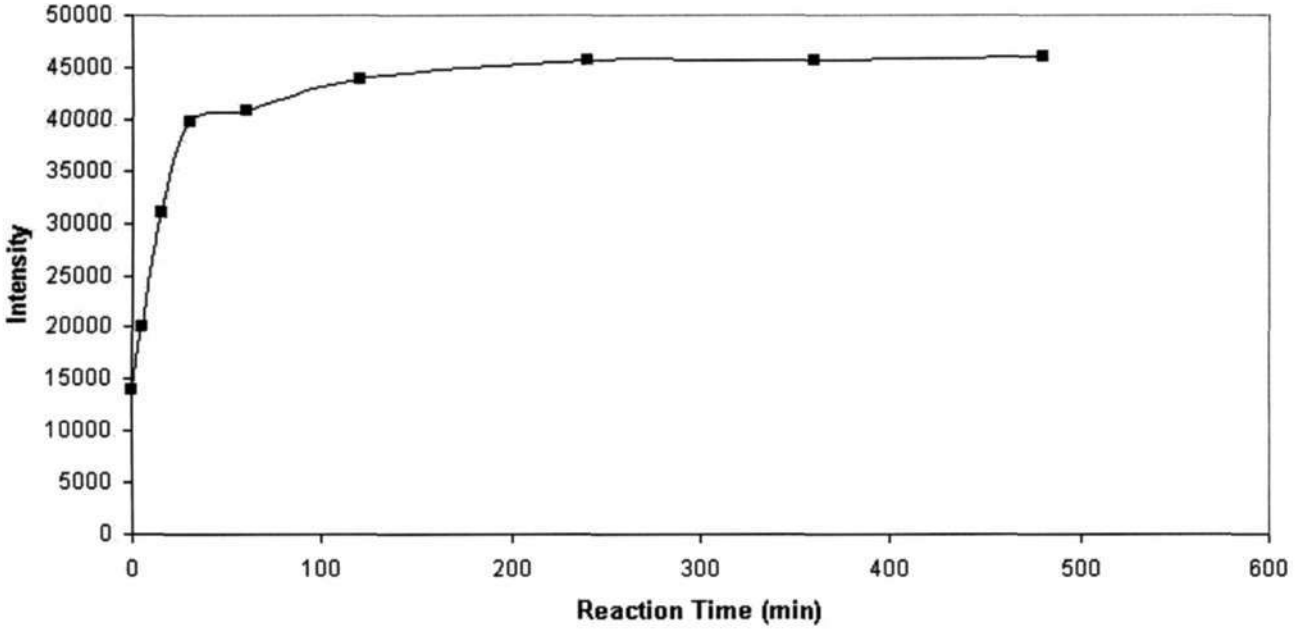

Figure 6.11: Effect of Reaction Time on the Response of As(V)

A summary of the optimized operating conditions for the HG-ICP-AES and ICP-AES were given in Table 6.4.

Table 6.4: Operating Conditions of the HG-ICP-AES System

ICP-AES	
ICP Spectrometer	Varian Libery 150 AX Turbo
Power, kW	1.00
Ar Plasma Flow, L/min	15.0
Auxiliary Ar Flow, L/min	1.50
Photomultiplier, V	800
Integration Time, sec	1
Nebulizer, kPa	240
Wavelength, nm	193.7
Hydride Generation	
$NaBH_4$ Concentration, %m/v	2
$NaBH_4$ Flow Rate, ml/min	1.60
Sample Flow Rate, ml/min	2.40
L-Cysteine Concentration, %m/v	0.5

Buffered Reaction Media

The effect of pH on the arsine generation from As(III) and As(V) was investigated by recording intensity obtained from the test solution (75 μg/l As(III) and As(V)) in a series of buffered solutions. But, from previous works[106,107,114,121,123] it can be seen that a wide range of buffers, pHs and reducing media were used and therefore there is no standard matrix or constant pH which is employed for the determination of As(III) in the presence of As(V).

Hence, in this study two (2) buffer solutions were tested for the selective reduction of As(III) using the continuous hydride generation method with less interference from As(V) using 2% $NaBH_4$ stabilized with 0.1 M NaOH. Referring to Figure 5.1 which

shows a speciation diagram for arsenic, consideration was given for the pH ranges of 1-6 in this work.

The two buffer solutions studied were acetic acid - acetate and citric acid - citrate solutions.

Sodium Acetate - Acetic Acid Buffered Reaction Media

The responses of As(III) and As(V) in the acetate buffer are shown in Figure 6.12. At low pH, both arsenic species show high intensities which decrease with the increase of pH. Especially, arsine generation from As(V) falls continuously with increasing pH (up to pH 4.8) and at around pH 4.8 it becomes negligible with respect to that of As(III). The reason for this is, as pH is increased the rate of reaction As(V) $\longrightarrow$ As(III) (reduction) decreases until it becomes much slower than the hydrolysis of $NaBH_4$.[106] Similarly, As(III) intensity decreases at higher pH due to the hydrolysis of alkaline $NaBH_4$ and subsequently consuming the protons.

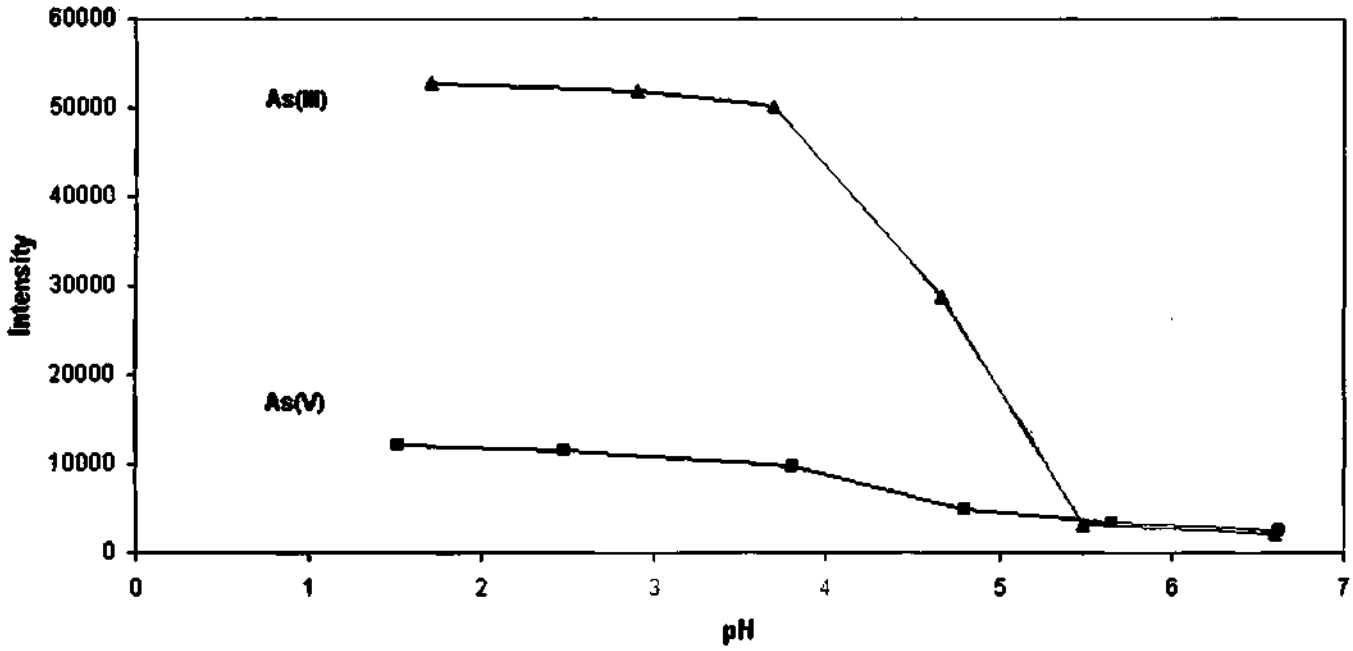

Figure 6.12: Effect of Acetate Buffering on the Responses of As(III) and As(V)

Sodium Citrate - Citric Acid Buffered Reaction Media

Sodium citrate - citric acid buffered reaction media was also studied with the same procedure as acetate buffered reaction media and the results are shown in Figure 6.13. Note: the data point on Figure 6.13 at pH 4.8 was obtained with citrate concentration of 0.06 M.

From the above two buffered reaction media, sodium citrate - citric acid buffered reaction media was used for analysis of As(III) in seaweed kelp samples maintaining the pH at 4.8.

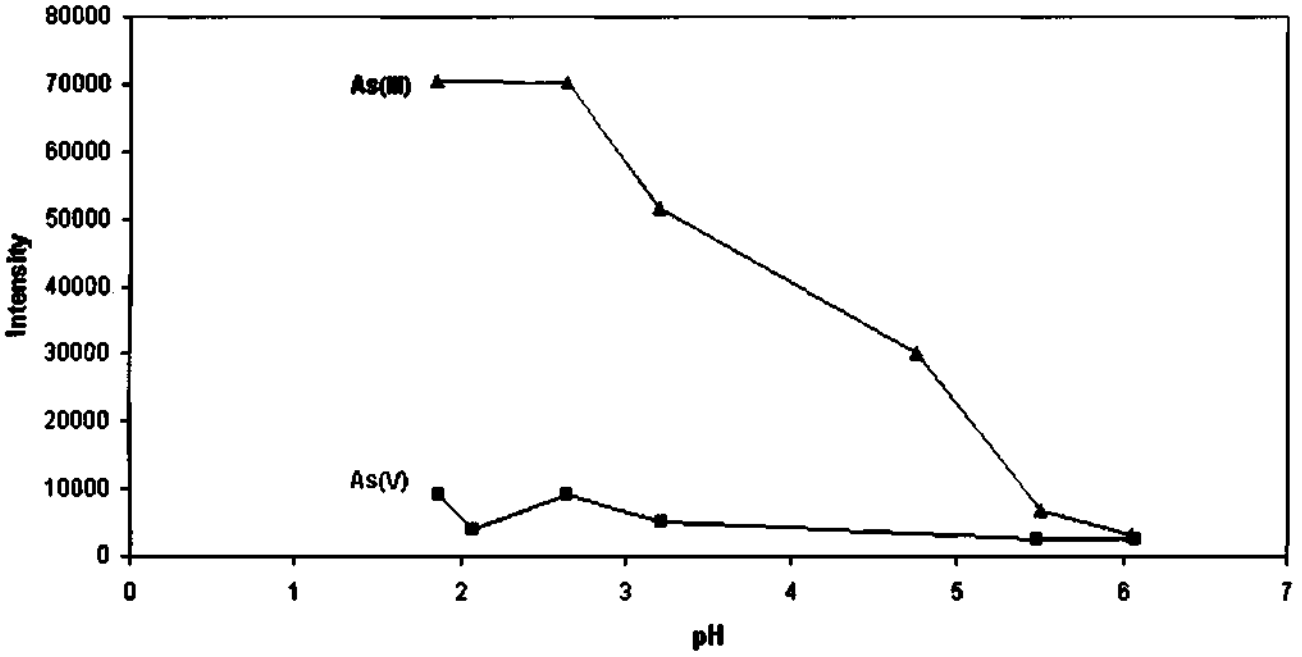

Figure 6.13: Effect of Citrate Buffering on the Responses of As(III) and As(V)

Buffer Concentration

The effect of changing the buffer concentration (that is the sum of the concentrations of citric acid and sodium citrate used to prepare the buffer solution) at pH 4.8 was studied on the signal of As(III) and As(V) and the results are shown in Figure 6.14. Increasing the buffer concentration results in an increment of intensity of As(III) by about 50% and 30% for As(V) from 0.16 M to 0.95 M. A concentration of 0.95 M buffer concentration (0.6 M sodium citrate) was thus chosen.

The increasing of intensity when the buffer concentration was increased was due to the fact that at a higher buffer concentration, the buffer capacity will be increased. The increased buffer capacity will not change its pH appreciably by reacting with alkaline $NaBH_4$. However, at lower buffer concentration the solution became alkaline during the reaction and lower intensity was obtained.

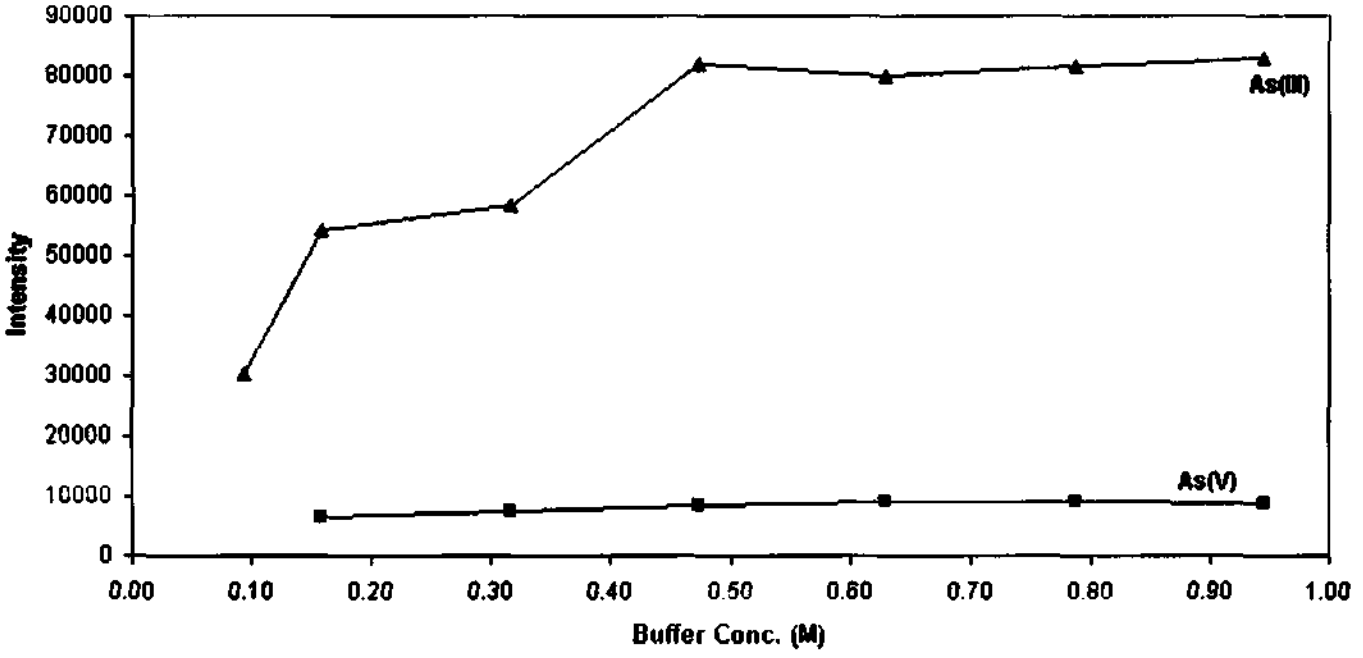

Figure 6.14: Effect of Changing Buffer Concentration on the Responses of As(III) and As(V) at pH 4.8

6.6.2 Interference Study

Even though hydride generation is a relatively simple and sensitive method, it suffers interferences that arise from the presence of species (especially transition metal ions) in the sample solution. These species change the signal by consuming the reductant, slowing the reduction kinetics and/or by forming volatile hydrides other than arsenic.

An experiment was performed to discover the extent to which the proposed HG-ICP-AES method is susceptible to interferences using five (5) selected elements. The elements studied were: Cu(II), Ni(II), Mn(II), Fe(III) and Se(IV). The reason for choosing these elements was that, as previously indicated in the literature (chapter 2), Cu(II)/Mn(II)/Fe(III) are some of the many constituents in seaweed and Cu(II), Ni(II) Fe(III) and Se(IV) are the most severe interferents that are encountered in real

samples in the hydride generation method.[148] The interferences were examined without L-cysteine, in the presence of 0.5% L-cysteine, in the buffered reaction media and using the masking agent EDTA.

In this work, 0.1, 10 and 100 ppm solutions of the interferents were prepared from their chlorides or oxides (see section 6.2) and an appropriate quantity of As(III) solution was added to give a final As(III) concentration of 75 μg/l. The signals obtained were compared with those where no interfering ion had been added. The results obtained for As(III) are shown in Table 6.5 as % deviation of the interference free response for interfering ion with and without L-cysteine and with the masking agent whereas Table 6.6 shows results for the buffered reaction media. As most previous work[9] shows, if the variation in the measurement is more than $\pm$10% then it will be considered due to the interference effect of the element.

From Table 6.5 it can be seen that there was severe interference observed from Cu(II), Fe(III) at 10 ppm and 100 ppm concentration, while Ni(II) interfere in all concentration ranges studied in the absence of L-cysteine. However, no interference was observed from Se(IV) and Mn(II) up to 10 ppm concentration of the interferent.

When L-cysteine was used, interefernces from Cu(II), Mn(II), Ni(II) and Fe(III) were reduced. This is in agreement with Hengwu Chen et al.[7] who found the reduction of interference in the presence of L-cysteine in the hydride generation process for the determination of arsenic. This study also supports the view that the presence of L-cysteine reduces interference from some elements. But, in case of Se(IV), the interferent effect was increased in the presence of L-cysteine.

Even though interferences are reduced by different reagents, the mechanism of the interference reduction is not clear. Welz and Melcher[109,149,150] proposed a mechanism that interference reduction is related to the precipitation of the interfering element that occur when the transition metal ion react with $NaBH_4$ formed prior to complete re-

Table 6.5: Effect of Metal Ions on As(III) (75 μg/l)

		Deviation (As(III), %)[a]		
Interferent	Conc. (ppm)	No L-Cysteine	0.5% L-Cysteine	0.02 M EDTA
Cu(II)	0.1	0	- 1	- 12
	10	- 35	- 12	- 19
	100	- 50	- 13	- 22
Ni(II)	0.1	- 38	- 2	- 65
	10	- 83	- 11	- 68
	100	- 87	- 75	- 85
Se(IV)	0.1	0	- 3	- 29
	10	- 2	- 58	- 23
	100	- 11	- 32	- 32
Fe(III)	0.1	+ 4	0	- 7
	10	- 84	- 2	- 14
	100	- 88	- 20	- 10
Mn(II)	0.1	0	0	- 18
	10	- 10	+ 6	- 20
	100	- 21	+ 7	- 29

[a]Results are average of 3 replicates

duction of arsenic species. The precipitate then adsorbs and decomposes the hydride formed. However, Brown et al[151] and Yamamoto et al[152] suggested that interfering ions react with $NaBH_4$ and form metal borides, not the elemental metal. On the other hand, Aggett and Hayashi[153] suggested that interferences occur in solution via the formation of a soluble species formed between interferent in low oxidation state, stabilized by the $NaBH_4$, and arsine.

The enhancement of signal and reduction of interference by L-Cysteine was studied by I.D. Brindle and X.C. Le.[154] In one mechanism they suggested that there is no reaction between L-Cysteine and the transition metals but they noted a reaction occuring between L-Cysteine and $NaBH_4$. In this reaction, the thiol group of L-Cysteine reacts with $NaBH_4$ which was used to enhance the signal and reduced the interference. The proposed reaction between L-Cysteine and $NaBH_4$ is:

$$BH_4^- + HS - R \rightarrow BH_3 - S - R^- + H_2 \tag{6.1}$$

They suggested that the intermediate complex may be more efficient in hydride forming reaction.

A masking agent, EDTA, was studied for its ability to reduce the interference. As can be seen from the results of Table 6.5, in the presence of 0.02 M EDTA there was a decrease in interference from Cu(II) and Fe(III) from 10 ppm to 100 ppm. But for the rest of the elements there was no decrease in the level of the interference in the presence of EDTA.

In the citric acid - citrate matrix, interference increases with the increase of concentration in the species studied in the absence of EDTA. In the presence of 0.02 M EDTA, interference from Cu(II), Ni(II), Fe(III) and Mn(II) decrease in all concentration ranges studied. This is due to EDTA is complexing the transition elements (Cu(II), Ni(II),

Table 6.6: Effect of Metal Ions on As(III) (75 μg/l) on a Citrate Buffered Medium

		Deviation (As(III), %)[a]	
Interferent	Conc. (ppm)	No EDTA	0.02 M EDTA
No Cu(II)	0	0	+ 5
Cu(II)	0.1	- 5	+1
	10	- 6	- 2
	100	- 20	- 6
No Ni(II)	0	0	+ 6
Ni(II)	0.1	- 5	+ 2
	10	- 8	- 1
	100	- 12	- 4
No Se(IV)	0	0	+ 4
Se(IV)	0.1	- 6	- 3
	10	- 10	- 11
	100	- 18	- 16
No Fe(III)	0	0	+ 6
Fe(III)	0.1	- 5	+ 1
	10	- 20	- 4
	100	- 38	- 10
No Mn(II)	0	0	+ 5
Mn(II)	0.1	- 4	+ 1
	10	- 9	- 3
	100	- 19	- 8

[a]Results are average of 3 replicates

Fe(III) and Mn(II)) . But Se(IV) shows interference at 10 and 100 ppm concentration.

The following table shows the formation constants, α_4 and conditional formation constants for the interferences (Cu(II), Ni(II), Fe(III) and Mn(II)) studied. In Appendix 2 the calculations used are shown.

Table 6.7: Values for EDTA Complexes with Interferents

Cation	K_{MY}	α_4	K'_{MY}
Cu(II)	6.3×10^{18}	1.5×10^{-7}	9.5×10^{11}
Ni(II)	4.2×10^{18}	1.5×10^{-7}	6.3×10^{11}
Fe(III)	1.3×10^{25}	1.5×10^{-7}	2.0×10^{18}
Mn(II)	6.2×10^{13}	1.5×10^{-7}	9.3×10^{6}

In conclusion, the low level of these and other interfering elements in the seaweed kelp means that they are unlikely to cause analytical problems. In this work L-cysteine (which provides milder conditions) and short reaction coil were used in the analysis of samples to reduce interferences. Short reaction coil was used to favour the very fast hydride generation reaction, while suppressing the interferent that reacts slowly with the reductant.

The experimental results that was used to draw the graphs for optimization of parameters and interference studies were given in Appendix 2.

6.6.3 Method Performance

The method developed in this study was checked for its performance by investigating the detection limit (limit of detection and limit of quantification) and precision.

Detection Limit

The limit of detection (LOD), the lowest concentration level that can be determined to be statistically different from a blank is defined as: LOD = 3SD/m, where m is

the slope of the calibration graph, corresponding to 99% confidence level and SD is the standard deviation of blank measurements. The limit of quantification (LOQ) is defined as the level above which quantitative result can be obtained with a specified degree of confidence. LOQ = 10SD/m. Based on these definitions the detection limit for the As(III) in 0.1 M HCl and As(III) in 0.95 M buffer concentration (0.6 M citrate buffered media) based on three (3) times the standard deviation of twenty (20) determinations of blank solution and under the operating conditions specified in Table 6.4 were LOD 0.6 μg/l and LOQ 2 μg/l for As(III) in 0.1 M HCl and LOD 0.8 μg/l and LOQ 3 μg/l for As(III) in 0.6 M sodium citrate buffered reaction media.

In Table 6.8, the results of this study were compared with the previous results for the detection limit of HG-ICP-AES systems as well as for the conventional nebulization. But, due to the wide range of hydride generation generators, differences in the optimized experimental conditions and confusion over the definition of the term detection limit, the comparison of the results might not be conclusive.

Table 6.8: Comparison of Detection Limits (μg/l)

	Solution Nebulization	HG- ICP-AES	Reference
As(III), 0.1 M HCl	40	0.6	This Work
As(III), 0.6 M Citrate	40	0.8	This Work
As	770	12.5	211
As	40	0.02	168
As(III)	-	0.62	200
As(III) + As(V), HCl	-	0.2 - 0.6	151
As(III) + As(V), Merc.[a]	-	0.3 - 0.7	151
As(III), Acetate	-	0.3 - 0.7	151
As(III), Citrate	-	0.3 - 0.7	151

[a]Mercaptoacetic acid matrix

Precision

The within-run precision (relative standard deviation) of the method developed was studied for 10 μg/l of twenty (20) determinations of As(III) in 0.1M HCl and the value was 4 %. Published values for overall relative standard deviation (RSD) ranges between 1 to 5 % and sometimes to 10 %.

Calibration Curve

Table 6.9 shows a typical calibration curve parameter for As(III) under the selected conditions of HG-ICP-AES found using this study.

Appendix 3 gives experimental results that were employed to calculate method performance of the HG - ICP - AES system.

Table 6.9: Calibration Parameters for As(III)(0.005 - 0.11 mg/l)

Parameter	As(III)
Slope (Sensitivity)	302 225
Standard error of slope	8 900
Intercept	2 270
Standard error of intercept	532
Regression Coefficient (r)[a]	0.9974

[a]Based on calibration with seven standards

6.6.4 Sample Analysis

The developed HG-ICP-AES method was employed for the analysis of two (2) types of commercial seaweed kelp tablets using the three digestion procedures. Also, two different calibration methods were used - the method of external standards and the method of standard additions. The following paragraphs explain the different calibration methods. Recovery efficiencies of arsenic for the three sample digestion methods were determined by spiking the samples.

(A) Microwave Digested Samples

The two types of seaweed kelp tablets were digested using the microwave digestion procedure as outlined in section 6.5. The samples were analysed using the developed method of HG-ICP-AES using:

External Standard Calibration Method

Six standard solutions (0.01, 0.03, 0.05, 0.07, 0.09 and 0.11 mg/l As(III)) were prepared and analysed using HG-ICP-AES to produce a calibration curve. The curve had a correlation coefficient (R^2) of 0.9979. Twelve replicates of each sample of Tablet A and

Tablet B were analysed using the standard calibration method. The results that were calculated by linear calibration curve are shown in Appendix 4.

Standard Addition Method

Additions of 0.0, 0.5, 1.0, 1.5, 2.0 and 2.5 ml of 0.8383 mg/l As(III) were made to solutions of Tablet A and Tablet B samples - the final volume in each case being 10 ml. Six replicate intensities were measured using HG-ICP-AES. Arsenic concentrations for the six replicate samples of Tablet A and Tablet B were calculated - see Appendix 4.

Recovery

Spike recoveries were studied on samples of Tablet A and Table B by spiking with enough As(III) to increase the concentration by 0.01 mg/l.

(B) Dry-Ashed Samples

Dry-ashed samples of Tablet A and Tablet B were used to analyse for arsenic content. See section 6.5 for the dry ashing procedure.

External Standard Calibration Method

Five standard solutions (0.005, 0.01, 0.03, 0.05 and 0.07 mg/l As(III)) and six standard solutions solutions (0.005, 0.01, 0.03, 0.05, 0.07 and 0.09 mg/l As(III)) were prepared and analysed using HG-ICP-AES to produce a calibration curve. The curve had a correlation coefficient (R^2) of 0.9997. Thirty replicates of each sample of Tablet A and Tablet B were analysed using the standard calibration method. The results that were calculated by linear calibration curve are shown in Appendix 4.

Recovery

Spike recoveries were studied on samples of Tablet A and Table B by spiking with enough As(III) to increase the concentration by 0.01 mg/l.

(C) Wet Digested Samples

Tablet A and Tablet B were digested using wet digestion procedure (section 6.5).

External Standard Calibration Method

Seven standard solutions (0.005, 0.01, 0.03, 0.05, 0.07, 0.09 and 0.11 mg/l As(III)) were prepared and analysed using HG-ICP-AES to produce a calibration curve. The curve had a correlation coefficient (R^2) of 0.9959. Eighteen replicates of each sample of Tablet A and Tablet B were analysed using the standard calibration method. The results that were calculated by linear calibration curve are shown in Appendix 4.

Standard Addition Method

Additions of 0.0, 0.25, 0.5, 0.75, 1.0 and 1.25 ml of 0.8383 mg/l As(III) were made to solutions of Tablet A and Tablet B samples - the final volume in each case being 5 ml. Six replicate intensities were measured using HG-ICP-AES. Arsenic concentrations for the six replicate samples of Tablet A and Tablet B were calculated - see Appendix 4.

Recovery

Spike recoveries were studied on samples of Tablet A and Table B by spiking with enough As(III) to increase the concentration by 0.01 mg/l.

Citrate - Buffered Reaction Media

A study for the analysis of As(III) was also conducted on the wet-digested samples using citrate buffered reaction media. Five standard solutions (0.005, 0.01, 0.03, 0.05 and 0.07 mg/l As(III)) were used to construct a calibration curve. Using the calibration curve, samples of Tablet A and Tablet B were analysed for As(III) by HG-ICP-AES for As(III) (see Appendix 4 for results).

Summary of Results

In all three cases a low recovery was attained, which might be due to the complex matrix of the sample. The mean concentrations determined by each of the digestion procedures (see Table 6.10) reveal differences in efficiencies in the procedures. Concerning dry ashing, a much lower arsenic value is to be expected due to possible losses by volatilization of arsenic species. On the other hand, volatilization losses would be expected to be less important with wet digestion procedures due to the lower temperatures used. Furthermore, microwave digestion is carried out in closed vessels which means that volatilization losses will be even less important. Another important outcome of using a closed digestion vessel is that the elevated temperatures attainable increase the rate of digestion. Also, another advantage of closed vessels is that the possibility of contamination is decreased.

The results show that, in each case, the microwave procedure gave the highest arsenic concentration. The next highest arsenic concentrations were found using the wet digestion procedure and the lowest concentrations were found from dry ashing. This order manifests the relative efficiencies of the procedures with microwave assisted digestion being the most efficient.

Another advantage of the microwave assisted digested procedure is its speed. A 30 minute cycle was used for microwave digestion (see Appendix 1 for details) whereas

more than 5 hours was required by the wet digestion method.

Moreover, as well as being very safe, rapid, convenient and less prone to contamination, microwave digestion avoids the close and constant operator attention which is required by the wet digestion method.

A study for the analysis of As(III) was also conducted on the wet-digested samples using the citrate buffered reaction medium. In this way the fraction of total arsenic existing as As(III) was found. The following table shows the results. From the results, 50 % of the arsenic in tablet A is shown to be As(III) and 32 %of the arsenic in tablet B is shown to be As(III).

Table 6.10: Experimental Results for Citrate Buffered Media for As(III)(in μg/g) in Wet Digested Kelp Tablets

Sample	Mean	Range
Tablet A	11.44	12.02 - 10.86
Tablet B	11.74	12.09 - 10.93

Table 6.11: Experimental Results for the Two Commercial Seaweed Kelp Tablets for Total As (in μg/g) and Recoveries (%) in the Three Digestion Procedures

	Microwave Digestion					Dry Ashing			Wet Digestion				
	Std. Calib. [a]		Std. Add. [b]			Std. Calib.			Std. Calib.		Std. Add.		
	Mean [c]	Range	Mean [d]	Range	Rec.[e]	Mean [f]	Range	Rec.	Mean [g]	Range	Mean[e]	Range	Rec.
Tablet A	**56.36**	59.67-50.78	**81.90**	84.03-77.81	56	**4.63**	5.65-3.69	56	**22.71**	25.94-19.45	46.44	47.48-44.72	64
Tablet B	**81.34**	92.69-74.21	**93.69**	97.88-91.13	63	**5.95**	6.39-5.44	65	**37.15**	48.31-26.06	59.40	66.87-54.39	63

[a]Results based on standard calibration curve
[b]Results based on standard addition method
[c]n = 12
[d]n = 6
[e]Recovery
[f]n = 30
[g]n = 18

6.7 Conclusion

A HG-ICP-AES method was used for the determination of arsenic in commercial seaweed kelp tablets. The method was developed by optimizing $NaBH_4$, HCl, L-cysteine, reaction coil length, reaction time and buffered reaction media. The study has shown that in the presence of the pre-reducing agent L-cysteine, the concentration of HCl was optimal at lower concentration than when L-cysteine was absent. This has advantage to ICP as reduced amount of hydrogen was generated. Moreover, there was an increase in signal at the low HCl concentration in the presence of L-Cysteine. L-cysteine has also the effect of decreasing interference in the hydride generation procedure from some elements.

A commercial seaweed kelp tablet was digested using three digestion procedures namely, microwave-assisted, dry ashing and wet digestion for the determination of arsenic using the developed method. The results show that the efficiency of digestion in microwave-assisted was the highest while that of dry ashing was the lowest.

Trivalent arsenic at pH 4.8 in 0.95 M buffer concentration (0.6 M sodium citrate) was determined from the samples that were digested using the wet digestion method and the result show 32 - 50% of the total arsenic were As(III).

The performance of the method was investigated for detection limit and precision (RSD%) which gave the result 0.6 μg/l (0.6 μg/g, 0.2 μ/g and 0.3 μ/g in the seaweed tablet that was digested using microwave, dry and wet digested samples respectively) and 4 respectively for As(III) in 0.1 M HCl reaction media. The developed method gave low recovery values for the 10 μg/l As(III) added spike in the microwave, dry ashing and wet digested samples.

References

[1] G. Lunde, *J. Sci. Food Agr.*, 1970, **21**, 242

[2] D. Beauchemin, K.W.M. Siu, J.W. McLaren and S.S. Berman, *J. Anal. At. Spectrom.*, 1989, **4**, 285

[3] M. Burguera and J.L. Burguera, *Talanta*, 1997, **44**, 1581

[4] T, Maruta and G. Sudoh, *Anal. Chim. Acta*, 1975, **77**, 37

[5] M. Burguera, J.L. Burguera, M.R. Brunetto, M. de la Guardia and A. Salvador, *Anal. Chim. Acta*, 1991, **161**, 105

[6] M. Burguera and J.L. Burguera, *J. Anal. At. Spectrom.*, 1993, **8**, 229

[7] H. Chen, I.D. Brindle and X.C.Le, *Anal. Chem.*, 1992, **64**, 667

[8] X.C. Le, W.R. Cullen and K.J. Reimer, *Anal. Chim. Acta*, 1994, **285**, 277

[9] R.K. Anderson, M. Thompson and E. Culbard, *Analyst*, 1986, **111**, 1153

[10] S. Surey - Gent and G. Morris, *Seaweed: A User's Guide*, Whittet Books, London, 1987

[11] C.S. Lobban, P.J. Harrison and M.J. Duncan, *The Physiological Ecology of Seaweeds*, Cambridge University Press, London, 1985

[12] H.G. Levine, *The Use of Seaweeds for Monitoring Coastal Waters, in Algae: As Ecological Indicators*, (ed. L.E. Shuber), Academic Press, London, 1984, pp. 189

[13] G.S. Campbell, *An Introduction to Environmental Biophysics*, Springer - Verlag, New York, 1977, 159pp

[14] J.A. DeBoer, *Nutrients, in The Biology of Seaweeds*, (eds. C.S. Lobban and M.J. Wynne), Blackwell Scientific Publications, Oxford, 1981, pp. 356

[15] M.J. Atkins and S.V. Smith, *Limnol. Oceanogr.*, 1983, 28, 568

[16] Environmental Health Critrea 18, *Arsenic*, World Health Organization, Geneva, 1981

[17] S. Bonotto, Cultivation of Plants. 2. Multicellular Plants, *In Marine Ecology*, (ed. O. Kinne), John Wiley, London, 1976, **Vol. III**, pp. 467

[18] T.A. Norton, A.C. Mathieson and M.Neushul, *Morphology and Environment*, in *The Biology of Seaweeds*, (eds. C.S. Lobban and M.J. Wynne), Blackwell Scientific Publications, Oxford, 1981, pp. 421

[19] J.R. Waaland, *Commercial Utilization*, in *The Biology of Seaweeds*, (eds. C.S. Lobban and M.J. Wynne), Blackwell Scientific Publications, Oxford, 1981, pp. 726

[20] F.C. Nachod and J.J. Zucherman, *Chemistry of the Non-Metals*, Walter de Gruyter, Berlin, 1977, 99. 307

[21] W.R. Cullen and K.J. Reimer, *Chem. Rev.*, 1989, **89, No. 4**, pp. 713

[22] A. El Moll, R. Heimburg, F. Lagarde, M.J.F. Leroy and E. Maier, *Fresenius J. Anal. Chem.*, 1996, **354**, 550

[23] W.L. Jolly, *The Chemistry of the Non-Metals*, Prentice - Hall, Englewood Cliffs, N,J., 1966, pp. 88

[24] P.J. Peterson, C.A. Girling, L.M. Benson and R. Zieve, Metalloids, In *Effects of Heavy Metal Pollution on Plants* (ed. N.W. Lepp), Applied Science Publishers, London, 1981, **Vol. 1**, p. 213ff

[25] A. Leonard, Arsenic, In *Metals and Compounds in the Environment* (ed. E. Merian), Weinheim - VCH, 1991, pp. 751

[26] J.C. Bailar, H.J. Emelus, R. Nyholm and A.F.T. Dickenson, *Comprehensive Inorganic Chemistry*, Pergamon, Oxford, 1973, **V-2**, pp 547.

[27] D.C. Chilvers and P.J. Peterson, In *Lead, Mercury, Cadmium and Arsenic in the Environment* (eds. T.C. Hutchinson and K.M. Meema), Wiley, New York, 1987, p.279 [Cited in reference 12]

[28] P.E. Gardiner, *J. Anal. At. Spectrom.*, 1988, **3**, 163

[29] J.C. Van Loon, *Anal. Chem.*, 1979, **51**, 1139A

[30] R. Cornelis and J. De Kimpe, *J. Anal. At. Spectrom.*, 1994, **9**, 945

[31] T.M. Florence, *Talanta*, 1982, **29**, 354

[32] P. Quevauviller, O.F.X. Donard, E.A. Maier and B. Griepink, *Mikrochim. Acta*, 1992, **109**, 169

[33] G.L. Christie, Speciation, In *Environmental Analytical Chemistry* (eds. F.W. Fifield and P.J. Haines), Blackie Academic and Proffessional, London, 1995, pp. 279

[34] J.J. Corr and E.H. Larsen, *J. Anal. At. Spectrom.*, 1996, **11**, 1215

[35] S.H. Ebdon, A.P. Walton and R.W. Ward, *Analyst*, 1988, **113**, 1159

[36] *Medical and Biological Effects of Environmental Pollutants - Arsenic*, National Academy of Sciences, Washington, 1977

[37] D. Heitkemper, J. Creed and J. Caruso, *J. Anal. At. Spectrom.*, 1989, **4**, 279

[38] J.M. Wood, *Science*, 1974, **183**, 1049

[39] R.A. Zingaro and N.R. Bottino, *Biochemistry of Arsenic: Recent Developments* (eds. W.H. Lederer, R.H. Fensterhein), Proceedings of the Arsenic Symposium, Gaithersburg, MD; Van Nostrand Reinhold, New York, 1983

[40] M.L. Magnuson, J.T. Creed and C.A. Brockhoff, *J. Anal. At. Spectr.*, 1996, **11**, 893

[41] K.A. Francesconi and J.S. Edmonds, *Oceanogr. Mar. Biol. Annu. Rev.*, 1993, **31**, 111 [Cited in reference 27]

[42] W.T. Frankenberger, *Environmental Chemistry of Arsenic*, Marcel Dekker, New York, 2002

[43] C.J. Sonderquist, D.G. Crosby and J.B. Bowers, *Anal. Chem.*, 1974, **46**, 155

[44] J.A. Cherry, A.U. Shaikh, D.E. Tallman and R.V. Nicholson, *J. Hydrol.*, 1979, **43**, 373

[45] W. Stumm and J.J. Morgan, *Aquatic Chemistry*, Wiley, New York, 1981, pp. 418

[46] D.R. Turner, M. Whitefield and A.G. Dickson, *Geochim. Cosmochim. Acta*, 1981, **45**, 855

[47] E.A. Crecelius, N.S. Bloom, C.E. Cowan and E.A. Jenne, *Speciation of Selenium and Arsenic in Natural Waters and Sediments: Arsenic Speciation*, Electric Power Research Institute, 1986, **Vol. 2** [Cited in reference 12]

[48] W.S. Broecker and T.H. Peng, *Tracers in the Sea*, Lamont-Doherty Geological Observatory Publications, Columbia University, New York, 1982 [Cited in reference 12]

[49] D. Dryssen and M. Wedborg, In *Chemistry and Biogeochemistry of Estuaries* (eds. E. Olausson and I. Cato), Wiley, New York, 1980, pp. 175

[50] A.I.A. Vogel, *Textbook of Qualitative Chemical Analysis*, Longmans, Green and OC., New York, 1951, pp. 162

[51] P.J. Craig, *Organometallic Compounds in the Environment: Principles and Reactions*, Longman, 1986, pp. 1 - 64, 198

[52] M.D. Baker, W.E. Innis, C.I. Mayfield, P.T.S. Wong and Y.K. Chau, *Environ. Technol. Lette.*, 1983, **4**, 89

[53] B.C. McBride and R.S. Wolfe, *Biochemistry*, 1971, **10**, 4312

[54] P.J. Craig, *Organometallic Compounds in the Environment: Principles and Reactions*, Longman, New York, 1985, pp. 1

[55] A.G. Howard, *Aquatic Environmental Chemistry*, Oxford University Press, New York, 1998, pp. 35

[56] J.R. Kramer and H.E. Allen, *Metal Speciation: Theory, Analysis and Application*, Lewis Publishers, Michigan, 1991

[57] S. Caroli, *Element Speciation in Bioinorganic Chemistry*, John Wiley, New York, 1996, **Vol. 135**

[58] V.P. Evangelou, *Environmental Soil and Water Chemistry: Principles and Applications*, Wiley, New York, 1998, pp. 229

[59] W.R. Penrose, H.B.S. Conacher, R. Black, J.C. Meranger, W. Miles, H.M. Cunningham and W.R. Squires, *Environ. Health Perspect.*, 1977, **19**, 53 [Cited reference in 7]

[60] *Drinking Water and Health*, Safe Drinking Water Committe: Advisory Centre on Toxicology, Assembly of Life Sciences and National Research Council, Publisher National Academy of Sciences, Washington, DC, 1977, pp. 316

[61] D.J.H. Phillips, *Aquat. Toxicol.*, 1990, **16**, 151

[62] R.S. Braman and C.C. Foreback, *Science*, 1973, **182**, 1247

[63] R. Conor, *Metal Contamination of Food*, Applied Science, London, 1980, pp. 123

[64] A.C. Chapman, *On the Presence of Compounds of Arsenic in Marine Crustaceans and Shell Fish*, 1926, 448 - 563

[65] A.J. Jones, *Yearb. Pharm.*, 1922, 388 (Chem. Abstr., 1922, 3932) [Cited reference in 57]

[66] J.S. Edmonds and K.A. Francesconi, *Nature* (London), 1981, **289**, 602

[67] J.S. Edmonds and K.A. Francesconi, *J. Chem. Soc. Perkin Trans. 1*, 1983, 2375

[68] M. Morita, T. Uehiro and K. Fuwa, *Anal. Chem.*, 1981, **53**(12), 1806

[69] G.E. Batley and G.K.C. Low, *Application of High-Performance Liquid Chromatography to Trace Element Speciation Studies, in Trace Element Speciation: Analytical Methods and Problems* (ed. G.E. Batley), CRC Press, Boca Raton, 1989, pp. 1

[70] L.E. Shuber, *Algae: As Ecological Indicators*, Academic Press, London, 1984

[71] X.C. Le, M. Ma and N.A. Wong, *Anal Chem.*, 1996, **68**(24) , 4501

[72] M. Bernhard, F.E. Brinckman and P.J. Sadler (ed.),*The Importance of Chemical "Speciation" in Environmental Processes*, Springer-Verlag, Berlin, 1986

[73] G. Lunde, *Acta Chemica Scand.*, 1972, **26**, 2642

[74] G. Lunde, *Nature*, 1969, **224**, 186

[75] National Council Canada, *Effects of Arsenic in the Canadian Environment*, NRCC, Canada, 1978

[76] D.W. Klump, In *Biological and Environmental Effects of Arsenic* (ed. A.F. Bruce), Elsevier, Amsterdam, 1983

[77] S. Sakao, Y. Ogawa and H. Urchida, *Anal. Chim. Acta*, 1997, **355**, 121

[78] V. Vlachos, A.T. Critchley, T.E. Bannatyne and A. Von Holy, *S. Afr. J. Bot.*, 1998, **64**(4), 223

[79] J.C. Van Loon and R.R. Barefoot, *Analyst*, 1992, **117**, 563

[80] Y. Shibata, M. Morita and J.S. Edmonds, *Agric. Biol. Chem.*, 1987, **51**, 391

[81] J.S. Edmonds, M. Morita and Y. J. Shibata, *Chem. Soc., Perkin Trans. 1*, 1987, 577

[82] W.A. Campell, *Chemistry in Britain*, 1965, **1**(5) 198

[83] A.S. Taylor, *Med. Times and Gaz.*, 1854, new ser. **8** 326 [Cited in reference 76]

[84] J.J. Berzelius, *Ann. Chem.*, 1837, **42**, 159 [Cited in reference 76]

[85] J. Hume, *Phil. Mag.*, 1809, **33**, 401 [Cited in reference 76]

[86] J. Marsh, *Edinb. Phil. J.*, 1836, **21**, 229; London Med. Gaz., 1836, 650 [Cited in reference 76]

[87] H. Reinsch, *J. Prakt. Chem.*, 1841, **24**, 244; *Phil. Mag.*, 1841, 3rd ser. **19**, 481 [Cited in reference 76]

[88] H. Gutzeit, *Pharm. Ztg*, 1879, **24**, 263 [Cited in reference 76]

[89] Y. Talmi and D.T. Bostick, *Journal of Chromatographic Science*, 1975, **13**, 231

[90] Ph. Quevauviller, E.A. Maier and B. Griepink, Quality Control of Results of Speciation Analysis, In *Element Speciation in Bioinorganic Chemistry* (ed. S. Caroli), John-Wiley, New York, 1996, **Vol. 135**, pp. 195

[91] Ph. Quevauviller, *Analyst*, 1995, **120**, 597

[92] S.J. Den, W.C. Wei, J. Mierzwa and M.H. Yang, *Journal of the Chinese Chemical Society*, 2002, **49**, 197

[93] G.E. Batley, T*race Element Speciation: Analytical Methods and Problems*, CRC Press, Boca Raton, Florida, 1989

[94] J.E. Portmann and J.P. Riley, *Anal. Chim. Acta*, 1964, **31**, 509

[95] M. Sperling, In *Encyclopedia of Analytical Chemistry: Applications, Theory and Instrumentation* (ed. R.A. Meyers), Wiley, Chichester, 2000, **Vol. 3**, pp. 2441

[96] T.T. Gorsuch, *Analyst*, 1959, **84**, 135

[97] R.J. Evans and S.L. Bandemer, *Anal. Chem.*, 1954, **26**, 595

[98] L. Ebdon and A. Fisher, Organometallic Compound Analysis in Environmental Sample, In *Encyclopedia of Analytical Chemistry: Applications, Theory and Instrumentation* (ed. R.A. Meyers), Wiley, Chichester, 2000, **Vol. 4**, pp. 3064

[99] W.B. Robbins and J.A. Caruso, *Anal. Chem.*, 1979, **51**, 889A

[100] B. Weiz, *Chem. Br.*, 1986, **22**, 130

[101] T. Nakahara, *Proc. Anal. At. Spectrosc.*, 1983, **6**, 163

[102] R. Godden and D.K. Thomerson, *Analyst* (London), 1980, **105**, 1137

[103] J. Dedina, *J. Prog. Anal. At. Spectrosc.*, 1988, **11**, 251

[104] P.G. Riby, S.J. Haswell and R.J. Grzeskowiak, *J. Anal. At. Spectrom.*, 1989, **4**, **181**

[105] C. Boampong, I.D. Brindle and C.M. Ceccarelli-Ponzoni, *J. Anal. At. Spectrom.*, 1987, **2**, 197

[106] J. Aggett and A.C. Aspell, *Analyst* (London), 1976, **101**, 341

[107] R.S. Braman, D.L. Johnson, C.C. Foreback, J.M. Ammons and J.L. Bricker, *Anal. Chem.*, 1977, **49**, 621

[108] R.J.A. Van Cleuvenbergen, W.E. Van Mol and F.C. Adams, *J. Anal. At. Spectrom.*, 1988, **3**, 169

[109] B. Weiz and M. Melcher, *Analyst* (London), 1984, **109**, 573

[110] B.J.A. Haring, W. Van Delft and C.M. Bom, *Fresenius' Z. Anal. Chem.*, 1982, **310**, 217

[111] A.E. Smith, *Analyst* (London), 1975, **100**, 300

[112] F.D. Pierce and H.R. Brown, *Anal. Chem.*, 1976, **48**, 693; 1977, **49**, 1417

[113] B. Weiz and M. Schuber-Jacobs, *J. Anal. At. Spectrom.*, 1986, **1**, 23

[114] A.G. Howard and M.H. Arbab-Zavar, *Analyst*, 1981, **106**, 213

[115] Y. Yamamoto and T. Kumamaru, *Fresenius'Z Anal. Chem.*, 1976, **282**, 139

[116] A. Brockmann, C. Nonn and A. Golloch, *J. Anal. At. Spectrom.*, 1993, **8**, 397

[117] J.E. Drinkwater, *Analyst*, 1976, **101**, 672

[118] K. De Doncker, R. Dumarey, R. Dams and J. Hoste, *Anal. Chim. Acta*, 1985, **169**, 339

[119] C.J. Hwang and S.J. Jiang, *Anal. Chim. Acta*, 1994, **289**, 205

[120] M. Thompson, S.J. Pahlavanpour, S.J. Walton and G.F. Kirkbright, *Analyst*, 1978, **103**, 705

[121] M.O. Andreae, A*nal. Chem.*, 1977, **49**, 820

[122] S.H. Vien and R.C. Fry, *Anal. Chem.*, 1988, **60**, 465

[123] T.A. Hinners, *Analyst*, 1980, **105**, 751

[124] M.H. Arbab-Zavar and A.G. Howard, *Analyst*, 1980, **105**, 744

[125] D.D. Siemer, P. Koteel and V. Jariwala, *Anal. Chem.*, 1976, **48**, 836

[126] P.D. Goulden and P. Brooksband, *Anal. Chem.*, 1974, **46**, 1431

[127] Y. Talmi and C. Feldman, The Determination of Traces of Arsenic: A Review Chapter 2, In *Arsenical Pesticides* (ed. E.A. Woolson), ACS Symp Ser. 7., Washington, , 1975, pp. 13

[128] G. Lunde, *Acta Chim. Scand.*, 1973, **15**, 377

[129] B.J. Ray and D.L. Johnson, *Anal. Chim. Acta*, 1972, **62**(1), 196

[130] G.F. Kirkbright and L. Ranson, *Anal. Chem.*, 1971, **43**, 1238

[131] F.J. Henry and T.M. Thorpe, *Anal. Chem.*, 1980, **52**, 80

[132] A.A. Grabinski, *Anal. Chem.*, 1981, **53**, 966

[133] E.A. Dietz and M.E. Perez, *Anal. Chem.*, 1976, **48**, 1088

[134] R. Rubio, A.Padr, J. Alberti and G. Rauret, *Anal. Chim. Acta*, 1993, **283**, 160

[135] W.D. Spall, J.G. Lynn and L.R. Gurlay, *Anal. Chem.*, 1986, **58**, 1340

[136] K.E. Lafreniere, V.A. Fassel and D.E. Eckels, *Anal. Chem.*, 1987, **59**, 897

[137] P.C. White, *Analyst* (London), 1984, **109**, 677

[138] C.H. Gast, J.C. Kraak, H. Poppe and F.J.M.J. Maessen, *J. Chromatogr.*, 1979, **185**, 549

[139] D.M. Fraley, D. Yates and S.E. Manahan, *Anal. Chem.*, 1979, **51**, 2225

[140] S. Branch, L. Ebdon and P. O'Neill, *J. Anal. At. Spectros.*, 1994, **9**, 33

[141] J.R. Dean, S. Munro, L. Ebdon, H.M. Crews and R.C. Massey, *J. Anal. At. Spectrom.*, 1987, **2**, 607

[142] D.L. Windsor and M.B. Denton, *J. Chromatogr. Sci.*, 1979, **7**, 492

[143] N.S. Chong and R.S. Houk, *Appl. Spectrosc.*, 1987, **41**, 66

[144] G.R. Ricci, L.S. ShepardG. Colovos and N.E. Hester, *Anal. Chem.*, 1981, **53**, 610

[145] C.T. Tye, S.J. Haswell, P. O'Neill and K.C.C. Bancroft, *Anal. Chim. Acta*, 1985, **169**, 195

[146] B.S. Chana and N.J. Smith, *Anal. Chim. Acta*, 1987, **197**, 177

[147] F.E. Brinckman, W.R. Blair, K.L. Jewett and W.P. Iverson, *J. Chromatogr. Sci.*, 1977, **15**, 493

[148] R.G. Smith, J.C.V. Loon, J.R. Knechtel, J.R. Fraser, A.E. Pitts and A.E. Hodges, *Anal. Chimca Acta*, 1977, **93**, 61

[149] B. Welz and M. Melcher, *Analyst*, 1984, **109**, 569

[150] B. Welz and M, Melcher, *Analyst*, 1984, **109**, 577

[151] R.M. Brown, R.C. Fry, J.L. Moyers, S.J. Northway, M.B. Denton, and G.S. Wilson, *Anal. Chem.*, 1981, **53**, 1560

[152] M. Yamamoto, Y.Yamamoto and T. Yamashiga, *Analyst*, 1984, **109**, 1461

[153] J. Aggett and Y. Hayashi, *Analyst*, 1987, **112**, 277

[154] I.D. Brindle and X.C. Le, *Anal. Chimca Acta*, 1990, **229**, 239

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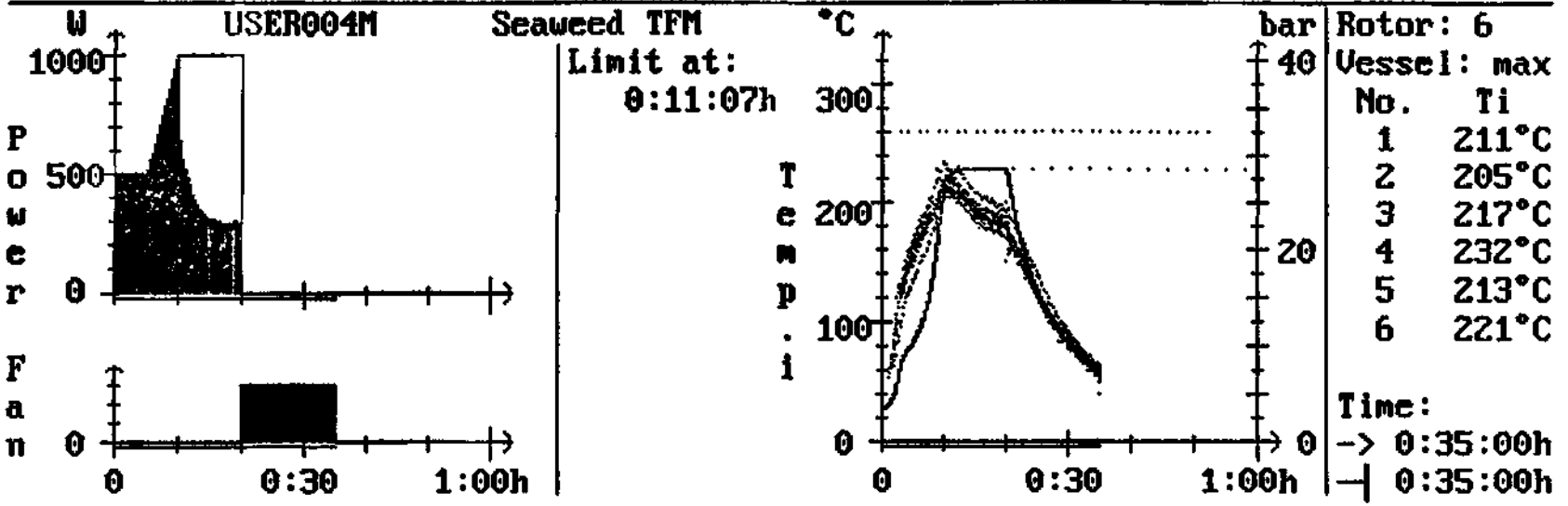


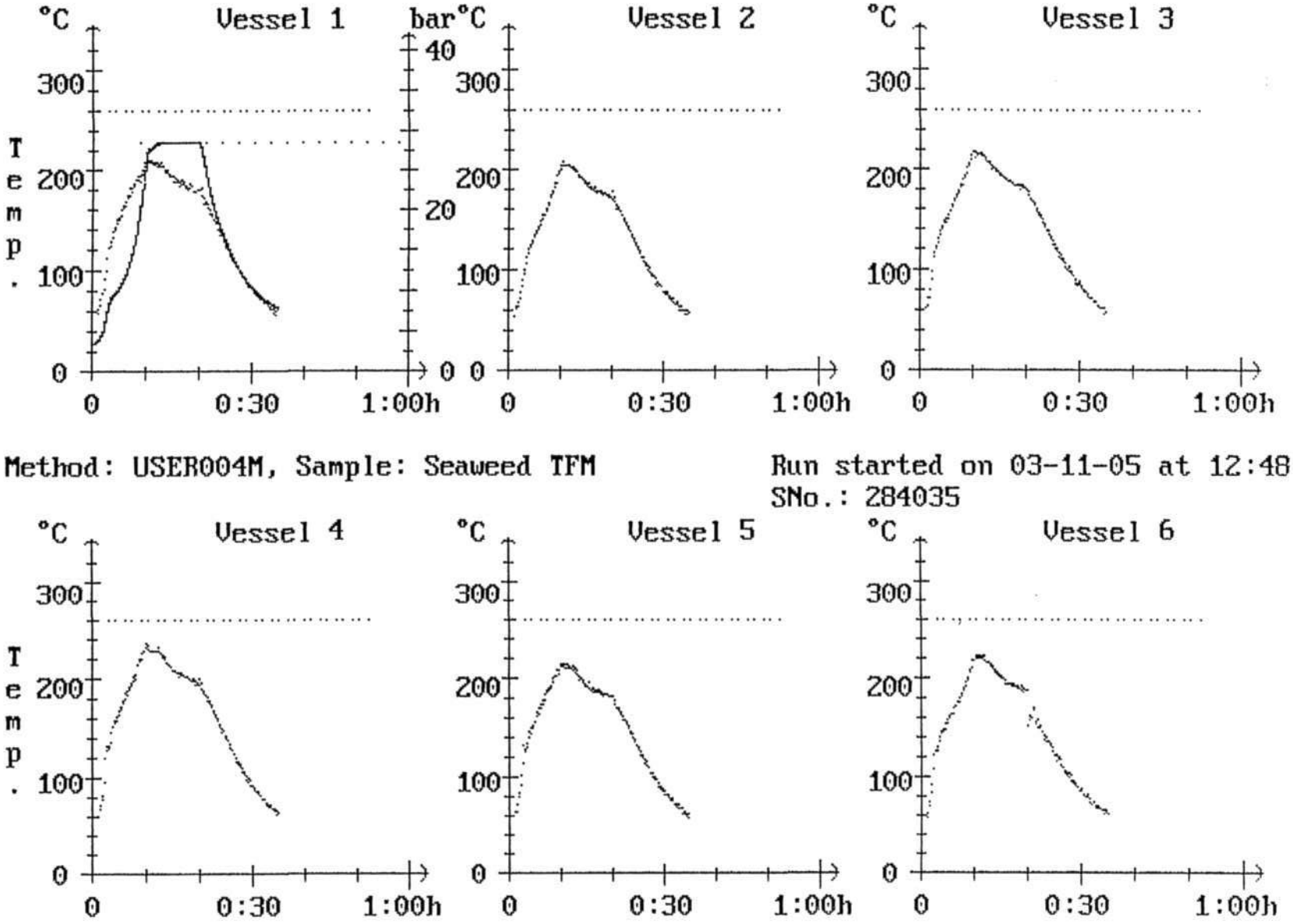
Method: USER004M
Note:

Sample (USER): Seaweed TFM
Note: Seaweed TFM vessel

Ph	Power W	Time mm:ss	Power W	Fan
1	500	5:00	500	0
2	500	5:00	1000	0
3	1000	10:00	1000	0
4	0	15:00	0	3
5	0	0:00	0	0
6	0	0:00	0	0
7	0	0:00	0	0
8	0	0:00	0	0

Ves	Weight max	Reag 1 HNO3	Reag 2 ———	Reag 3 ———	Remark
	0.500g	5.00 ml	0.00 ml	0.00 ml	
1					
2					
3					
4					
5					
6					

Run started on 03-11-05 at 12:48


°C
Vessel 1
bar°C
Vessel 2
°C
Vessel 3
Temp.
300
200
100
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40
20
0 0
0
0:30
1:00h
Method: USER004M, Sample: Seaweed TFM
Run started on 03-11-05 at 12:48
SNo.: 284035
°C
Vessel 4
°C
Vessel 5
°C
Vessel 6

Appendix 2

Experimental Results

1. Study of Experimental Conditions

Each data in the table represents mean of a three measurements.

A. $NaBH_4$

75 μg/l arsenic species, 1 M HCl

$NaBH_4(\%m/v)$	As(III)Intensity	As(V) Intensity
0.5	45 640	28 070
1.0	78 090	58 560
1.5	74 820	57 090
2.0	78 750	66 340
2.5	76 130	67 650
3.0	69 990	62 620

B. L-Cysteine

75 μg/l arsenic species, 1 M HCl, 2 % (m/v) $NaBH_4$

L-Cysteine (% m/v)	As(III)Intensity	As(V) Intensity
0.5	18 260	17 180
1.0	16 960	15 160
1.5	15 710	15 120
2.0	15 780	16 860
3.0	14 430	15 840
5.0	14 030	15 750
10.0	17 060	16 370

C. HCl

75 μg/l arsenic species, 2 % (m/v) $NaBH_4$, No L-cysteine

HCl (M)	As(III)Intensity	As(V) Intensity
0.01	7 273	3 794
0.10	28 040	9 741
0.50	62 660	35 320
1.0	64 840	41 610
2.0	64 150	49 090
4.0	66 000	46 820
5.0	66 220	50 890

D. HCl

75 μg/l arsenic species, 2 % (m/v) $NaBH_4$, 0.5 % L-cysteine

HCl (M)	As(III)Intensity	As(V) Intensity
0.01	18 440	23 110
0.10	43 370	46 770
0.50	36 130	38 150
1.0	20 720	19 490
2.0	20 540	24 190

E. Reaction Coil

75 μg/l arsenic species, 2 % (m/v) $NaBH_4$, 1 M HCl

Reaction Coil (m)	As(III)Intensity	As(V) Intensity
0	62 770	32 600
0.5	65 380	41 890
1	67 920	45 180
2	68 360	54 250
4	65 660	55 500
6	69 920	66 010

F. Reaction Time

75 μg/l As(V), 2 % (m/v) $NaBH_4$, 0.1 M HCl, 0.5 % L-cysteine

Reaction Time (min)	As(III)Intensity
0	14 060
5	20 130
15	31 040
30	39 860
60	40 860
120	43 850
240	45 780
360	45 730
480	46 140

G. Sodium Acetate - Acetic Acid Buffered Reaction Media

75 μg/l arsenic species, 2 % (m/v) $NaBH_4$

pH	As(III)Intensity	pH	As(V) Intensity
1.71	52 660	1.52	12 150
2.90	52 010	2.48	11 540
3.70	50 260	3.81	9 720
4.67	28 700	4.80	4 860
5.48	3 129	5.65	3 322
6.60	2 059	6.62	2 455

H. Sodium Citrate - Citric Acid Buffered Reaction Media

75 μg/l arsenic species, 2 % (m/v) $NaBH_4$

pH	As(III)Intensity	pH	As(V) Intensity
1.85	70 450	1.86	9 306
2.64	70 370	2.07	4 074
3.20	51 500	2.64	9 128
4.76	30 170	3.21	5 068
5.50	6 913	5.48	2 631
6.05	3 226	6.07	2 571

I. Buffer Concentration

75 μg/l arsenic species, 2 % (m/v) $NaBH_4$, pH $\sim$ 4.8

Na Citrate (M)	Citric Acid (M)	Buffer Conc.[a] (M)	As(III)Intensity	As(V) Intensity
0.06	0.03	0.09	30 170	
0.1	0.06	0.16	41 250	6 360
0.2	0.12	0.32	57 860	7 283
0.3	0.17	0.47	58 770	8 393
0.4	0.23	0.63	62 100	8 953
0.5	0.29	0.79	62 170	9 012
0.6	0.35	0.95	63 710	8 569

[a]Buffer Conc. = Sodium citrate + Citric acid

2. INTERFERENCE STUDY

2.1. L-Cysteine and EDTA

A. Cu(II)

75 μg/l As(III), 2 % (m/v) $NaBH_4$

	No L-Cysteine[a] Intensity	0.5 % L-cysteine [b] Intensity	0.02 M EDTA[a] Intensity
No Cu(II)	66 120	46 200	-
0.1 ppm Cu(II)	66 320	45 570	58 300
10 ppm Cu(II)	42 980	40 660	53 660
100 ppm Cu(II)	33 060	40 190	51 670

[a]In 1.0 M HCl
[b]In 0.1 M HCl

B. Ni(II)

75 μg/l As(III), 2 % (m/v) $NaBH_4$

	No L-Cysteine [a] Intensity	0.5 % L-cysteine [b] Intensity	0.02 M EDTA[a] Intensity
No Ni(II)	65 980	46 450	-
0.1 ppm Ni(II)	40 910	45 520	23 050
10 ppm Ni(II)	11 220	41 340	21 080
100 ppm Ni(II)	8 580	11 610	9 880

[a]In 1.0 M HCl
[b]In 0.1 M HCl

C. Se(IV)

75 μg/l As(III), 2 % (m/v) $NaBH_4$

	No L-Cysteine[a] Intensity	0.5 % L-cysteine[b] Intensity	0.02 M EDTA[a] Intensity
No Se(IV)	64 810	45 230	-
0.1 ppm Se(IV)	64 770	43 870	46 020
10 ppm Se(II)	63 510	30 760	49 900
100 ppm Se(IV)	57 680	19 000	44 070

[a]In 1.0 M HCl
[b]In 0.1 M HCl

D. Fe(III)

75 μg/l As(III), 2 % (m/v) $NaBH_4$

	No L-Cysteine[a] Intensity	0.5 % L-cysteine[b] Intensity	0.02 M EDTA[a] Intensity
No Fe(III)	65 140	45 530	-
0.1 ppm Fe(III)	67 750	45 610	60 580
10 ppm Fe(III)	10 420	44 620	56 020
100 ppm Fe(III)	7 820	40 980	58 630

[a]In 1.0 M HCl
[b]In 0.1 M HCl

E. Mn(II)

75 μg/l As(III), 2 % (m/v) $NaBH_4$

	No L-Cysteine[a] Intensity	0.5 % L-cysteine[b] Intensity	0.02 M EDTA[a] Intensity
No Mn(II)	65 450	45 910	-
0.1 ppm Mn(II)	65 310	45 750	53 670
10 ppm Mn(II)	58 910	48 660	52 360
100 ppm Mn(II)	51 710	49 120	46 470

[a]In 1.0 M HCl
[b]In 0.1 M HCl

2.2. CITRATE BUFFERED REACTION MEDIA

A. Cu(II)

75 μg/l As(III), 2 % (m/v) $NaBH_4$, 0.6 M Sodium Citrate, pH $\sim$ 4.8

	No EDTA Intensity	0.02 M EDTA Intensity
No Cu(II)	25 010	26 260
0.1 ppm Cu(II)	23 760	25 260
10 ppm Cu(II)	23 510	24 510
100 ppm Cu(II)	20 010	23 510

B. Ni(II)

75 μg/l As(III), 2 % (m/v) $NaBH_4$, 0.6 M Sodium Citrate, pH $\sim$ 4.8

	No EDTA Intensity	0.02 M EDTA Intensity
No Ni(II)	25 120	26 630
0.1 ppm Ni(II)	23 860	25 620
10 ppm Ni(II)	23 110	24 870
100 ppm Cu(II)	22 130	24 120

C. Se(IV)

75 μg/l As(III), 2 % (m/v) $NaBH_4$, 0.6 M Sodium Citrate, pH $\sim$ 4.8

	No EDTA Intensity	0.02 M EDTA Intensity
No Se(IV)	24 840	25 870
0.1 ppm Se(IV)	23 350	24 090
10 ppm Se(IV)	22 360	22 110
100 ppm Se(IV)	20 370	20 870

D. Fe(III)

75 μg/l As(III), 2 % (m/v) $NaBH_4$, 0.6 M Sodium Citrate, pH $\sim$ 4.8

	No EDTA Intensity	0.02 M EDTA Intensity
No Fe(III)	24 910	26 400
0.1 ppm Fe(III)	23 660	25 160
10 ppm Fe(III)	19 930	23 910
100 ppm Fe(III)	15 440	22 420

E. Mn(II)

75 μg/l As(III), 2 % (m/v) $NaBH_4$, 0.6 M Sodium Citrate, pH $\sim$ 4.8

	No EDTA Intensity	0.02 M EDTA Intensity
No Mn(II)	25 150	26 410
0.1 ppm Mn(II)	24,140	25 400
10 ppm Mn(II)	22 890	24 390
100 ppm Mn(II)	20 370	23 140

2.3. EDTA

The following table gives the equilibrium constants for EDTA.

$H_4Y \rightleftharpoons H^+ + H_3Y^-$	$K_1 = 1.02 \times 10^{-2}$
$H_3Y^- \rightleftharpoons H^+ + H_2Y^{2-}$	$K_2 = 2.14 \times 10^{-3}$
$H_2Y^{2-} \rightleftharpoons H^+ + HY^{3-}$	$K_3 = 6.92 \times 10^{-7}$
$HY^{3-} \rightleftharpoons H^+ + Y^{4-}$	$K_4 = 5.50 \times 10^{-11}$

Conditional and Formation Constants

$$M^{n+} + Y^{4-} \rightleftharpoons MY^{(n-4)} \tag{A-1}$$

$$Y = \frac{[MY^{(n-4)}]}{[M^{n}+][Y^{4-}]} \tag{A-2}$$

$$\alpha_4 = \frac{[Y^{4-}]}{C_T} \tag{A-3}$$

$$C_T = [Y^{4-}] + [HY^{3-}] + [H_2Y^{3-}] + [H_3Y^{-}] + [H_4Y] \tag{A-4}$$

$$\alpha_4 = \frac{K_1K_2K_3K_4}{[H^+]^4 + K_1[H^+]^3 + K_1K_2[H^+]^2 + K_1K_2K_3[H^+] + K_1K_2K_3K_4} \tag{A-5}$$

$$K'_{MY} = K_{MY}\alpha_4 = \frac{[MY^{(n-4)}]}{[M^{n+}]C_T} \tag{A-6}$$

Using the above constants and equations and at pH 4.8 ($[H^+] = 1.58 \times 10^{-5}$) α_4 was calculated to be 1.48×10^{-7}.

The calculation for the equilibrium concentration of interferents (example shown for 0.1 ppm ($1.7x10^{-6}$) M, 10 ppm ($1.7x10^{-4}$) M and 100 ppm ($1.7x10^{-3}$) M Ni(II)) in a solution of 0.02 M EDTA and pH 4.8 is shown as follows.

For 0.1 ppm Ni(II)

$$Ni^{2+} + Y^{4-} \rightleftharpoons NiY^{2-} \tag{A-7}$$

$$[NiY^{2-}] = 1.7x10^{-6}M - [Ni^{2+}] \approx 1.7x10^{-6}M \tag{A-8}$$

$$K'_{MY} = \alpha_4 K_{MY} = \frac{[NiY^{2-}]}{[Ni^{2+}]C_T} = (4.2x10^{18})(1.45x10^{-7}) = 6.09x10^{11} \tag{A-9}$$

Substituting $[NiY^{2-}] = 1.7 \times 10^{-6}$ and $C_T \approx 0.02$ M and solving for $[Ni^{2+}]$ gives 1.4×10^{-16} M.

Similarly, using the same procedure as above the calculated values of 10 and 100 ppm Ni(II) is 1.4×10^{-14} and 1.4×10^{-13} M respectively.

The same calculation can be employed for the other interferents (Mn(II), Fe(III) and Cu(II)).

Appendix 3

Method Performance

A. Detection Limit

A.1. In 0.5 % L-Cysteine Media

The following table gives results for the calculation of detection limit.

	As(III), ppm	Intensity	Corr. Intensity	SD	% RSD
Blank	0	2 891	0	64.28	2.223
Standard 1	0.005	4 582	1 691	54.55	1.754
Standard 2	0.01	5 658	2 767	87.43	2.419
Standard 3	0.03	10 210	7 319	95.89	1.605
Standard 4	0.05	16 070	13 179	93.72	1.057
Standard 5	0.07	22 870	19 979	335.2	2.824
Standard 6	0.09	29 570	26 679	135.9	0.9136
Standard 7	0.11	36 620	33 729	370.4	1.925

$$Y = 302225X - 621.14; r = 0.9974 \quad \text{(B-1)}$$

Twenty (20) intensity determinations of blank

2 868	2 861	2 973	2 875	2 962
2 876	2 924	2 888	2 815	2 949
2 918	2 851	2 796	2 800	2 978
2 915	2 838	3 028	2 818	2 892

A.2. In Citrate Buffered Reaction Media

	As(III), ppm	Intensity	Corr. Intensity	SD	% RSD
Blank	0	3 365	0	70.19	2.08
Standard 1	0.005	5 048	1 683	42.38	0.8396
Standard 2	0.01	6 657	3 292	265.3	3.985
Standard 3	0.03	11 690	8 325	141.7	1.212
Standard 4	0.05	17 240	13 875	89.37	0.5184
Standard 5	0.07	21 890	18 525	455.1	2.079

$$Y = 263623 + 367; r = 0.9985 \quad \text{(B-2)}$$

Twenty (20) intensity determinations of blank

3 320	3 375	3 365	3 365	3 279
3 328	3 422	3 383	3 412	3 332
3 299	3 454	3 391	3 352	3 393
3 280	3 446	3 259	3 341	3 556

B. Reproducibility

Twenty (20) determinations of 0.01 ppm of As(III) in the 0.5 % L-Cysteine and 0.1 M HCl gave the following concentration results.

0.01119	0.01116	0.01154	0.01110	0.01184
0.01092	0.01123	0.01132	0.01087	0.01034
0.01079	0.01160	0.01172	0.01185	0.01178
0.01143	0.01197	0.01176	0.01182	0.01157

$$Mean = 0.01139 \tag{B-3}$$

$$SD = 0.000435 \tag{B-4}$$

$$\%RSD = 4 \tag{B-5}$$

APPENDIX 4

SAMPLE ANALYSIS

The following formulas were used:

$$Recovery = \frac{A - B}{T} * 100 \quad \text{(C-1)}$$

where A = Conc. of spiked sample; B = Conc. of sample and T = Known value of spike and for concentration using standard addition methods:

$$C_x = \frac{b * C_s}{mV_x} \quad \text{(C-2)}$$

where C_x = Conc. of unknown solution.; b = Intercept; m = slope; C_s = Std. Conc.; V_x = Vol. of unknown solution

1. MICROWAVE DIGESTED SAMPLES

1.1. CALIBRATION

The following results were used to calculate calibration curve

	As(III), ppm	Intensity	Corr. Intensity
Blank	0	3 024	0
Standard 1	0.01	5 368	2 344
Standard 2	0.03	8 170	5 146
Standard 3	0.05	12 050	9 026
Standard 4	0.07	14 650	11 626
Standard 5	0.09	17 280	14 256
Standard 6	0.11	20 450	17 426

$$Y = 155286.69X + 560.11; r = 0.9979 \quad \text{(C-3)}$$

TABLET A

The following results are for Tablet A based on calibration equation (C-3)

Sample Weight (g)	Intensity	Corr. Intensity	Conc., ppm	D.F.	Actual Conc. (μg/g)
0.5	12 850	9 826	0.05967	1 000	59.67
0.5	12 770	9 746	0.05915	1 000	59.15
0.5	12 590	9 566	0.05800	1 000	58.00
0.5	12 620	9 596	0.05819	1 000	58.19
0.5	12 640	9 616	0.05832	1 000	58.32
0.5	12 610	9 586	0.05812	1 000	58.12
0.5	12 650	9 626	0.05838	1 000	58.38
0.5	12 630	9 606	0.05825	1 000	58.25
0.5	11 950	8 926	0.05387	1 000	53.87
0.5	11 470	8 446	0.05078	1 000	50.78
0.5	11 720	8 696	0.05239	1 000	52.39
0.5	11 530	8 506	0.05117	1 000	51.17

1.2. STANDARD ADDITION METHODS FOR TABLET A

Volume (ml)	Intensity 1	Intensity 2	Intensity 3	Intensity 4	Intensity 5	Intensity 6
0.0	13 480	10 650	12 010	12 280	12 260	12 100
0.5	19 140	17 100	17 960	18 050	18 150	18 130
1.0	23 910	21 190	22 470	22 520	22 850	22 960
1.5	29 690	26 190	27 680	28 040	28 480	28 150
2.0	37 910	32 690	35 940	35 620	35 810	34 460
2.5	45 030	39 840	41 740	42 250	41 810	42 310

REGRESSION EQUATION FOR TABLET A

$$Y = 12562.29X + 12490.48, r = 0.9953 \quad \text{(C-4)}$$

$$Y = 11298.29X + 10487.14, r = 0.9962 \quad \text{(C-5)}$$

$$Y = 11874.29X + 11457.14, r = 0.9961 \quad \text{(C-6)}$$

$$Y = 11890.29X + 11597.14, r = 0.9964 \quad \text{(C-7)}$$

$$Y = 11792.00X + 11820, r = 0.9980 \quad \text{(C-8)}$$

$$Y = 11727.43X + 11692.38, r = 0.9966 \quad \text{(C-9)}$$

CALCULATED CONCENTRATION OF TABLET A

Replicates	Calculated Conc. (ppm)	D.F.	Actual conc. (μg/g)
1	0.08335	1 000	83.35
2	0.07781	1 000	77.81
3	0.08089	1 000	80.89
4	0.08176	1 000	81.76
5	0.08403	1 000	84.03
6	0.08358	1 000	83.58

1.3. RECOVERY FOR TABLET A

Spike + Sample Intensity	Corrected Intensity	Spike+Sample Conc. (ppm)	Unspiked Conc.(ppm)	Recovery (%)
13 740	10 716	0.06540	0.05967	57
13 530	10 506	0.06405	0.05915	49
13 550	10 526	0.06418	0.05800	62
13 560	10 536	0.06424	0.05819	61
13 450	10 426	0.06353	0.05832	52
13 770	10 746	0.06559	0.05812	75
13 290	10 266	0.06250	0.05838	41
13 530	10 506	0.06405	0.05825	58
13 020	9 996	0.06076	0.05387	69
12 260	9 236	0.05587	0.05078	51
12 560	9 536	0.05780	0.05239	54
12 240	9 216	0.05574	0.05117	46

TABLET B

The following results are for tablet B based on calibration equation (C-3)

Sample Weight (g)	Intensity	Corr. Intensity	Conc., ppm	D.F.	Actual Conc. (μg/g)
0.5	15 950	12 926	0.07963	1 000	79.63
0.5	15 660	12 636	0.07777	1 000	77.77
0.5	15 700	12 676	0.07802	1 000	78.02
0.5	15 890	12 866	0.07925	1 000	79.25
0.5144	16 260	13 236	0.08163	972	79.34
0.5144	15 880	12 856	0.07918	972	76.97
0.5144	15 690	12 666	0.07796	972	75.78
0.5144	15 440	12 416	0.07635	972	74.21
0.5053	18 130	15 106	0.09367	990	92.69
0.5053	17 610	14 586	0.09032	990	89.38
0.5053	17 010	13 986	0.08646	990	85.55
0.5053	17 310	14 286	0.08839	990	87.46

1.4. STANDARD ADDITION METHODS FOR TABLET B

Volume (ml)	Intensity 1	Intensity 2	Intensity 3	Intensity 4	Intensity 5	Intensity 6
0.0	15 070	16 770	15 720	16 170	16 060	15 800
0.5	18 850	20 290	20 110	19 510	20 300	19 710
1.0	23 140	26 000	24 330	25 250	24 050	23 590
1.5	31 820	36 290	33 900	33 090	34 810	34 190
2.0	36 420	39 130	38 280	38 250	38 320	38 320
2.5	44 620	48 530	45 520	47 660	48 260	46 730

REGRESSION EQUATION FOR TABLET B

$$Y = 11950.86X + 13381.43, r = 0.9915 \quad \text{(C-10)}$$

$$Y = 12892X + 15053.33, r = 0.9890 \quad \text{(C-11)}$$

$$Y = 12176X + 14423.33, r = 0.9930 \quad \text{(C-12)}$$

$$Y = 12657.71X + 14166.19, r = 0.9913 \quad \text{(C-13)}$$

$$Y = 12904X + 14170, r = 0.9862 \quad \text{(C-14)}$$

$$Y = 12633.14X + 13931.90, r = 0.9883 \quad \text{(C-15)}$$

CALCULATED CONCENTRATION OF TABLET B

Replicates	Calculated Conc. (ppm)	D.F.	Actual conc. (μg/g)
1	0.09386	1 000	93.86
2	0.09788	1 000	97.88
3	0.09930	972	96.52
4	0.09382	972	91.19
5	0.09205	990	91.13
6	0.09245	990	91.53

1.5. RECOVERY FOR TABLET B

Spike + Sample Intensity	Corrected Intensity	Spike+Sample Conc. (ppm)	Unspiked Conc.(ppm)	Recovery (%)
16 890	13 866	0.08569	0.07963	61
16 440	13 416	0.08279	0.07777	50
16 710	13 686	0.08453	0.07802	65
17 000	13 976	0.08639	0.07925	71
16 960	13 936	0.08614	0.08163	45
16 870	13 846	0.08556	0.07918	64
16 760	13 736	0.08485	0.07796	69
16 410	13 386	0.08259	0.07635	62
19 130	16 106	0.10011	0.09367	64
18 660	15 636	0.09708	0.09032	68
18 130	15 106	0.09367	0.08646	72
18 360	15 336	0.09515	0.08839	68

2. DRY ASHED SAMPLES

2.1. CALIBRATION CURVE I

The following results were used to calculate calibration curve

	As(III), ppm	Intensity	Corr. Intensity
Blank	0	5 087	0
Standard 1	0.005	9 038	3 951
Standard 2	0.01	11 770	6 683
Standard 3	0.03	24 100	19 013
Standard 4	0.05	36 040	30 953
Standard 5	0.07	47 580	42 493

$$Y = 602978.14X + 600.27, r = 0.9997 \quad \text{(C-16)}$$

2.1. CALIBRATION CURVE II

The following results were used to calculate calibration curve

	As(III), ppm	Intensity	Corr. Intensity
Blank	0	7 804	0
Standard 1	0.005	11 060	3 256
Standard 2	0.01	13 470	5 666
Standard 3	0.03	26 220	18 416
Standard 4	0.05	38 250	30 446
Standard 5	0.07	50 020	42 216
Standard 6	0.09	60 760	52 956

$$Y = 593847.58X + 217.84, r = 0.9997 \quad \text{(C-17)}$$

TABLET A

The following results are for Tablet A based on calibration equations (C-16) and (C-17)

Sample Weight (g)	Intensity	Corr. Intensity	Conc., ppm	D.F.	Actual Conc. (μg/g)
Method Blank	5 085	0	*	*	*
0.2500	11 880	6 795	0.01027	400	4.11
0.2500	11 830	6 745	0.01019	400	4.08
0.2500	12 030	6 945	0.01052	400	4.21
0.2507	12 180	7 095	0.01077	399	4.30
0.2507	12 150	7 065	0.01072	399	4.28
0.2507	12 090	7 005	0.01062	399	4.24
0.2511	12 570	7 485	0.01142	398	4.55
0.2511	12 850	7 765	0.01188	398	4.73
0.2511	12 820	7 735	0.01183	398	4.71
0.2538	13 260	8 175	0.01256	394	4.95
0.2538	13 450	8 365	0.01288	394	5.07
0.2538	13 290	8 205	0.01261	394	4.97
0.2521	13 970	8 885	0.01374	397	5.45
0.2521	13 610	8 525	0.01314	397	5.21
0.2521	13 450	8 365	0.01288	397	5.11
Method Blank	5 322	0	*	*	*
0.2518	11 060	5 738	0.00930	397	3.69
0.2518	11 320	5 998	0.00973	397	3.87
0.2518	11 530	6 208	0.01009	397	4.01
0.2507	11 350	6 028	0.00978	399	3.90
0.2507	11 410	6 088	0.00988	399	3.94

Continued...

Sample Weight (g)	Intensity	Corr. Intensity	Conc., ppm	D.F.	Actual Conc. (μg/g)
0.2507	11 830	6 508	0.01059	399	4.23
0.2519	12 490	7 168	0.01170	397	4.65
0.2519	12 740	7 418	0.01212	397	4.81
0.2519	12 300	6 978	0.01138	397	4.52
0.2519	12 650	7 328	0.01197	397	4.75
0.2519	12 810	7 488	0.01224	397	4.86
0.2519	12 680	7 358	0.01202	397	4.77
0.2503	13 940	8 618	0.01415	400	5.65
0.2503	13 880	8 558	0.01404	400	5.61
0.2503	13 770	8 448	0.01386	400	5.54

2.2. RECOVERY FOR TABLET A

Spike + Sample Intensity	Corrected Intensity	Spike+Sample Conc. (ppm)	Unspiked Conc.(ppm)	Recovery (%)
15 170	10 085	0.01573	0.01027	55
15 350	10 265	0.01603	0.01019	58
15 200	10 115	0.01578	0.01052	53
15 580	10 495	0.01641	0.01077	56
15 530	10 445	0.01633	0.01072	56
15 770	10 685	0.01672	0.01062	61
15 340	10 255	0.01601	0.01142	46
15 330	10 245	0.01600	0.01188	41
15 500	10 415	0.01628	0.01183	44
17 010	11 925	0.01878	0.01256	62

Continued...

Spike + Sample Intensity	Corrected Intensity	Spike+Sample Conc. (ppm)	Unspiked Conc.(ppm)	Recovery (%)
17 390	12 305	0.01941	0.01288	65
17 240	12 155	0.01916	0.01261	66
17 560	12 238	0.01930	0.01374	56
17 500	12 178	0.01920	0.01314	61
17 670	12 348	0.01948	0.01288	66
13 920	8 598	0.01411	0.00930	48
14 320	8 998	0.01479	0.00973	51
14 710	9 388	0.01544	0.01009	54
15 580	10 258	0.01691	0.00978	71
15 210	9 888	0.01628	0.00988	64
14 980	9 658	0.01590	0.01059	53
16 060	10 738	0.01772	0.01170	60
16 150	10,828	0.01787	0.01212	57
15,750	10 428	0.01719	0.01138	58
15 750	10 428	0.01719	0.01197	52
16 100	10 778	0.01778	0.01224	55
15 600	10 278	0.01694	0.01202	49
16 970	11 648	0.01925	0.01415	51
16 670	11 348	0.01874	0.01404	47
17 040	11 718	0.01937	0.01386	55

TABLET B

The following results are for Tablet B based on calibration equations (C-16) and (C-17).

Sample Weight (g)	Intensity	Corr. Intensity	Conc., ppm	D.F.	Actual Conc. (μg/g)
Method Blank	5 085	0	*	*	*
0.2506	14 200	9 115	0.01412	399	5.63
0.2506	14 430	9 345	0.01450	399	5.79
0.2506	14 110	9 025	0.01397	399	5.58
0.2517	14 670	9 585	0.01490	397	5.92
0.2517	14 490	9 405	0.01460	397	5.80
0.2517	14 700	9 615	0.01495	397	5.94
0.2515	14 490	9 405	0.01460	398	5.81
0.2515	14 840	9 755	0.01518	398	6.04
0.2515	14 900	9 815	0.01528	398	6.08
0.2509	14 550	9 465	0.01470	399	5.86
0.2509	13 920	8 835	0.01366	399	5.44
0.2509	14 330	9 245	0.01434	399	5.71
0.2532	14 470	9 385	0.01457	395	5.75
0.2532	14 270	9 185	0.01424	395	5.62
0.2532	14 490	9 405	0.01460	395	5.77
Method Blank	5 322	0	*	*	*
0.2515	14 040	8 718	0.01431	398	5.69
0.2515	14 080	8 758	0.01438	398	5.72
0.2515	14 280	8 958	0.01472	398	5.85
0.2507	14 260	8 938	0.01468	399	5.86
0.2507	14 560	9 238	0.01519	399	6.06

Continued...

Sample Weight (g)	Intensity	Corr. Intensity	Conc., ppm	D.F.	Actual Conc. (μg/g)
0.2507	14 720	9 398	0.01546	399	6.17
0.2514	14 820	9 498	0.01563	398	6.22
0.2514	14 980	9 658	0.01590	398	6.32
0.2514	14 820	9 498	0.01563	398	6.22
0.2515	15 080	9 758	0.01606	398	6.39
0.2515	15 030	9 708	0.01598	398	6.35
0.2515	14 950	9 628	0.01585	398	6.30
0.2502	14 990	9 668	0.01591	400	6.36
0.2502	14 760	9 438	0.01553	400	6.21
0.2502	14 660	9 338	0.01536	400	6.14

2.3. RECOVERY FOR TABLET B

Spike + Sample Intensity	Corrected Intensity	Spike+Sample Conc. (ppm)	Unspiked Conc.(ppm)	Recovery (%)
18 090	13 005	0.02057	0.01412	65
18 530	13 445	0.02130	0.01450	68
18 640	13 555	0.02148	0.01397	75
18 760	13 675	0.02168	0.01490	68
18 800	13 715	0.02175	0.01460	71
18 710	13 625	0.02160	0.01495	67
18 260	13 175	0.02085	0.01460	63
18 500	13 415	0.02125	0.01518	61
18 380	13 295	0.02105	0.01528	58
18 540	13 455	0.02132	0.01470	66

Continued...

Spike + Sample Intensity	Corrected Intensity	Spike+Sample Conc. (ppm)	Unspiked Conc.(ppm)	Recovery (%)
18 250	13 165	0.02084	0.01366	72
18 170	13 085	0.02071	0.01434	64
18 910	13 588	0.02154	0.01457	70
18 660	13 338	0.02112	0.01424	69
18 710	13 388	0.02121	0.01460	66
17 580	12 258	0.02027	0.01431	60
17 750	12 428	0.02056	0.01438	62
17 820	12 498	0.02068	0.01472	60
18 680	13 358	0.02213	0.01468	74
18 040	12 718	0.02105	0.01519	59
17 910	12 588	0.02083	0.01546	54
19 030	13 708	0.02272	0.01563	71
18 770	13 448	0.02228	0.01590	64
18 840	13 518	0.02240	0.01563	68
18 960	13 638	0.02260	0.01606	65
19 030	13 708	0.02272	0.01598	67
19 280	13 958	0.02314	0.01585	73
17 930	12 608	0.02086	0.01591	50
18 360	13 038	0.02159	0.01553	61
18 540	13 218	0.02189	0.01536	65

3. WET DIGESTED SAMPLES

3.1. CALIBRATION

The following results were used to calculate calibration curve

	As(III), ppm	Intensity	Corr. Intensity
Blank	0	2 369	0
Standard 1	0.005	3 109	740
Standard 2	0.01	3 615	1 246
Standard 3	0.03	5 976	3 607
Standard 4	0.05	8 865	6 496
Standard 5	0.07	11 870	9 501
Standard 6	0.09	14 880	12 511
Standard 7	0.11	19 250	16 881

$$Y = 147937.25X - 376.89, r = 0.9959 \quad \text{(C-18)}$$

TABLET A

The following results are for tablet A based on calibration equation (C-18)

Sample Weight (g)	Intensity	Corr. Intensity	Conc., ppm	D.F.	Actual Conc. (μg/g)
Method Blank	4,702	0	*	*	*
1.0026	12 020	7 318	0.05201	499	25.94
1.0026	11 600	6 898	0.04918	499	24.52
1.0026	11 900	7 198	0.05120	499	25.54
1.0019	10 130	5 428	0.03924	499	19.58
1.0019	10 090	5 388	0.03897	499	19.45
1.0019	10 140	5 438	0.03931	499	19.62
1.0113	10 980	6 278	0.04498	494	22.24
1.0113	11 300	6 598	0.04715	494	23.31
1.0113	11 200	6 498	0.04647	494	22.98
1.0022	11 170	6 468	0.04627	499	23.08
1.0022	10 960	6 258	0.04485	499	22.38
1.0022	11 310	6 608	0.04722	499	23.56
1.0018	11 060	6 358	0.04553	499	22.72
1.0018	11 040	6 338	0.04539	499	22.65
1.0018	11 600	6 898	0.04918	499	24.54
1.0121	11 160	6 458	0.04620	494	22.82
1.0121	10 750	6 048	0.04343	494	21.46
1.0121	11 050	6 348	0.04546	494	22.46

3.2. STANDARD ADDITION METHODS FOR TABLET A

Volume (ml)	Intensity 1	Intensity 2	Intensity 3	Intensity 4	Intensity 5	Intensity 6
0.0	17 470	16 810	16 540	16 500	16 230	16 850
0.25	28 010	28 920	28 600	29 240	29 160	29 030
0.50	38 420	38 480	38 630	39 010	39 100	38 410
0.75	46 710	46 680	46 160	47 720	47 190	46 600
1.00	48 140	49 110	49 270	48 980	47 870	48 270
1.25	62 750	62 250	63 660	62 950	63 990	63 920

REGRESSION EQUATION FOR TABLET A

$$Y = 33723.43X + 19172.86; r = 0.9849 \quad \text{(C-19)}$$

$$Y = 33825.14X + 19234.29; r = 0.9870 \quad \text{(C-20)}$$

$$Y = 34873.14X + 18680.95; r = 0.9873 \quad \text{(C-21)}$$

$$Y = 34306.29X + 19291.90; r = 0.9828 \quad \text{(C-22)}$$

$$Y = 34630.86X + 18945.71; r = 0.9784 \quad \text{(C-23)}$$

$$Y = 34429.71X + 18994.76; r = 0.9832 \quad \text{(C-24)}$$

CALCULATED CONCENTRATION OF TABLET A

Replicates	Weight (g)	Calculated Conc. (ppm)	D.F.	Actual conc. (μg/g)
1	1.0037	0.09532	498	47.47
2	1.0037	0.09534	498	47.48
3	1.0037	0.08981	498	44.72
4	1.0018	0.09428	499	47.05
5	1.0018	0.09172	499	45.77
6	1.0018	0.09250	499	46.16

3.3. RECOVERY FOR TABLET A

Spike + Sample Intensity	Corrected Intensity	Spike+Sample Conc. (ppm)	Unspiked Conc.(ppm)	Recovery (%)
12 920	8 218	0.05810	0.05201	61
12 720	8 018	0.05675	0.04918	76
12 880	8 178	0.05783	0.05120	66
10 990	6 288	0.04505	0.03924	58
10 840	6 138	0.04404	0.03897	51
11 150	6 448	0.04613	0.03931	68
11 880	7 178	0.05107	0.04498	61
12 130	7 428	0.05276	0.04715	56
12 040	7 338	0.05215	0.04647	57
12 210	7 508	0.05330	0.04627	70
11 910	7 208	0.05127	0.04485	64
12 180	7 478	0.05310	0.04722	59
11 950	7 248	0.05154	0.04553	60
12 150	7 448	0.05289	0.04539	75
12 560	7 858	0.05566	0.04918	65
12 140	7 438	0.05283	0.04620	66
11 820	7 118	0.05066	0.04343	72
11 980	7 278	0.05174	0.04546	63

TABLET B

The following results are for tablet B based on calibration equation (C-18)

Sample Weight (g)	Intensity	Corr. Intensity	Conc., ppm	D.F.	Actual Conc. (μg/g)
Method Blank	4,702	0	*	*	*
1.0037	16 270	11 568	0.08074	498	40.22
1.0037	16 030	11 328	0.07912	498	39.41
1.0037	16 180	11 478	0.08013	498	39.92
1.0018	14 210	9 508	0.06682	499	33.35
1.0018	14 310	9 608	0.06749	499	33.69
1.0018	14 450	9 748	0.06844	499	34.16
1.0029	15 380	10 678	0.07473	499	37.26
1.0029	15 600	10 898	0.07621	499	38.00
1.0029	15 720	11 018	0.07703	499	38.40
1.0097	15 660	10 958	0.07662	495	37.94
1.0097	15 660	10 958	0.07662	495	37.94
1.0097	15 490	10 788	0.07547	495	37.37
1.0008	18 630	13 928	0.09670	500	48.31
1.0008	18 420	13 718	0.09528	500	47.60
1.0008	17 930	13 228	0.09196	500	45.95
1.0122	12 130	7 428	0.05276	494	26.06
1.0122	12 320	7 618	0.05404	494	26.70
1.0122	12 240	7 538	0.05350	494	26.43

3.4. STANDARD ADDITION METHODS FOR TABLET B

Volume (ml)	Intensity 1	Intensity 2	Intensity 3	Intensity 4	Intensity 5	Intensity 6
0.0	12 080	12 390	13 040	13 170	13 680	13 570
0.25	21 390	21 510	21 830	21 390	23 050	22 730
0.50	24 660	25 370	25 600	26 630	27 210	26 650
0.75	32 600	33 360	33 700	32 680	32 570	32 710
1.00	34 340	34 190	34 060	34 750	34 390	34 250
1.25	40 630	41 120	42 300	42 010	41 440	40 310

REGRESSION EQUATION FOR TABLET B

$$Y = 21661.71X + 14078.10; r = 0.9856 \quad \text{(C-25)}$$

$$Y = 21677.71X + 14441.43; r = 0.9830 \quad \text{(C-26)}$$

$$Y = 21838.86X + 14772.38; r = 0.9826 \quad \text{(C-27)}$$

$$Y = 21752X + 14843.33; r = 0.9899 \quad \text{(C-28)}$$

$$Y = 20363.43X + 15996.19; r = 0.9830 \quad \text{(C-29)}$$

$$Y = 19922.29 + 15918.57; r = 0.9824 \quad \text{(C-30)}$$

CALCULATED CONCENTRATION OF TABLET B

Replicates	Weight (g)	Calculated Conc. (ppm)	D.F.	Actual conc. (μg/g)
1	1.0026	0.1090	499	54.39
2	1.0026	0.1117	499	55.74
3	1.0026	0.1134	499	56.59
4	1.0019	0.1144	499	57.09
5	1.0019	0.1317	499	65.72
6	1.0019	0.1340	499	66.87

3.5. RECOVERY FOR TABLET B

Spike + Sample Intensity	Corrected Intensity	Spike+Sample Conc. (ppm)	Unspiked Conc.(ppm)	Recovery (%)
17 320	12 618	0.08784	0.08074	71
16 840	12 142	0.08462	0.07912	55
17 240	12 543	0.08733	0.08013	72
14 990	10 292	0.07212	0.06682	53
14 980	10 274	0.07200	0.06749	45
15 250	10 547	0.07384	0.06844	54
16 150	11 447	0.07993	0.07473	52
16 590	11 889	0.08291	0.07621	67
16 810	12 113	0.08443	0.07703	74
16 470	11 772	0.08212	0.07662	55
16 620	11 920	0.08312	0.07662	65
16 540	11 838	0.08257	0.07547	71
19 550	14 845	0.10289	0.09670	62
19 410	14 709	0.10197	0.09528	67
18 950	14 249	0.09887	0.09196	69
13 200	8 493	0.05996	0.05276	72
13 220	8 520	0.06014	0.05404	61
13 230	8 529	0.06020	0.05350	67

4. CITRATE BUFFERED REACTION MEDIA (WET DIGESTED SAMPLES)

4.1. CALIBRATION CURVE

The following results were used to calculate calibration curve

	As(III), ppm	Intensity	Corr. Intensity
Blank	0	3 365	0
Standard 1	0.005	5 048	1 683
Standard 2	0.01	6 657	3 292
Standard 3	0.03	11 690	8 325
Standard 4	0.05	17 240	13 875
Standard 5	0.07	21 890	18 525

$$Y = 263623.15X + 367.03; r = 0.9959 \quad \text{(C-31)}$$

TABLET A

The following results are for tablet A based on calibration equation (C-31)

Sample Weight (g)	Intensity	Corr. Intensity	Conc., ppm	D.F.	Actual Conc. (μg/g)
1.0026	9 788	6 423	0.02297	499	11.46
1.0026	9 724	6 359	0.02273	499	11.34
1.0026	9 472	6 107	0.02177	499	10.86
1.0026	9 567	6 202	0.02213	499	11.04
1.0026	9 944	6 579	0.02356	499	11.75
1.0026	9 594	6 229	0.02224	499	11.09
1.0019	9 667	6 302	0.02251	499	11.24
1.0019	9 918	6 553	0.02347	499	11.71
1.0019	9 740	6 375	0.02279	499	11.37
1.0019	9 557	6 192	0.02210	499	11.03
1.0019	9 674	6 309	0.02254	499	11.25
1.0019	9 748	6 383	0.02282	499	11.39
1.0113	9 855	6 490	0.02323	494	11.48
1.0113	10 100	6 735	0.02416	494	11.94
1.0113	9 790	6 425	0.02298	494	11.36
1.0113	10 140	6 775	0.02431	494	12.02
1.0113	9 988	6 623	0.02373	494	11.73
1.0113	10 020	6 655	0.02385	494	11.79

TABLET B

The following results are for sample A based on calibration equation (C-31)

Sample Weight (g)	Intensity	Corr. Intensity	Conc., ppm	D.F.	Actual Conc. (μg/g)
1.0037	10 050	6 685	0.02397	498	11.94
1.0037	10 090	6 725	0.02412	498	12.01
1.0037	9 891	6 526	0.02336	498	11.64
1.0037	10 050	6 685	0.02397	498	11.94
1.0037	10 130	6 765	0.02427	498	12.09
1.0037	9 980	6 615	0.02370	498	11.81
1.0018	10 020	6 655	0.02385	499	11.90
1.0018	9 971	6 606	0.02367	499	11.81
1.0018	10 070	6 705	0.02404	499	12.00
1.0018	9 934	6 569	0.02353	499	11.74
1.0018	9 967	6 602	0.02365	499	11.80
1.0018	9 961	6 596	0.02363	499	11.79
1.0029	9 982	6 617	0.02371	499	11.82
1.0029	9 779	6 414	0.02294	499	11.44
1.0029	9 511	6 146	0.02192	499	10.93
1.0029	9 949	6 584	0.02358	499	11.76
1.0029	9 694	6 329	0.02262	499	11.28
1.0029	9 833	6 468	0.02314	499	11.54